The background of the cover is a microscopic image of green algae cells. The cells are elongated and spindle-shaped, with a distinct outer wall and internal organelles. They are arranged in a somewhat regular pattern, with some cells appearing to be part of a filamentous structure. The color is a vibrant green, and the lighting is bright, highlighting the cellular details.

Fourth Edition

Robert Edward Lee

# Phycology

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# Phycology

Phycology is the study of algae, the primary photosynthetic organisms in freshwater and marine food chains. As a food source for zooplankton and filter-feeding shellfish, the algae are an extremely important group.

Since the publication of the first edition in 1980, this textbook has established itself as a classic resource on phycology. This revised edition maintains the format of previous editions, whilst incorporating the latest information from nucleic acid sequencing studies. Detailed life-history drawings of algae are presented alongside information on the cytology, ecology, biochemistry, and economic importance of selected genera.

Phycology is suitable for upper-level undergraduate and graduate students following courses in phycology, limnology or biological oceanography. Emphasis is placed on those algae that are commonly covered in phycology courses, and encountered by students in marine and freshwater habitats.

ROBERT LEE has had a long and varied career, teaching worldwide in countries including South Africa and Iran, as well as at Harvard Medical School, and Colorado State University, where he currently works as a Teaching Coordinator in the Department of Biomedical Sciences.



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# Phycology

Fourth edition

Robert Edward Lee  
*Colorado State University, USA*



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To Patricia, Nicole, Alana, and Christian



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## Preface to the first edition

It was that eccentric British soldier of fortune Col. Meinertzhagen, in his *Birds of Arabia*, who expressed the sentiment that prefaces should be kept short because few people ever read them. Accordingly, I would like to take a brief opportunity to express my gratitude to the people who offered encouragement and assistance during the preparation of this book. I would like to thank Adele Strauss Wolbarst, Robert Cnoops, Charmaine Slack, Sophia Skiordis, Caroline Mondel, Jill Keetley-Smith, Heather Edwards, Gail Arbeter, and the Lending Library at Boston Spa, England, for help while most of this manuscript was being prepared at the University of the Witwatersrand. For general encouragement while at Pahlavi (Shiraz) University and for providing assistance during the last turbulent and chaotic

year of imperial rule in Iran, while the manuscript was being finished, I would like to thank Mark Gettner, Brian Coad, and Mumtaz Bokhari.

When photographs or drawings have been taken directly from the original material, this is indicated by stating in the legend that it is *from* the original work. Most of the drawings have been redrawn to suit my tastes, and these drawings are indicated by stating that the work is *after* the original. In some cases I have made drawings from photographs or have incorporated a number of drawings in one, in which case I state that the finished drawing is *adapted* from the original work or works.

I have used the metric system in this book, and the fine-structural illustrations are expressed in micrometers ( $\mu\text{m}$ ) and nanometers (nm).



# Part I

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Introduction



# Basic characteristics of the algae

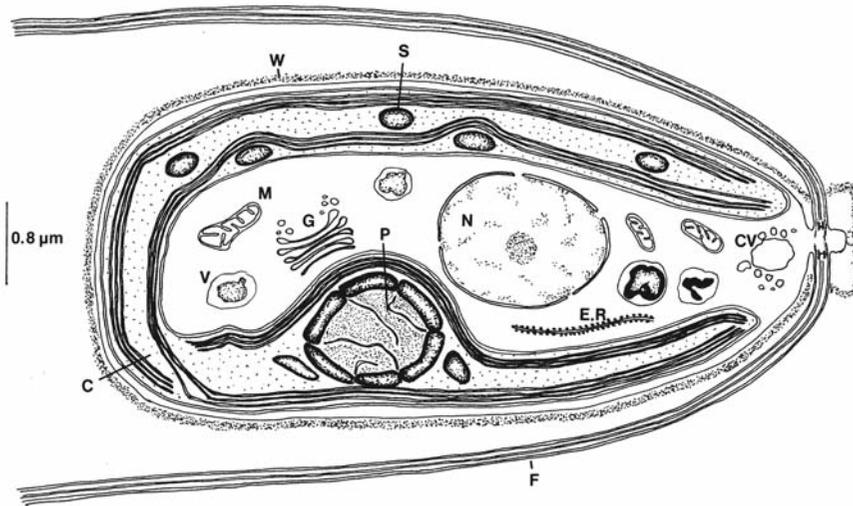
Phycology or algology is the study of the algae. The word **phycology** is derived from the Greek word *phykos*, which means “seaweed.” The term **algology**, described in Webster’s dictionary as the study of the algae, has fallen out of favor because it resembles the term *algogenic* which means “producing pain.” The algae are **thallophytes** (plants lacking roots, stems, and leaves) that have chlorophyll *a* as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cells. This definition encompasses a number of plant forms that are not necessarily closely related, for example, the cyanobacteria which are closer in evolution to the bacteria than to the rest of the algae.

Algae most commonly occur in water, be it freshwater, marine, or brackish. However, they can also be found in almost every other environment on earth, from the algae growing in the snow of some American mountains to algae living in lichen associations on bare rocks, to unicellular algae in desert soils, to algae living in hot springs. In most habitats they function as the primary producers in the food chain, producing organic material from sunlight, carbon dioxide, and water. Besides forming the basic food source for these food chains, they also form the oxygen necessary for the metabolism of the consumer organisms. In such cases humans rarely directly consume the algae as such, but harvest organisms higher up in the food chain (i.e., fish, crustaceans, shellfish). Some algae, particularly the reds and browns, are harvested and eaten as a vegetable, or the mucilages are extracted from the thallus for use as gelling and thickening agents.

## Structure of the algal cell

There are two basic types of cells in the algae, **prokaryotic** and **eukaryotic**. Prokaryotic cells lack membrane-bounded organelles (plastids, mitochondria, nuclei, Golgi bodies, and flagella) and occur in the cyanobacteria (Fig. 2.11). The remainder of the algae are eukaryotic and have organelles.

A eukaryotic cell (Fig. 1.1) is often surrounded by a cell wall composed of polysaccharides that are partially produced and secreted by the Golgi body. The plasma membrane (plasmalemma) surrounds the remaining part of the cell; this membrane is a living structure responsible for controlling the influx and outflow of substances in the protoplasm. Locomotory organs, the flagella, propel the cell through the medium by their beating. The flagella are enclosed in the plasma membrane and have a specific number and orientation of microtubules. The nucleus, which contains the genetic material of the cell, is surrounded by a double membrane with pores in it. The contents of the nucleus are a nucleolus, chromosomes, and the background material or karyolymph. The chloroplasts have membrane sacs called thylakoids that carry out the light reactions of photosynthesis. The thylakoids are embedded in the stroma where the dark reactions of carbon fixation take place. The stroma has small 70S ribosomes, DNA, and in some cases the storage product. Chloroplasts are surrounded by the two membranes of the chloroplast envelope. Sometimes chloroplasts have a dense proteinaceous area, the pyrenoid, which is associated with storage-product formation.



**Fig. 1.1** Drawing of a cell of the green alga *Chlamydomonas* showing the organelles present in a eukaryotic algal cell. (C) Chloroplast; (CV) contractile vacuole; (E.R.) endoplasmic reticulum; (F) flagella; (G) Golgi body; (M) mitochondrion; (N) nucleus; (P) pyrenoid; (S) starch; (V) vacuole; (W) wall.

Double-membrane-bounded mitochondria have 70S ribosomes and DNA, and contain the respiratory apparatus. The Golgi body consists of a number of membrane sacs, called cisternae, stacked on top of one another. The Golgi body functions in the production and secretion of polysaccharides. The cytoplasm also contains large 80S ribosomes and lipid bodies.

### Flagella

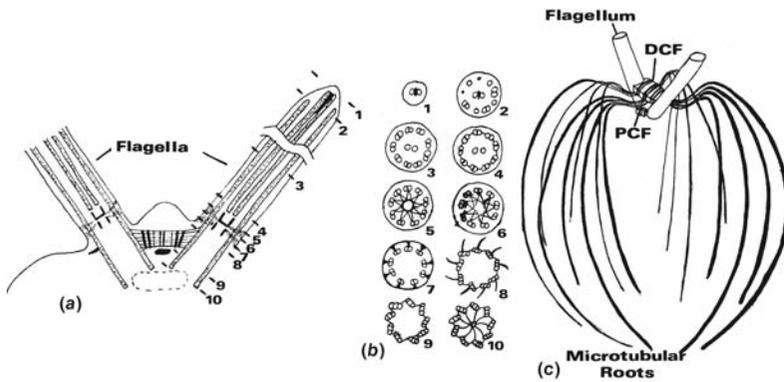
The flagella of the green alga *Chlamydomonas* have been used as a model of flagellar structure. Flagella structure has been highly conserved throughout evolution, images from *Chlamydomonas* are virtually indistinguishable from flagella (or cilia – a term for a short flagellum) of mammalian cells including human sperm and certain epithelia (Johnson, 1995). *Chlamydomonas* has been chosen because of the ease of growing the organism and because the flagella can be detached from the cells by pH shock or blending. Since the flagella are not essential for viability of the cell, it is relatively easy to isolate mutations affecting flagella synthesis by the cells.

A flagellum consists of an **axoneme** of nine doublet microtubules that surround two central

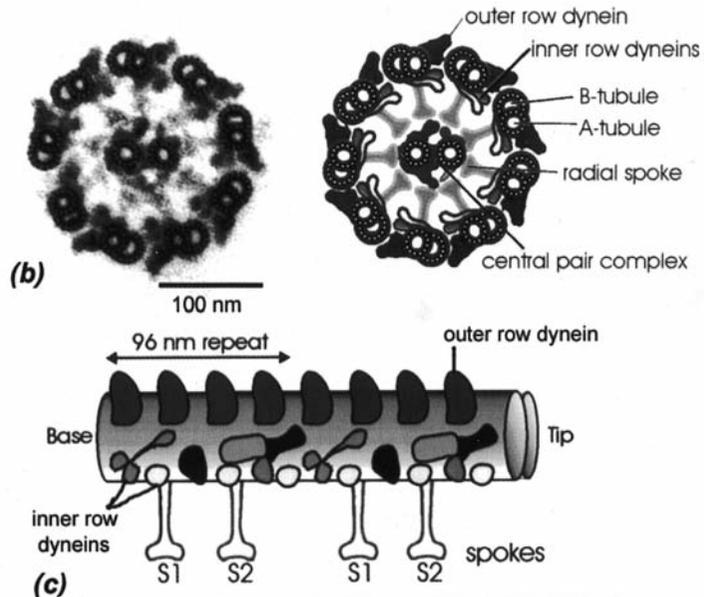
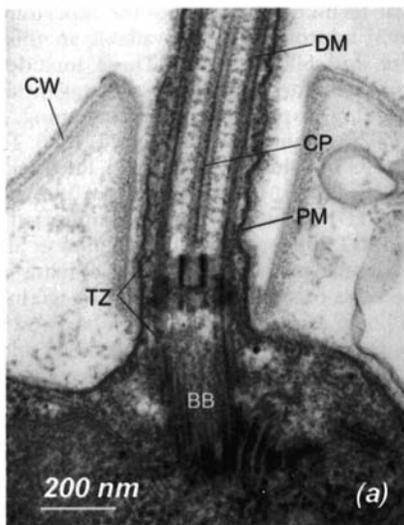
microtubules, with all of the microtubules encased in the plasma membrane (Figs. 1.2, 1.3). On entering the cell body, the two central microtubules end at a dense plate, whereas the nine peripheral doublets continue into the cell, usually picking up an additional structure that transforms them into triplets. The flagellum passes through a tunnel in the cell wall called the **flagellar collar**.

The central pair of microtubules are single microtubules with 13 protofilaments while the outer microtubules are doublets with the A-tubule consisting of 13 protofilaments and the B-tubule having 11 protofilaments. The central-pair microtubules resemble cytoplasmic microtubules, in that they are more labile than the outer doublet microtubules. The axoneme microtubules are composed of  $\alpha$ - and  $\beta$ -tubulin which make up 70% of the protein mass of the axoneme (Dutcher, 1995). **Radial spokes**, each consisting of a thin stalk and head, project from the A-tubule of the outer microtubule doublets (Figs. 1.2, 1.3).

Inner and outer **dynein arms** attach to the A-tubule of the outer microtubule doublet and extend to the B-tubule of the adjacent outer microtubule doublet. Dynein is a mechanoenzyme that hydrolyzes ATP with the resulting energy used by dynein to move along the B-tubule of the adjacent outer microtubule doublet (Fig. 1.3). In this action, the B-tubule is called the **track** while the A-tubule is called the **cargo**. The resulting displacement of outer microtubule



**Fig. 1.2** The flagellar system in the green alga *Chlamydomonas*. (a) A diagrammatic drawing of a section of the flagellar system. The numbers refer to cross sections of the flagellar system in (b). (c) Diagrammatic drawing of the whole flagellar apparatus. The two flagella are joined by the proximal connecting fiber (PCF) and distal connecting fiber (DCF). (After Ringo, 1967.)

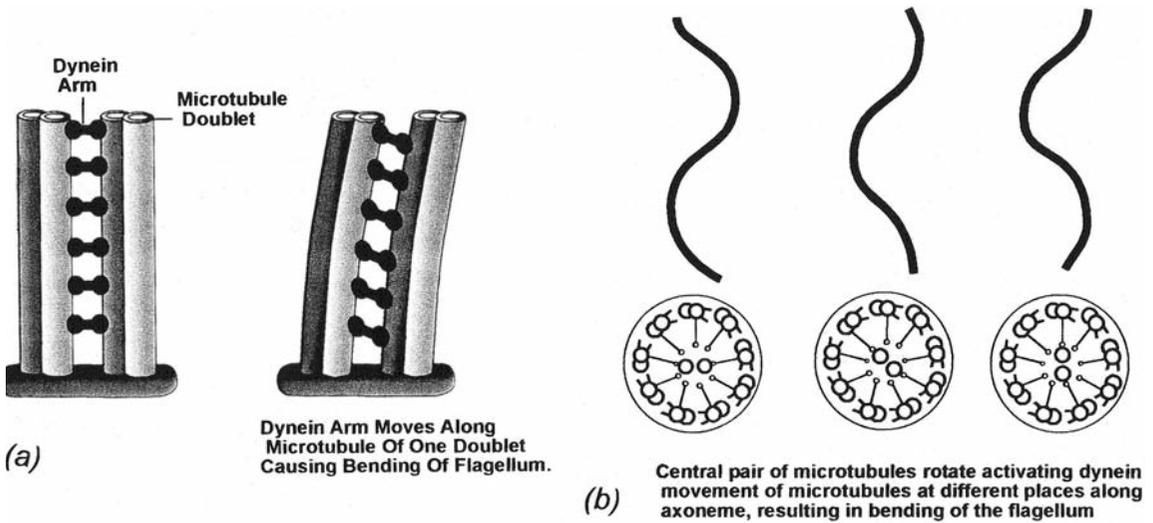


**Fig. 1.3** *Chlamydomonas* flagella. (a) Transmission electron micrograph through the anterior region of a *Chlamydomonas reinhardtii* cell including the cell wall (CW), double microtubules (DM), central pair microtubules (CP), plasma membrane (PM), transition zone (TZ), and basal body (BB). (b) Thin section through an isolated demembrated flagellar axoneme showing the main components. (c) Diagrams of dyneins and related structures seen along the A-tubule of each doublet. (From Mitchell, 2000.)

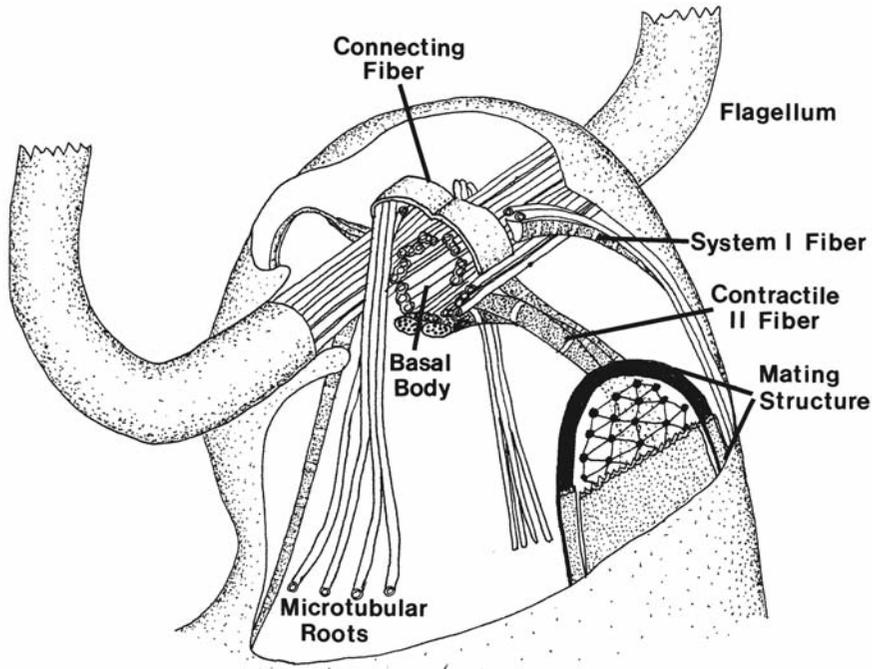
doublets in relation to each other causes bending of the flagellum (Mitchell, 2000). Kinesin proteins cause the central pair of microtubules to rotate within the axoneme (Fig. 1.4). As the central pair of microtubules rotates, the microtubules interact with the individual radial spokes inducing sliding between adjacent microtubule doublets,

asymmetric bending of the flagellum and propagation of flagellar waves (Johnson, 1995).

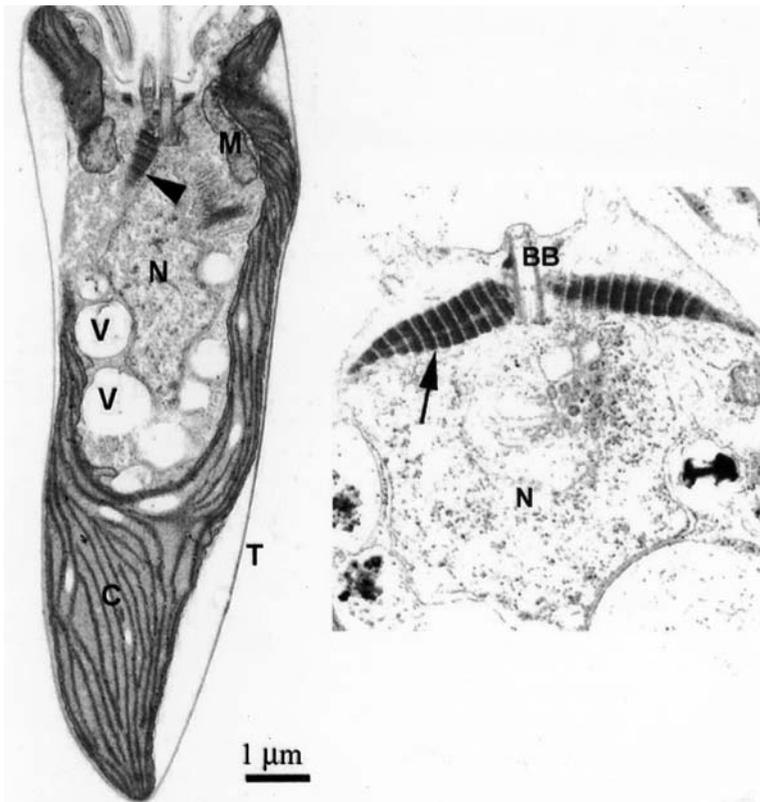
There are also other structures between the microtubules in the basal region of the flagellum (**basal body**). Attached to the basal body there can be either microtubular roots or striated fibrillar roots. The former type of root consists of a group of microtubules running from the basal body into the protoplasm (Figs. 1.2, 1.4), whereas the latter consists of groups of fibers that have striations along their length (Figs. 1.4, 1.6) The gamete of the green seaweed *Ulva lactuca* (sea lettuce) has both types of flagellar roots (Fig. 1.5) (Melkonian, 1980; Andersen et al., 1991). There are four **microtubular roots** composed of microtubules arranged in a cruciate pattern, and **fibrous roots (rhizoplasts)** composed of a bundle



**Fig. 1.4** Bending of flagella occurs by the rotating central pair of microtubules activating dynein movement of specific outer doublet microtubules.



**Fig. 1.5** Schematic three-dimensional reconstruction of the flagellar apparatus of a female gamete of *Ulva lactuca* showing the four cruciately arranged microtubular roots and the fibrous contractile roots. (Adapted from Melkonian, 1980.)



**Fig. 1.6** Transmission electron micrographs of striated roots (rhizoplasts) in the green alga *Scherffelia dubia* (Chlorophyta). Arrow and arrowhead point to a striated root. (BB) Basal body; (C) chloroplast; (M) mitochondrion; (N) nucleus; (V) vacuole. (From Vierkotten et al., 2004.)

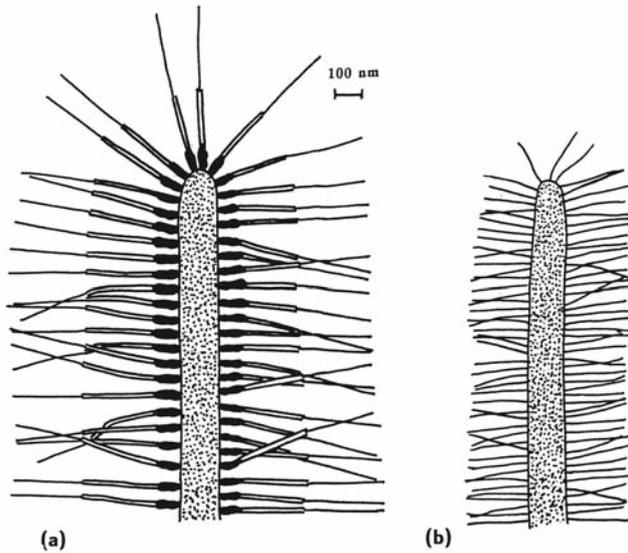
of filaments (Fig. 1.6). There are two types of fibrous roots: (1) **system I fibrous roots** composed of 2 nm filaments cross-striated with a periodicity of approximately 30 nm and (2) **system II fibrous roots** composed of 4–8 nm filaments usually cross-striated with a periodicity greater than 80 nm. System I fibrous roots are non-contractile while system II fibrous roots are contractile when appropriately stimulated (Moestrup, 2000; Brugerolle and Mignot, 2003).

The flagellar membrane may have no **hairs** (**mastigonemes**) on its surface (**whiplash** or **acronematic flagellum**) or it may have hairs on its surface (**tinsel** or **hairy** or **pantonematic** or **Flimmergeissel**). There are two types of flagellar hair (Fig. 1.7):

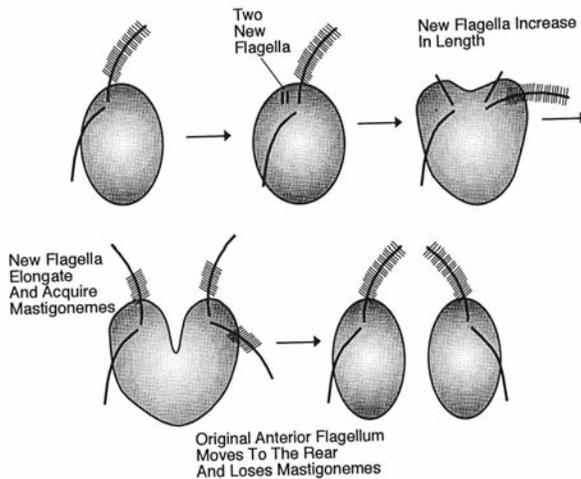
**1 Non-tubular flagellar hairs** made up of solid fibrils 5–10 nm wide and 1–3  $\mu\text{m}$  long that are composed of glycoproteins. These hairs are flexible and wrap around the flagellum increasing the surface area and efficiency of propulsion.

**2 Tubular flagellar hairs** about 2  $\mu\text{m}$  long composed of three regions: (1) a tapering basal region 200 nm long attached to the flagellar membrane, (2) a microtubular shaft 1  $\mu\text{m}$  long, and (3) a few 0.52  $\mu\text{m}$ -long terminal filaments (Andersen et al., 1991).

The bases of the hairs do not penetrate the flagellar membrane but are stuck to it. Development of the tubular hairs begins in the space between the inner and outer membrane of the nuclear envelope (perinuclear continuum) where the basal and microtubular regions are assembled. These then pass to the Golgi apparatus, where the terminal filaments are added. Finally the hairs are carried to the plasma membrane in Golgi vesicles, where they are discharged and attached to the flagellar membrane. Tripartite tubular hairs occur in the Heterokontophyta. The term **stramenopile** (straw hair) has been used to include all protists with tubular hairs (van der Auwera and deWachter, 1997). In addition to the algae in the Heterokontophyta, the



**Fig. 1.7** Drawings of the types of hairs on algal flagella. (a) Tripartite hairs (example *Ascophyllum* sperm). Each hair is composed of a basal region attached to the flagellar membrane, the microtubular shaft, and a terminal hair. (b) Non-tubular hairs (example *Chlamydomonas* gamete). ((a) adapted from Bouck, 1969; (b) from Snell, 1976.)



**Fig. 1.8** The sequence of flagellar transformation during cell division.

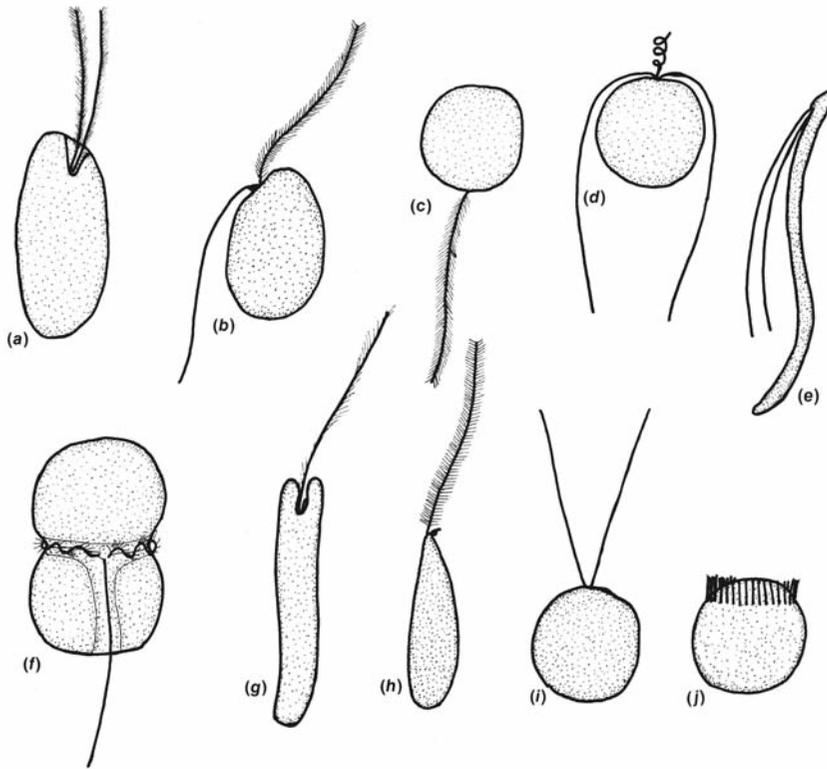
stramenopiles include the fungal oomycetes, hyphochytridomycetes, thraustochytrids, and the bicosoecids and labyrinthulids.

The remainder of the algae have non-tubular hairs if hairs occur on the flagella (Moestrup, 1982). In addition to hairs, a number of different scale types occur on the surface of the flagella. These will be discussed in the chapters on the individual algal groups.

Flagella progress through a set of developmental cycles during cell division (Fig. 1.8). A biflagellate cell with an anterior flagellum covered with tubular hairs (tinsel flagellum), and a posterior smooth flagellum (whiplash flagellum), will be used as an example. Before the onset of cell

division, two new flagella appear next to the anterior flagellum. These two new flagella elongate while the original anterior flagellum moves toward the posterior of the cell and loses its tubular hairs, to become the posterior smooth flagellum of one of the daughter cells. The two new flagella at the anterior end of the cell acquire tubular hairs and become the tinsel flagella of the daughter cells. Thus, each daughter cell has one new anterior tinsel flagellum, and one posterior smooth whiplash flagellum that was originally a flagellum in the parent cell (Beech and Wetherbee, 1990; Melkonian et al., 1987).

Algal cells can have different arrangements of flagella (Fig. 1.9). If the flagella are of equal length,



**Fig. 1.9** The shape of eukaryotic motile algal cells and their flagella. The drawings represent the common arrangement of flagella in the groups. There are a number of modifications in structure that are not included here. (a) Cryptophyta; (b) most of the Heterokontophyta; (c) Bacillariophyceae of the Heterokontophyta; (d) Prymnesiophyta; (e) Chlorophyta; (f) Dinophyta; (g) Euglenophyta; (h) Eustigmatophyceae of the Heterokontophyta; (i, j) Chlorophyta.

they are called **isokont** flagella; if they are of unequal length, they are called **anisokont flagella**; and if they form a ring at one end of the cell, they are called **stephanokont** flagella. **Heterokont** refers to an organism with a hairy and a smooth flagellum (Moestrup, 1982).

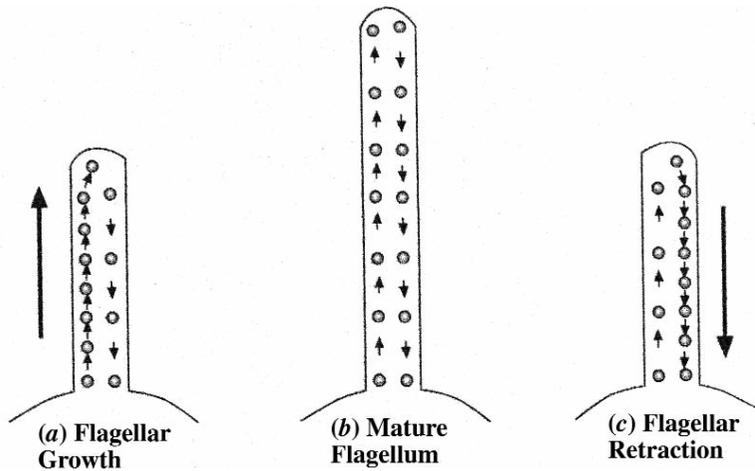
Flagella can be of different length in the same cell. This is controlled by **intraflagellar transport**, defined as the *bi-directional movement of particles along the length of the flagellum between the axoneme and the flagellar membrane* (Beech, 2003). A mature flagellum that is not elongating has a steady disassembly of the flagellum that is countered by an equally steady assembly provided by intraflagellar transport (Fig. 1.10). A change in length of the

flagellum is produced by an imbalance in the assembly or disassembly of flagellar components (Rosenbaum and Witman, 2002). Thus, disassembly occurs faster than assembly in flagellar retraction. The opposite occurs during flagellar growth. The differences in length of flagella arise from the shorter flagellum being delayed in the initial stages of construction. The assembly rate of the shorter flagellum is the same as the longer flagellum. There may be a gate at the base of the flagellum that regulates the passage of flagellar precursors into the basal body and the flagellum (Schoppmeier and Lechtreck, 2003).

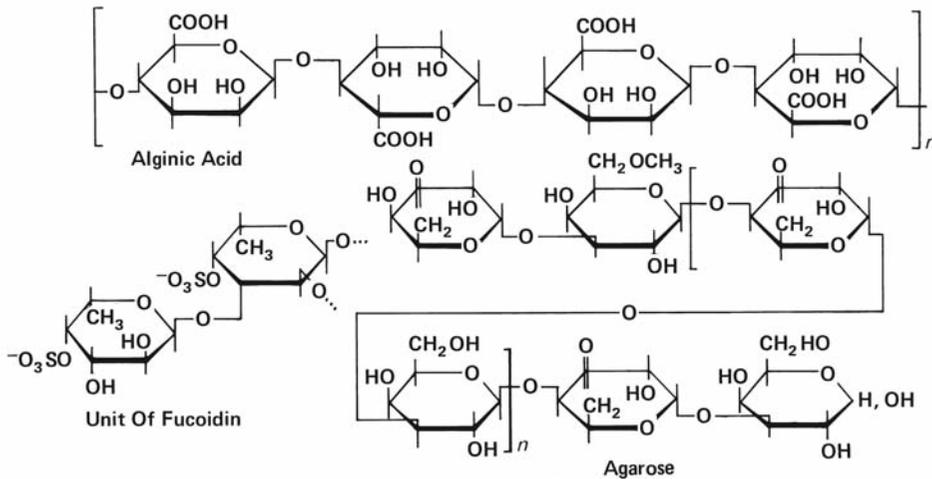
### Cell walls and mucilages

In general, algal cell walls are made up of two components: (1) the fibrillar component, which forms the skeleton of the wall, and (2) the amorphous component, which forms a matrix within which the fibrillar component is embedded.

The most common type of fibrillar component is **cellulose**, a polymer of 1,4 linked  $\beta$ -D-glucose. Cellulose is replaced by a **mannan**, a polymer of 1,4 linked  $\beta$ -D-mannose, in some siphonaceous



**Fig. 1.10** (a) Intraflagellar transport results in more assembly of flagellar subunits than disassembly during flagellar growth. (b) A mature flagellum has an equal amount of assembly and disassembly of flagellar subunits. (c) There is more disassembly of flagellar subunits during flagellar retraction.



**Fig. 1.11** Structural units of alginic acid, fucoidin, and agarose. (After Percival and McDowell, 1967.)

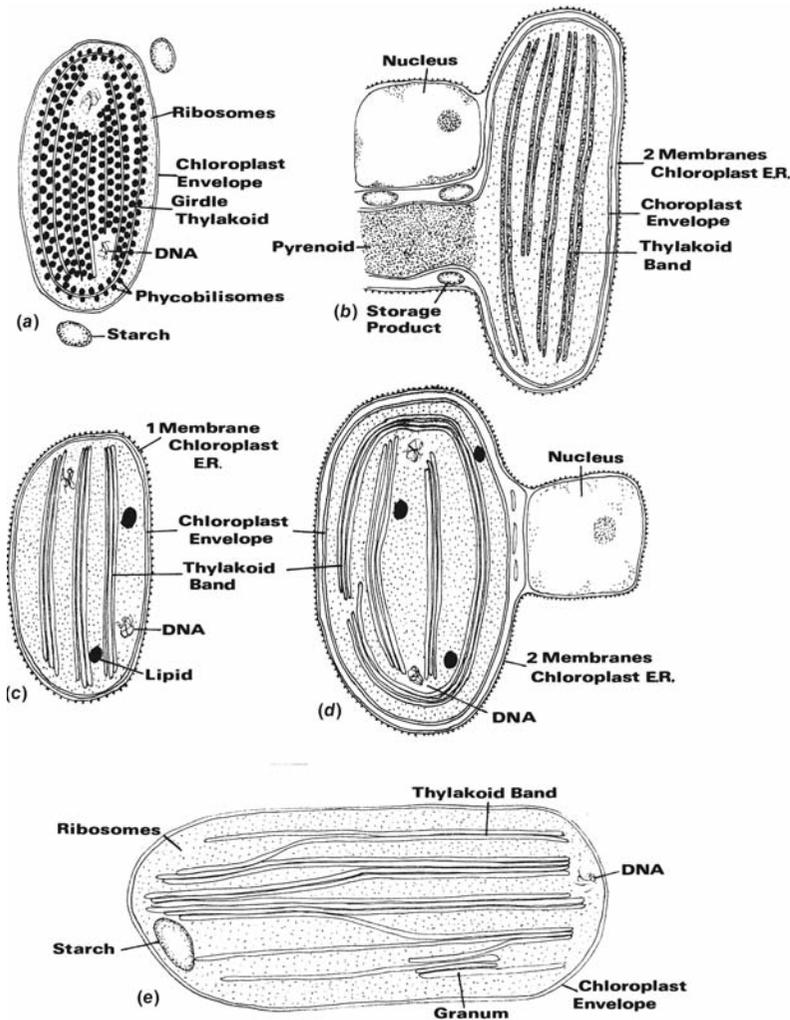
greens, and in *Porphyra* and *Bangia* in the Rhodophyta. In some siphonaceous green algae and some Rhodophyta (*Porphyra*, *Rhodochorton*, *Laurencia*, and *Rhodymenia*), fibrillar xylans of different polymers occur.

The amorphous mucilaginous components occur in the greatest amounts in the Phaeophyceae and Rhodophyta, the polysaccharides of which are commercially exploited. **Alginic acid** (Fig. 1.11) is a polymer composed mostly of  $\beta$ -1,4 linked D-mannuronic acid residues with variable amounts of L-guluronic acid. Alginic acid is present in the intercellular spaces and cell walls of the

Phaeophyceae. **Fucoidin** (Fig. 1.11) also occurs in the Phaeophyceae and is a polymer of  $\alpha$ -1, 2,  $\alpha$ -1, 3, and  $\alpha$ -1, 4 linked residues of L-fucose sulfated at C-4. In the Rhodophyta the amorphous component of the wall is composed of galactans or polymers of galactose, which are alternatively  $\beta$ -1,3 and  $\beta$ -1,4 linked. These galactans include **agar** (made up of **agaropectin** and **agarose**, Fig. 1.11) and **carrageenan** (Fig. 4.15).

### Plastids

The basic type of plastid in the algae is a **chloroplast**, a plastid capable of photosynthesis. **Chromoplast** is synonymous with chloroplast; in the older literature a chloroplast that has a color other than green is often called a chromoplast. A **proplastid** is a reduced plastid with few if any



**Fig. 1.12** Types of chloroplast structure in eukaryotic algae. (a) One thylakoid per band, no chloroplast endoplasmic reticulum (Rhodophyta). (b) Two thylakoids per band, two membranes of chloroplast E.R. (Cryptophyta). (c) Three thylakoids per band, one membrane of chloroplast E.R. (Dinophyta, Euglenophyta). (d) Three thylakoids per band, two membranes of chloroplast E.R. (Prymnesiophyta and Heterokontophyta). (e) Two to six thylakoids per band, no chloroplast E.R. (Chlorophyta).

thylakoids. A proplastid will usually develop into a chloroplast although in some heterotrophic algae it remains a proplastid. A **leucoplast** or **amyloplast** is a colorless plastid that has become adapted for the accumulation of storage product.

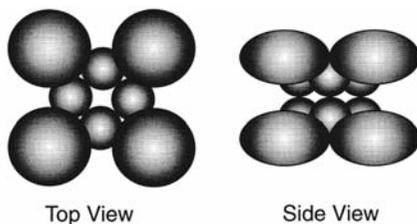
In the Rhodophyta and Chlorophyta, the chloroplasts are bounded by the double membrane of the chloroplast envelope (Fig. 1.12(a), (e)). In the other eukaryotic algae, the chloroplast envelope is surrounded by one of two membranes of **chloroplast endoplasmic reticulum** (chloroplast E.R.), which has ribosomes attached to the outer face of the membrane adjacent to the cytoplasm. The chloroplast E.R. is the remnant of the food vacuole membrane and/or the plasma membrane involved in the original endosymbiosis leading

to the chloroplasts in a secondary endosymbiosis. In the Euglenophyta and Dinophyta, there is one membrane of chloroplast E.R. (Fig. 1.12(c)). In the Cryptophyta, Prymnesiophyta, and Heterokontophyta, there are two membranes of chloroplast E.R., with the outer membrane of chloroplast E.R. usually continuous with the outer membrane of the nuclear envelope, especially if the chloroplast number is low (Fig. 1.12 (b), (d)).

The basic structure of the photosynthetic apparatus in a plastid consists of a series of flattened membranous vesicles called **thylakoids** or **discs**, and a surrounding matrix or **stroma**. The thylakoids contain the chlorophylls and are the sites of the photochemical reactions; carbon dioxide fixation occurs in the stroma. The thylakoids can

be free from one another or grouped to form **thylakoid bands**. In the cyanobacteria and Rhodophyta (Fig. 1.12(a)), the thylakoids are usually free from one another, with **phycobilisomes** (containing the phycobiliproteins) on the surface of the thylakoids. The phycobilisomes on the surface of one thylakoid alternate with those on the surface of an adjacent thylakoid. The phycobilisomes appear as 35-nm granules when phycoerythrin predominates, or as discs when phycocyanin predominates. In the more primitive members of the Rhodophyta the thylakoids terminate close to the chloroplast envelope, whereas in advanced members of the Rhodophyta peripheral thylakoids are present, which enclose the rest of the thylakoids. In the Cryptophyta, the chloroplasts contain bands of two thylakoids (Fig. 1.12(b)); the phycobiliproteins are dispersed within the thylakoids. In the Euglenophyta and Heterokontophyta the thylakoids are grouped in bands of three with a girdle or peripheral band running parallel to the chloroplast envelope. In the Dinophyta, Prymnesiophyta, and Eustigmatophyceae, the thylakoids are also in bands of three, but there is no girdle band (Fig. 1.12(c), (d)). In the Chlorophyta, the thylakoids occur in bands of two to six, with thylakoids running from one band to the next. The above grouping of algal thylakoids into bands occurs under normal growth conditions. Abnormal growth conditions commonly cause lumping of thylakoids and other variations in structure.

A pyrenoid (Fig. 1.12(b)) is a differentiated region within the chloroplast that is denser than the surrounding stroma and may or may not be traversed by thylakoids. A pyrenoid is frequently associated with storage product. Pyrenoids contain



**Fig. 1.13** The structure of Form I variation of Rubisco showing the eight large subunits and eight small subunits.

ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco), the enzyme that fixes carbon dioxide (Jenks and Gibbs, 2000; Nagasato et al., 2003). Consequently, the size of the pyrenoid will vary depending on how much Rubisco is present.

Rubisco exists in two forms (Jenks and Gibbs, 2000; Zhang and Lin, 2003):

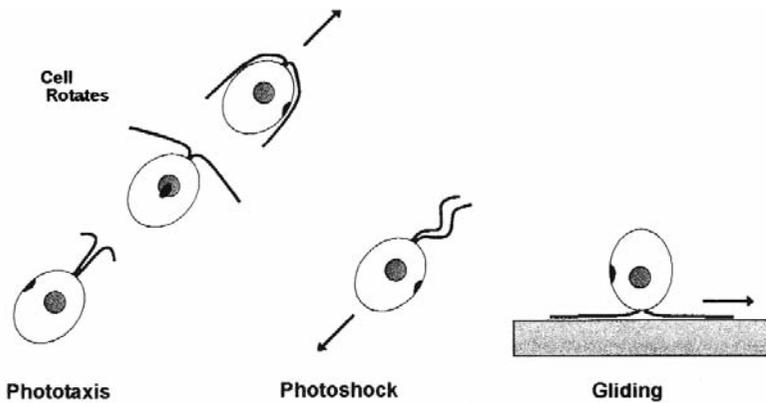
- 1 Form I occurs in some bacteria, the cyanobacteria, in all green plants and non-green plants. Form I is composed of eight large subunits and eight small subunits (Fig. 1.13). Form I has a high affinity for  $\text{CO}_2$  and a low catalytic efficiency (low rate of  $\text{CO}_2$  fixation). In green algae, euglenoids, and green plants, the large subunit is coded by chloroplast DNA and the small subunit by nuclear DNA. In the cyanelle (endosymbiotic cyanobacterium) of *Cyanophora paradoxa* and in some non-green algae, both subunits are coded by chloroplast DNA.
- 2 Form II occurs in some eubacteria and in the dinoflagellates and is composed of two large subunits. Form II has a low affinity for  $\text{CO}_2$  and a high catalytic efficiency.

The common ancestor of all ribulose-1,5-bisphosphate carboxylase was probably similar to Form II and was adapted to the anaerobic conditions and high  $\text{CO}_2$  concentrations prevailing in the ancient earth (Haygood, 1996). Form I evolved as the earth's atmosphere became oxygenated, and  $\text{CO}_2$  concentration declined and with it the need for a greater affinity for  $\text{CO}_2$ . The greater affinity for  $\text{CO}_2$  in Form I, however, came at the price of reduced catalytic efficiency.

Chloroplasts contain small (30–100 nm), spherical lipid droplets between the thylakoids (Fig. 1.12 (c), (d)). These lipid droplets serve as a pool of lipid reserve within the chloroplast.

Many motile algae have groups of tightly packed carotenoid lipid-globules that constitute an orange-red eyespot or stigma (Fig. 5.2) that is involved in response to light. Motile algae exhibit three types of responses to light (Kawai and Kreimer, 2000): phototaxis, photophobia, and gliding (Fig. 1.14).

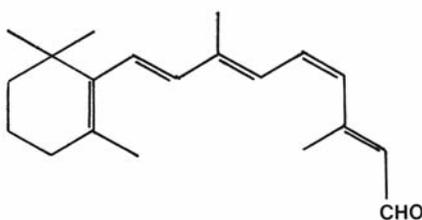
- 1 **Phototaxis.** In phototaxis, the orientation of cell movement is effected by the **direction** and



**Fig. 1.14** Three types of flagellar orientation in *Chlamydomonas*. In **phototaxis**, the cells swim forward and rotate. Phototaxis requires that cells swim forward in a spiral path that causes rotation of the symmetrically placed eyespot. In **photoshock**, the cell has a transient avoidance response that causes the cell to swim backwards. In **gliding**, the leading flagellum and passive flagellum are 180° apart.

intensity of light. The cells move toward the light in **positive phototaxis** and away from the light in **negative phototaxis**. The photoreceptor in the green alga *Chlamydomonas* is **chlamyrodopsin** (Fig. 1.15) in the plasma membrane over the eyespot. Chlamyrodopsin contains an all-*trans*, 6-*S-trans* retinal chromophore that undergoes a 13-*trans* to *cis* isomerization during illumination (Hegemann, 1997). The eyespot periodically shades the photoreceptor as the cell rotates during swimming. The eyespot has a different structure in the different groups of algae and will be covered in the appropriate chapters. Eyespots have certain basic characteristics (Kawai and Kreimer, 2000): (1) Eyespots usually have carotenoid-rich lipid globules packed in a highly ordered hexagonal arrangement. (2) Eyespots are usually single structures in peripheral positions, most often oriented perpendicular to the axis of the swimming path.

Phototaxis in *Chlamydomonas* is controlled by the beating of each flagellum. The flagellum closest to the eyespot is the *cis* flagellum while



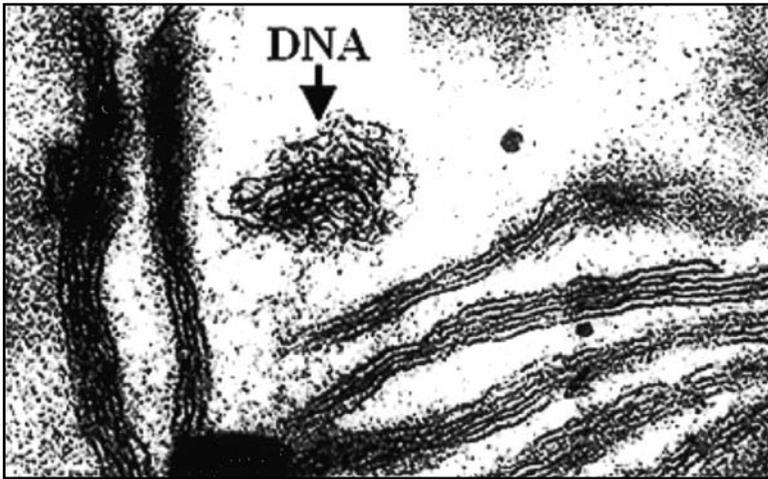
**Fig. 1.15** The structure of chlamyrodopsin, the photoreceptor in *Chlamydomonas*.

the *trans* flagellum is furthest from the eyespot. The light is received by the photoreceptor which controls the opening and closing of calcium channels, and the level of intraflagellar calcium concentration. The calcium concentration within the flagellum effects the interactions of the radial spokes with the central pair of microtubules (Mitchell, 2000). When the plasma membrane of *Chlamydomonas* is made permeable, *Chlamydomonas* cells swim normally at  $10^{-8}$  M calcium in the medium. Decreasing the calcium to  $10^{-9}$  M reduces the stroke velocity of the *trans* flagellum, while increasing the calcium to  $10^{-7}$  M reduces the stroke velocity of the *cis* flagellum.

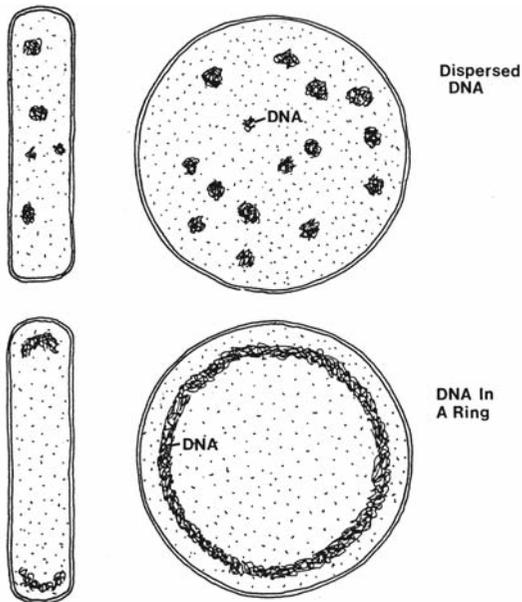
- 2 Photophobia (photoshock).** Photophobia is a change in direction of movement of the cell caused by a rapid change in light intensity, **irrespective of the direction of the light**. Swimming cells stop and change the beat pattern from the normal asymmetric flagellar stroke to a symmetrical stroke that propels the cell backward (Fig. 1.14). At the end of the photophobic response, the cells tumble and resume swimming in a new direction.

Laboratory experiments with *Chlamydomonas* link photophobic responses to increases in calcium above  $10^{-6}$  M (Mitchell, 2000). Unlike phototaxis, interactions between radial spokes and central-pair microtubules are not necessary for a photophobic reaction.

- 3 Gliding (quiescence).** In gliding, the flagella stop beating and adhere to a surface or an



**Fig. 1.16** Transmission electron micrograph of DNA in the chloroplast of the dinoflagellate *Prorocentrum micans*. (From Laatsch et al., 2004.)



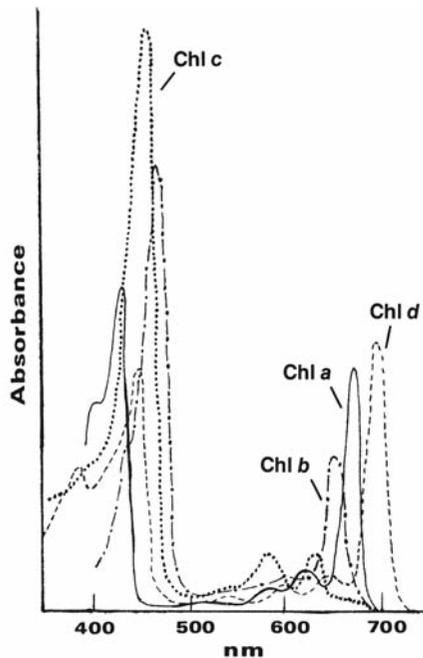
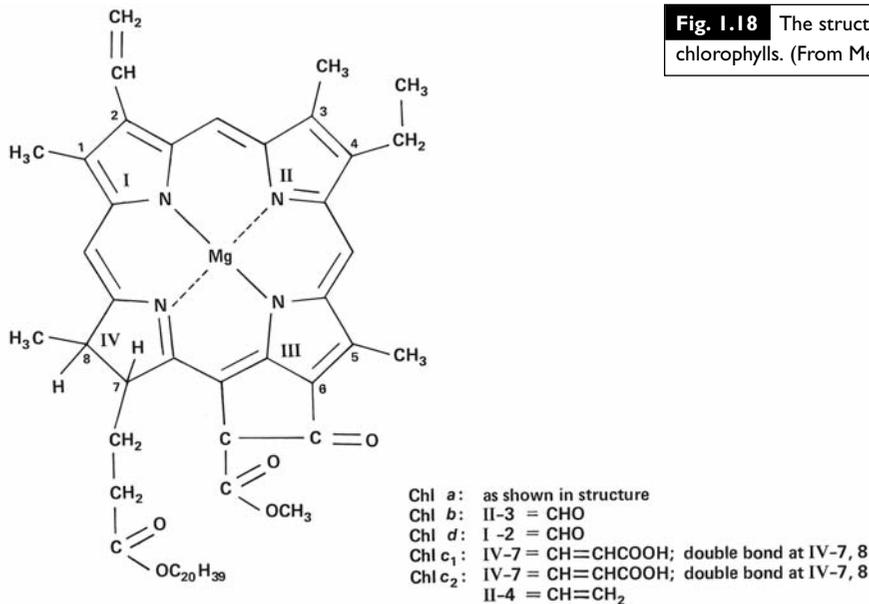
**Fig. 1.17** Semidiagrammatic drawing of the two types of distribution of DNA in algal chloroplasts. Side and face views of the plastids are drawn. (Adapted from Coleman, 1985.)

air/water interface (Mitchell, 2000). The cells can glide over the surface with one flagellum actively leading and the other passively trailing (Fig. 1.14). Cells may switch direction by changing which flagellum is active. Gliding motility may be a common phenomenon among organisms that live in the thin film of water on soil particles.

In the Chlorophyta (Fig. 5.2), Cryptophyta (Fig. 9.4) and most of the Heterokontophyta (Fig. 10.1), the eyespot occurs as lipid droplets in the chloroplast. In the Euglenophyta (Fig. 6.2), Eustigmatophyceae (Fig. 12.1), and Dinophyta (Figs. 7.21, 7.22, 7.23), the eyespot occurs as a group of membrane-bounded lipid droplets, free of the chloroplast.

Most chloroplasts contain prokaryotic DNA in an area of the chloroplast devoid of 70S ribosomes (Figs. 1.16 and 1.17). The DNA is an evolutionary remnant of the cyanobacterium involved in the endosymbiosis leading to the chloroplast. The individual DNA microfibrils are circular, are attached to the chloroplast membranes, and lack basic proteins (**histones**). The algae can be divided into two general groups according to the distribution of DNA in the plastids (Coleman, 1985). In the first group, the clumps of DNA (**nucleoids**) are scattered throughout the plastids. This group includes the Cryptophyta, Dinophyta, Prymnesiophyta, Eustigmatophyceae, Rhodophyta, and Chlorophyta. In the second group, the DNA occurs in a ring just within the girdle lamella. This group includes the Chrysophyceae, Bacillariophyceae, Raphidophyceae, and Xanthophyceae (with the exception of *Vaucheria* and three genera known to lack girdle lamellae – *Bumilleria*, *Bumilleriopsis*, and *Pseudobumilleriopsis*). The Euglenophyta fit into neither group, showing a variable distribution of chloroplast DNA.

The photosynthetic algae have chlorophyll in their chloroplasts. **Chlorophyll** is composed of a



**Fig. 1.19** The absorption spectra of chlorophylls *a*, *b*, *c*, and *d*.

porphyrin-ring system that is very similar to that of hemoglobin but has a magnesium atom instead of an iron atom (Fig. 1.18). The algae have four types of chlorophyll, *a*, *b*, *c* (*c*<sub>1</sub> and *c*<sub>2</sub>), and *d*. Chlorophyll *a* is the primary photosynthetic pigment (the light receptor in photosystem I of the

light reaction) in all photosynthetic algae and ranges from 0.3% to 3.0% of the dry weight. Chlorophyll *a* is insoluble in water and petroleum ether but soluble in alcohol, diethyl ether, benzene, and acetone. The pigment has two main absorption bands *in vitro*, one band in the red light region at 663 nm and the other at 430 nm (Fig. 1.19).

Whereas chlorophyll *a* is found in all photosynthetic algae, the other algal chlorophylls have a more limited distribution and function as accessory photosynthetic pigments. Chlorophyll *b* is found in the Euglenophyta and Chlorophyta (Fig. 1.18). Chlorophyll *b* functions photosynthetically as a light-harvesting pigment transferring absorbed light energy to chlorophyll *a*. The ratio of chlorophyll *a* to chlorophyll *b* varies from 2:1 to 3:1. The solubility characteristics of chlorophyll *a* are similar to chlorophyll *b*, and *in vitro* chlorophyll *b* has two main absorption maxima in acetone or methanol, one at 645 nm and the other at 435 nm (Fig. 1.19).

Chlorophyll *c* (Fig. 1.18) is found in the Dinophyta, Cryptophyta, and most of the Heterokontophyta. Chlorophyll *c* has two spectrally different components: chlorophyll *c*<sub>1</sub> and *c*<sub>2</sub>. Chlorophyll *c*<sub>2</sub> is always present, but chlorophyll *c*<sub>1</sub> is absent in the Dinophyta and Cryptophyta. The ratio of chlorophyll *a* to chlorophyll *c* ranges from

1.2:2 to 5.5:1. Chlorophyll *c* probably functions as an accessory pigment to photosystem II. The pigment is soluble in ether, acetone, methanol, and ethyl acetate, but is insoluble in water and petroleum ether. Extracted chlorophyll *c*<sub>1</sub> has main absorption maxima at 634, 583, and 440 nm in methanol, whereas chlorophyll *c*<sub>2</sub> has maxima at 635, 586, and 452 nm.

Chlorophyll *d* (Fig. 1.18) occurs in some cyanobacteria (Murakami et al., 2004). It has three main absorption bands at 696, 456, and 400 nm.

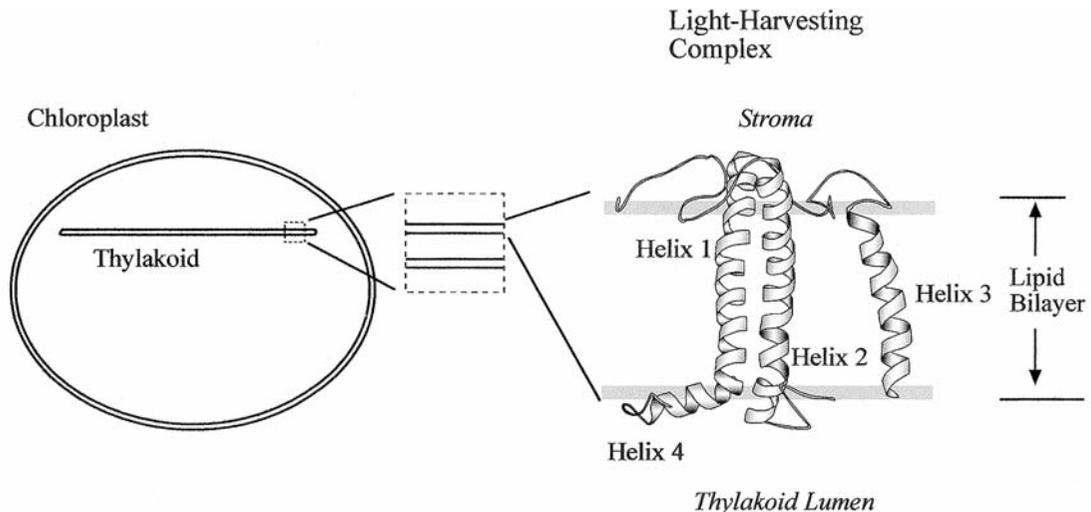
The photosynthetically active pigments of algae are gathered in discrete pigment-protein complexes which can be divided functionally into two groups (Grossman et al., 1990):

- 1 the **photochemical reaction center** containing chlorophyll *a*, where light energy is converted into chemical energy;
- 2 the **light-harvesting complexes** that serve as antennae to collect and transfer available light energy to the reaction center.

The light-harvesting complexes use different antennae pigment complexes to capture light energy. All of the light-harvesting complexes are

composed of three-membrane spanning helices (Fig. 1.20).

- 1 Green algae and higher plants use chlorophyll *a/b* binding proteins.
- 2 Brown and golden-brown algae, (diatoms, chrysophytes, dinoflagellates, brown algae, and related groups) use a fucoxanthin chlorophyll *a/c* complex that is an integral part of the thylakoid membrane. The ratio of fucoxanthin to chlorophyll in this complex is approximately 2:1 and the characteristic brown or golden-brown color of these algae is due to the high level of fucoxanthin in these cells. Due to chlorophyll *c* and special xanthophylls, these organisms are especially suited to harvest blue and green light, which are the most abundant at increasing ocean depths. This light-harvesting complex also is composed of three membrane-spanning helices and is closely related to the light-harvesting complex in the first group (Caron et al., 1996).
- 3 Cyanobacteria, cryptophytes and red algae use the phycobilisome as the major light-harvesting complex.



**Fig. 1.20** The basic structure of the light-harvesting complex in all eukaryotic plants. Three transmembrane helices traverse the membrane. The similarity of the light-harvesting complex in all eukaryotic plants is an argument for the chloroplast arising from a single endosymbiotic event. (Modified from Kuhlbrandt et al., 1994.)

**Carotenoids** are yellow, orange, or red pigments that usually occur inside the plastid but may be outside in certain cases. In general, naturally occurring carotenoids can be divided into two classes: (1) oxygen-free hydrocarbons, the **carotenes**; and (2) their oxygenated derivatives, the **xanthophylls**. The most widespread carotene in the algae is  $\beta$ -carotene (Fig. 1.21). There are a large number of different xanthophylls, with the Chlorophyta having xanthophylls that most closely resemble those in higher plants. Fucoxanthin (Fig. 1.21) is the principal xanthophyll in the golden-brown algae (Chrysophyceae, Bacillariophyceae, Prymnesiophyceae, and Phaeophyceae), giving these algae their characteristic color. Like the chlorophylls, the carotenoids are soluble in alcohols, benzene, and acetone but insoluble in water.

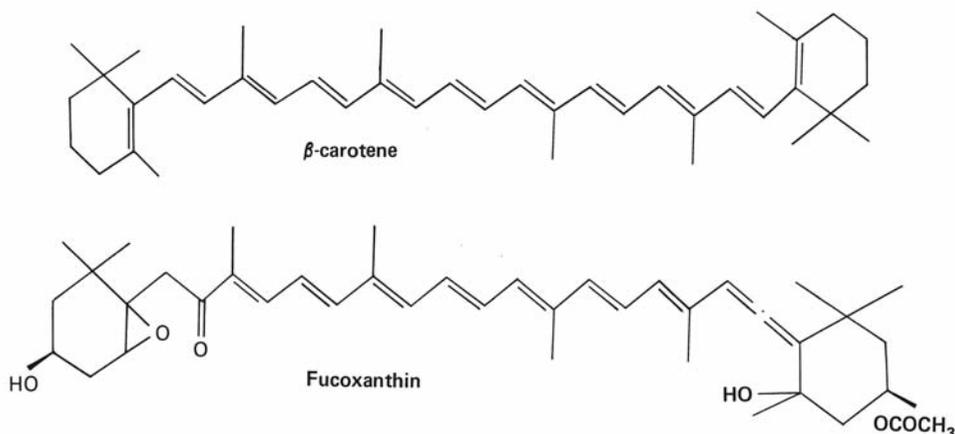
The cyanobacteria and chloroplasts of the Rhodophyta and Cryptophyta have evolved membrane-peripheral antenna complexes containing phycobiliproteins that transfer light energy to photosystem II reaction centers. Like chlorophyll *b/c/d*, the phycobiliproteins expand the range of light energy that can be utilized in photosynthesis. Light tends to become blue-green as it courses down the water column, and this light is better absorbed by the biliproteins than chlorophyll *a*.

**Phycobiliproteins** are water-soluble blue or red pigments located *on* (Cyanophyta, Rhodophyta) or *inside* (Cryptophyta) thylakoids of algal

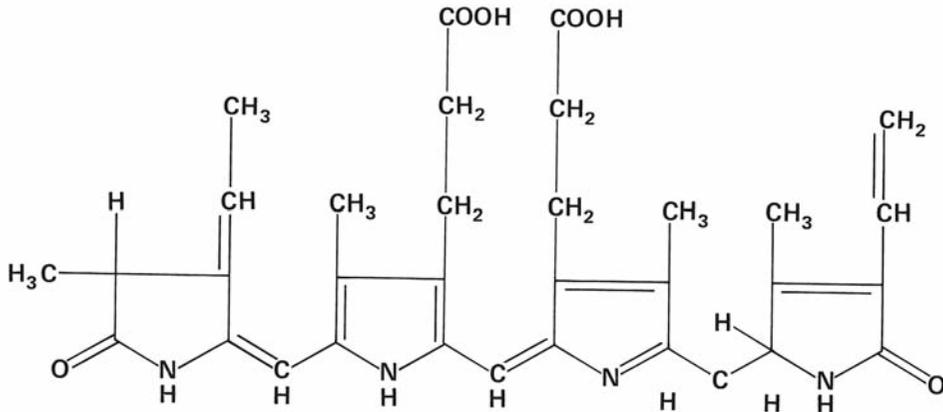
chloroplasts (Glazer, 1982). They are described as **chromoproteins** (colored proteins) in which the **prosthetic group** (non-protein part of the molecule) or **chromophore** is a tetrapyrrole (bile pigment) known as **phycobilin**. The prosthetic group is tightly bound by covalent linkages to its **apoprotein** (protein part of the molecule) (see Fig. 1.21). Because it is difficult to separate the pigment from the apoprotein, the term **phycobiliprotein** is used. There are two different apoproteins,  $\alpha$  and  $\beta$ , which together form the basic unit of the phycobiliproteins. To either  $\alpha$  or  $\beta$  are attached the colored chromophores. The major “blue” chromophore occurring in **phycocyanin** and **allophycocyanin** is **phycocyanobilin**, and the major “red” chromophore occurring in **phycoerythrin** is **phycoerythrobilin** (Fig. 1.22).

The general classification of phycobiliproteins is based on their absorption spectra. There are three types of phycoerythrin: R-phycoerythrin and B-phycoerythrin in the Rhodophyta, and C-phycoerythrin in the Cyanophyta. There are also three types of phycocyanin: R-phycocyanin from the Rhodophyta and C-phycocyanin and allophycocyanin from the Cyanophyta. In addition, in the Cryptophyta there are three spectral types of phycoerythrin and three spectral types of phycocyanin.

The basic subunit of a phycobilisome consists of apoproteins  $\alpha$  and  $\beta$ , each of which is attached to a chromophore (Anderson and Toole,



**Fig. 1.21** The structure of  $\beta$ -carotene and fucoxanthin.



**Fig. 1.22** The structure of phycoerythrin.

1998; Samsonoff and MacColl, 2001) (Fig. 1.23). In the core of the phycobilisome  $\alpha$  and  $\beta$  are attached to allophycocyanins, which are closest to chlorophyll in the energy transfer pathway. In the outer rods,  $\alpha$  and  $\beta$  are attached to phycoerythrin or phycocyanin. In the core of the phycobilisome  $\alpha$  and  $\beta$  are attached to allophycocyanin. The  $\alpha$ ,  $\beta$  molecules are assembled into hexamers ( $\alpha_1$ ,  $\beta_1$ ) cylindrical in shape. The hexamers that make up the core of the phycobilisome are assembled in pairs, with the hexamers of the rods radiating from the core. The hexamers are joined together by linker polypeptides. The linker polypeptides are basic whereas the hexamers are acidic; this suggests that electrostatic interactions are important in assembling phycobiliproteins. There are high-molecular-weight polypeptides that anchor the phycobilisome to the area of the thylakoid membrane that contains the reaction center and associated chlorophylls.

The pathway of energy transfer (Glazer et al., 1985) is

In intact cells, the overall efficiency of energy transfer from the phycobilisome to chlorophyll *a* in the thylakoids exceeds 90% (Porter et al., 1978).

Chromatic adapters change their pigment components under different light wavelengths (Fig. 1.24). For example, the cyanobacterium *Synechocystis* grown in green light produces phycoerythrin (red in color), phycocyanin (blue), and allophycocyanin (blue-green) in a molar ratio of about 2:2:1; when it is grown in red light, the ratio is about 0.4:2:1. The phycobilisome structure changes appropriately, with the peripheral rods having more phycoerythrin hexamers under green light, and less phycocyanin hexamers. The allophycocyanin core hexamers stay the same.

Depriving cells of nitrogen results in an ordered degradation of phycobilisomes (Fig. 1.25). There is a progressive degradation of hexamer rod and linker polypeptides followed by the core peptides. New phycobilisomes are rapidly synthesized on the addition of nitrogen to the medium. Phycobilisomes are, thus, an important source of internal nitrogen and offer the algae that have

phycoerythrin

( $\lambda_{\max} = 565$ )

or

phycoerythrocyanin

( $\lambda_{\max} = 568$ )

→ phycocyanin →

( $\lambda_{\max} = 620-638$ )

allophycocyanin →

( $\lambda_{\max} = 650$ )

allophycocyanin B

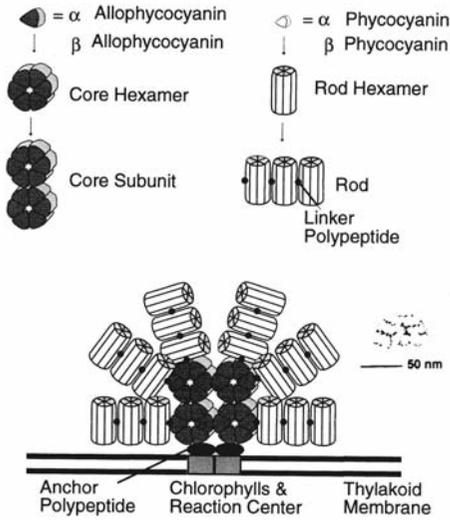
( $\lambda_{\max} = 670$ )

or

high-molecular-weight polypeptide

( $\lambda_{\max} = 665$ )

→ chlorophyll *a*



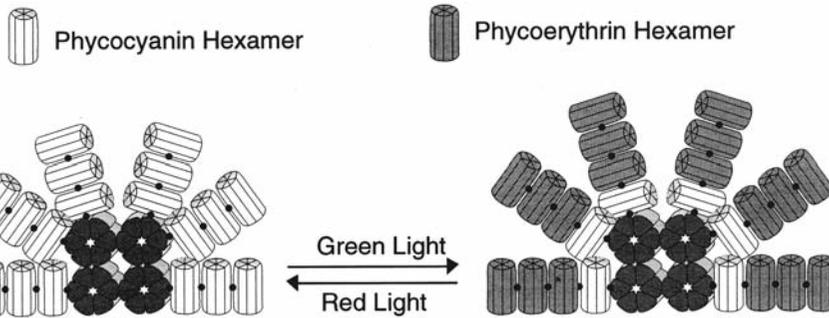
**Fig. 1.23** Drawing of a phycobilisome from the cyanobacterium *Synechococcus*. (Adapted from Grossman et al., 1993.)

phycobilisomes (cyanobacteria, cryptophytes, and red algae) an important ecological advantage in the open ocean, which is predominantly nitrogen limited (Vergara and Niell, 1993).

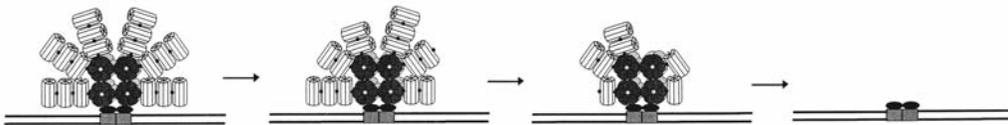
**Mitochondria and peroxisomes**

There are two types of mitochondria in algal cells (Leipe et al., 1994). Mitochondria with **flat lamellae cristae** occur in the red algae, green algae, euglenoids, and cryptophytes (Fig. 1.26). Mitochondria with **tubular cristae** occur in heterokonts and haptophytes.

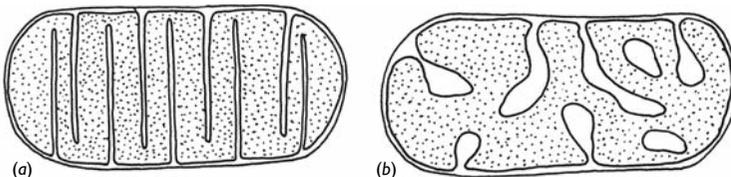
Glycolate, the major substrate of photorespiration, can be broken down by either **glycolate dehydrogenase** in the mitochondria, or by **glycolate oxidase** in **peroxisomes**, single membrane-bounded bodies in the cytoplasm (for the reactions, see the chapter on Chlorophyta). The distribution of the two enzymes is as follows (Betsche et al., 1992; Iwamoto et al., 1996):



**Fig. 1.24** Chromatic adaptation in a phycobilisome of a cyanobacterium.



**Fig. 1.25** Phycobilisome breakdown under conditions of nitrogen deprivation. (Adapted from Grossman et al., 1993.)



**Fig. 1.26** Drawings of the two types of mitochondria that occur in the algae. (a) Mitochondrion with flat lamellar cristae. (b) Mitochondrion with tubular cristae.

- 1 Glycolate dehydrogenase occurs in the cyanobacteria, cryptophytes, euglenoids, diatoms, and the green algae with the exception of the Charophyceae.
- 2 Glycolate oxidase occurs in the glaucophytes, red algae, brown algae, and the Charophyceae in the green algae and higher plants.

### Division of chloroplasts and mitochondria

Chloroplasts and mitochondria divide by pinching in half to form two new organelles. A plastid-dividing (PD) ring or mitochondrion-dividing (MD) ring surrounds the organelle in the area of fission (Fig. 1.27) (Miyagishima et al., 2003; Osteryoung and Nunnari, 2003). Each ring is composed of two parts, an outer ring in the protoplasm outside of the chloroplast and an inner ring in the stroma inside the inner membrane of the chloroplast. These rings are also called FtsZ (filamentous temperature-sensitive) rings after a counterpart that is present when bacteria divide. The similarity is indicative of the endosymbiotic origin of chloroplasts and mitochondria from bacteria. The plastid-dividing ring appears in the area of division and begins to contract after a microbody has migrated to the plastid-dividing ring (Fig. 1.27). The plastid-dividing

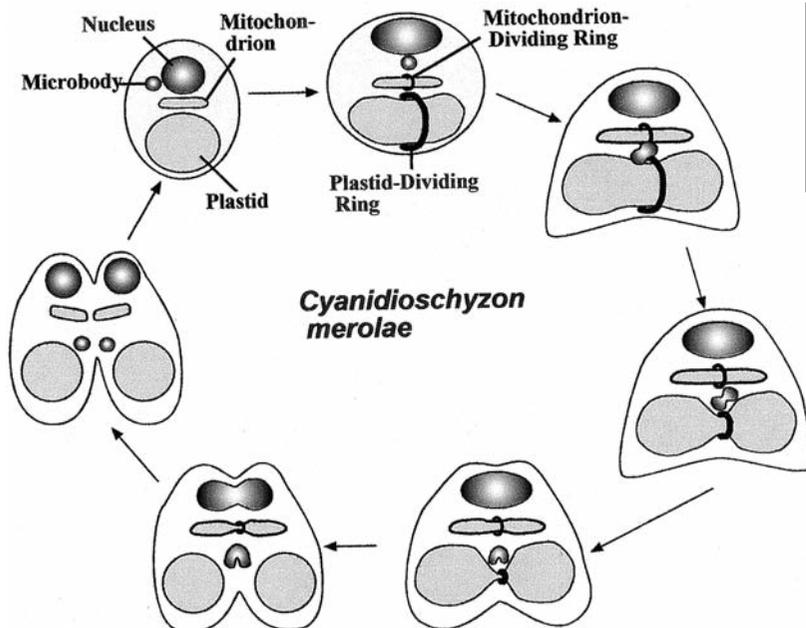
ring contracts around the area of plastid fission in association with GTPase proteins called dynamins. The PD ring disappears after fission is completed.

### Storage products

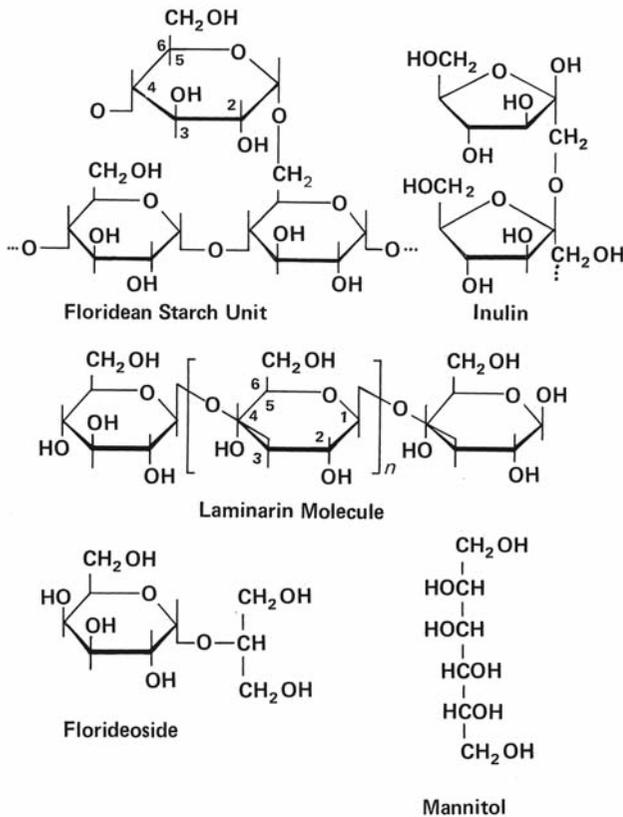
The storage products that occur in the algae are as follows:

#### High-molecular-weight compounds

- 1  $\alpha$ -1,4 Linked glucans
  - a **Floridean starch** (Fig. 1.28): This substance occurs in the Rhodophyta and is similar to the amylopectin of higher plants. It stains red-violet with iodine, giving a color similar to that of the stain reaction of animal glycogen. Floridean starch occurs as bowl-shaped grains from 0.5 to 25  $\mu\text{m}$  outside the chloroplast, inferring the host in the original endosymbiosis took over formation of storage product. This differs from the Chlorophyta where starch is produced in the chloroplast. Despite the differing locations of starch synthesis, the Rhodophyta and Chlorophyta use a common pathway in the synthesis of starch (Patron and Keeling, 2005).



**Fig. 1.27** Diagrammatic representation of the behavior of the plastid-dividing ring and mitochondrion-dividing ring in the unicellular red alga *Cyanidioschyzon merolae*.



**Fig. 1.28** The structure of floridean starch, inulin, laminarin, and floridoside. (After Percival and McDowell, 1967.)

- b **Myxophycean starch:** Found in the Cyanophyta, myxophycean starch has a similar structure to glycogen. This reserve product occurs as granules ( $\alpha$ -granules), the shape varying between species from rod-shaped granules to 25-nm particles to elongate 31- to 67-nm bodies.
- c **Starch:** In the Chlorophyta, starch is composed of amylose and amylopectin. It occurs inside the chloroplast in the form of starch grains (Fig. 1.12(e)). In the Cryptophyta, starch has an unusually high content of amylose and occurs as grains between the chloroplast envelope and the chloroplast E.R. (Fig. 1.12(b)). In the Dinophyta also, starch occurs in the cytoplasm outside of the chloroplast, but its structure is not known.
- 2  **$\beta$ -1,3 Linked glucans**
- a **Laminarin** (Fig. 1.28): In the Phaeophyceae, laminarin consists of a related group of predominantly  $\beta$ -1,3 linked glucans containing 16 to 31 residues. Variation in the molecule is introduced by the number of 1 $\rightarrow$ 6 linkages, the degree of branching, and the occurrence of a terminal mannitol molecule. The presence of a high proportion of C-6 interresidue linkages and of branch points seems to determine the solubility of the polysaccharide in cold water: the greater the number of linkages, the higher the solubility. Laminarin occurs as an oil-like liquid outside of the chloroplasts, commonly in a vesicle surrounding the pyrenoid.
- b **Chrysolaminarin (leucosin):** In the Chrysophyceae, Prymnesiophyta, and Bacillariophyceae, chrysolaminarin consists of  $\beta$ -1,3 linked D-glucose residues with two 1 $\rightarrow$ 6 glycosidic bonds per molecule. Chrysolaminarin occurs in vesicles outside of the chloroplast and has more glucose residues per molecule than laminarin.
- c **Paramylon:** In the Euglenophyta, Xanthophyceae, and Prymnesiophyta (*Pavlova mesolychnon*), paramylon occurs as

water-soluble, single-membrane-bounded inclusions of various shapes and dimensions outside of the chloroplast (Fig. 6.2).

Paramylon consists solely of  $\beta$ -1,3 linked glucose residues, and the molecule is about as large as that of chrysolaminarin.

- 3 **Fructosans:** *Acetabularia* (Chlorophyta) has an inulin-like storage product consisting of a series of 1,2 linked fructose units terminated by a glucose end group (Fig. 1.28).

### Low-molecular-weight compounds

- 1 **Sugars:** Chlorophyta and Euglenophyta form sucrose as a reserve product; trehalose is found in the Cyanophyta and at low levels in the Rhodophyta.
- 2 **Glycosides:** The glycerol glycosides, floridoside (Fig. 1.28) and isofloridoside, are widely distributed in the Rhodophyta.
- 3 **Polyols:** Mannitol (Figs. 1.28, 4.4) occurs in Rhodophyta and Phaeophyceae. It is also present in lower green algae, where it replaces sucrose as a photosynthetic product. Free glycerol occurs widely in the algae and is an important photosynthetic product in several zooxanthellae (endosymbiotic algae in animals) and in some marine Volvocales, especially *Dunaliella*.

### Contractile vacuoles

The ability of algal cells to adjust to changes in the salinity of the medium is an important aspect of the physiology of these cells. In cells with walls, this osmoregulation is accomplished with the aid of turgor pressure, whereas in naked cells it is accomplished by means of contractile vacuoles and/or regulation of the solutes present in the cells. In the latter case, cells increase the internal concentration of osmotically active molecules and ions when the concentration of dissolved solutes increases in the external medium. Likewise, the internal concentration of such molecules decreases when the concentration of dissolved salts in the external medium decreases.

Most algal flagellates have two contractile vacuoles in the anterior end of a cell (Fig. 1.1). A contractile vacuole will fill with an aqueous solution (**diastole**) and then expel the solution outside of

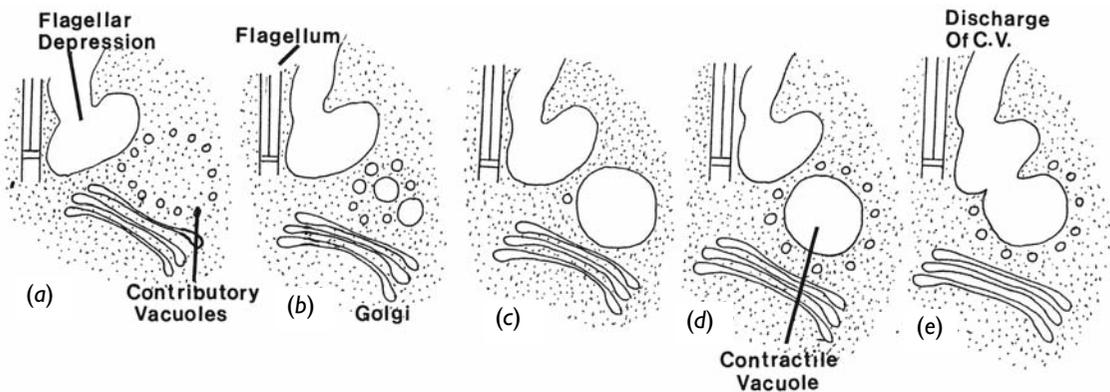
the cell and contract (**systole**). The contractile vacuole rhythmically repeats this procedure. If there are two contractile vacuoles, they usually fill and empty alternately. Contractile vacuoles occur more frequently in freshwater than marine algae, a phenomenon that gives credence to the theory that the contractile vacuoles maintain a water balance in the cells. The algal cells in freshwater have a higher concentration of dissolved substances in their protoplasm than in the surrounding medium so that there is a net increase of water in the cells. The contractile vacuoles act to expel this excess water. An alternate theory on the function of the contractile vacuoles is that they remove waste products from the cells. The Dinophyta have a structure similar to a contractile vacuole, called a pusule, which may have a similar function but is more complex.

The contractile vacuoles of the Cryptophyta are characteristic of the algae (Fig. 1.29). In the Cryptophyta, the contractile vacuole occurs in a fixed anterior position next to the flagellar depression (Patterson and Hausmann, 1981). At the beginning of the filling phase (**diastole**), there is no distinct contractile vacuole, only a region filled with small (*ca.* 0.5- $\mu$ m diameter) contributory vacuoles. These vacuoles fuse to form a large irregular vacuole which subsequently rounds up. The contributory vacuoles destined to form the next contractile vacuole now appear around the rounded contractile vacuole. The contractile vacuole fuses with the plasma membrane of the flagellar pocket and discharges its contents outside the cell. The area of the plasma membrane that fuses with the contractile vacuole does not have a periplast (specialized plates within the plasma membrane). This area is, instead, bounded by microtubules. The membrane of the contractile vacuole is recovered by the cell as small vesicles with an electron-dense coat, and the membrane components are reutilized by the cell. These vesicles plus the contractile vacuole occur in the **spongione** or area around the contractile vacuole. In freshwater algae the contractile vacuole cycle lasts for 4 to 16 seconds, whereas in marine species the cycle can last for up to 40 seconds.

Algal flagellates use a combination of contractile vacuoles and osmoregulation to control the water content of their cells. In the chrysophyte

Table 1.1 | Types of nutrition found in the algae

Type of nutrition	Principle source of energy for growth	Principal source of carbon for growth
<i>Autotrophic</i>		
Photoautotrophic	Light	Carbon dioxide
Chemoautotrophic	Oxidation of organic compounds	Carbon dioxide
<i>Heterotrophic</i>		
Photoheterotrophic	Light	Organic compounds
Chemoheterotrophic	Oxidation of organic compounds	Organic compounds



**Fig. 1.29** Semidiagrammatic illustration of the behavior of the contractile vacuole (C.V.) complex during filling and discharge in the Cryptophyta. (Adapted from Patterson and Hausmann, 1981.)

*Poteroiochromonas malhamensis* (*Ochromonas malhamensis*), the internal level of isofloridoside ( $O\text{-}\alpha\text{-D-galactopyranosyl-1}\rightarrow\text{1-glycerol}$ ) is proportional to the external osmotic value as long as the external solute concentration exceeds 75 mOsm (Wessel and Robinson, 1979). Below this external solute concentration, the influx of water into the cytoplasm is counterbalanced by means of the contractile vacuoles (Kauss, 1974).

## Nutrition

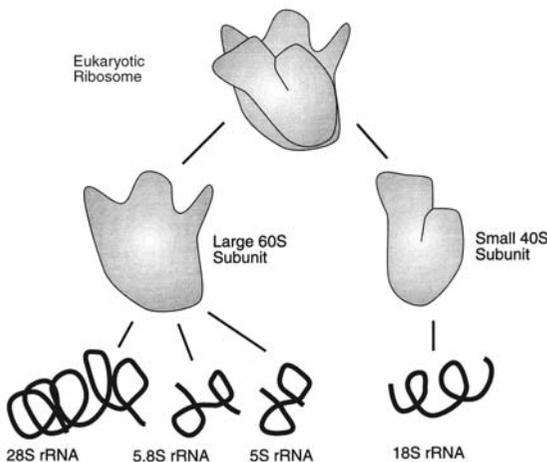
Algae can be either **autotrophic** (**lithotrophic** or **holophytic**) or **heterotrophic** (**organotrophic**) (Table 1.1). If they are **autotrophic**, they use inorganic compounds as a source of carbon. Autotrophs can be **photoautotrophic** (**photolithotrophic**),

using light as a source of energy, or **chemoautotrophic** (**chemolithotrophic**), oxidizing inorganic compounds for energy. If they are **heterotrophic**, the algae use organic compounds for growth. Heterotrophs can be **photoheterotrophs** (**photoorganotrophs**), using light as a source of energy, or **chemoheterotrophs** (**chemoorganotrophs**), oxidizing organic compounds for energy. Heterotrophic algae may be **phagocytotic** (**holozoic**), absorbing food particles whole into food vesicles for digestion, or they may be **osmotrophic**, absorbing nutrients in a soluble form through the plasma membrane. If the algae live heterotrophically on dead material, they are **saprophytic**; if they live off a live host, they are **parasitic**. Some algae, particularly the flagellates, are **auxotrophic**, requiring a small amount of an organic compound, but not as an energy source. These algae usually require a vitamin. Some photosynthetic algae are **mixotrophic** (**facultatively heterotrophic**), capable of also using organic compounds supplied in the medium.

## Gene sequencing and algal systematics

Specific sequences of nucleotides in DNA of the cell code for cell constituents. It is possible to isolate DNA from cells, multiply certain DNA segments and determine the nucleotide sequences of that DNA. Each species has differences in the nucleotides that make up the DNA and differences in nucleotides that can be used to produce an evolutionary history of the cell. The DNA nucleotides that are most commonly sequenced to produce phylogenies are those of ribosomal DNAs (rDNAs). These rDNA nucleotides make up the genes that code for the rRNAs. rRNAs occur in ribosomes and there are three types of ribosomes, each made up of a large and small subunit:

- 1 **Prokaryotic ribosomes.** The large 70S subunit contains 5S and 23S rRNAs as well as 34 ribosomal proteins. The small 30S subunit contains a single 16S rRNA and 21 proteins.
- 2 **Eukaryotic ribosomes** (Fig. 1.30). The large 60S subunit contains 28S, 5.8S, and 5S rRNAs, and 49 proteins. The small 40S subunit contains 18S rRNA and 33 proteins.
- 3 **Mitochondrial ribosomes.** These are similar, although not the same, as prokaryotic ribosomes. They are not used to produce algal phylogenies, mostly because mitochondria



**Fig. 1.30** The components of a eukaryotic ribosome.

have been transferred between eukaryotic hosts and, therefore, do not reflect the evolutionary history of the organism (Stiller and Hall, 1997).

The rDNA for the 18S rRNA of the small subunit of the eukaryotic ribosome is the form of rDNA usually sequenced to determine the phylogeny of eukaryotic organisms. The nucleotides coding for all of the ribosomal subunits are encompassed within a single operon and transcribed by a single RNA polymerase (Kawai et al., 1997). The procedure for determining the nucleotide sequences is available in any basic biochemistry book.

The rDNA for the 5S rRNA has been also used in phylogeny studies. Although less nucleotides are in the rDNA coding for 5S rRNA, making it easier to sequence, the data have been suspect because of large deviations in the nucleotides (Ragan, 1994). The DNA coding for other molecules, such as ribulose biphosphate carboxylase/oxygenase (Freshwater et al., 1994; Fujiwara et al., 1994) and actin (Bhattacharya and Ehling, 1995), have also been used in determining phylogeny.

Gene sequencing has been the most active field of phycological systematics in the last decade and has provided important new information on the relationships between algae. However, as stated by Manhart and McCourt (1992):

... molecular data are not a magic bullet for species problems. They are data, no more, no less. Some molecular data are informative, and others are misleading. Molecular data are fraught with many of the same difficulties as morphological data ...

## Classification

There are four distinct groups within the algae. The remainder of the text is divided into four parts based on these four groups.

- 1 Prokaryotes. The cyanobacteria are the only prokaryotic algae.
- 2 Eukaryotic algae with chloroplasts surrounded by the two membranes of the chloroplast envelope.

- 3 Eukaryotic algae with the chloroplast surrounded by one membrane of chloroplast endoplasmic reticulum.
- 4 Eukaryotic algae with the chloroplast surrounded by two membranes of chloroplast endoplasmic reticulum.

The standard botanical classification system is used in the systematics of the algae:

Phylum – phyta  
 Class – phyceae  
 Order – ales  
 Family – aceae  
 Genus  
 Species

Group 1 Prokaryotic algae

**Cyanophyta** (cyanobacteria) (Chapter 2): chlorophyll *a*; phycobiliproteins.

Group 2 Eukaryotic algae with chloroplasts surrounded only by the two membranes of the chloroplast envelope.

**Glaucophyta** (Chapter 3): algae that represent an intermediate position in the evolution of chloroplasts; photosynthesis is carried out by modified endosymbiotic cyanobacteria.

**Rhodophyta** (red algae) (Chapter 4): chlorophyll *a*; phycobiliproteins; no flagellated cells; storage product is floridean starch.

**Chlorophyta** (green algae) (Chapter 5): chlorophylls *a* and *b*; storage product, starch, is found inside the chloroplast.

Group 3 Eukaryotic algae with chloroplasts surrounded by one membrane of chloroplast endoplasmic reticulum.

**Euglenophyta** (euglenoids) (Chapter 6): chlorophylls *a* and *b*; one flagellum with a spiraled row of fibrillar hairs; proteinaceous pellicle in strips under the plasma membrane; storage product is paramylon; characteristic type of cell division.

**Dinophyta** (dinoflagellates) (Chapter 7): mesokaryotic nucleus; chlorophylls *a* and *c*<sub>1</sub>; cell commonly divided into an epicone and a hypocone by a girdle; helical transverse flagellum; thecal plates in vesicles under the plasma membrane.

**Apicomplexa** (Chapter 8): heterotrophic flagellates with colorless plastids.

Group 4 Eukaryotic algae with chloroplasts surrounded by two membranes of chloroplast endoplasmic reticulum.

**Cryptophyta** (cryptophytes) (Chapter 9): nucleomorph present between inner and outer membrane of chloroplast endoplasmic reticulum; starch formed as grains between inner membrane of chloroplast endoplasmic reticulum and chloroplast envelope; chlorophyll *a* and *c*; phycobiliproteins; periplast inside plasma membrane.

**Heterokontophyta** (heterokonts) (Chapters 10–21): anterior tinsel and posterior whiplash flagellum; chlorophyll *a* and *c*; fucoxanthin; storage product usually chrysolaminarin occurring in vesicles.

**Chrysophyceae** (golden-brown algae) (Chapter 10)

**Synurophyceae** (Chapter 11)

**Eustigmatophyceae** (Chapter 12)

**Pinguiphyceae** (Chapter 13)

**Dictyochophyceae** (silicoflagellates) (Chapter 14)

**Pelagophyceae** (Chapter 15)

**Bolidophyceae** (Chapter 16)

**Bacillariophyceae** (diatoms) (Chapter 17)

**Raphidophyceae** (chloromonads) (Chapter 18)

**Xanthophyceae** (yellow-green algae) (Chapter 19)

**Phaeothamniophyceae** (Chapter 20)

**Phaeophyceae** (brown algae) (Chapter 21)

**Prymnesiophyta** (haptophytes) (Chapter 22): two whiplash flagella; haptonema present; chlorophyll *a* and *c*; fucoxanthin; scales common outside cell; storage product chrysolaminarin occurring in vesicles.

Data from molecular studies indicate that the red algae diverged about 1400 million years ago from the common line leading to higher plants (Saunders and Hommersand, 2004). This was followed by divergence of the green algae and, then, multiple independent secondary endosymbioses evolving to those algae with chloroplast endoplasmic reticulum. The host phagocytic organisms leading to the euglenoids was probably a kinetoplastid, that leading to the dinoflagellates was probably an apicomplexan, and that leading to the photosynthetic cryptophytes and haptophytes was a colorless cryptophyte and haptophyte,

**Table 1.2** First appearance of algae in the geological time scale

Era	Period	Epoch	Millions of years ago	First appearance of algal fossil
Cenozoic	Quaternary	Holocene		
		Pleistocene	1.8	
	Tertiary	Pliocene	5.5	
		Miocene	25.0	Xanthophyta
		Oligocene	36.0	
		Eocene	53.5	Euglenophyta
Palaeocene	65.0			
Mesozoic	Cretaceous		135	Chrysophyta
	Jurassic		191–205	
	Triassic		235–245	Bacillariophyta
Paleozoic	Permian		275–290	Prymnesiophyta
		Carboniferous	360–380	
	Devonian		405–430	
		Silurian	435–460	Stoneworts (Chlorophyta)
	Ordovician		500–530	
	Cambrian		570–610	
Proterozoic	Precambrian		3000	Cyanophyta, Rhodophyta, Chlorophyta

respectively. The host organisms leading to the heterokonts have not been identified.

## Algae and the fossil record

The cyanobacteria are the oldest group of algae with definite fossil remains in the form of stromatolites (Fig. 2.53), dating back about 2700 million years. When the cyanobacteria evolved, the atmosphere contained little or no oxygen and was composed primarily of methane ( $\text{CH}_4$ ), ammonia ( $\text{NH}_3$ ), and other reduced compounds. Photosynthesis by the cyanobacteria eventually built up the oxygen content of the atmosphere to what it is today (20%). The first eukaryotic algae appeared in a form similar to the extant

Glaucochyta, with endosymbiotic cyanobacteria instead of chloroplasts (see Chapter 3). It is difficult to fix this date exactly because these first algae were composed of soft tissues and would not have been preserved. In order to appear in the fossil record, algae would usually have to be large or to have some calcified ( $\text{CaCO}_3$ ) or silicified ( $\text{SiO}_2$ ) structures, which are preserved in sedimentary rocks. The appearance of fossil members of the algal classes in the geological timetable is presented in Table 1.2. This table does not purport to show when the algal groups first evolved, but shows only where fossil specimens appear in the geological timetable. The fossil members of each of the algal classes are discussed in the chapter on the particular class.



**Fig. 1.31** Two prominent phycologists of the twentieth century, Felix Eugen Fritsch (left) and Gilbert Morgan Smith (right). Photograph taken by Ralph Lewin.

**Felix Eugen Fritsch** Born April 26, 1879, in Hampstead, United Kingdom, died May 23, 1954. Dr. Fritsch was educated at the University of London and the University of Munich where he received his Ph.D. From 1902 to 1911, he was an assistant professor at University College London; from 1905 to 1906, a lecturer at Birkbeck College; and from 1907 to 1911, a lecturer at East London College. In 1911, he became head of the Department of Botany at Queen Mary College, University of London, where he stayed until retirement in 1948. Dr. Fritsch was the author of a number of books, by far the best known being *The Structure and Reproduction of the Algae*, which today is still the most comprehensive treatise on the algae as a whole.

**Gilbert Morgan Smith** Born January 6, 1885 in Beloit, Wisconsin. Died July 11, 1959. Dr. Smith received his BS from Beloit College (1907) and his Ph.D. from the University of Wisconsin (1913). From 1913 to 1925, he was at the University of Wisconsin, ultimately as an associate professor. From 1925 until his retirement, he was a professor at Stanford University. Dr. Smith is best known for his books on algae, which include *Phytoplankton of the Inland Lakes of Wisconsin*, *The Freshwater Algae of the United States*, *Cryptogamic Botany*, and *Marine Algae of the Monterey Peninsula*.

## REFERENCES

- Andersen, R. A., Barr, D. J. S., Lynn, D. H., Melkonian, M., Moestrup, O., and Sleight, M. A. (1991). Terminology and nomenclature of the cytoskeletal elements associated with the flagellar/ciliary apparatus in protists. *Protoplasma* 164:1-8.
- Anderson, L. K., and Toole, C. M. (1998). A model for early events in the assembly pathway of cyanobacterial phycobilisomes. *Mol. Microbiol.* 30:467-74.
- Beech, P. L. (2003). The long and short of flagellar length control. *J. Phycol.* 39:837-39.
- Beech, P. L., and Wetherbee, R. (1990). Direct observations on flagellar transformation in *Mallomonas splendens* (Synurophyceae). *J. Phycol.* 26:90-5.
- Betsche, T., Schaller, D., and Melkonian, M. (1992). Identification and characterization of glycolate oxidase and related enzymes from the endocytotic alga *Cyanophora paradoxa* and from pea leaves. *Plant Physiol.* 98:887-93.
- Bhattacharya, D., and Ehltling, J. (1995). Actin coding regions: gene family evolution and use as a phylogenetic marker. *Arch. Protistenkd.* 145:155-64.
- Bouck, G. B. (1969). Extracellular microtubules. The origin, structure, and attachment of flagellar hairs in *Fucus* and *Ascomyllum* antherozoids. *J. Cell Biol.* 40:446-60.
- Brugerolle, G., and Mignot, J.-P. (2003). The rhizoplast of chrysoomonads, a basal body-nucleus connector that polarises the dividing spindle. *Protoplasma* 222:13-21.
- Caron, L., Douady, D., Quinet-Szely, M., deGoër, S., and Berkaloff, C. (1996). Gene structure of a chlorophyll *a/c*-binding protein from a brown alga: Presence of an intron and phylogenetic implications. *J. Mol. Evol.* 43:270-80.
- Coleman, A. W. (1985). Diversity of plastid DNA configuration among classes of eukaryote algae. *J. Phycol.* 21:1-16.
- Dutcher, S. K. (1995). Flagellar assembly in two hundred and fifty easy-to-follow steps. *Trends Genet.* 11:398-404.
- Freshwater, D. W., Fredericq, S., Butler, B. S., Hommersand, M. H., and Chase, M. W. (1994). A gene phylogeny of the red algae (Rhodophyta) based on plastid *rbcL*. *Proc. Natl. Acad. Sci., USA* 91:7281-5.
- Fujiwara, S., Sawada, M., Someya, J., Minaka, N., Kawachi, M., and Inouye, I. (1994). Molecular phylogenetic analysis of *rbcL* in the Prymnesiophyta. *J. Phycol.* 30:863-71.
- Glazer, A. N. (1982). Phycobilisomes: Structure and dynamics. *Annu. Rev. Microbiol.* 36:173-98.

- Glazer, A. N., Yeh, S. W., Webb, S. P., and Clark, J. H. (1985). Disk-to-disk transfer as the rate-limiting step for energy flow in phycobilisomes. *Science* 227:419–23.
- Grossman, A., Manodori, A., and Snyder, D. (1990). Light-harvesting proteins of diatoms: Their relationship to the chlorophyll *a/b* binding protein of higher plants and their mode of transport into plastids. *Mol. Gen. Genetics* 224:91–100.
- Grossman, A. R., Schaffer, M. R., Chiang, G. G., and Collier, J. L. (1993). The phycobilisome, a light-harvesting complex response to environmental conditions. *Microbiol. Rev.* 57:725–49.
- Hegemann, P. (1997). Vision in microalgae. *Planta* 203:265–74.
- Jenks, A., and Gibbs, S. P. (2000). Immunolocalization and distribution of Form II RUBISCO in the pyrenoid and chloroplast stroma of *Amphidinium carterae* and Form I RUBISCO in the symbiont-derived plastids of *Peridinium foliaceum* (Dinophyceae). *J. Phycol.* 36:127–38.
- Johnson, K. A. (1995). Keeping the beat: form meets function in the *Chlamydomonas* flagellum. *BioEssays* 17:847–54.
- Iwamoto, K., Suzuki, K., and Ikawa, T. (1996). Purification and characterization of glycolate oxidase from the brown alga *Spatoglossum pacificum* (Phaeophyta). *J. Phycol.* 32:790–8.
- Kauss, H. (1974). Osmoregulation in *Ochromonas*. In *Membrane Transport in Plants*, ed. U. Zimmermann, and J. Daintz, pp. 90–4. Berlin: Springer-Verlag.
- Kawai, H., and Kreimer, G. (2000). Sensory mechanisms. Phototaxes and light perception in algae. In *The Flagellates*, ed. B. S. C. Leadbeater, and J. C. Green, pp.124–46. London:Taylor and Francis.
- Kawai, H., Nakayama, T., Inouye, I., and Kato, A. (1997). Linkage of 5S ribosomal DNA to other rDNAs in the chromophytic algae and related taxa. *J. Phycol.* 33:505–11.
- Kuhlbrandt, W., Wang, D. N., and Fujiyoshi, Y. (1994). Atomic model of plant light-harvesting complex by electron crystallography. *Nature* 367:614–21.
- Laatsch, T., Zauner, S., Stoebe-Maier, B., Kowallik, K. V., and Maier, U.-G. (2004). Plastid-derived single gene minicircles of the dinoflagellate *Ceratium horridum* are located in the nucleus. *Mol. Biol. and Evol.* 21:1318–22.
- Lee, R. E. (1977). Evolution of algal flagellates with chloroplast endoplasmic reticulum from the ciliates. *S. Afr. J. Sci.* 73:179–82.
- Leipe, D. D., Wainright, P. O., Gunderson, J. H., Porter, D., Patterson, D. J., Valois, F., Himmerich, S., and Sogin, M. L. (1994). The stramenopiles from a molecular perspective: 16S-like rRNA sequences from *Labyrinthuloides minuta* and *Cafeteria roenbergensis*. *Phycologia* 33:369–77.
- Manhart, J. R., and McCourt, R. M. (1992). Molecular data and species concepts in the algae. *J. Phycol.* 28:730–7.
- Meeks, J. C. (1974). Chlorophylls. In *Algal Physiology and Biochemistry*, ed. W. D. P. Stewart, pp. 161–75. Berkeley: Univ. Calif. Press.
- Melkonian, M. (1980). Flagellar roots, mating structure and gametic fusion in the green alga *Ulva lactuca* (Ulvales). *J. Cell Sci.* 46:149–69.
- Melkonian, M., Reize, I. B., and Preisig, H. R. (1987). Maturation of a flagellum/basal body requires more than one cell cycle in algal flagellates: studies on *Nephroselmis olivaea* (Prasinophyceae). In *Algal Development, Molecular and Cellular Aspects*, ed. W. Wiessner, D. G. Robinson, and R. C. Starr, pp. 102–13. Heidelberg: Springer.
- Mitchell, D. R. (2000). *Chlamydomonas* flagella. *J. Phycol.* 36:261–73.
- Miyagishima, S., Nishida, K., and Kuriowa, T. (2003). An evolutionary puzzle: chloroplast and mitochondrial division rings. *Trends Plant Sci.* 8:432–8.
- Moestrup, Ø. (1982). Flagellar structure in algae: A review, with new observations particularly on the Chrysophyceae, Phaeophyceae (Fucophyceae), Euglenophyceae, and *Reckertia*. *Phycologia* 21:427–528.
- Moestrup, O. (2000). The flagellate cytoskeleton. In *The Flagellates*, ed. B. S. C. Leadbeater, and J. C. Green, pp.69–94. London:Taylor and Francis.
- Murakami, A., Miyashita, H., Iseki, M., Adachi, K., and Mimuro, M. (2004). Chlorophyll *d* in an epiphytic cyanobacterium of red algae. *Science* 303:1633.
- Nagasato, C., Yoshikawa, S., Yamashita, M., Kawai, H., and Motomura, T. (2003). Pyrenoid formation associated with the cell cycle in the brown alga, *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). *J. Phycol.* 39:1172–80.
- Osteryoung, K. W., and Nunnari, J. (2003). The division of endosymbiotic organelles. *Science* 302:1698–1704.
- Patron, N. J., and Keeling, P. J. (2005). Common evolutionary origin of starch biosynthesis enzymes in green and red algae. *J. Phycol.* 41:1131–41.
- Patterson, D. J., and Hausmann, K. (1981). The behavior of contractile vacuole complexes of cryptophycean flagellates. *Br. Phycol. J.* 16:429–39.

- Percival, E., and McDowell, R. H. (1967). *Chemistry and Enzymology of Marine Algal Polysaccharides*. New York: Academic Press.
- Porter, G., Tredwell, C. J., Searle, G. F. W., and Barber, J. (1978). Picosecond time-resolved energy transfer in *Porphyridium cruentum*. *Biochim. Biophys. Acta* 501:232–45.
- Ragan, M. A. (1994). 18S ribosomal DNA sequences indicate a monophyletic origin of Charophyceae. *J. Phycol.* 30:490–500.
- Raven, J. (1997). CO<sub>2</sub> concentrating mechanisms: a direct role for thylakoid lumen acidification? *Plant Cell and Environ.* 20:147–54.
- Ringo, D. L. (1967). Flagellar motion and fine structure of the flagellar apparatus in *Chlamydomonas*. *J. Cell Biol.* 33:543–71.
- Rosenbaum, J. L., and Witman, G. B. (2002). Intraflagellar transport. *Nat. Rev. Mol. Cell Biol.* 3:815–25.
- Samsonoff, W. A., and MacColl, R. (2001). Biliproteins and phycobilisomes from cyanobacteria and red algae at extremes of habitat. *Arch. Microbiol.* 176:402–5.
- Saunders, G. W., and Hommersand, M. H. (2004). Assessing red algal supraordinal diversity and taxonomy in the context of contemporary systematic data. *Amer. J. Bot.* 91:1494–1507.
- Schoppmeier, J., and Lechtreck, K.-F. (2003). Flagellar regeneration in *Spermatozopsis similes* (Chlorophyta). *J. Phycol.* 39:918–22.
- Snell, W. J. (1976). Mating in *Chlamydomonas*: A system for the study of specific cell adhesion. I. Ultrastructural and electrophoretic analyses of flagellar surface components involved in adhesion. *J. Cell Biol.* 68:48–69.
- Stiller, J. W., and Hall, B. D. (1997). The origin of red algae: implication for plastid evolution. *Proc. Natl. Acad. Sci. USA* 94:4520–5.
- Sukenik, A., Tchernov, D., Kaplan, A., Huertas, E., Lubian, L. M., and Livne, A. (1997). Uptake, efflux, and photosynthetic utilization of inorganic carbon by the marine eustigmatophyte *Nannochloropsis* sp. *J. Phycol.* 33:969–74.
- van der Auwera, G., and deWachter, R. (1997). Complete large subunit ribosomal RNA sequences from the heterokont algae *Ochromonas danica*, *Nannochloropsis salina*, and *Tribonema aequale*, and phylogenetic analysis. *J. Mol. Evol.* 45:84–90.
- Vergara, J. J., and Niell, F. X. (1993). Effects of nitrate availability and irradiance on internal nitrogen constituents in *Corallina elongata* (Rhodophyta). *J. Phycol.* 29:285–93.
- Vierkotten, L., Simon, A., and Becker, B. (2004). Preparation and characterization of protoplasts from the prasinophyte *Scherffelia dubia* (Chlorophyta). *J. Phycol.* 40:1106–11.
- Wessel, D., and Robinson, D. G. (1979). Studies on the contractile vacuole of *Poterioochromonas malhamensis* Peterfi. I. The structure of the alveolate vesicles. *Eur. J. Cell Biol.* 19:60–6.
- Zhang, H., and Lin, S. (2003). Complex structure of the Form II RUBISCO in the dinoflagellate *Prorocentrum minimum* (Dinophyceae). *J. Phycol.* 38:1160–71.



# Part II

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## The prokaryotic algae

The cyanobacteria or blue-green algae form a natural group by virtue of being the only prokaryotic algae. Prokaryotic algae have an outer plasma membrane enclosing protoplasm containing photosynthetic thylakoids, 70S ribosomes, and DNA fibrils not enclosed within a separate membrane. Chlorophyll *a* is the main photosynthetic pigment, and oxygen is evolved during photosynthesis.



# Cyanobacteria

## CYANOPHYCEAE

The Cyanophyceae or **blue-green algae** are, today, usually referred to as the **cyanobacteria** (blue-green bacteria). The term cyanobacteria acknowledges that these prokaryotic algae are more closely related to the prokaryotic bacteria than to eukaryotic algae. For the last quarter century, cyanobacteria were thought to have evolved about 3.5 billion years ago. These reports were based on interpretation of microfossils, difficult at best with such small organisms. It now appears that these investigators selected specimens that fit the assumptions of the authors, with most phylogenists now rejecting their claims. Based on other reports, the actual time of evolution of cyanobacteria is thought to be closer to 2.7 billion years ago (Buick, 1992; Brasier et al., 2002; Dalton, 2002).

Cyanobacteria have chlorophyll *a* (some also have chlorophyll *b* or *d*), phycobiliproteins, glycogen as a storage product, and cell walls containing amino sugars and amino acids.

At one time, the occurrence of chlorophyll *b* in cyanobacteria was used as a criterion to place the organisms in a separate group, the **Prochlorophyta**. Modern nucleic-acid sequencing, however, has shown that chlorophyll *b* evolved a number of times within the cyanobacteria and the term Prochlorophyta has been discarded (Palenik and Haselkorn, 1992; Urbach et al., 1992).

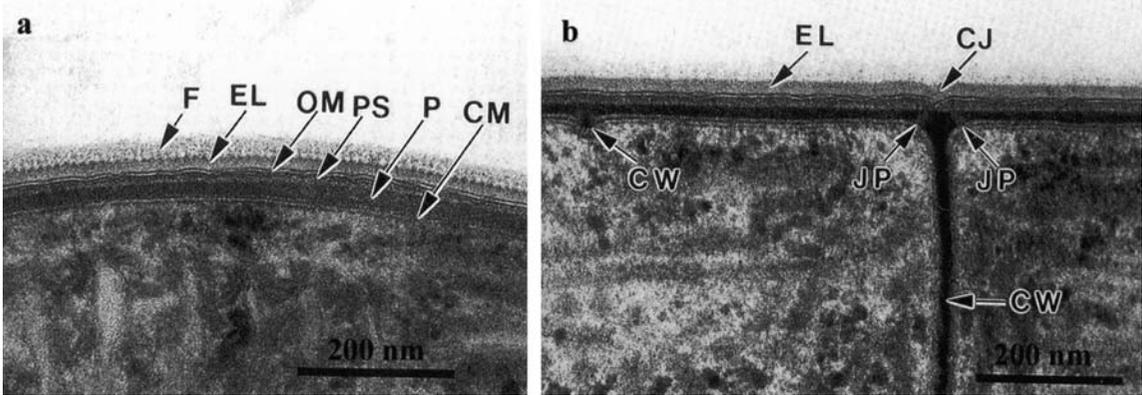
## Morphology

The simplest morphology in the cyanobacteria is that of unicells, free-living (see Figs. 2.19(c), 2.20) or enclosed within a mucilaginous envelope (Figs. 2.48, 2.56(a), (b)). Subsequent evolution resulted in the formation of a row of cells called a **trichome** (Fig. 2.16). When the trichome is surrounded by a sheath, it is called a **filament** (Fig. 2.10). It is possible to have more than one trichome in a filament (Figs. 2.56(e), 2.58(b)). The most complex thallus is the branched filament (Fig. 2.58(a)). Such a branched filament can be **uniserial** (composed of a single row of cells) or **multiserial** (composed of one or more rows of cells).

## Cell wall and gliding

The cell wall of cyanobacteria is basically the same as the cell wall of Gram-negative bacteria (Fig. 2.1). A **peptidoglycan layer** is outside of the **cell membrane**. The peptidoglycan is an enormous polymer composed of two sugar derivatives, *N*-acetylglucosamine and *N*-acetylmuramic acid, and several different amino acids (Fig. 2.2). Outside of the peptidoglycan is a **periplasmic space**, probably filled with a loose network of peptidoglycan fibrils. An **outer membrane** surrounds the periplasmic space.

Some cyanobacteria are capable of gliding, that is, *the active movement of an organism on a solid*



**Fig. 2.1** Transmission electron micrographs of sections of the wall of the cyanobacterium *Phormidium uncinatum*. The cell wall (CW) contains layers similar to those of a Gram-negative bacterium, e.g., the cytoplasmic membrane (CM), peptidoglycan layers (P), periplasmic space (PS) and outer membrane (OM). In addition, the cyanobacterium contains the additional two external layers typical of a motile cell, the serrated external layer (EL) and hair-like fibers (F). (CJ) Circumferential junction; (JP) junctional pore. (From Hoiczky and Baumeister, 1995.)

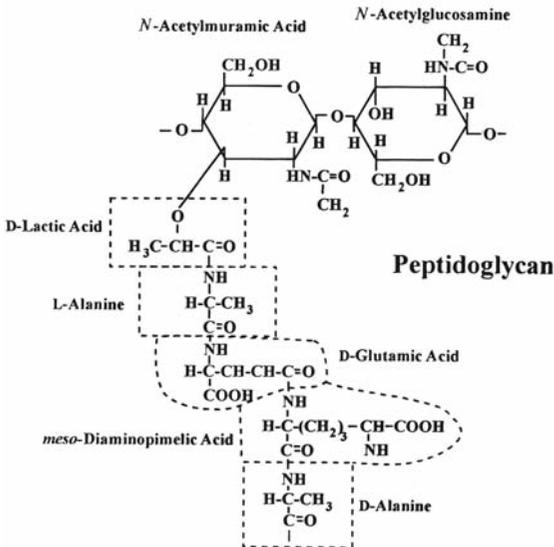
substrate where there is neither a visible organ responsible for the movement nor a distinct change in the shape of the organism (Jarosch, 1962). Gliding is a slow uniform motion (up to  $600 \mu\text{m s}^{-1}$  in *Oscillatoria*; Bhaya, 2004) at a direction parallel

to the long axis of the cell and is occasionally interrupted by reversals in direction. Gliding is accompanied by a steady secretion of slime, which is left behind as a mucilaginous trail. Some cyanobacteria (*Phormidium*, *Oscillatoria*) rotate during gliding while other cyanobacteria (*Anabaena*) do not rotate.

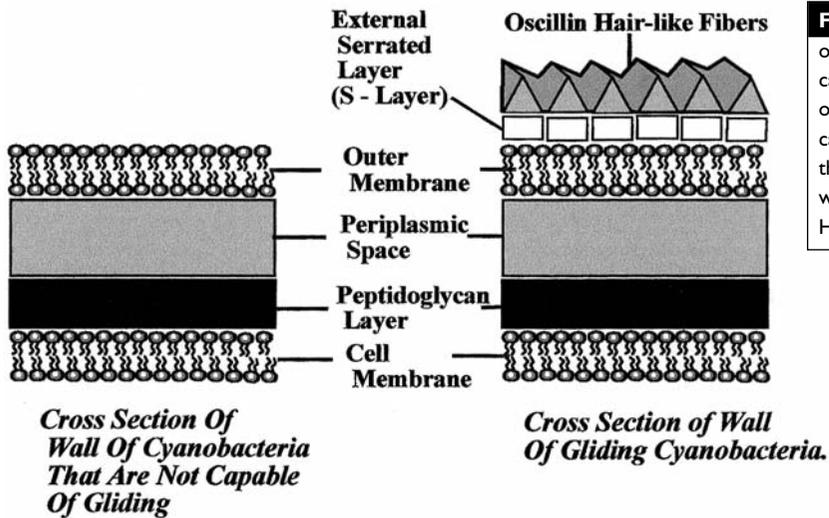
The cell wall of gliding bacteria has two additional layers outside of the cell wall (Figs. 2.1, 2.3, 2.4). A **serrated external layer (S-layer)** and a layer of **hair-like fibers** occur outside of the outer membrane of the cell wall of gliding cyanobacteria. The hair-like fibers of the outermost layer are composed of a rod-like glycoprotein called **oscillin** (Hoiczky and Baumeister, 1998; Hoiczky, 2000).

The cross walls of neighboring cells of gliding cyanobacteria contain **junctional pores** that are 15 nm in diameter and radiate outward from the cytoplasm at an angle of about  $30\text{--}40^\circ$  relative to the plane of each septum (Figs. 2.1, 2.5, 2.6). The number of rows of junctional pores around each side of the septum varies from one circumferential ring in *Phormidium* to several rows of pores that girdle the septum in *Anabaena*. The junctional pore is 70–80 nm long and spans the entire multi-layered cell wall. The junctional pore is composed of a tube-like base and an outer pore complex.

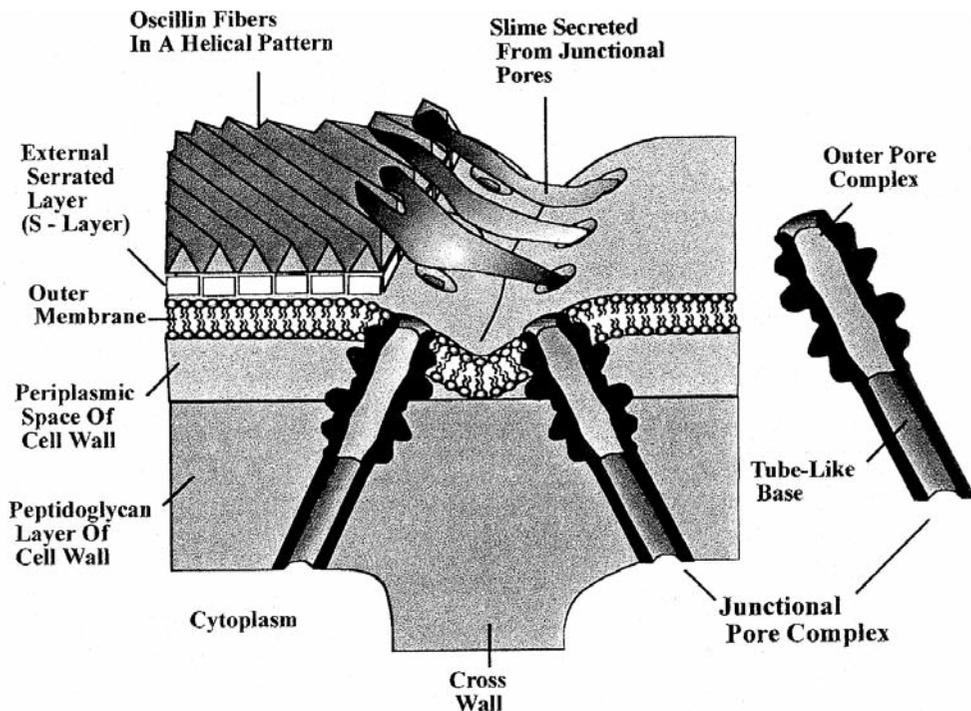
Gliding occurs by slime secretion through the circumferential junctional pores on one side of the septum (Hoiczky and Baumeister, 1998; Hoiczky, 2000). The slime passes along the surface of the oscillin fibers of the outer layer of the cell wall and onto the adjacent substrate, propelling the filament forward. The orientation of the



**Fig. 2.2** The structure of a peptidoglycan molecule in the cell wall of cyanobacteria.

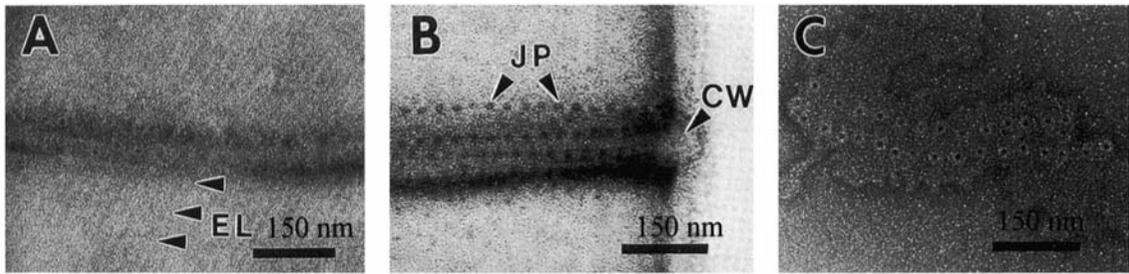


**Fig. 2.3** Cross sections of a wall of a cyanobacterium that is not capable of gliding and a cross section of a wall of a cyanobacterium that is capable of gliding. Cyanobacteria that can glide have an additional two wall layers on the outside. (From Hoiczky and Baumeister, 1995.)



**Fig. 2.4** A model of the junctional pore complex of a cyanobacterium. Extrusion of slime through the circumferentially arranged junctional pores on one side of the cross wall results in forward movement of the filament in contact with the substrate. The arrangement of the oscillin fibers in the outer layer of the cell wall determines whether the filament rotates as it glides over the surface. In the drawing, the oscillin fibers are spiraled so the filament rotates as it glides. (Modified from Hoiczky and Baumeister, 1998.)

oscillin fibers of the outer layer determines whether the filament rotates during gliding. In *Anabaena*, the spiral oscillin fibers produce a clockwise rotation while in *Oscillatoria princeps* and *Lyngbya aeruginosa* the oscillin fibers are spiraled in the opposite direction and produce a counterclockwise rotation during gliding



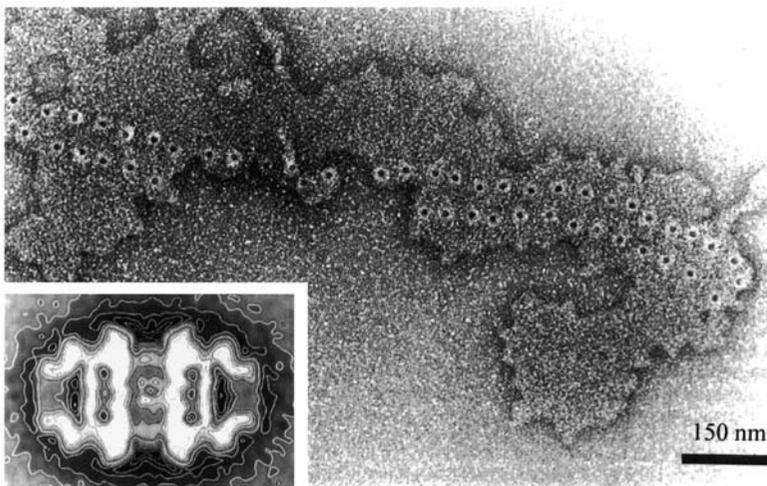
**Fig. 2.5** Transmission electron micrographs of *Phormidium uncinatum*. (A) Isolated cell wall with the outer membrane and external layer (EL) still attached. On both sides of the cross wall, the ring-shaped counterpart of the junctional pores with their central pores are visible. (B) Negatively stained isolated wall showing the junctional pores (JP) filled with slime. (CW) cross wall. (C) Isolated outer membrane with the ring-shaped parts of the junctional pores. (From Hoiczky and Baumeister, 1995.)

(Hoiczky and Baumeister, 1995) (Fig. 2.7). In *Phormidium*, the oscillin fibers are not spiraled and the filament does not rotate during gliding.

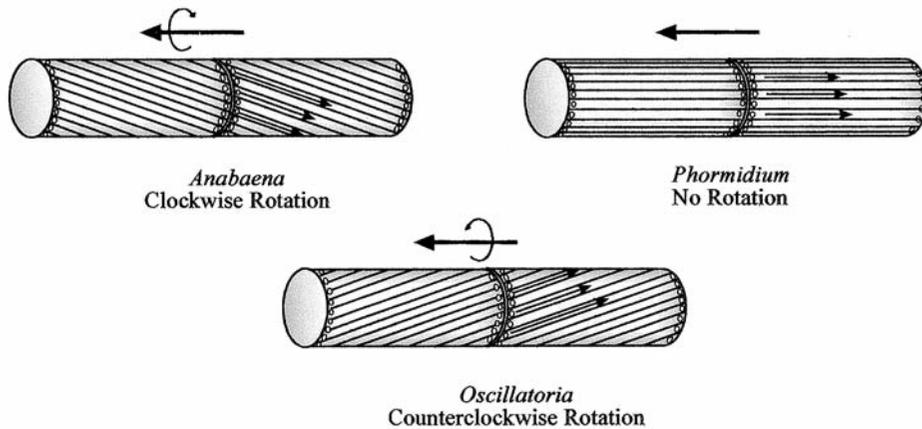
The arrangement of hair-like fibers thus serves as a passive screw as the slime passes over their surface in gliding. Reversal of gliding occurs when slime stops coming out of the ring of junctional pores on one side of the septum, and when slime begins coming out of the ring of junctional pores on the other side of the septum.

## Pili and twitching

Pili are proteinaceous appendages that project from the surface of cyanobacterial cells (Fig. 2.8). There are two types of pili in the unicellular cyanobacterium *Synechocystis* (Bhaya, 2004). The cell is covered uniformly with a layer of thin-brush-like pili with an average diameter of 3–4 nm and a length of 1  $\mu\text{m}$ . Cells also have thick flexible pili with a diameter of 6–8 nm and length of 4–5  $\mu\text{m}$  that often make connections with other cells. The pili are composed of 500 to 1000 units of the polypeptide **pilin**. Each pilin unit consists of between 145 and 170 amino acids (Bhaya et al., 1999). The pilin molecule is similar to the oscillin molecule involved in gliding. *Synechocystis* is able to move across a surface at 1 to 2  $\mu\text{m s}^{-1}$  using a mechanism called twitching that utilizes change in configuration of the pili (Wall and Kaiser, 1999). The pili probably move the cell body along a sur-



**Fig. 2.6** Structure of the organelles of the junctional-pore complex in *Phormidium uncinatum*. Transmission electron micrograph of a negatively stained isolated outer membrane patch showing the ring-shaped orifices of the junctional pores that would be circumferentially arranged in the cross wall in the cell. Inset shows a number of superimposed images of junctional-pore complexes. (From Hoiczky, 2000.)



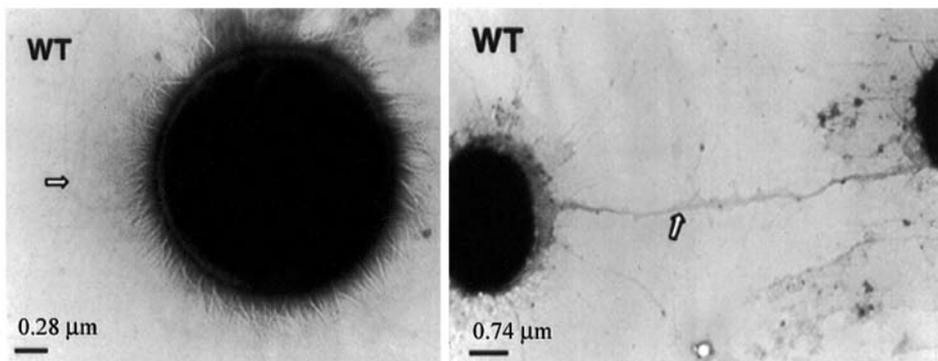
**Fig. 2.7** Rotation of the cyanobacterial filament depends on the orientation of the oscillin protein. Mucilage is secreted from the pores near the cross walls. The mucilage flows along the oscillin fibers causing rotation if the oscillin is helically oriented. There is no rotation if the oscillin is not helically oriented.

face by a reiterative process of pili extension, adhesion, and retraction (Bhaya, 2004).

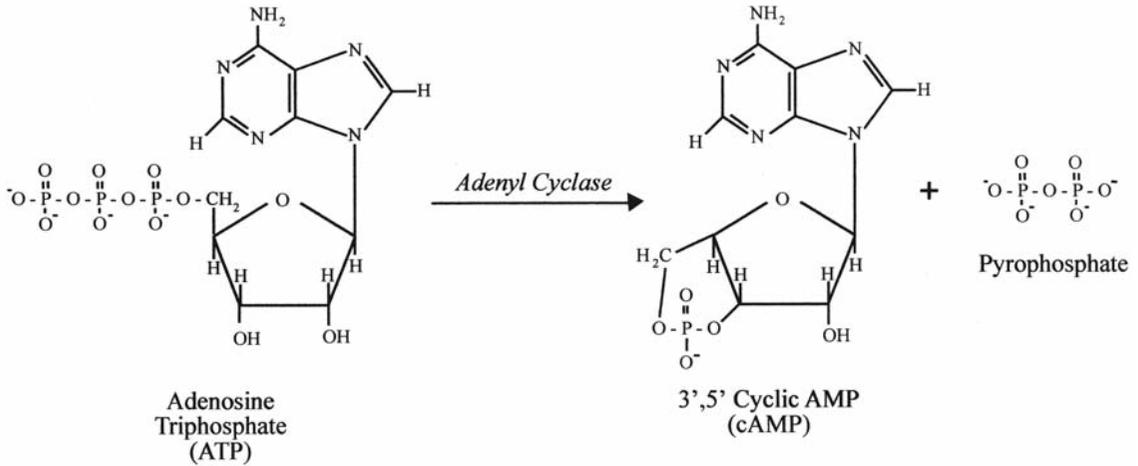
*Synechocystis* exhibits both positive and negative phototaxis in blue light (450 nm wavelength) but not in red or far-red light (Terauchi and Ohmori, 2004). Blue light stimulates the production of cyclic adenosine monophosphate (cAMP), a common second messenger in biological systems (Fig. 2.9).

## Sheaths

A sheath (capsule or extracellular polymeric substances (EPS)) composed of mucilage and a small amount of cellulose is commonly present in cyanobacteria (Nobles et al., 2001) (Figs. 2.10, 2.11). The sheath protects cells from drying. Active growth appears necessary for sheath formation, a fact that may explain its sometimes poor development around spores and akinetes. The sheath of *Gloeotheca* sp. is composed of polysaccharides with neutral sugars and uronic acids including galactose, glucose, mannose, rhamnose, 2-O-methyl-D-xylose, glucuronic acid and galacturonic acids (Weckesser et al., 1987). The



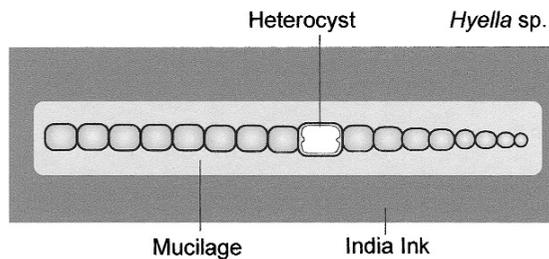
**Fig. 2.8** Transmission electron micrographs of negatively stained whole cells of *Synechocystis* showing pili. (WT) Wild type. (From Bhaya et al., 1999.)



**Fig. 2.9.** The enzyme adenyl cyclase catalyzes the formation of cAMP from ATP.

sheath of *Gloeothece* contains only 2% protein and a trace of fatty acids and phosphate. The commercial applications of cyanobacterial EPS have been reviewed by De Philippis and Vincenzini (1998). Sheaths are often colored, with red sheaths found in algae from highly acid soils and blue sheaths characteristic of algae from basic soils (Drouet, 1978). Yellow and brown sheaths are common in specimens from habitats of high salt content, particularly after the algae dry out.

The sheath excludes India ink so the easiest way to visualize the sheath is to place a small amount of India ink in the water (Fig. 2.10). Production of a sheath is dependent on environmental conditions. A shortage of CO<sub>2</sub> results in cessation of sheath production and release of



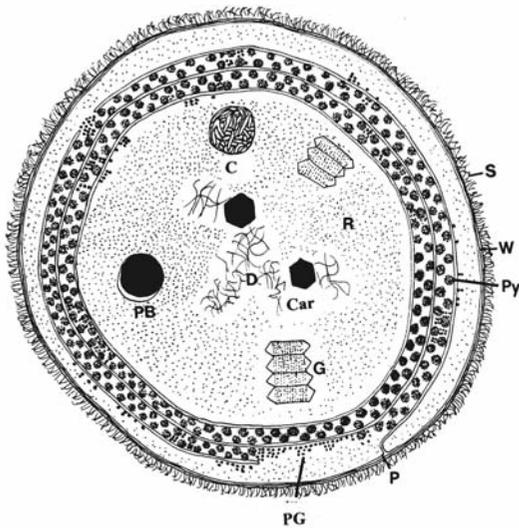
**Fig. 2.10** A drawing of a filament of *Hyella* sp. in India ink. This method clearly shows the sheath around the filament.

the sheath. An excess of fixed carbon results in formation of a sheath (Otero and Vincenzini, 2004).

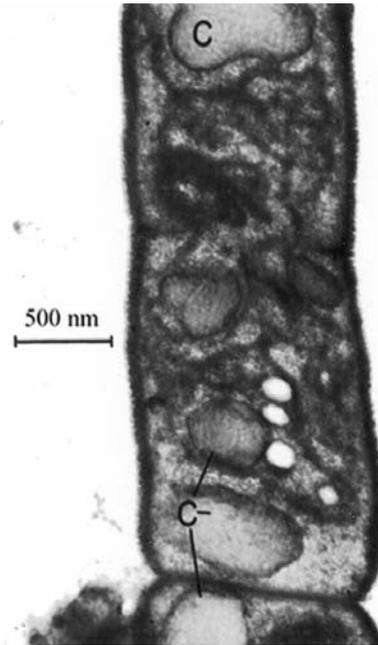
## Protoplasmic structure

Many of the protoplasmic structures found in the bacteria occur in the cyanobacteria. In the central protoplasm are the **circular fibrils of DNA** which are not associated with basic proteins (**histones**) (Figs. 2.11 and 2.14). The amount of DNA in unicellular cyanobacteria varies from  $1.6 \times 10^9$  to  $8.6 \times 10^9$  daltons. This is similar to the genome size in bacteria ( $1.0 \times 10^9$  to  $3.6 \times 10^9$  daltons) and is larger than the genome size in mycoplasmas ( $0.4 \times 10^9$  to  $0.5 \times 10^9$  daltons) (Herdman et al., 1979). The peripheral protoplasm is composed principally of **thylakoids** and their associated structures, the **phycobilisomes** (on the thylakoids, containing the phycobiliproteins) and **glycogen granules**. The **70S ribosomes** are dispersed throughout the cyanobacterial cell but are present in the highest density in the central region around the nucleoplasm (Allen, 1984).

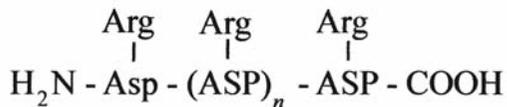
**Cyanophycin** is a non-ribosomally synthesized protein-like polymer that occurs in the cytoplasm in structured granules that are not surrounded by a membrane (Fig. 2.13) (Aboulmagd et al., 2000; Sherman et al., 2000). Cyanophycin is a polymer that consists of equimolar amounts of arginine and aspartic acid arranged as a polyaspartate



**Fig. 2.11** Drawing of the fine-structural features of a cyanobacterial cell. (C) Cyanophycin body (structured granule); (Car) carboxysome (polyhedral body); (D) DNA fibrils; (G) gas vesicles; (P) plasmalemma; (PB) polyphosphate body; (PG) polyglucan granules; (Py) phycobilisomes; (R) ribosomes; (S) sheath; (W) wall.



**Fig. 2.13** Transmission electron micrograph of a section of a cell of *Plectonema boryanum* showing cyanophycin bodies (C). (From Lawry and Simon, 1982.)



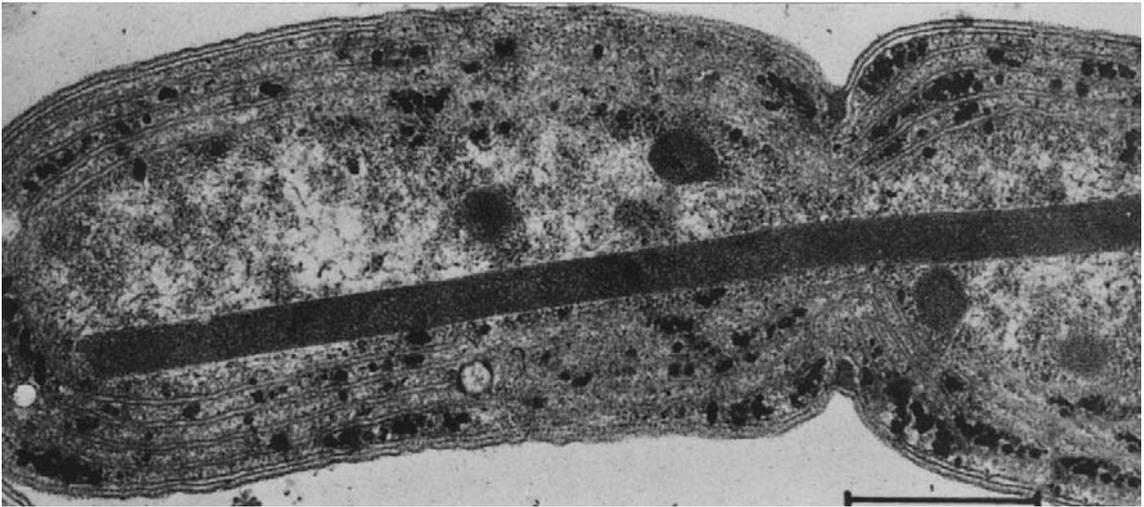
**Fig. 2.12** Cyanophycin is composed of equimolar amounts of arginine (Arg) and aspartic acid (Asp) arranged as a polyaspartate backbone.

backbone (Fig. 2.12). Cyanophycin functions as a temporary nitrogen reserve in nitrogen-fixing cyanobacteria, accumulating during the transition from the exponential to the stationary phase and disappearing when balanced growth resumes. Nitrogen is stored in phycobilisomes in cyanobacteria that do not fix nitrogen (Li et al., 2001a).

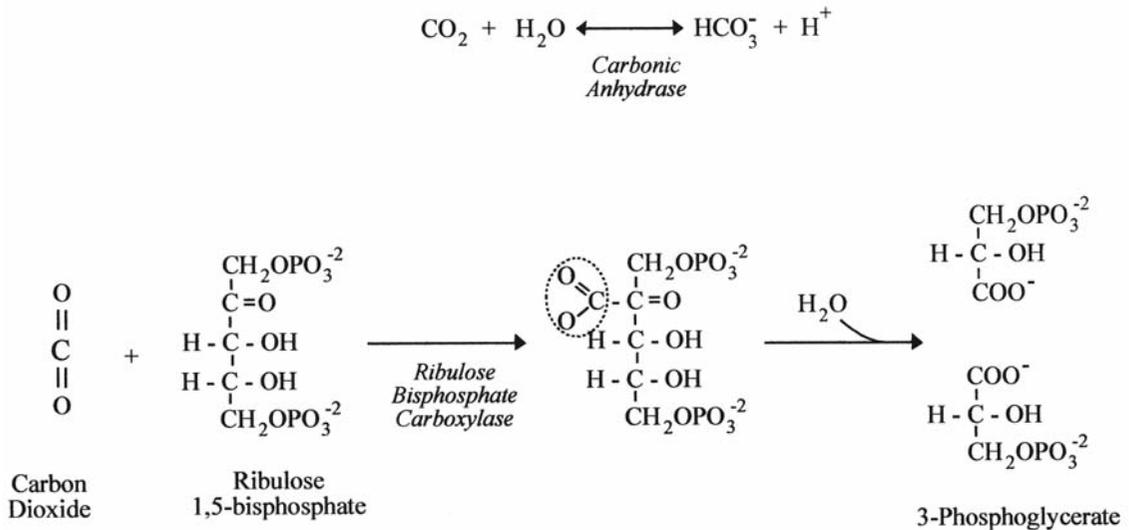
**Carboxysomes** (polyhedral bodies) (Fig. 2.14) are similar to the carboxysomes in bacteria and contain the carbon dioxide-fixing enzyme **ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)**. There are two types of carboxysomes,

$\alpha$ -carboxysomes and  $\beta$ -carboxysomes, which differ in their protein composition. Cyanobacteria with  $\alpha$ -carboxysomes occur in environments where dissolved carbon is not limiting (e.g., oligotrophic oceanic waters), whereas cyanobacteria with  $\beta$ -carboxysomes occur in environments where dissolved carbon is limiting (e.g., mats, films, estuaries, and alkaline lakes with higher densities of photosynthetic organisms) (Badger et al., 2002).

Carboxysomes also contain the enzyme **carbonic anhydrase** that converts  $\text{HCO}_3^-$  into carbon dioxide, the only form of carbon that is fixed by Rubisco (Fig. 2.15). Bicarbonate ( $\text{HCO}_3^-$ ) is transported into the cell and carboxysome. Carbonic anhydrase in the carboxysome converts  $\text{HCO}_3^-$  into  $\text{CO}_2$  which is fixed by Rubisco into carbohydrates. The amount of a cell occupied by carboxysomes increases as the inorganic carbon ( $\text{HCO}_3^-$ ,  $\text{CO}_2$ ) in the medium decreases (Turpin et al., 1984). Heterocysts (Fig. 2.4) lack ribulose-1,5-bisphosphate carboxylase/oxygenase and the ability to fix carbon dioxide. Heterocysts also lack carboxysomes (Winkenbach and Wolk, 1973).



**Fig. 2.14** Transmission electron micrograph of a section of a dividing cell of *Anacystis nidulans* showing thylakoids in the peripheral cytoplasm, DNA microfibrils, ribosomes, and a long carboxysome in the central cytoplasm. Bar = 0.5  $\mu\text{m}$ . (From Gantt and Conti, 1969.)

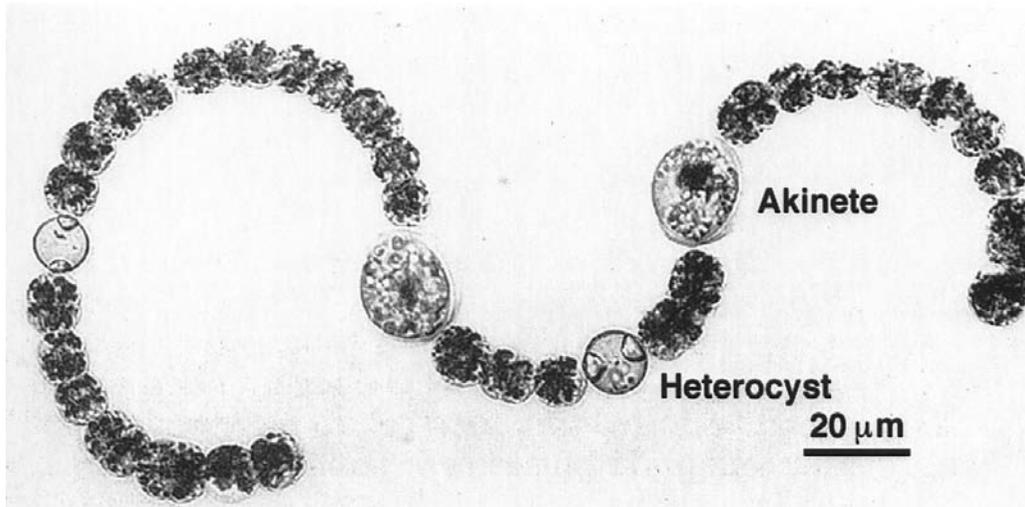


**Fig. 2.15** Carboxysomes contain the enzymes carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase/oxygenase. Carbonic anhydrase in the carboxysome converts  $\text{HCO}_3^-$  into  $\text{CO}_2$  which is fixed by ribulose-1,5-bisphosphate carboxylase/oxygenase into carbohydrates.

**Polyphosphate bodies (metachromatic or volutin granules)** (Fig. 2.11) are spherical and appear similar to lipid bodies of eukaryotic cells in the electron microscope. Polyphosphate bodies

contain stored phosphate, the bodies being absent in young growing cells or cells grown in a phosphate-deficient medium, but present in older cells (Tischer, 1957).

**Polyglucan granules ( $\alpha$ -granules)** (Fig. 2.11) are common in the space between the thylakoids in actively photosynthesizing cells. These granules contain a carbohydrate, composed of 14 to 16



**Fig. 2.16** Light micrograph of *Anabaena crassa* showing vegetative cells, akinetes, and heterocysts. (From Li et al., 1997.)

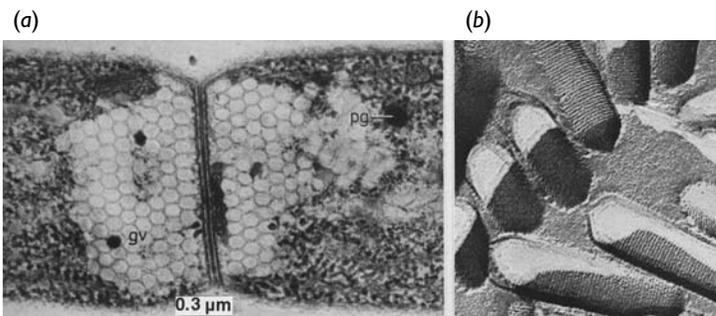
glucose molecules, that is similar to amylopectin (Hough et al., 1952; Frederick, 1951).

## Gas vacuoles

A gas vacuole is composed of gas vesicles, or hollow cylindrical tubes with conical ends, in the cytoplasm of cyanobacteria (Figs. 2.11, 2.17) (Walsby, 1994; Oliver, 1994). Gas vesicles do not have true protein-lipid membranes, being composed exclusively of protein ribs or spirals arranged similarly to the hoops on a barrel. It is possible to collapse the gas vesicles by applying pressure to the cells, the collapsed vesicles having the two halves stuck together. The membrane of

the gas vesicle is quite rigid, with the gas inside it at a pressure of 1 atm. The membrane is permeable to gases, allowing the contained gas to equilibrate with gases in the surrounding solution. The membrane must, however, be able to exclude water. It has been postulated that the inner surface must be **hydrophobic**, thereby preventing condensation on it of water droplets, and restraining, by surface tension, water creeping through the pores. At the same time these molecules must present a **hydrophilic** surface at the outer (water-facing) surface in order to minimize the interfacial tension, which would otherwise result in the collapse of the gas vacuole.

Cyanobacteria possessing gas vacuoles can be divided into two physiological-ecological groups. In the first group are those algae having vacuoles only at certain stages of their life cycle, or only in certain types of cells. In *Gloetrichia ghosei* and in certain

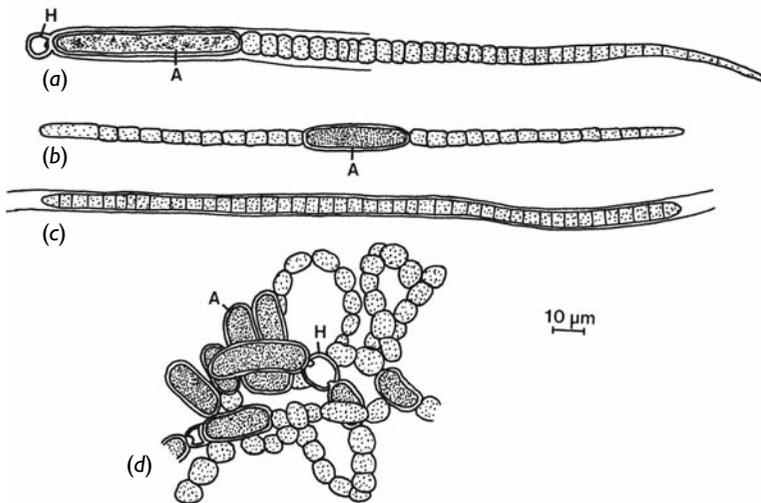


**Fig. 2.17** (a) Transmission electron micrograph of two cells of *Oscillatoria redekei* showing a cross wall separating areas of gas vacuoles (gv); (pg) lipid droplet. (From Whitton and Peat, 1969.) (b) Freeze-etch preparation of gas vacuoles. (From Jones and Jost, 1970.)

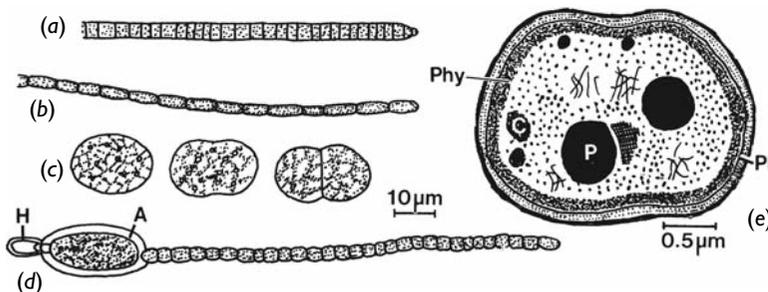
species of *Tolypothrix* and *Calothrix*, gas vesicles appear only in hormogonia. The hormogonia float when they are released, and it is possible that the buoyancy provided is of significance in dispersal of these stages. The second group consists of planktonic cyanobacteria, including species of *Anabaena* (Figs. 2.16, 2.18(d), 2.57(b)), *Gloeotrichia* (Fig. 2.18(a)), *Microcystis* (Figs. 2.48, 2.56(b)), *Aphanizomenon* (Fig. 2.18(b)), *Oscillatoria* (Figs. 2.19(a), (b), 2.34(a), (b)), *Trichodesmium* (Figs. 2.31, 2.56(g)), and *Phormidium* (Figs. 2.18(c), 2.56(c)). These algae derive positive buoyancy from their gas vesicles, and as a consequence form blooms floating near the water surface. The loss of buoyancy, and subsequent sinking of these algae in the water column, can be due to different factors. In *Anabaena flos-aquae* (Fig. 2.18(d)), the loss of buoyancy is caused by the loss of gas vesicles owing to increased turgor pressure, whereas in

*Oscillatoria agardhii* (Fig. 2.19(a)), buoyancy is lost through cessation of gas vesicle production and an increase in cell mass. In *Microcystis aeruginosa* (Fig. 2.56(b)), loss of buoyancy can be due to entrapment of whole colonies in a colloidal precipitate composed of organic material and iron salts. The colloidal precipitate is formed in certain lakes when dissolved iron in the anoxic water of the hypolimnion in stratified lakes becomes oxidized on mixing with aerated water of the epilimnion (Oliver et al., 1985).

There is a direct relationship between buoyancy and light quantity in nitrogen-fixing cyanobacteria such as *Anabaena flos-aquae* (Fig. 2.18(d)) (Spencer and King, 1985). The relationship is complex and also involves the concentration of ammonium ions ( $\text{NH}_4^+$ ) in the water. Buoyancy in *Anabaena flos-aquae* increases under low irradiance (less than  $10 \mu\text{E m}^{-2}$



**Fig. 2.18** (a) *Gloeotrichia echinulata*. (b) *Aphanizomenon flos-aquae*. (c) *Phormidium inundatum*. (d) *Anabaena flos-aquae*. (A) Akinete; (H) heterocyst. (After Prescott, 1962.)



**Fig. 2.19** (a) *Oscillatoria agardhii*. (b) *O. limnetica*. (c) *Synechococcus aeruginosus*. (d) *Cyndrospermum majus*. (A) Akinete; (H) heterocyst. (e) Drawing of the fine structure of *Gloeobacter violaceus*. (C) Cyanophycin granule; (P) polyphosphate body; (Phy) probable layer of phycobiliproteins; (Pl) plasmalemma. ((c),(d) after Prescott, 1962; (e) after Rippka et al., 1974.)

s<sup>-1</sup>), absence of NH<sub>4</sub><sup>+</sup>, and low CO<sub>2</sub> concentrations. Such conditions occur in many stagnant eutrophic lakes during the summer. In these lakes, rapid growth of algae has depleted the NH<sub>4</sub><sup>+</sup> and CO<sub>2</sub>. The water transmits little light because of the large standing crop of algae. Under these conditions, *A. flos-aquae* and other nitrogen-fixing cyanobacteria having gas vacuoles increase their buoyancy and rise close to the surface of the water. Here they are able to outcompete other algae because of their ability to fix nitrogen in water that has little available nitrogen. Cyanobacteria that do not fix nitrogen have reduced growth, and therefore reduced buoyancy, and sink in the water column. Once established, a bloom of buoyant, nitrogen-fixing, cyanobacteria tends to be self-perpetuating in that increased mass of the bloom maintains the reduced light and CO<sub>2</sub> levels required for maximum buoyancy.

Structures other than gas vesicles can cause significant variation in cell density, and therefore buoyancy (Konopka et al., 1987). Polyphosphate granules may have a density of 2 g cm<sup>-3</sup> or greater, and glycogen (which is accumulated under high light intensities) has a density of about 1.5 cm<sup>-3</sup>. Both have a higher density than water (1 g cm<sup>-3</sup>) and can cause cells to sink (Booker and Walsby, 1981; McCausland et al., 2005).

## Pigments and photosynthesis

The major components of the photosynthetic light-harvesting system of the cyanobacteria are **chlorophyll *a*** in the thylakoid membrane, and the **phycobiliproteins**, which are water-soluble chromoproteins assembled into macromolecular aggregates (phycobilisomes) attached to the outer surface of the thylakoid membranes. Some cyanobacteria contain chlorophyll *b* and the cyanobacterium *Acaryochloris marina* (Fig. 2.20) contains chlorophyll *d*. At one time those cyanobacteria containing chlorophyll *b* (*Prochlorococcus* (Fig. 2.20), *Prochlorothrix*, *Prochloron*) were thought to be a distinct evolutionary group and they were placed in the Prochlorophyta. Evolutionary trees based on nucleic acid sequencing have shown that chlorophyll *b* arose a number of times and that these cyanobacteria are spread throughout the group

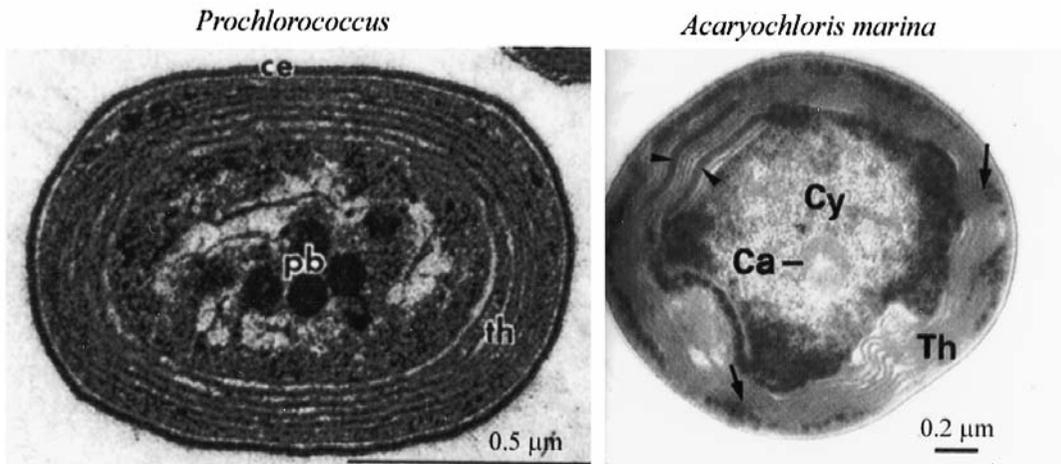
(Palenik and Haselkorn, 1992; Urback et al., 1992). In contrast, *Acaryochloris marina* (Fig. 2.20), the only cyanobacterium known to have chlorophyll *d*, appears to be only distantly related to other cyanobacteria (Miller et al., 2005; Miyashita et al., 2003). The absorption spectrum of chlorophyll *d* (Fig. 1.19) is shifted toward far-red wavelengths and *A. marina* exists in environments where there is an abundance of these wavelengths of light (e.g., under red algae).

The carotenoids of the cyanobacteria differ from those of the eukaryotic algae in having **echinone** (4-keto- $\beta$ -carotene) and **myxoxanthophyll**, which eukaryotic algae do not have; in lacking **lutein**, the major xanthophyll of chloroplasts; and in having much higher proportions of  $\beta$ -carotene than are found in eukaryotic algae (Goodwin, 1974).

The Cyanophyceae have four phycobiliproteins: **C-phycocyanin** (absorption maximum at a wavelength [ $\lambda$ ] of 620 nm), **allophycocyanin** ( $\lambda_{\max}$  at 650 nm), **C-phycoerythrin** ( $\lambda_{\max}$  at 565 nm), and **phycoerythrocyanin** ( $\lambda_{\max}$  at 568 nm). All cyanobacteria contain the first two, whereas C-phycoerythrin and phycoerythrocyanin occur only in some species. The phycobiliproteins of the cyanobacteria change in concentration in response to light quality and growth conditions. Cyanobacteria that produce the red phycoerythrin and the blue phycocyanin in white light, suppress phycoerythrin synthesis in red light and phycocyanin synthesis in green light (**complementary chromatic adaptation**; see Tandeau de Marsac, 1977).

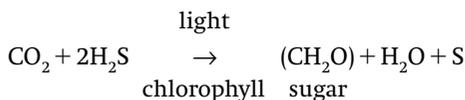
In the evolution of the cyanobacteria, the thylakoids probably originated by invaginations of the plasmalemma; some cyanobacteria today have thylakoids that are continuous with the plasmalemma. An example of the primitive condition may be *Gloeobacter violaceus* (Fig. 2.19(e)), a unicellular cyanobacteria that lacks thylakoids but has chlorophyll *a*, carotenoids, and phycobiliproteins. In this alga the pigments, and presumably photosynthesis, are associated with the plasmalemma (Rippka et al., 1974).

Many cyanobacteria have the ability to photosynthesize under aerobic or anaerobic conditions. Under aerobic conditions, electrons for photosystem I are derived from photosystem II. Under



**Fig. 2.20** Transmission electron micrographs of a section of *Prochlorococcus*, a chlorophyll *b* containing cyanobacterium, and of *Acaryochloris marina*, a chlorophyll *d* containing cyanobacterium. *Prochlorococcus* is the smallest known photosynthetic organism. (Ca) Carboxysome (polyhedral body); (Cy) cytoplasm; (pb) polyhedral body; (th) thylakoids. (Arrow) channel-like structures perforating thylakoids. (Arrowheads) areas of accumulation of phycobiliproteins. (*Prochlorococcus* micrograph from Partensky, Hess and Vault, 1999; *Acaryochloris* micrograph is from Marquardt et al., 2000.)

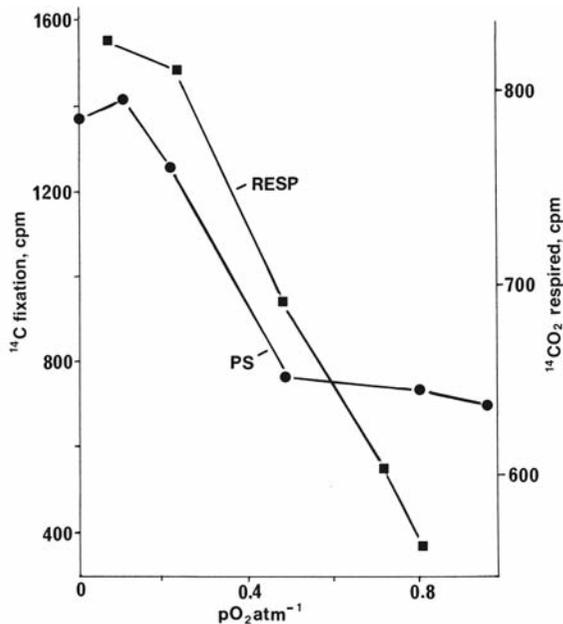
anaerobic conditions, in the presence of sulfur, electrons are derived by the reduction of sulfur:



These cyanobacteria are **facultative phototrophic anaerobes** and fill an important ecological niche in aquatic systems (Padan, 1979). Eukaryotic algae are restricted to photoaerobic habitats, whereas photosynthetic bacteria are restricted to photoanaerobic habitats. In habitats that fluctuate between the above conditions, cyanobacteria with facultative anaerobic photosynthesis have a clear selective advantage. An example of this is the Solar Lake, Elat, Israel, where in the winter high levels of sulfide are found in the anaerobic bottom layers of water of the thermally stratified lake. *Oscillatoria limnetica* (Fig. 2.19(b)) occurs in these highly anaerobic bottom layers, where sulfide functions as an electron donor for photosynthesis. In the spring, the lake overturns, with all of the water becoming aerobic, *Oscillatoria limnetica* activates aerobic

photosynthesis (Fig. 2.28) and carries out photosynthesis aerobically. Thus *O. limnetica*, by utilizing combined anoxygenic and oxygenic photosynthesis, is the dominant phototroph of the Solar Lake, with its fluctuating photoaerobic and photoanaerobic conditions. The interlinking position of the cyanobacteria in the phototropic world is compatible with the fact that they are among the oldest organisms, dating back to the Precambrian Period. Significantly, two of the sulfide-rich ecosystems containing high numbers of cyanobacteria – that is, hot sulfur springs and the marine littoral sediments – may represent old ecosystems that may predate the oxidized biosphere.

Photosynthesis in many cyanobacteria is stimulated by lowered oxygen concentration, the oxygen competing with carbon dioxide for the enzyme ribulose-1, 5-bisphosphate carboxylase/oxygenase (Fig. 2.21) (Stewart and Pearson, 1970; Weller et al., 1975). This phenomenon probably reflects an adaptation to the absence of free oxygen in the atmosphere of Precambrian times when the cyanobacteria first evolved. After the evolution of the oxygen-evolving cyanobacteria, the oxygen in the atmosphere gradually built up, creating a protective ozone (O<sub>3</sub>) layer in the atmosphere at the same time. The ozone layer removed most of the harmful ultraviolet radiation from the sun and allowed the evolution of more radiation-sensitive organisms. The cyanobacteria are relatively insensitive to radiation, having a system that repairs radiation damage (Bhattacharjee, 1977).



**Fig. 2.21** Graph showing stimulation of photosynthesis (●-●) and respiration (■-■) by low concentrations of atmospheric O<sub>2</sub> in *Anabaena flos-aquae*. (After Stewart and Pearson, 1970.)

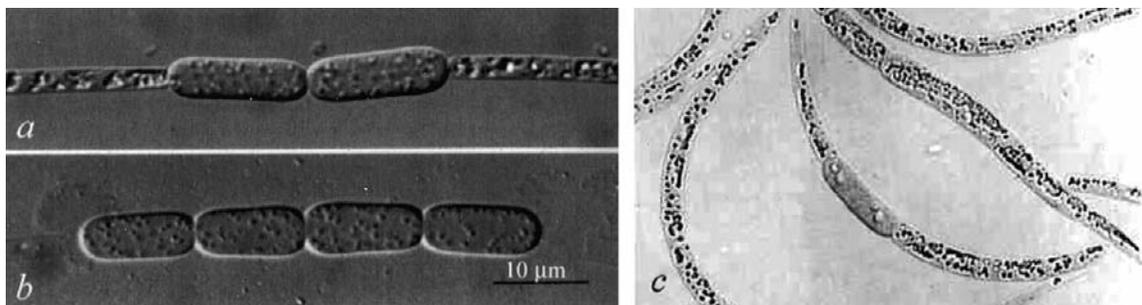
## Akinetes

Akinetes are generally recognized by their larger size relative to vegetative cells and conspicuous granulation due to high concentrations of glycogen and cyanophycin (Figs. 2.22, 2.23) (Meeks et al., 2002). The most consistent property of akinetes is their greater resistance to cold compared with

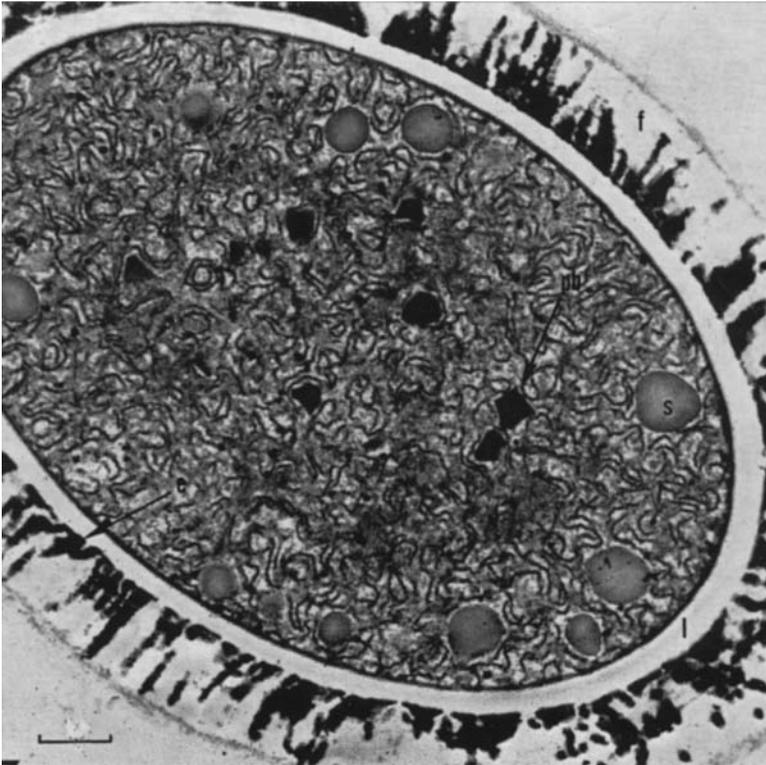
vegetative cells. Akinetes have often been compared to endospores in Gram-positive bacteria. Akinetes, however, are neither as metabolically quiescent nor as resistant to various environmental extremes. Akinetes only occur in cyanobacteria that form heterocysts.

In *Aphanizomenon* (Fig. 2.18(b)) (Wildman et al., 1975), the development of akinetes from vegetative cells involves an increase in cell size, the gradual disappearance of gas vacuoles, and an increase in cytoplasmic density and number of ribosomes and cyanophycin granules. Akinetes of *Nostoc* lose 90% of their photosynthetic and respiratory capabilities, as compared with vegetative cells. The loss occurs even though there is little change in phycocyanin and chlorophyll, the main photosynthetic pigments (Chauvat et al., 1982). Mature akinetes are usually considerably larger than vegetative cells, contain protoplasm full of food reserves, and have a normal cell wall surrounded by a wide three-layered coat (Jensen and Clark, 1969; Cmiec et al., 1986) (Figs. 2.16, 2.18, 2.19(d), 2.22, 2.23). Loss of flotation by an increase in cytoplasmic density causes filaments with akinetes to sink and overwinter in bottom sediments. In akinete germination, there is a reverse of the above events (Fig. 2.24).

A wide range of physicochemical factors have been reported to stimulate akinete differentiation; for example, phosphate deficiency, low temperature, carbon limitation and reduction in the availability of light energy (Li et al., 1997; van Dok and Hart, 1995).

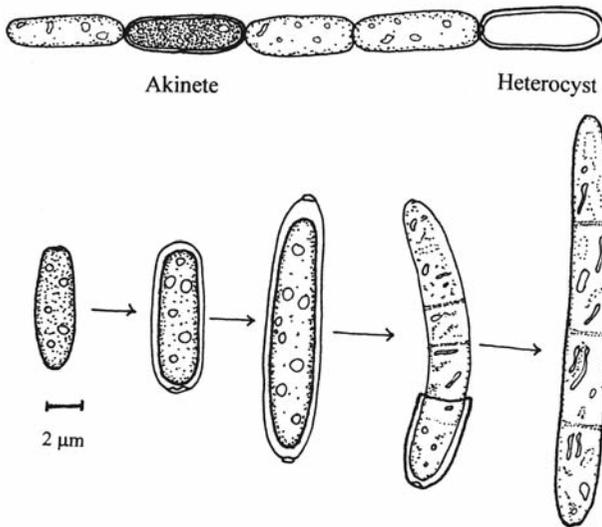


**Fig. 2.22** Light micrographs of akinetes in *Raphidiopsis mediterranea* (a),(b) and *R. curvata* (c). ((a),(b) from Watanabe et al., 2003; (c) from Li et al., 2001a.)



**Fig. 2.23** Electron micrograph of a mature akinete of *Cylandrospermum* sp. with a thick layered wall (f,l) and cytoplasm full of proteinaceous cyanophycin (structured) granules (s), polyhedral bodies, and ribosomes. (From Clark and Jensen, 1969.)

*Cylandrospermopsis raciborskii*



**Fig. 2.24** The germination of an akinete of *Cylandrospermopsis raciborskii*. (Modified from Moore et al., 2004.)

## Heterocysts

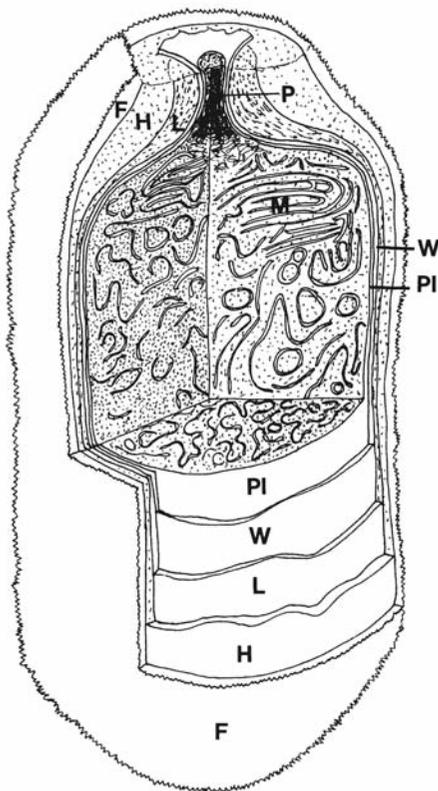
Heterocysts are larger than vegetative cells and appear empty in the light microscope (whereas

akinetes appear full of storage products) (Figs. 2.16, 2.18(a), 2.19(d), 2.25, 2.35). Heterocysts are photosynthetically inactive, they do not fix  $\text{CO}_2$ , nor do they produce  $\text{O}_2$ . They also exhibit a high rate of respiratory  $\text{O}_2$  consumption and are

surrounded by a thick, laminated cell wall that limits ingress of atmospheric gases, including  $O_2$ . The internal environment of heterocysts is, therefore, virtually anoxic, which is ideal for nitrogenase, a notoriously  $O_2$  sensitive enzyme.

Heterocysts are formed at regular intervals from vegetative cells by the dissolution of storage granules, the deposition of a multilayered envelope outside of the cell wall, the breakdown of photosynthetic thylakoids, and the formation of new membranous structures (Fig. 2.25) (Kulasooriya et al., 1972).

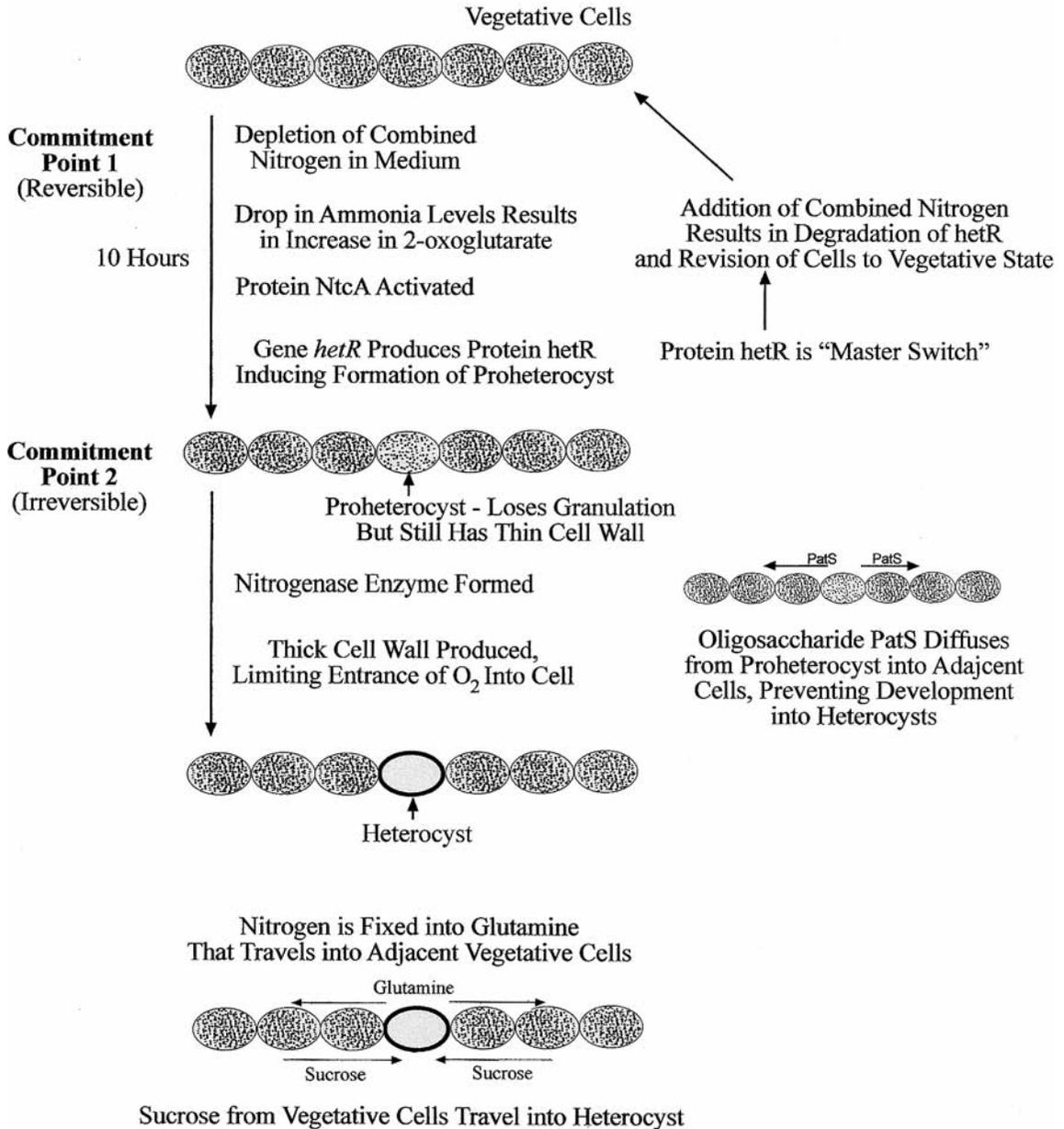
The differentiation of heterocysts in *Anabaena* is triggered by nitrogen deprivation (ammonia, nitrate, nitrite) in two steps: the first step is reversible and the second step is irreversible (Adams, 2000; El-Shehawy and Kleiner, 2003).



**Fig. 2.25** Three-dimensional view of a heterocyst. The envelope has homogeneous (H), fibrous (F), and laminated (L) layers. (M) Membranes; (P) pore channel; (PI) plasmalemma; (W) cell wall. (After Lang and Fay, 1971.)

**Commitment point 1.** 2-oxoglutarate ( $\alpha$ -keto-glutarate) (Fig. 2.26) is the substrate used for incorporation of ammonium in cyanobacteria. The absence of combined nitrogen leads to an increase in intracellular 2-oxoglutarate since cyanobacteria lack 2-oxoglutarate dehydrogenase and the ability to breakdown 2-oxoglutarate. The increase in intracellular 2-oxoglutarate activates the protein NtcA and results in a drop in the amount of the calcium-binding protein CcbP, and a rise in  $Ca^{2+}$  in the cells (Zhao et al., 2005). Heterocysts have 10 times more  $Ca^{2+}$  than vegetative cells. The rise in  $Ca^{2+}$  up-regulates the *hetR* gene that forms **hetR**, a serine-type protease, which induces the vegetative cell to change into a heterocyst. *The hetR protein is considered the “master switch” in heterocyst development.* The production of hetR protein constitutes *commitment point 1*. A lack of combined nitrogen over a period of about 10 hours results in continued production of hetR protein and the cell becoming a **proheterocyst**, which under the light microscope appears less granulated than vegetative cells but still lacks a thick cell wall. The hetR protein also induces the production of an **oligopeptide** called PatS. This oligopeptide diffuses to adjacent cells where it prohibits the formation of hetR protein in the cells and assures that the adjacent cells do not transform into proheterocysts. This sets up the spacing seen in *Anabaena* where heterocysts are formed equidistant from each other. Commitment point 1 is reversible. A proheterocyst will revert back to a vegetative cell if combined nitrogen is added to the medium, causing a switch “off” of the *hetR* gene and formation of hetR protein. Differentiation of a vegetative cell to a proheterocyst also involves loss of photosystem II activity, thus eliminating photosynthetic generation of  $O_2$  (which inhibits nitrogen fixation).

**Commitment point 2.** The second stage can not be reversed by the addition of combined nitrogen and comprises the transformation of a proheterocyst into a mature heterocyst. This involves the formation of a thick cell wall containing glycolipids and polysaccharides to reduce diffusion of  $O_2$ . The nitrogen-fixing enzyme **nitrogenase** is activated when 11 kilobases are removed between repeat sequences flanking nitrogenase (*nif*) genes.

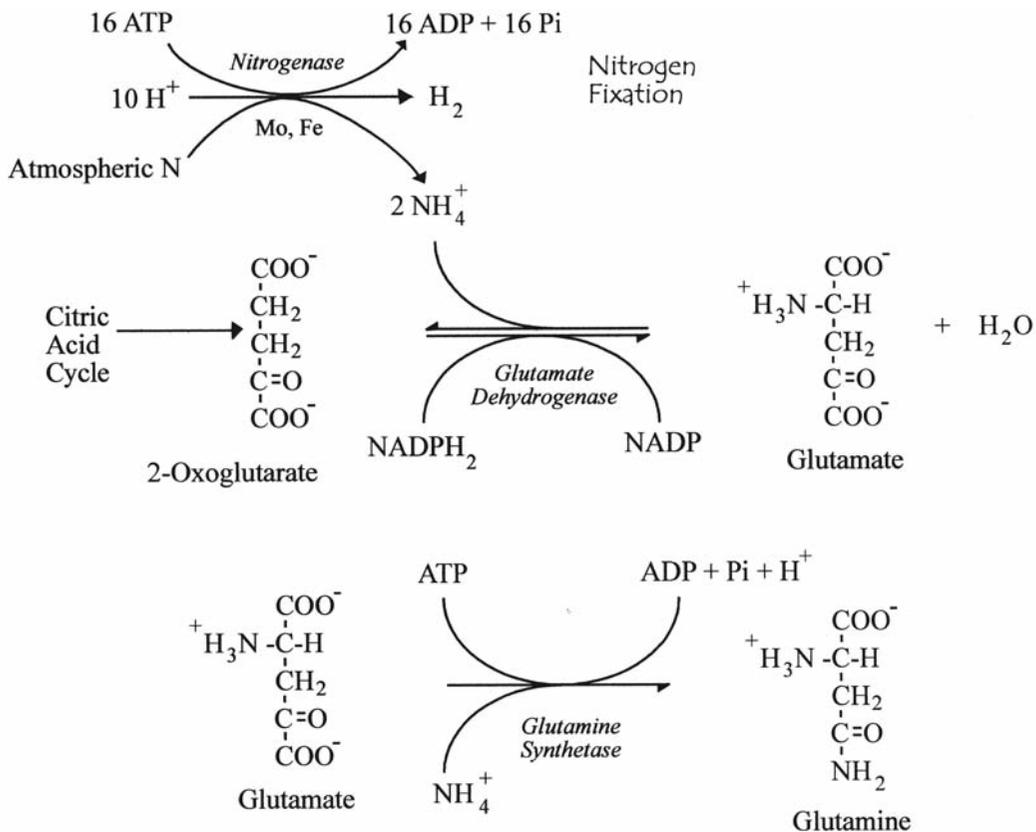


**Fig. 2.26** Summation of events leading to the formation of heterocysts.

This process of heterocyst development is interesting in that it may represent one of the earliest examples of pattern formation in evolution. It is probable that akinetes were the evolutionary precursors in heterocysts (Meeks et al., 2002). Akinetes are only seen in heterocyst-forming

cyanobacteria, akinetes contain glycolipids characteristic of heterocysts, and the cell wall of heterocysts is identical to that of akinetes (and unlike that of vegetative cells). Heterocyst differentiation is a terminal event and is a basic form of programmed cell death or apoptosis.

Apart from the exceptional cases of germination, heterocysts are unable to divide. Heterocysts



**Fig. 2.27** The chemistry of nitrogen fixation and the subsequent incorporation of the fixed nitrogen into glutamate and glutamine.

have a limited period of physiological activity and appear to have a limited life. Senescent heterocysts undergo vacuolation and usually break off from the filament, causing fragmentation of the filament.

Heterocysts are dependent on a supply of substrates from adjacent vegetative cells through cytoplasmic connections (**microplasmodesmata**). These cytoplasmic connections probably convey nitrogen fixed in the form of glutamine (Fig. 2.27) by the heterocysts to vegetative cells. The vegetative cells transfer photosynthate to the heterocysts since the heterocysts are incapable of carbon fixation.

## Nitrogen fixation

Cyanobacteria are **diazotrophs** (able to fix atmospheric nitrogen). All known nitrogen-fixing organ-

isms are prokaryotes. In nitrogen fixation,  $N_2$  from the atmosphere is fixed by the enzyme **nitrogenase** into ammonium using ATP as a source of energy (Fig. 2.27). The process is one of the most metabolically expensive processes in biology, requiring 16 ATP for each molecule of  $N_2$  fixed. The amount of biologically fixed nitrogen produced is in excess of  $2 \times 10^{13} \text{ g year}^{-1}$ . In contrast, lightning discharge, the primary abiotic source of fixed nitrogen, accounts for  $5 \times 10^{12} \text{ g year}^{-1}$  (Raymond et al., 2004).

The ammonium fixed by nitrogen fixation is added to **2-oxoglutarate** (from the citric acid cycle) by the enzyme glutamate dehydrogenase to form **glutamate** (glutamic acid) (Fig. 2.27). Addition of a second ammonium to glutamate produces **glutamine**, the molecule that is transferred from one cyanobacterial cell to another.

In bacteria, nitrogenase is composed of two components, dinitrogenase reductase (iron protein) and dinitrogenase (molybdenum-iron protein) encoded by the *nif* HDK operon (Henson et al.,

2004). The situation is probably similar in the cyanobacteria.

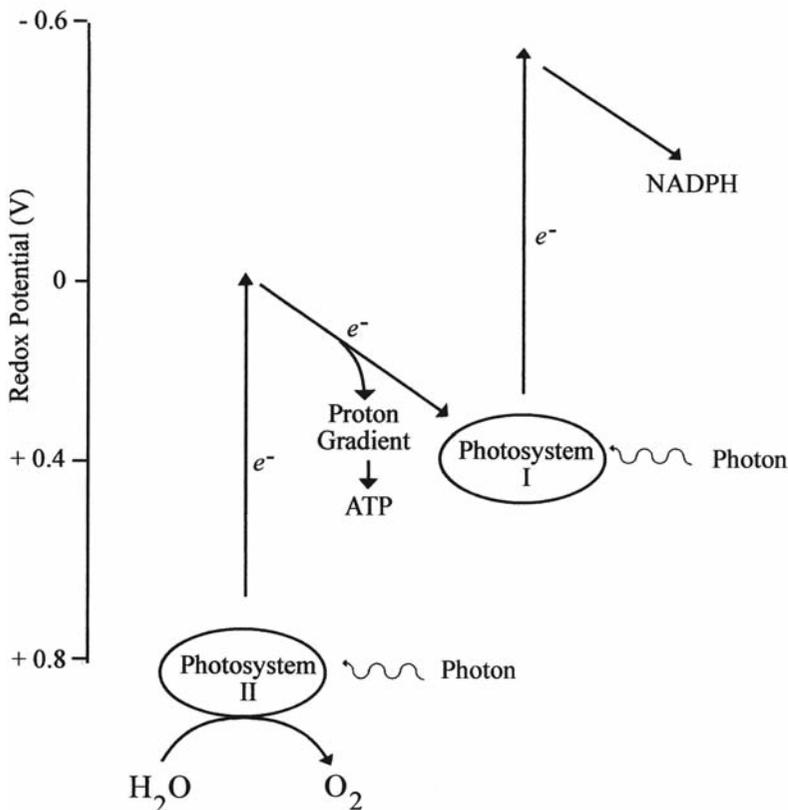
Hydrogen gas is also produced in nitrogen fixation (Fig. 2.27) and has drawn interest as a renewable energy source as hydrogen fuel-cell technology for motor vehicles becomes more of a practical reality (Schutz et al., 2004).

Nitrogenase, the nitrogen-fixing enzyme, is very sensitive to inactivation by oxygen. Cyanobacteria have evolved three different mechanisms designed to exclude oxygen from the area of the cells containing nitrogenase:

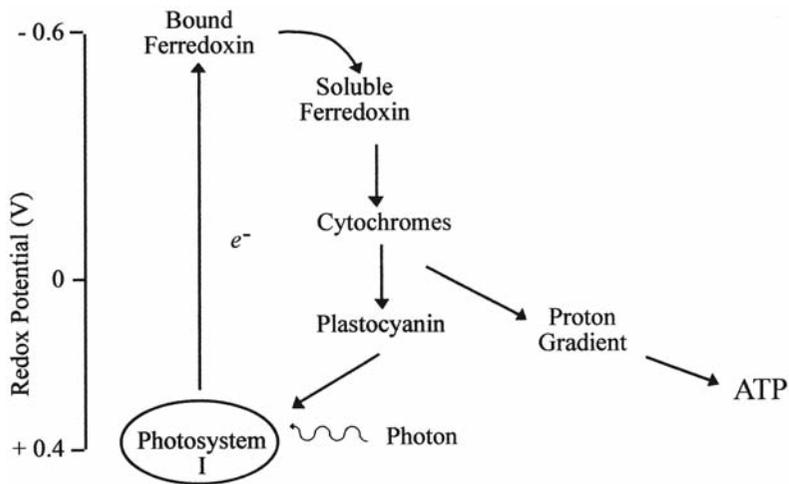
- 1 *Heterocystous cyanobacteria*: These cyanobacteria occur primarily in fresh and brackish water and fix nitrogen in heterocysts. Heterocysts are surrounded by a glycolipid layer which is impermeable to  $O_2$  (Staal et al., 2003). Heterocysts lack photosystem II (Fig. 2.28) and, therefore, the ability to evolve  $O_2$ . Heterocysts do have cyclic photophosphorulation (Fig. 2.29) and can produce the ATP necessary for

nitrogen fixation. Heterocysts also have a form of myoglobin called **cyanoglobin** that scavenges oxygen, preventing inhibition of nitrogenase (Potts et al., 1992). Under anaerobic conditions, in an atmosphere of nitrogen and carbon dioxide, both vegetative cells and heterocysts can fix nitrogen.

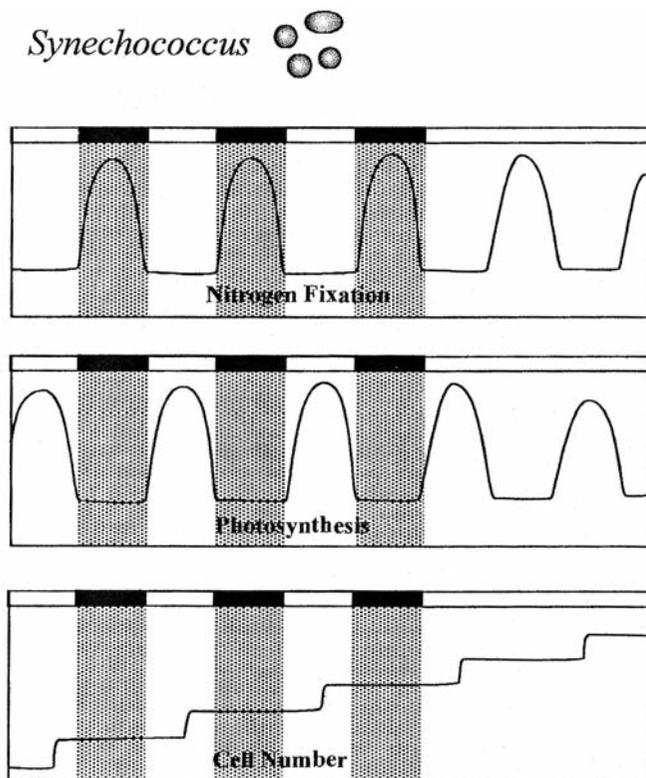
- 2 *Non-filamentous cyanobacteria that fix nitrogen in the dark but not in the light*: These cyanobacteria fix nitrogen in the dark when photosynthesis is not producing nitrogenase-inhibiting oxygen. If *Synechococcus* is grown under a 12-hour light: 12-hour dark cycle, most of the nitrogen fixation occurs during the dark period (Fig. 2.30). If the cells are subjected to continuous illumination, an endogenous timing cycle entrained by cell division continues to alternate the level of photosynthesis and nitrogen fixation (Mitsui et al., 1986; Chen et al., 1996). Nitrogenase activity peaks at the same time that it had in the previous dark period



**Fig. 2.28** Non-cyclic photophosphorylation. Heterocysts in cyanobacteria lack photosystem II and do not produce oxygen.



**Fig. 2.29** Cyclic photophosphorylation. Heterocysts in cyanobacteria produce ATP by cyclic photophosphorylation.

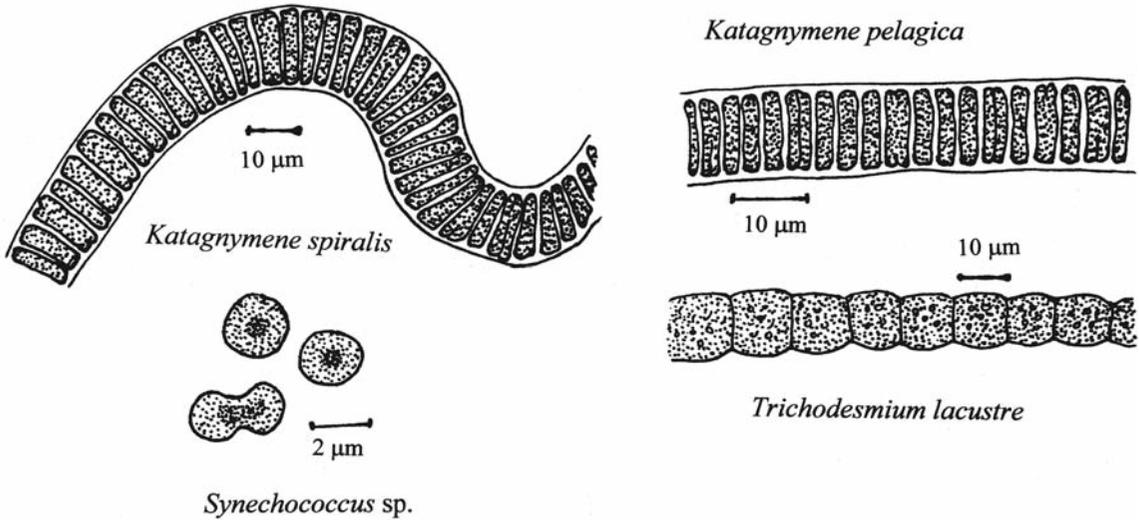


**Fig. 2.30** Illustration of the relationship between photosynthesis and nitrogen fixation in a culture of *Synechococcus* sp. growing under a 12-hour light: 12-hour dark photoperiod. Under continuous illumination, an endogenous clock maintains the cycles. (After Mitsui et al., 1986.)

(Fig. 2.30). At the beginning of the next light period, oxygen is produced by photosynthesis and the nitrogenase is inactivated. New nitrogenase must be synthesized at the

beginning of the next dark period for nitrogen fixation to occur.

3 *Trichodesmium* (Figs. 2.31, 2.56(g)) and *Katagnymene* (Fig. 2.31): These cyanobacteria



**Fig. 2.31** Some nitrogen-fixing cyanobacteria that lack heterocysts.

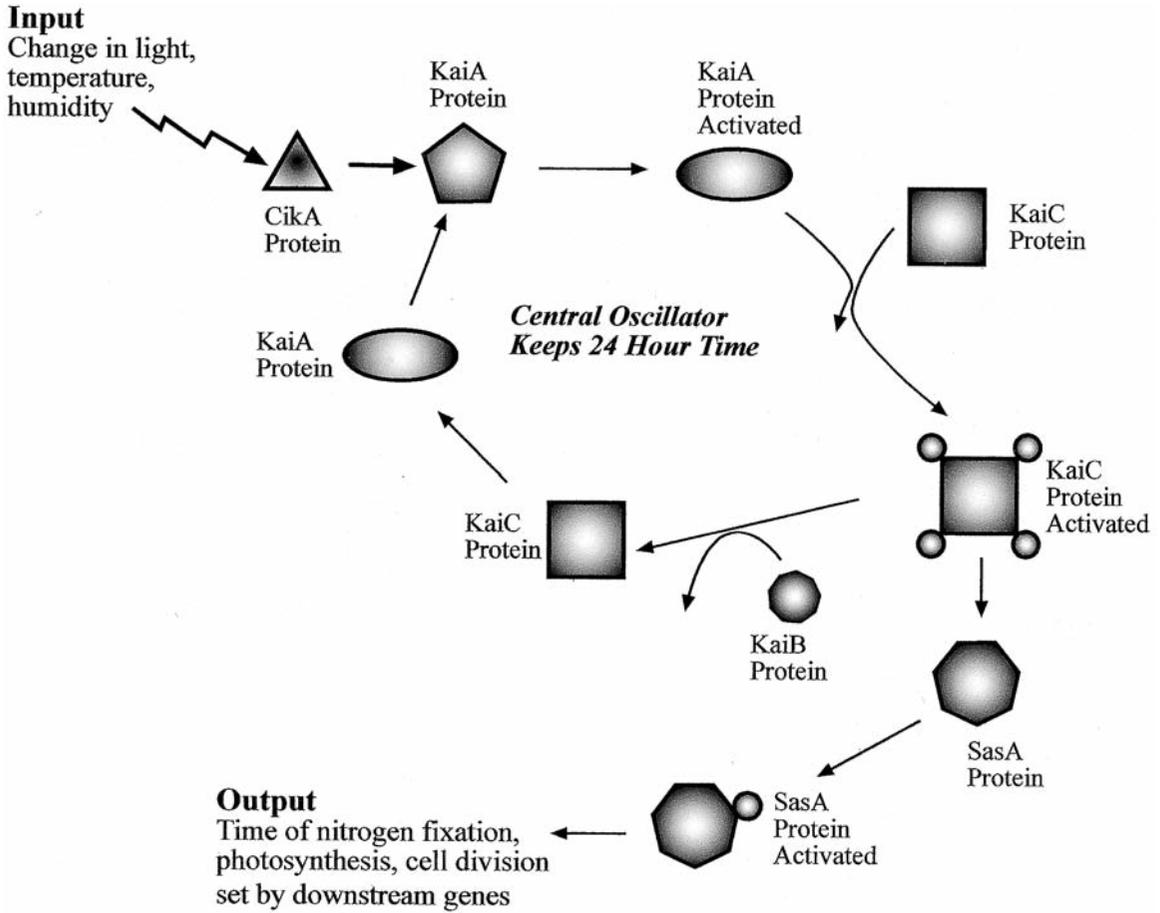
are the major bloom-forming, nitrogen-fixing, organisms in the oceans, responsible for fixing one-quarter of the total nitrogen in the oceans of the world (Bergman and Carpenter, 1991). These filamentous cyanobacteria do not have heterocysts yet fix nitrogen in the light under aerobic conditions (Bergman et al., 1997). Within the filaments, 10 to 15% of the cells (called **diazocytes**) are specialized to fix nitrogen, while the others do not (Lundgren et al., 2001). Cells that fix nitrogen are adjacent to one another and have a denser thylakoid network with fewer gas vacuoles and cyanophycin granules (Fredriksson and Bergman, 1997). The tropical seas where *Trichodesmium* and *Katagnymene* live are relatively low in dissolved oxygen and this may assist the nitrogen-fixing cells in maintaining anaerobic conditions in the protoplasm where nitrogenase is present (Staal, Meysman and Stal, 2003).

*Trichodesmium* and *Katagnymene* represent the most ancient type of nitrogen-fixing cyanobacteria (Berman-Frank et al., 2001).

## Circadian rhythms

The cyanobacteria have circadian rhythms in photosynthesis, nitrogen fixation, and cell division similar to those in eukaryotic organisms (Fig. 2.30). The requirements of a circadian rhythm are: (1) approximate 24-hour cycles in biological processes even in the absence of an environmental cycle; (2) synchronization with the environment through light or environmental cues; and (3) maintenance of a nearly constant period over a range of physiologically relevant temperatures (Golden, 2003).

The details of the cyanobacterial circadian rhythm have been elucidated in *Synechococcus elongatus* (Williams et al., 2002). The key components of the timekeeping complex are the proteins KaiA, KaiB, and KaiC coded by the genes *KaiA*, *KaiB*, and *KaiC* (Figs. 2.32, 2.33). Interactions between these three proteins maintain the rhythm at about 24 hours. In the real world, the clock is reset daily by cycles of light and darkness, temperature and humidity. The major portal into the clock is the protein kinase CikA. Environmental change to CikA changes the configuration of the protein KaiA which causes phosphorylation of the protein KaiC (Fig. 2.32), resetting the clock. The output that sets cycles of nitrogen fixation, photosynthesis, etc. begins when KaiC interacts with the protein SasA (*Synechococcus* adaptive sensor). This results in information being relayed downstream to produce the observed effect.

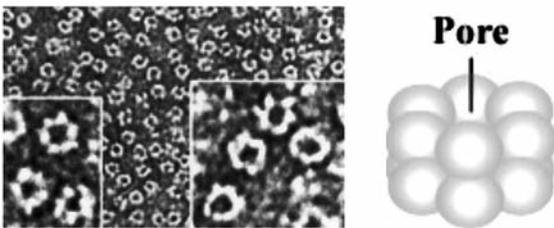


**Fig. 2.32** Diagrammatic representation of the cyanobacterial circadian clock.

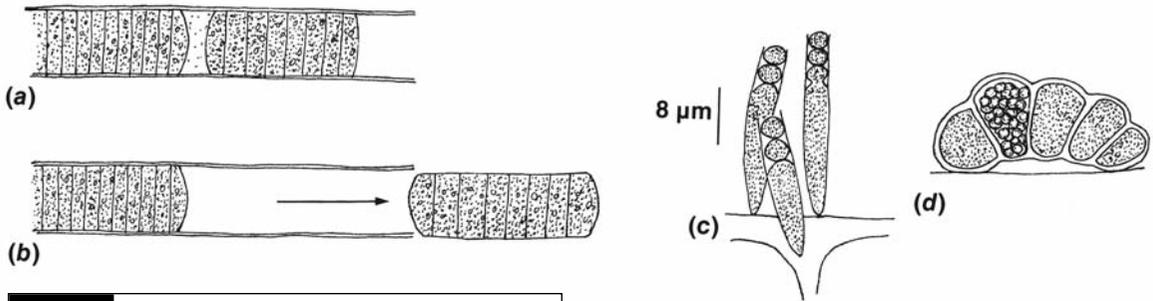
### Asexual reproduction

Asexual reproduction occurs by the formation of hormogonia or baecocytes or fragmentation of colonies (Fig. 2.34).

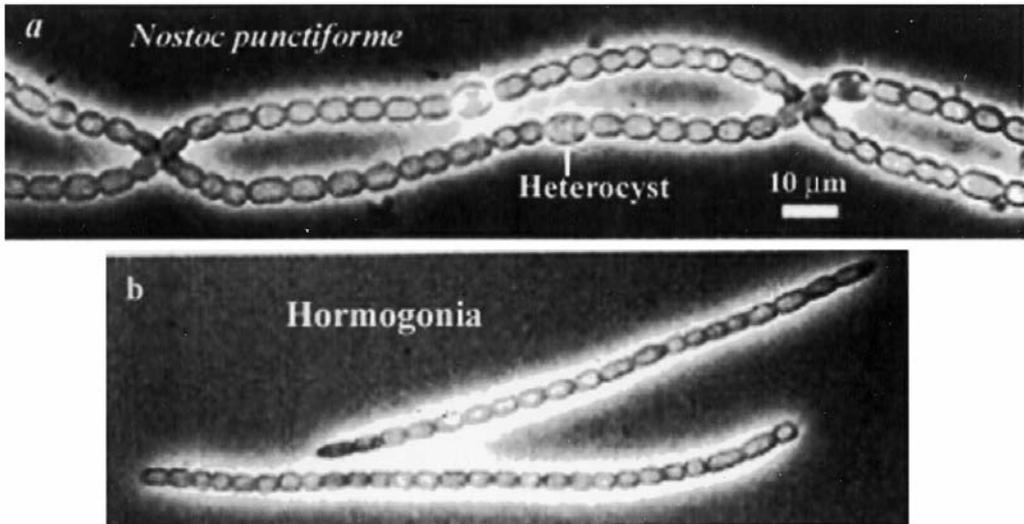
**Hormogonia** (or hormogones), which are characteristic of all truly filamentous cyanobacteria, are *short pieces of trichome that become detached from the parent filament and move away by gliding, eventually developing into a separate filament.* Hormogonia are distinguished from vegetative filaments by their gliding motility, the small size of their cells (Figs. 2.34(a), (b), 2.35), and the absence of heterocysts (Meeks and Elhai, 2002). In some species, hormogonia contain gas vacuoles, which control buoyancy. In some filamentous algae, such as *Oscillatoria* and *Cylindrospermum*, the entire filament may break



**Fig. 2.33** KaiC protein from *Synechococcus elongatus*, one of the circadian clock proteins in cyanobacteria. *Left:* Transmission electron micrograph of negatively stained KaiC protein showing the hexamer formed in an ATP-containing solution. *Right:* Model of a KaiC hexamer. (From Mori et al., 2002.)



**Fig. 2.34** (a),(b) Formation of a hormogonium in *Oscillatoria*. (c) Baeocyte formation in *Chamaesiphon incrustans*. (d) Baeocyte formation in *Dermocarpa pacifica*. (After Smith, 1950.)

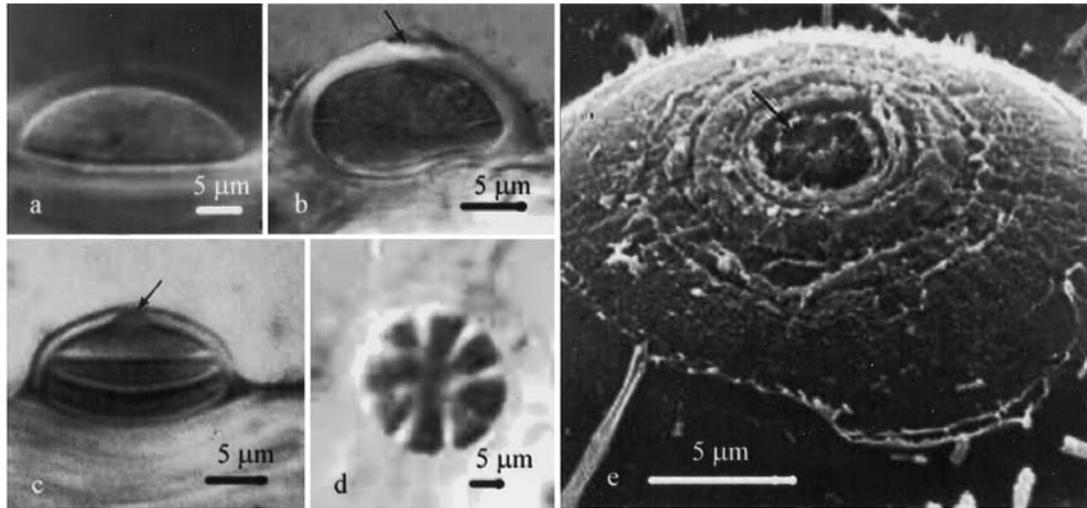


**Fig. 2.35** *Nostoc punctiforme*. (a) Vegetative cells. (b) Hormogonia. The hormogonia lack heterocysts and are smaller than vegetative cells. (From Meeks and Elhai, 2002.)

up (Fig. 2.34(a), (b)), whereas in others the hormogonia are produced at the tips of special branches. In some algae, specialized separation discs or necridia (Fig. 2.34) are involved in the breaking of the hormogone from the parent filament, whereas in others, the filament just fractures.

The one common factor in initiation of hormogonium differentiation appears to be a change in some environmental parameter such as an increase or decrease of a nutrient or a change in the quantity of light.

Hormogonia of many strains display positive phototaxis, which is important in the colonization of illuminated portions of the habitats by these photoautotrophic organisms (Meeks and Elhai, 2002). The acquisition of motility in hormogonia comes at a price. Hormogonia lack heterocysts and are unable to fix nitrogen. Phycobiliprotein synthesis ceases, leading to an attrition in light gathering. Hormogonia cells continue to photosynthesize and assimilate exogenous ammonium, although the rates of  $\text{CO}_2$  fixation and  $\text{NH}_4^+$  incorporation are only 70% and 62%, respectively, of those of vegetative cells. Much of the metabolic output is devoted to the synthesis and secretion of mucilage required for gliding motility. Hormogonia remain in the



**Fig. 2.36** Baeocyte formation in *Dermocarpella gardneri*. (a)–(c). Light micrographs of lateral views showing parallel divisions. (d) Top view showing radial divisions. (e) Scanning electron micrograph showing apical pore through which baeocytes escape. (From Montejano and Leon-Tejera, 2002.)

gliding stage for 36 to 48 hours. Heterocysts begin to develop at this stage in the absence of combined nitrogen. Conversion to vegetative filaments is complete after 96 hours.

**Baeocytes** (endospores) are formed by some coccoid (spherical) cyanobacteria (Figs. 2.34, 2.36). The protoplasm divides several times in different planes without growth between successive divisions. The resulting baeocytes are smaller than the original cell. Baeocytes are similar to bacterial endospores. In *Dermocarpella*, the baeocytes are released through an apical pore and enlarge to mature organisms (Fig. 2.36) (Montejano and Leon-Tejera, 2002).

## Growth and metabolism

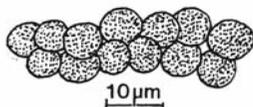
In the cyanobacteria there are three nutritional types: (1) **facultative chemoheterotrophs**, or those organisms capable of growing in the dark on an organic carbon source and of growing phototrophically in the light (only a portion of the cyanobacteria exhibit this condition); (2) **obligate phototrophs**, or organisms that can grow only in the light on an inorganic medium (some of

these are actually **auxotrophs**, requiring a small amount of an organic compound that is not used as a source of carbon, invariably meaning a vitamin); (3) **photoheterotrophs**, or those cells that are able to use organic compounds as a source of carbon in the light but not in the dark (Stanier, 1973).

Facultative chemoheterotrophs are able to grow in the dark on a very narrow range of substrates, being confined to glucose, fructose, and one or two disaccharides. This range of substrates is so small because the pentose phosphate pathway is the sole energy-yielding dissimilatory pathway. The tricarboxylic acid cycle lacks the enzyme  $\alpha$ -ketoglutarate (2-oxoglutarate) dehydrogenase and succinyl CoA synthetase, rendering it incomplete, and glycolysis likewise appears to be incomplete. Although the tricarboxylic acid cycle does not provide energy, it does provide carbon skeletons from the portions of it that are functional.

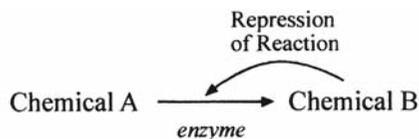
The pentose phosphate pathway does not operate in the *light* in the Cyanophyceae (although it is highly operational in the dark), being inhibited by ribulose-1,5-diphosphate, a product of light metabolism.

Even though some cyanobacteria grow in the dark, they grow very slowly. It is probable that the rate of chemoheterotrophic growth is always limited by the rate of dark ATP synthesis through oxidation of glucose-6-phosphate in the pentose phosphate pathway, a rate that is evidently not very great.



*Chlorogloea fritschii*

**Fig. 2.37** Cyanobacteria, such as *Chlorogloea fritschii* (left), lack feedback inhibition of enzyme activity (right).



### Feedback Repression of Enzyme Activity

### Lack of feedback control of enzyme biosynthesis

Cyanobacteria, such as *Chlorogloea fritschii* (Fig. 2.37), lack metabolic control of many pathways by repression and derepression of enzyme biosynthesis as occurs in many other organisms. In other organisms, as an end product of a pathway builds up to excess, the end product represses an enzyme involved in its synthesis, thereby directing precursors to other parts of the cell's metabolism, where they can be better used. When the amount of end product falls, the enzyme is derepressed, allowing manufacture of the end product again.

Cyanobacteria often release considerable quantities of nitrogenous and organic substances into the medium, with most of the compounds being excreted as peptides (Fogg, 1942, 1952; Walsby, 1974). The secretion of nitrogenous substances represents a considerable waste of potential metabolites which have been formed from carbon dioxide with an expenditure of ATP and reducing potential (e.g., NADH). Such an excretion, however, could well be a necessary consequence of ill-controlled amino acid biosynthesis. Unable accurately to adjust the synthesis of each amino acid to the needs of protein synthesis, the cells would inevitably have to synthesize an excess of amino acids to ensure that protein synthesis would proceed smoothly. This excess would then form the basis of the extracellular peptide material associated with the cyanobacteria.

The general lack of repression and derepression of enzyme synthesis in the cyanobacteria implies that the algal metabolic pathways developed before the control systems responsible

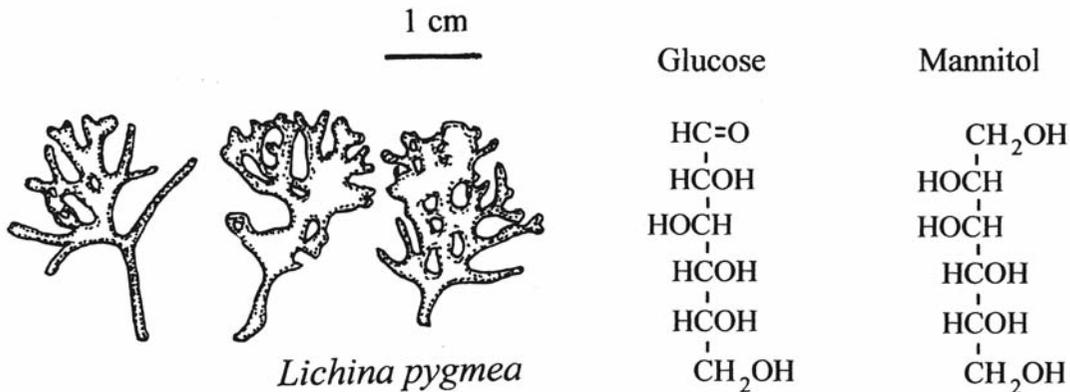
for their regulation. The failure to develop transcriptional or regulatory control places the cyanobacteria under a selective disadvantage relative to other organisms possessing such metabolic regulators. They would be unable to divert biosynthetic capacity away from surplus synthesis to more necessary activity. Such a selective advantage would survive only in microorganisms less open to competition than most, or, in other words, in organisms in a particular ecological niche or in an environment with an abundant energy source. Autotrophic microorganisms such as the cyanobacteria fall into this category. Their source of energy (light) is at times more than sufficient, and in certain cases they occur in eccentric ecological situations that extremes of temperature or pH make relatively inhospitable.

### Symbiosis

The cyanobacteria occur in basically two types of associations: those in which the cyanobacterium is *extracellular* and those in which it is *intracellular*.

#### Extracellular associations

Cyanobacteria in extracellular associations all show a decrease in growth, assimilation of CO<sub>2</sub> and assimilation of NH<sub>4</sub><sup>+</sup> (Meeks and Elhai, 2002). Conversely, there is an increase in the rate of N<sub>2</sub> fixation that accompanies the increased numbers of heterocysts. The heterocysts and vegetative cells of *Nostoc* within leaf cavities of *Azolla* are up to fourfold larger than the vegetative cells of free-living cyanobacteria. In the symbiosis, *Nostoc* receives hexose sugars from the host, while *Nostoc* provides fixed nitrogen to the host. There is also a five- to ten-fold increase in the production of



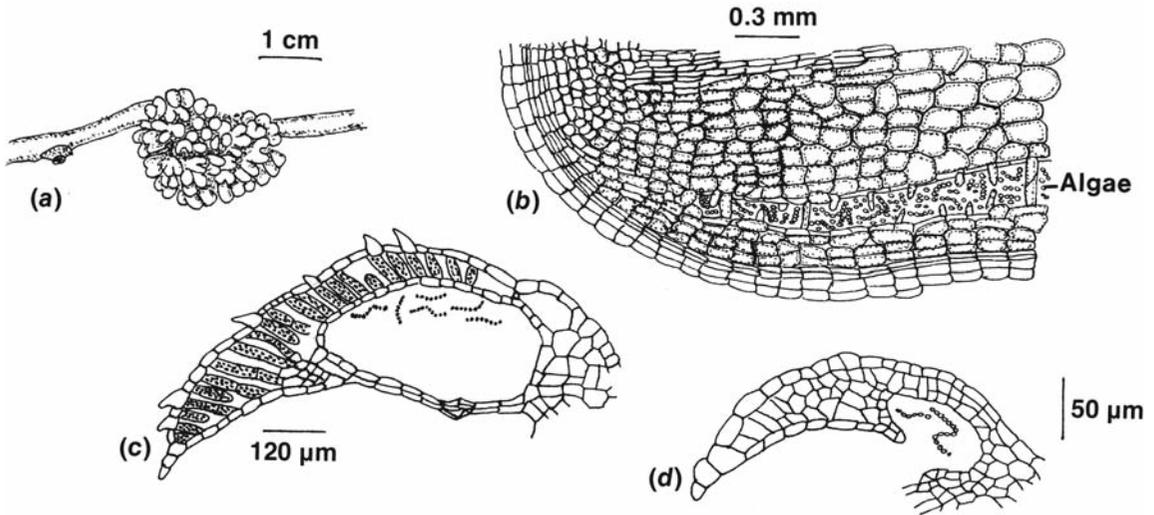
**Fig. 2.38** *Lichina pygmaea* is a lichen containing a cyanobacteria as the phycobiont. *Lichina* occurs on rocks near the high-tide mark and looks like a stubby seaweed with its club-shaped branches making a sward of uniform height. The cyanobacterial phycobiont transfers glucose to the mycobiont which converts the glucose to mannitol.

motile hormogonia that serve as infective units in the establishment of the symbioses (Meeks, 1998).

The most common type of extracellular association is that with fungi to form lichens. In most lichens, there is a single algal component (**phycobiont**), which is a green alga, cyanobacterium, or, as in species of *Verrucaria*, a member of the Xanthophyceae. Cyanobacteria occur in about 8% of the species of lichens (Fig. 2.38). Almost all of the fungal partners (**mycobionts**) are ascomycetes, but certain imperfect fungi and basidiomycetes also occur in lichens (Ahmadjian and Hale, 1973). Because the mycobionts frequently reproduce sexually whereas the phycobionts usually do not, the lichens are classified with the fungi. In the symbiotic association, the phycobiont fixes carbon in photosynthesis and liberates it as glucose, which the mycobiont converts into mannitol and assimilates (Fig. 2.38). Approximately 40% of the photosynthate of the alga is secreted as glucose in the lichen association (Smith et al., 1970). Whereas the benefits to the fungus in the association are obvious, the benefits to the alga are probably limited to some protection against desiccation.

The water fern *Azolla* (Fig. 2.40(a), (b)) has cavities in the dorsal lobe of the leaf that are occupied by *Anabaena azollae* (Fig. 2.39(c), (d)). This cyanobacterium fixes nitrogen (Peters and Mayne, 1974), some of which is excreted into the cavity and taken up by the cells of the *Azolla*. It is possible to obtain alga-free *Azolla* plants by treating them with antibiotic or subjecting them to growth conditions that allow the fern to outgrow the alga. The *Anabaena* is apparently unable to live outside of the host. The heterocyst frequency (compared to vegetative cells) in *A. azollae* is about 30%, which is very high, the highest for free-living *Anabaena* being about 8% (Hill, 1975). The host *Azolla* somehow modifies the surface properties of the *Anabaena*. The alga in the fern cavity has different surface antigens, as compared to when it is growing free in culture (Gates et al., 1980). Nitrogen fixation by the cyanobacterial symbionts of *Azolla* has for many years been utilized to advantage in the Far East; here the water fern has been used as a green manure in rice fields, where about 3 kg of atmospheric nitrogen per hectare per day is fixed by *Azolla* symbionts (Swaminathan, 1984; Canini et al., 1992).

Cycad roots are frequently infected with cyanobacteria that cause distortions of the roots (coralloid roots) (Figs. 2.39(a), (b), 2.40(c)). In these roots the cyanobacteria occupy a clearly defined cortical zone midway between the pericycle and the epidermis. The cyanobacteria occur in the intercellular spaces and are surrounded by a



**Fig. 2.39** (a) Root nodules of the cycad *Dioon spinulosum*. (b) Part of a longitudinal section of a nodule of *Encephalartos* with an algal zone. Longitudinal sections through the dorsal lobe of a mature (c) and an immature (d) leaf of the water fern *Azolla* illustrating filaments of *Anabaena* in leaf cavities. ((a),(b) after Spratt, 1915; (c),(d) after Smith, 1955.)

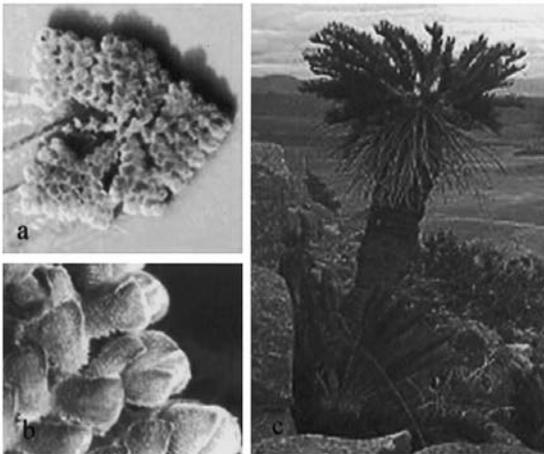
sheath and a multilayered wall, whereas the adjacent cycad cells have a reduced chromosome number and secrete slime (Storey, 1968; Caiola, 1975). These cyanobacteria are able to fix nitrogen and contribute a portion of the

fixed nitrogen to the cycad cells (Watanabe and Kiyohara, 1963).

### Intracellular associations

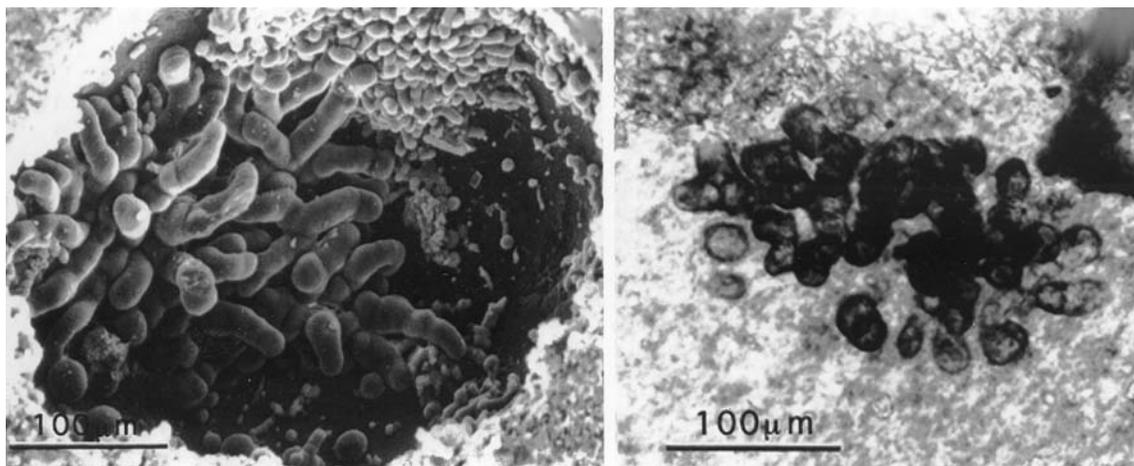
Intracellular associations involving cyanobacteria are usually more specialized than extracellular associations, and it is not possible to culture the cyanobacteria away from the host. Pascher (1914) coined the term **cyanelle** for intracellular cyanobacteria in symbioses, and the term **cyanome** for the host cell. Cyanelles are present in a variety of cyanomes; such a variety that it is apparent that they are the result of a number of different symbiotic establishments and are not derived from a single event. It is probable that one of these endosymbiotic associations led to the development of chloroplasts in some of the algal groups. This line of evolution is considered under Glaucophyta (see beginning of Chapter 3).

Colonies of the coral *Montastraea cavernosa* contain endosymbiotic coccoid cyanobacteria in vacuoles in the epithelial cells of the coral (Lesser et al., 2004). The cyanobacteria are capable of nitrogen fixation. Anaerobic conditions (necessary for function of the nitrogen-fixing enzyme nitrogenase) are created by the Mehler reaction (Fig. 2.41) where glycerol provided by dinoflagellate zooxanthellae is reduced.



**Fig. 2.40** Plants that have symbiotic cyanobacteria. (a,b) The water fern *Azolla* (c) The cycad *Encephalartos*.





**Fig. 2.43** Left: The endolith *Hyella immanis* inside a carbonate ooid sand grain. Right: The fossil endolith *Eohyella dichotoma* penetrating an ooid sand grain from the Late Proterozoic. (From Al-Thukair and Golubic, 1991.)

and sandstones, respectively, sustaining the greatest growth. Most of the cyanobacteria in the littoral zone are nitrogen fixing, and they make a significant contribution to the productivity of rocky shores and coral reefs (Mague and Holm-Hansen, 1975).

**Ooids** are spherical (0.2–2.0 mm in diameter), concentrically laminated, carbonate grains that form by carbonate accretion in agitated, shallow tropical marine environments. *Hyella* spp. are cyanobacterial endoliths that bore into, and live in, ooids (Fig. 2.43) (Al-Thukair and Golubic, 1991). Extant *Hyella* spp. are similar to extinct *Eohyella* in 800 million-year-old ooids (Fig. 2.43).

### Open ocean cyanobacteria

In the open ocean, most of the total photosynthetic capacity is made up of **pico-phytoplankton** (*phytoplankton cells unable to pass through a filter with 2- $\mu$ m-diameter holes*). The pico-phytoplankton is made up principally of tiny coccoid cyanobacteria at concentrations of around 10000 cells per milliliter (Glover et al., 1986). The coccoid cyanobacteria *Synechococcus* (Figs. 2.19(c), 2.31), *Synechocystis* (Fig. 2.8), and *Prochlorococcus* are the major organisms present (Ferris and Palenik, 1998). Although these cyanobacteria are small, they can be easily seen

because their phycobiliproteins undergo autofluorescence in a fluorescence microscope. The picophytoplankton cyanobacteria have negligible sinking rates; thus they are ideally suited for planktonic life. The high surface:volume ratios, combined with steep diffusion gradients that are set up around small cells, allow them to take up nutrients at a high rate. Furthermore, a given amount of photosynthetic pigment dispersed in small cells absorbs more light than an equivalent amount of pigment packaged in large cells. Therefore, these cells grow best at low light intensities of less than  $\frac{1}{50}$  of full sunlight. The picophytoplanktonic cyanobacteria are more evident in nutrient-poor offshore waters where larger phytoplankton are less successful, because the larger phytoplankton are less able to utilize low concentrations of nutrients. Although the picophytoplanktonic cyanobacteria occur throughout the euphotic zone, they are concentrated at the bottom of the zone, not because they sediment out, but because they grow best under these conditions; they use the low irradiance efficiently to support growth and benefit from nutrients transported up from richer waters below. These algae contain large amounts of phycoerythrin that allows them to absorb the blue-green light penetrating into deep water (Fogg, 1986).

Cyanobacteria larger than picophytoplankton often form a significant part of oceanic phytoplankton. Massive development of filaments of the nitrogen-fixing *Trichodesmium* (Figs. 2.31,

2.56(g) (Carpenter et al., 1992) occurs in certain tropical waters. Each colony of *Trichodesmium* consists of a mass of filaments that secrete a flocculent mucilage which supports bacterial colonies; these in turn are fed on by different protozoa. The large surface area produced by the algal filaments forms a miniature ecosystem (Andersen, 1977). *Trichodesmium* is a major component of the Caribbean Sea plankton, comprising 60% of the total chlorophyll *a* in the upper 50 m and about 20% of the primary production. It is also an important source of nitrogen, fixing 1.3 mg of nitrogen per square meter per day (Carpenter and Price, 1977). The cells produce gas vacuoles which, under calm conditions, cause the cells to accumulate at the water surface, giving rise to the phenomenon known to sailors as “sea sawdust,” or long orange or gray windrows of algae. One such bloom stretched 1600 km along the Queensland coast of Australia, extending from the shore to the Great Barrier Reef, and occupying an area of 52 000 km<sup>2</sup> (Ferguson-Wood, 1965). *Trichodesmium* also occurs in the Red Sea and it was probably the color produced by blooms of the alga that gave the Red Sea its name (Hoffman, 1999).

*Trichodesmium* moves up in the water column by means of gas vesicles, and moves down in the water column by carbohydrate ballasting (Romans et al., 1994). The cells become progressively heavier from morning to evening as carbohydrates and polyphosphate bodies are produced. In the Caribbean, *Trichodesmium* cells have been found down to 200 m. The gas vesicles of this cyanobacterium are much stronger and more difficult to collapse than those found in any freshwater alga (Walsby, 1978). The gas vesicles are able to stand up to 20 atm of pressure, enabling *Trichodesmium* to rise from great depths.

### Freshwater environment

Freshwater blooms of cyanobacteria are common. Most freshwater blooms of cyanobacteria consist of *Microcystis* (Figs. 2.48, 2.56(b)), *Anabaena* (Figs. 2.16, 2.18(d), 2.57(b)), *Aphanizomenon* (Fig. 2.57(b)), *Gloeotrichia* (Fig. 2.18(a)), *Lyngbya* (Fig. 2.42(b), (c)) or *Oscillatoria* (Fig. 2.19(a), (b)). Although they occur in lakes over the whole year, it is usually only in late summer and early autumn that they reach bloom proportions.

This because of (Tang et al., 1997):

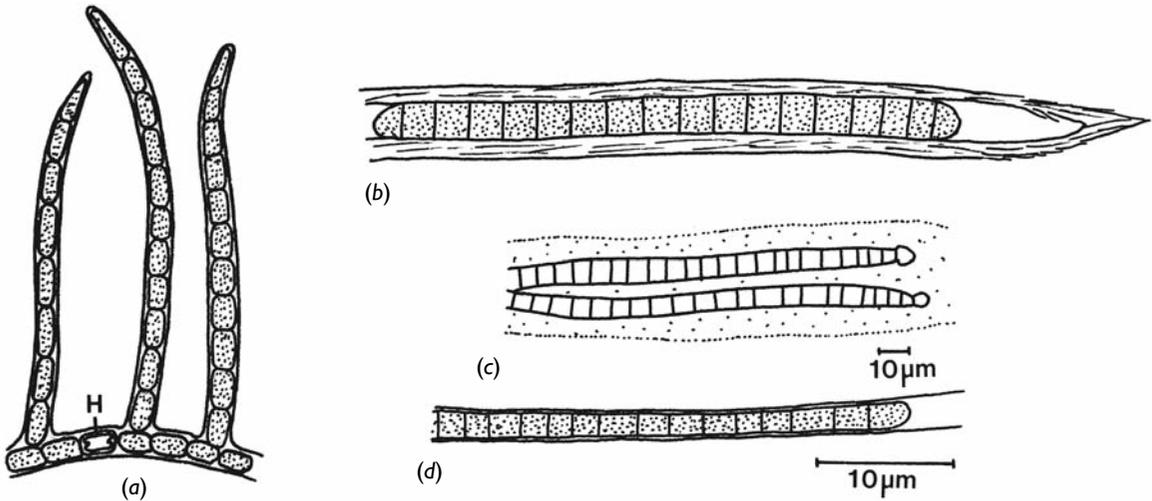
- 1 the superior light-capturing abilities of cyanobacteria when self-shading is the greatest.
- 2 their high affinity for nitrogen and phosphorus when nutrient limitation is most severe.
- 3 their ability to regulate their position in the water column by gas vacuoles to take advantage of areas richer in nutrients and/or light.
- 4 their higher temperature optima for growth and photosynthesis (greater than 20 °C).

Similar to marine environments, freshwater plankton is dominated by the picoplankton cyanobacteria, particularly species of *Synechococcus* (Fig. 2.31) (Postius and Ernst, 1999).

Even in the Arctic and Antarctic, cyanobacteria dominate the algal flora in the late summer. These cyanobacteria are **psychrotrophs**, able to tolerate the severe conditions during the winter and then grow in the warmer summer months (as contrasted to **psychrophiles** that are able to grow at temperatures less than 15 °C; (Tang et al., 1997; Nadeau and Castenholz, 2000).

### Hot-spring cyanobacteria

Cyanobacteria are important in the colonization of non-acidic hot springs throughout the world. Some cyanobacteria have the ability to grow at temperatures as high as 70 to 73 °C, a much higher temperature tolerance than occurs in eukaryotic algae. In thermal environments above 56 to 60 °C, both photosynthetic and non-photosynthetic eukaryotic algae are always absent. In acid springs (pH less than 4), no cyanobacteria are present, and at temperatures above 56 °C in these springs there are no photosynthetic organisms at all (Brock, 1973). *Mastigocladus laminosus* (Fig. 2.44(a)) is a cyanobacterium that occurs in thermal springs throughout the world, whereas other cyanobacteria such as *Synechococcus lividus* and *Oscillatoria terebriformis* have more restricted ranges (Castenholz, 1973). The cyanobacteria normally occur as mats mixed with flexibacteria, the cyanobacteria usually being more prevalent in the upper portion of the mat. The **thermophilic** cyanobacteria are especially adapted to live at elevated temperatures.



**Fig. 2.44** (a) *Mastigocladus laminosus*. (H) Heterocyst. (b) *Porphyrosiphon notarisi*. (c) *Microcoleus vaginatus*. (d) *Plectonema notatum*. ((c),(d) after Prescott, 1962.)

*Synechococcus lividus* can grow at temperatures up to 73 °C but ceases to grow in culture when the temperature is lowered to 54 °C, with optimum growth occurring from 60 to 63 °C (Meeks and Castenholz, 1971); the cells will die if kept at 30 °C for 10 days. Enzymes isolated from thermal algae are more stable at higher temperatures than those from other organisms. For example, NADPH<sub>2</sub>-cytochrome *c* reductase extracted from the thermal alga *Aphanocapsa thermalis* showed unimpaired activity after heating to 85 °C for 5 minutes, whereas that from *Anabaena cylindrica* was completely inactivated by a similar treatment.

### Terrestrial environment

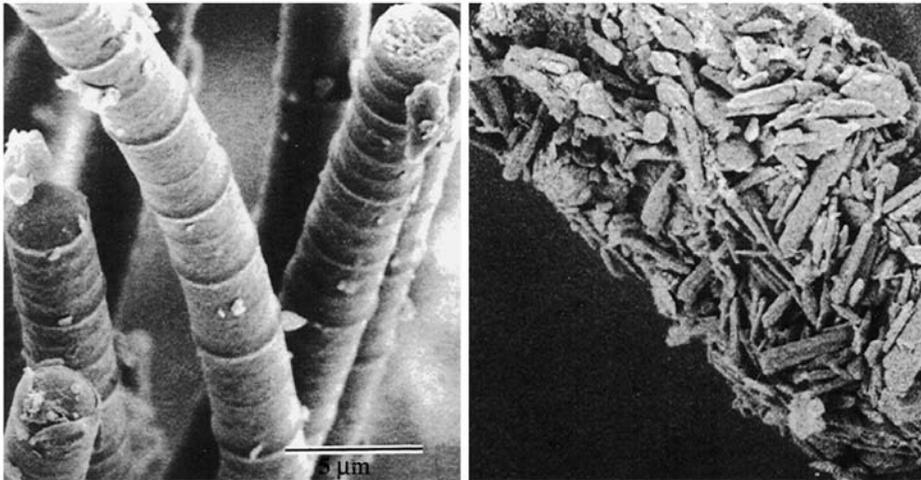
Terrestrial cyanobacteria play a major role as primary colonizers in the establishment of a soil flora and in the accumulation of humus. They do this in four main ways:

- 1 They bind sand and soil particles and prevent erosion. They do this with their gelatinous sheaths and by their growth pattern which produces closely intertwined rope-like bundles in and among soil particles. Genera that commonly perform this function are *Porphyrosiphon* (Fig. 2.44(b)) which covers large eroded areas in Brazil (Drouet, 1937), *Microcoleus* (Figs. 2.44(c), 2.45), *Plectonema*

(Fig. 2.44(d)), *Schizothrix* and *Scytonema* (Figs. 2.58(c), (d), 2.59).

- 2 They help to maintain moisture in the soil. Booth (1941), in studies in Oklahoma, found that soil with an algal covering had a moisture content of 8.9% compared to 1.3% in the absence of algae.
- 3 They are important as contributors of combined nitrogen through nitrogen fixation. In grasslands, the soil surface between crowns of grasses may support extensive zones of cyanobacteria and lichens that include cyanobacteria as their phycobiont constituting up to 20% of the ground cover (Kapustka and DuBois, 1987).
- 4 It has been suggested that cyanobacteria assist higher plant growth by supplying growth substances.

**Anhydrobiotics** are organisms that can withstand the removal of the bulk of their intracellular water for extended periods of time. The cosmopolitan terrestrial cyanobacterium *Nostoc commune* is able to tolerate acute water stress and can survive in the air-dry state for many years. Approximately 0.1 g of blackened air-dried colonies becomes an olive-green rubbery mass of more than 20 g wet weight within 30 min of rehydration. In *Nostoc commune* (Fig. 2.46), rehydration rather than desiccation appears to be the fatal event. To protect the cells during rehydration, a water stress protein and large amounts of the sugar trehalose are

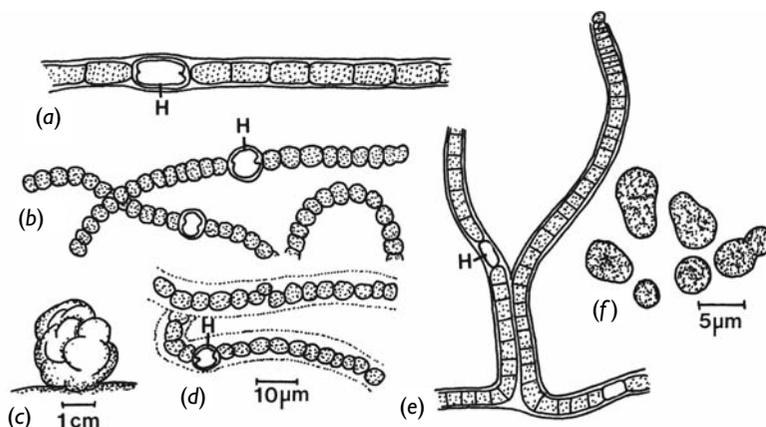


**Fig. 2.45** Scanning electron micrographs of *Microcoleus vaginatus*. Left: Filament. Right: Filament with clay particles and sand grains embedded in the slime surrounding the cell walls. (From Belnap and Gardner, 1993.)

synthesized that stabilize the phospholipid bilayers of cellular membranes (Potts, 1996, 1999; Qiu et al., 2004).

Cyanobacteria comprise the dominant component of the soil photosynthetic community in hot and cold arid regions where higher plant vegetation is absent or restricted. Desert **cryptobiotic crusts** are initiated by the growth of cyanobacteria in the soil during episodic events of available moisture. The only cyanobacteria that are initial colonizers are those that have heterocysts, and are therefore able to fix nitrogen; and those cyanobacteria that produce **scytonemin**, a sunscreen that

accumulates in the cyanobacterial sheaths and absorbs some of the strong sunlight in the near ultraviolet (370–384 nm) (Dillon and Castenholz, 1999). *Microcoleus vaginatus* (Fig. 2.45) makes up over 90% of cryptobiotic crusts in the arid soil of the Colorado Plateau in the United States (Belnap and Gardner, 1993). Filaments of *M. vaginatus* are surrounded by thick mucilaginous sheaths that can absorb eight times their weight in water, increasing the water capacity of sandy soils. Clay particles and sand grains become trapped in the cyanobacterial sheaths (Fig. 2.45). The clay particles are negatively charged and bind positive cationic nutrients (e.g.,  $K^+$ ,  $Ca^{2+}$ ) preventing them from leaching into the subsoil. Subsequently, lichens, fungi and moss establish themselves in the crust, enriching and stabilizing the soil.



**Fig. 2.46** (a) *Aulosira fertilissima*. (H) Heterocyst. (b) *Nostoc commune*. (c,d) *N. verrucosum*. (e) *Scytonema hofmanni*. (f) *Chamaesiphon* sp., showing formation of exospores. ((a), (e) after Desikachary, 1959; (b) after Prescott, 1962; (f) after Waterbury and Stanier, 1977.)

Nitrogen-fixing cyanobacteria make a major contribution to the fertility of paddy fields (Vaishampayan et al., 2001). In many Eastern countries, peasant farmers do not fertilize their fields; the nitrogen is fixed by cyanobacteria, thus permitting a moderate harvest when in their absence there would be a poor one. There are about 100 million km<sup>2</sup> of paddy fields, of which some are in southern Europe and the United States, but about 95% are in India and the Far East. Usually rice is grown on land that is submerged under 10 cm or so of water for 60 to 90 days during the growing season, and then allowed to dry to facilitate harvest. The warm conditions demanded by the rice, the availability of nutrients, the reducing conditions in the soil, and the cyanobacterial ability to withstand desiccation all favor growth of cyanobacteria. Over 70% of algal species in Indian paddy soils are cyanobacteria (Pandey, 1965). In paddy soils in India there is a succession of cyanobacteria over the growing season of the rice (Singh, 1961). Early in the rainy season (starting at the end of June), the soil becomes covered with a thick patchy growth composed of a variety of algae. In July, the fields are flooded, and the top 20 cm of soil and algae are mixed to form a muddy suspension. The rice seedlings are transplanted, and after about a fortnight the soil and algae have settled. By the middle of September, there is an extensive brownish-yellow gelatinous growth on the soil, composed primarily of *Aulosira fertilissima*

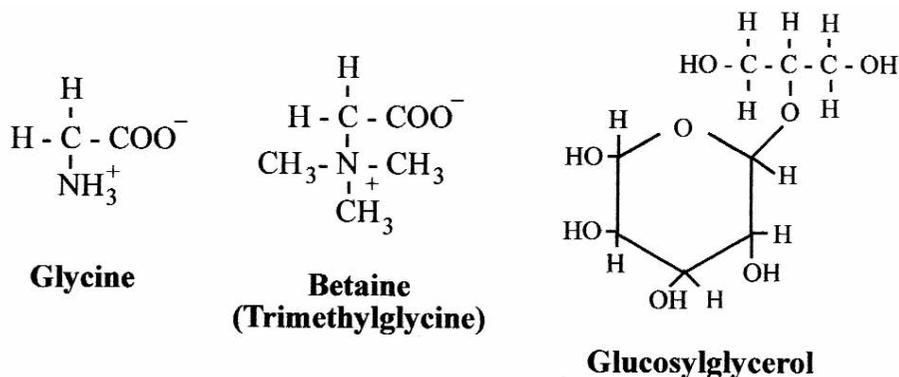
(Fig. 2.46(a)), the most important nitrogen-fixing species in the fields. This species is dominant for about 3 months and forms a papery growth on the soil surface when the fields are dry.

From the above discussion, it is plain that cyanobacteria occur in most environments on earth. There is, however, one important exception: cyanobacteria are usually absent in waters or soils with a pH below 5, and they are uncommon between pH 5 and 6 (Brock, 1973).

### Adaption to silting and salinity

In salt marshes and mud flats, felts of cyanobacteria, particularly *Microcoleus chthonoplastes* (Fig. 2.42(e)) are important in stabilizing mud surfaces. Filaments of *M. chthonoplastes* are phototactic and chemotactic, resulting in migration to the surface of the mud at a rate of about 7 mm per 24 h. The movement of the filaments is an adaption that allows the cyanobacterium to survive during silting (Whale and Walsby, 1984). *M. chthonoplastes* is **euryhaline**, it is able to survive in a wide range of salinities in the estuarine environment by producing glycosylglycerol (Fig. 2.47) as an osmolyte to counteract the osmolarity of the surrounding environment. It also synthesizes trehalose to stabilize the phospholipid membrane bilayers of the cell (Karsten, 1996).

In **hypersaline** environments, such as the Great Salt Lake in Utah, **halotolerant** and **halophilic** cyanobacteria can adapt to the high salinity in three ways (Fulda et al., 1999):



**Fig. 2.47** The chemical structure of three osmoregulatory compounds in cyanobacteria.

- 1 Active export of inorganic ions in the protoplasm leading to relatively unchanged internal salt concentrations.
- 2 Accumulation of organic osmoprotective compounds, such as glucosylglycerol, glycine, and betaine (trimethylglycine) (Fig. 2.47), to maintain the osmotic equilibrium (Ferjani et al., 2003).
- 3 Expression of a set of salt-stress proteins such as the protein flavodoxin.

## Cyanotoxins

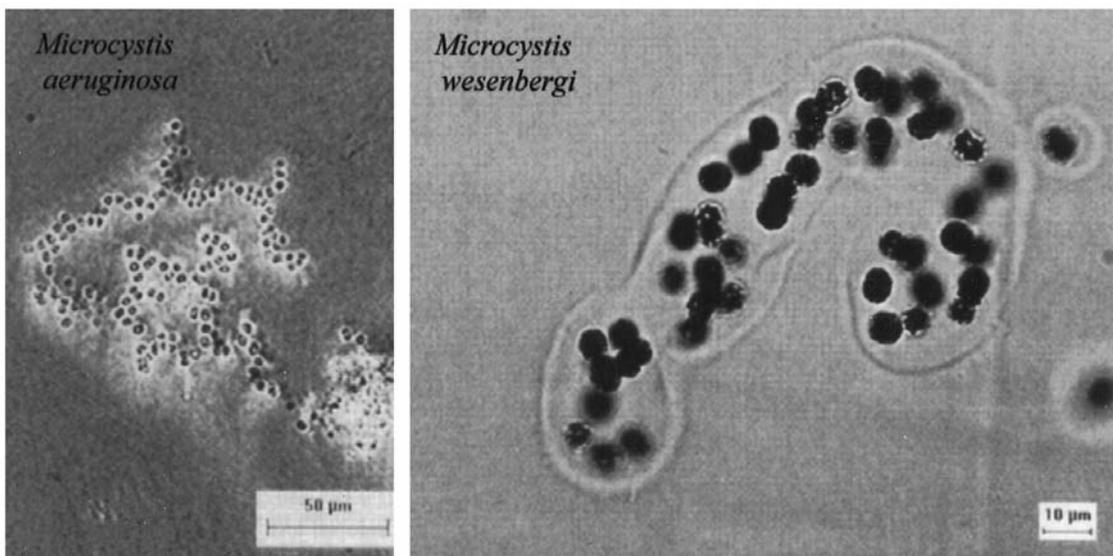
Some of the cyanobacteria produce toxins (cyanotoxins). Physiologically, there are basically two types of cyanotoxins: neurotoxins and hepatotoxins (Carmichael, 1992; Bell and Cobb, 1994; Codd et al., 1999).

**Neurotoxins** – The neurotoxins are alkaloids (nitrogen-containing compounds of low molecular weight) that block transmission of the signal from neuron to neuron and neuron to muscle in animals and man. Symptoms include staggering, muscle twitching, gasping and convulsions. The neurotoxins can be fatal at high concentrations due to respiratory arrest caused by failure of

the muscular diaphragm. The two neurotoxins produced by cyanobacteria are **anatoxin** and **saxitoxin** (Fig. 23.3). Anatoxins are synthesized by species of *Anabaena* (Figs. 2.16, 2.18(d), 2.57(b)), *Aphanizomenon* (Fig. 2.18(b)), *Oscillatoria* (Figs. 2.19(a), (b), 2.34(a), (b)) and *Trichodesmium* (Figs. 2.31, 2.56(g)) (Negri et al., 1997).

**Hepatotoxins** – The hepatotoxins are inhibitors of protein phosphatases 1 and 2A (Arment and Carmichael, 1996) and affect the animal by causing bleeding in the liver. Clinical signs include weakness, vomiting, diarrhea, and cold extremities. Cyanobacteria produce two types of hepatotoxins, the **microcystins** and **nodularins** (Fig. 23.2), that are produced along a similar pathway (Rinehart et al., 1994). Microcystins are synthesized by species of *Microcystis* (Figs. 2.48, 2.56(b)), *Anabaena* (Figs. 2.16, 2.18(d), 2.57(b)), *Nostoc* (Figs. 2.35, 2.46(b)–(d), 2.57(a)), *Nodularia* (2.42(a)), and *Oscillatoria* (Figs. 2.19(a), (b), 2.34(a), (b)) while the nodularins are produced by species of *Nodularia* (Fig. 2.42(a)) (Kotak et al., 1995; Bolch et al., 1999).

The cyanotoxins are mostly important in freshwaters where the cyanobacteria are ingested in drinking water by animals, with the algae dying and releasing their toxins in the intestinal tracts. The cyanotoxins are responsible for the loss of



**Fig. 2.48** Light micrographs of two species of *Microcystis*. (From Bittencourt-Oliveira et al., 2001.)

large numbers of stock each year throughout the world, usually in the warm summer months when the blooms of cyanobacteria are visible in the water. Seldom will man drink from such an unattractive water source. As such, poisoning of man by cyanotoxins is relatively rare, partly because of the odor from geosmin and MIB (Fig. 2.50) produced by cyanobacteria.

The cyanotoxins function as anti-herbivore chemicals by inhibiting invertebrate grazers in the aquatic environment. Grazing by invertebrates causes *Microcystis aeruginosa* (Figs. 2.48, 2.56(b)) to increase production of cyanotoxin (Jang et al., 2003). Cyanotoxins also can inhibit the growth of other algae. This is called an **allelopathic interaction** where one organism affects the growth of a second organism. An example of this is the inhibition of the freshwater dinoflagellate *Peridinium gatunense* by the microcystin produced by the cyanobacterium *Microcystis* (Figs. 2.48, 2.56) in Lake Kinneret (Sea of Galilee), Israel. The *Microcystis* cyanotoxins abolish carbonic anhydrase activity in the dinoflagellate and inhibit growth (Sukenik et al., 2002). The microcystin is classified as an **allelochemical** and acts as an **algicide**.

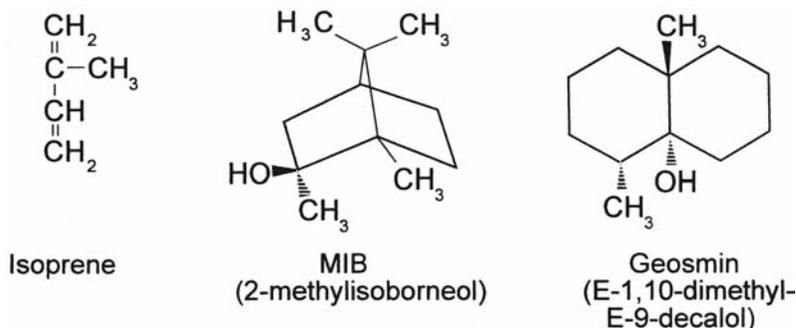
Another allelopathic interaction involves the zebra mussel (*Dreissena polymorpha*) (Fig. 2.49) and the cyanobacterium *Microcystis*. Zebra mussels, native to the Black and Caspian seas, were discovered in the United States near Detroit in 1988. Since then zebra mussels have spread throughout the Great Lakes of North America, displacing native shellfish. The zebra mussel has been beneficial in low-nutrient lakes (but not in lakes high in nutrients) because they graze on *Microcystis*, probably because the mussels have nothing else to eat (Raikow et al., 2004).



**Fig. 2.49** Zebra mussels; each mussel is about the size of a pistachio nut.

## Cyanobacteria and the quality of drinking water

**Geosmin** (E-1,10-dimethyl-E-9-decalol) and **MIB** (2-methylisoborneol) (Fig. 2.50) are two terpenoids (isoprenoids) that are produced by some cyanobacteria (Watson, 2003). These terpenoids are called **volatile organic compounds (VOCs)**, have a highly potent earthy, musty or muddy aroma and account for most of the odors in drinking water. They have odor threshold concentrations of  $\sim 10 \text{ ng l}^{-1}$ . Terpenoids as a class have extensive olfactory properties, which are exploited commercially in the food, beverage, and perfume industries. Both geosmin and MIB resist conventional water treatment, and their bioaccumulation in fish and shellfish causes off-flavor in farmed and wild stocks. Neither geosmin nor MIB are toxic to vertebrates (including humans). The low incidence of human



**Fig. 2.50** The chemical structure of the terpenoids MIB and geosmin. The terpenoids are constructed of multiples of 5-carbon isoprene or 2-methyl-1,3 butadiene. Terpenoids containing three isoprene units (e.g., MIB and geosmin) are called sesquiterpenoids.

poisonings by cyanotoxins has been attributed to the avoidance of water containing cyanobacteria because of the odors produced by geosmin and MIB. Both compounds are commonly found in freshwater and solid habitats, but are rare in offshore marine environments, and can be used as landmass indicators. These volatile organic compounds produced by cyanobacteria act as infochemicals by attracting nematodes to cyanobacterial colonies where the nematodes feed and deposit eggs (Hockelmann et al., 2004).

## Utilization of cyanobacteria as food

Cyanobacteria are used as human food and animal food supplements (Belay et al., 1966). In China, the *Spirulina* (Figs. 2.51, 2.56(f)) industry is supported by the State Science and Technology Commission as a natural strategic program (Li and Qi, 1997). In 1996 there were more than 90 *Spirulina* factories with a total production of 400 tons of *Spirulina* dry powder and a total production area of 1 million square meters. Besides *Spirulina* pills and capsules, there are also pastries, blocks, and *Spirulina*-filled chocolate blocks.

*Nostoc* (Figs. 2.46(b), (c), (d)) is a cyanobacterium that has been gathered for food for over two

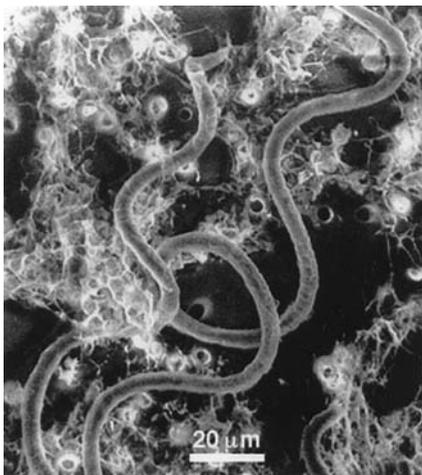
thousand years in China (Gao and Ye, 2003). It is called “hair vegetable” because of its hair-like appearance. The Chinese word for “hair vegetable” (Facai) sounds like another Chinese word that means fortunate and get rich, adding a spiritual value to the cyanobacterial food.

In Japan, *Aphanothece sacrum*, *Nostoc verrucosum* (Fig. 2.46(d)), *N. commune* (Fig. 2.46(b), (c)), and *Brachytrichia* have been used as side dishes since ancient time. Bernal Diaz del Castillo, who accompanied Cortez to Mexico, described in 1521 how people living in the area of Mexico City “sell some small cakes made from a sort of ooze which they get out of a great lake, which curdles and from which they make bread having a flavor something like cheese.”

*Aphanizomenon flos-aquae* (Fig. 2.18(b)) has been harvested from Klamath Lake in California since the early 1980s and sold as a food and health supplement. In 1998,  $10 \times 10^6$  kg (dry weight) was marketed with a value of \$100 million (Carmichael et al., 2000). Other cyanobacteria, such as *Spirulina* (Figs. 2.51, 2.56(f)), are readily available at health-food stores in the developed world, although one wonders how healthy these cyanobacteria really are in regard to the potential for this group of algae to produce cyanotoxins.

## Cyanophages

Cyanophages are viruses that infect and commonly kill cyanobacteria (Suttle and Chan, 1994). Cyanophages can be extremely numerous, concentrations in excess of 100 000 viruses have been observed in surface seawater off the coast of Texas. Normally, however, cyanophages occur at concentrations of  $\frac{1}{10}$  that of the cyanobacterial host cells. Most cyanobacteria are actually resistant to attack by cyanophages with the cyanophage population being maintained by infection of the relatively rare cyanobacteria that are susceptible to infection. Cyanobacteria in the open ocean are more susceptible to infection than cyanobacteria from inshore waters. High temperature and phosphate limitation increase the probability of cyanobacterial infection by cyanophages (Wilson et al., 1996).



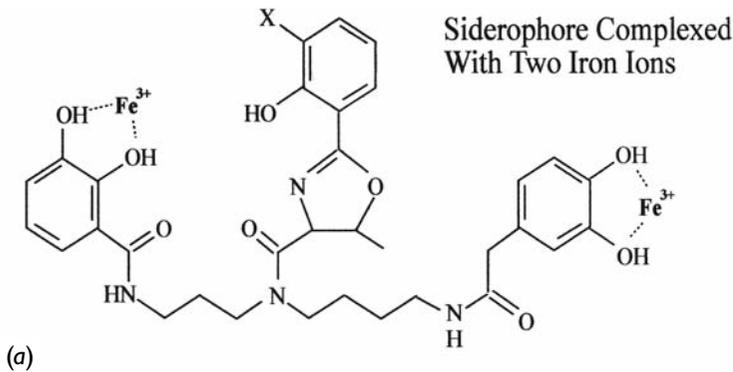
**Fig. 2.51** Scanning electron micrograph of *Spirulina platensis*. (From El-Bestaway et al., 1996.)

## Secretion of antibiotics and siderophores

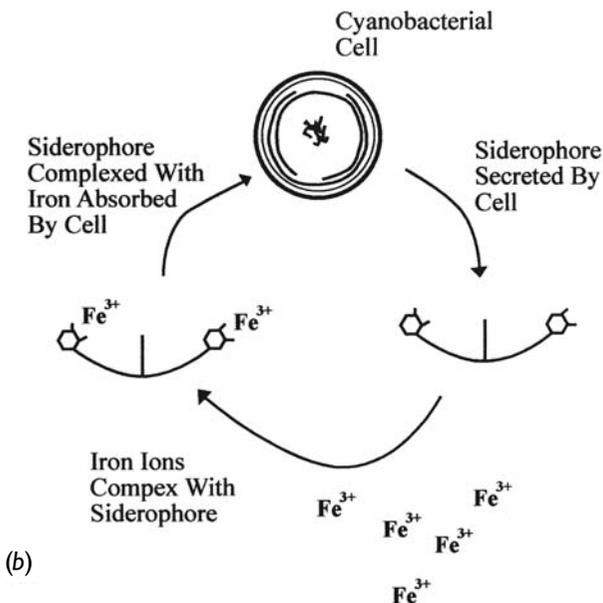
Some cyanobacteria, such as *Nostoc* (Figs. 2.35, 2.46(b), (c), (d), 2.57(a)) secrete antibiotics called **bacteriocins**, which kill related strains of the alga (Flores and Wolk, 1986). A bacteriocin is a proteinaceous antibiotic that is active against prokaryotic strains closely related to the organism that produces the antibiotic. Other cyanobacteria secrete antibiotics that are active against a wide range of cyanobacteria and eukaryotic algae. *Scytonema hofmanni* (Fig. 2.46(e)) produces such an antibiotic (Mason et al., 1982; Gleason and Paulson, 1984). This antibiotic, called

**cyanobacterin**, is a chlorine-containing  $\lambda$ -lactone (Pignatello et al., 1983). All of these antibiotics probably play an active role in the survival of the producing organism by inhibiting growth of competing organisms.

The obligate requirement for iron, coupled with the low solubility of iron in many aquatic habitats, has led to the evolution of a mechanism for iron acquisition, at the cost of cellular energy and nutritional stores. A number of cyanobacteria release extracellular ferric-specific chelating agents ("siderophores") during periods of low iron availability. Siderophores function as extracellular ligands that aid in solubilization and assimilation of  $\text{Fe}^{3+}$  (Fig. 2.52) (Wilhelm et al., 1996; Barbeau et al., 2003).



**Fig. 2.52** (a) A siderophore complexed with two ions of iron. (b) A cyanobacterial cell secretes a siderophore which complexes with iron ions in the medium. The siderophore complexed iron ions are absorbed by the cell.



## Calcium carbonate deposition and fossil record

Many species of cyanobacteria have calcium carbonate in the enveloping mucilage of the cells. In freshwater these algae usually grow in water where carbonate crystallizes out by non-biological physicochemical mechanisms, and the crystals of calcite become trapped in the mucilage of the algae. Normally, only 1% to 2% of the calcium carbonate is actively precipitated by the cells (Pentecost, 1978). There are a large number of different forms of carbonate deposits attributed to the Cyanophyceae (for a review, see Golubić, 1973).

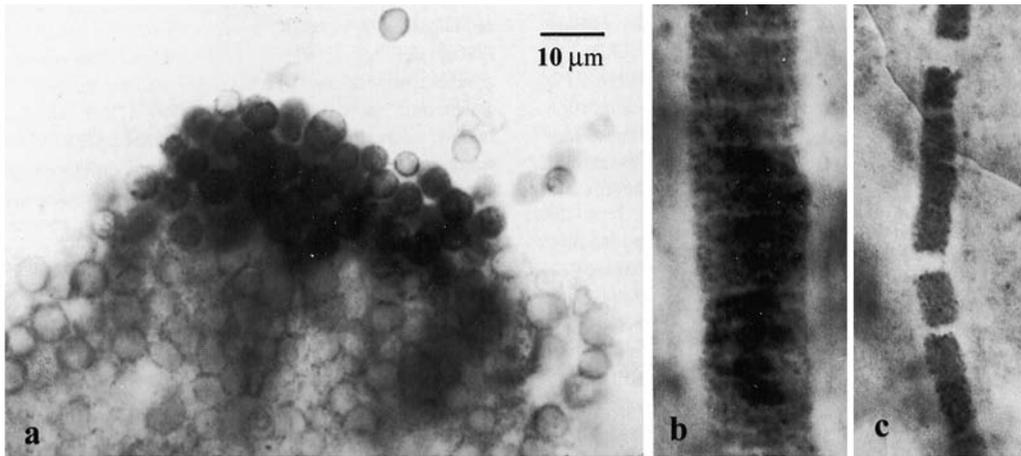
In the marine environment, cyanophycean depositions are the result of trapping and binding of the sediments as well as carbonate precipitation. **Stromatolithic** heads are probably the best known of these forms (Figs. 2.53, 2.55). These heads are firmly gelatinous to almost cartilaginous in texture, predominantly hemispherical, and show fine concentric lamination. In the shallow subtidal waters of south Florida and the Bahamas, they are produced by a single species of *Schizothrix* (Monty, 1967). During the day there is growth of algal filaments, resulting in the algae covering the surface of the stromatolite head. During the night, growth ceases, and sediments accumulate on the surface

of the head, forming sediment-rich laminae up to 100  $\mu\text{m}$  thick. In the early part of the day, the algae penetrate through this deposited sediment, and grow a hyaline layer, 200  $\mu\text{m}$  thick, with a low concentration of entrapped sediment. These alternating periods of growth and deposition give a laminated structure to the stromatolite (Reid et al., 2000) (Fig. 2.55). Stromatolites first formed 3500 million years ago although the first stromatolites were formed by non-biological deposition of  $\text{CaCO}_3$  (Arp et al., 1999) with the first stromatolites formed by cyanobacteria (Fig. 2.54) appearing about 2700 million years ago (Buick, 1992; Dalton, 2002; Brasier et al., 2002).

The production of laminae in stromatolites depends on fluctuations ultimately derived from the physical movements of the earth, sun, and moon, and requires some kind of rhythmicity that causes discontinuity in the accretionary process. The periodicity of laminae is due primarily to the daily photosynthetic cycle of the organisms in the stromatolites. In addition, stromatolites are **heliotropic** and grow toward sunlight (Awramik and Vanyo, 1986). This heliotropism allows the calculation of the extent of a year's deposition in a stromatolite. The yearly cycle of movement of the sun causes the sun to be higher in the sky in the summer and lower in the winter. The heliotropism of the stromatolites causes the stromatolites to grow in a sine waveform over the course of a



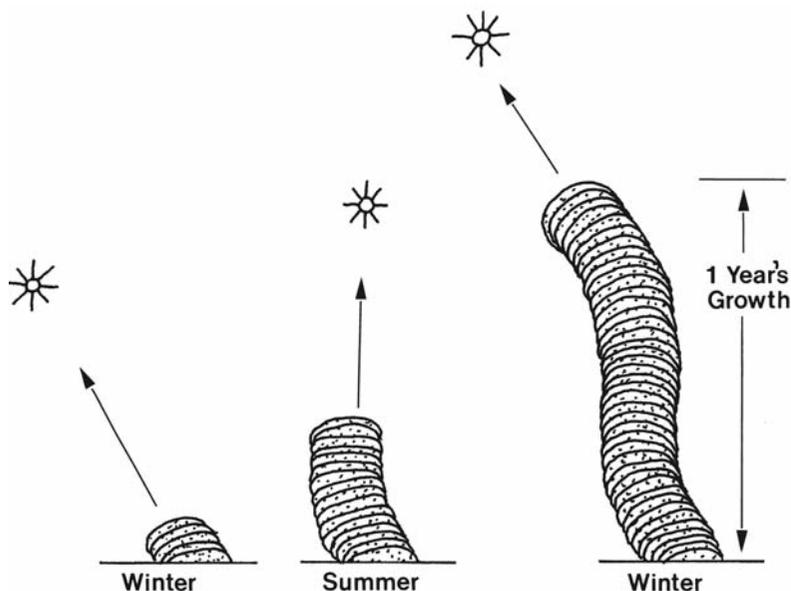
**Fig. 2.53** Cyanobacterial stromatolites in the process of formation in Shark Bay, Western Australia. (From Logan, 1961.)



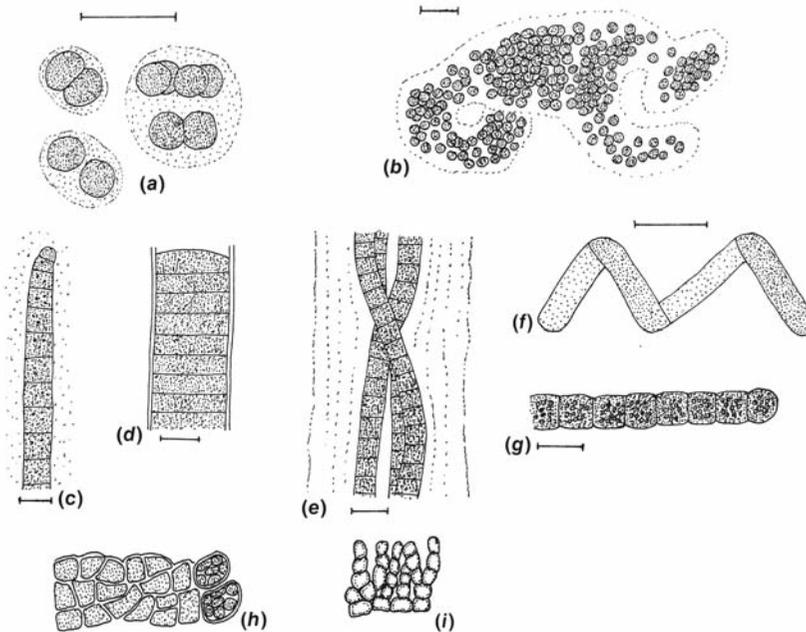
**Fig. 2.54** Fossil cyanobacteria preserved in silicified stromatolites of the 1400 million-year-old Gaoyuzhang formation of northern China. (a) *Eoentophysalis belcherensis*. (b) *Palaeolyngbya barghooriana*. (c) *Oscillatorioopsis*. (From Golubic and Seong-Joo, 1999.)

year (Fig. 2.55). Counting the number of laminae in one sine wave has allowed paleontologists to calculate the number of days in a year for a geological period. Such studies have shown that the solar year has varied considerably. For example, approximately 1000 million years ago, the solar year consisted of approximately 435 days (Vanyo and Awramik, 1985).

Up to 2000 million years ago, there were no grazing and boring organisms; thus stromatolites grew uncontested. Without competition, Precambrian stromatolites freely populated enormous areas and most likely grew in water down to a depth of 10 m. The occurrence and size of stromatolites declined dramatically after the evolution of grazing and boring organisms. Today stromatolites grow only in warm waters that are inhospitable to grazers and borers – waters such as the hypersaline waters in Shark Bay, Australia (Fig. 2.53), or in waters with a high tidal current that inhibit borers and grazers, such as in the Bahamas where stromatolites grow to a height of 2 m (Dill et al., 1986).



**Fig. 2.55** Diagrammatic representation of the growth of a stromatolite over the period of a year. A year's growth is represented by an S-shaped curve.



**Fig. 2.56** (a) *Gloeotheca magna*. (b) *Microcystis aeruginosa*. (c) *Phormidium autumnale*. (d) *Lyngbya birgei*. (e) *Hydrocoleus* sp. (f) *Spirulina major*. (g) *Trichodesmium lacustre*. (h,i) *Pleurocapsa minor*, surface view showing cells with endospores (h) and vertical section of thallus showing erect threads (i). Bar = 10  $\mu\text{m}$ .

## Classification

Nucleic acid sequencing of cyanobacteria is beginning to elucidate the evolutionary relationships among cyanobacteria (Honda et al., 1999; Nadeau et al., 2001; Turner, 1997). These studies have shown that there is little in common between the morphology of cyanobacteria and their evolutionary relationship. The one exception is the cyanobacteria with heterocysts, which all appear to be closely related. With the classification of cyanobacteria in such a state of flux, I have chosen to simplify the classification by dividing the cyanobacteria into three orders:

- Order 1 Chroococcales:** single cells or cells loosely bound into gelatinous irregular colonies.
- Order 2 Oscillatoriales:** filamentous cyanobacteria.
- Order 3 Nostocales:** filamentous cyanobacteria with heterocysts.

## Chroococcales

This order included basically unicellular cyanobacteria which are held together in palmelloid colonies by mucilage. *Gloeotheca* (Fig. 2.56(a)), *Microcystis* (Figs. 2.48, 2.56(b)), *Synechococcus* (Figs. 2.19(c), 2.31), and *Synechocystis* (Fig. 2.8) are some of the genera in this order. Although the organisms in this order have a similar morphology, studies involving nucleic acid base composition have shown that the order is actually composed of several distinct and widely separated groups (Honda et al., 1999).

*Prochlorococcus marinus* (Fig. 2.20) is the dominant photosynthetic organism in tropical and temperate oceans. The cyanobacterium is 0.5  $\mu\text{m}$  in diameter, making it the smallest photosynthetic organism. The cells have chlorophyll *a* and *b*, and lack phycobilin pigments. The complete genome of *Prochlorococcus marinus* has been determined (Dufresne et al., 2003).

*Gloeobacter violaceus* (Fig. 2.19(e)), a cyanobacterium without internal thylakoids, appears to be the most primitive cyanobacterium based on nucleic acid sequencing studies (Honda et al., 1999).

## Oscillatoriales

These are filamentous cyanobacteria without heterocysts. Representative cyanobacteria are

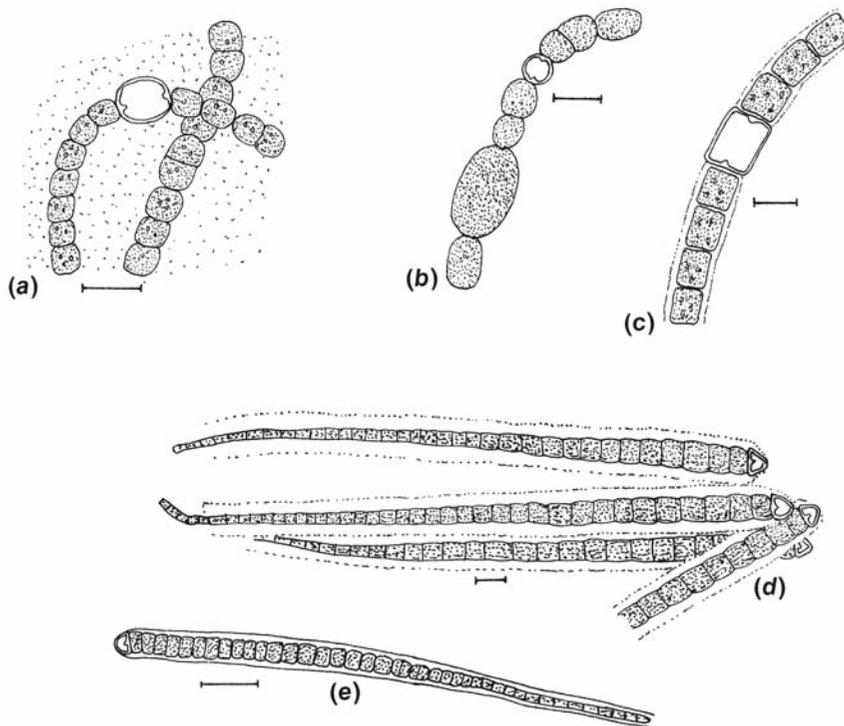
*Oscillatoria* (Figs. 2.17, 2.34(a), (b), 2.19(a), (b)), *Trichodesmium* (Figs. 2.31, 2.56(g)), *Phormidium* (Figs. 2.13, 2.18(c), 2.56(c)), *Lyngbya* (Fig. 2.56(d)), *Hydrocoleus* (Fig. 2.56(e)), and *Spirulina* (Fig. 2.51).

*Pleurocapsa* (Fig. 2.56(h) (i)) is composed of an erect and a prostrate system and is therefore **heterotrichous**. *Pleurocapsa* is a widely distributed **lithophyte** (grows on rocks) in the marine and freshwater environment. Initially it consists of a filament creeping over the surface. Later the filament branches and forms a pseudoparenchymatous disc. Development may stop here, or the prostrate basal disc cells may form erect threads (Fig. 2.56(i)). Endospores are formed internally by the division of a vegetative cell in three planes.

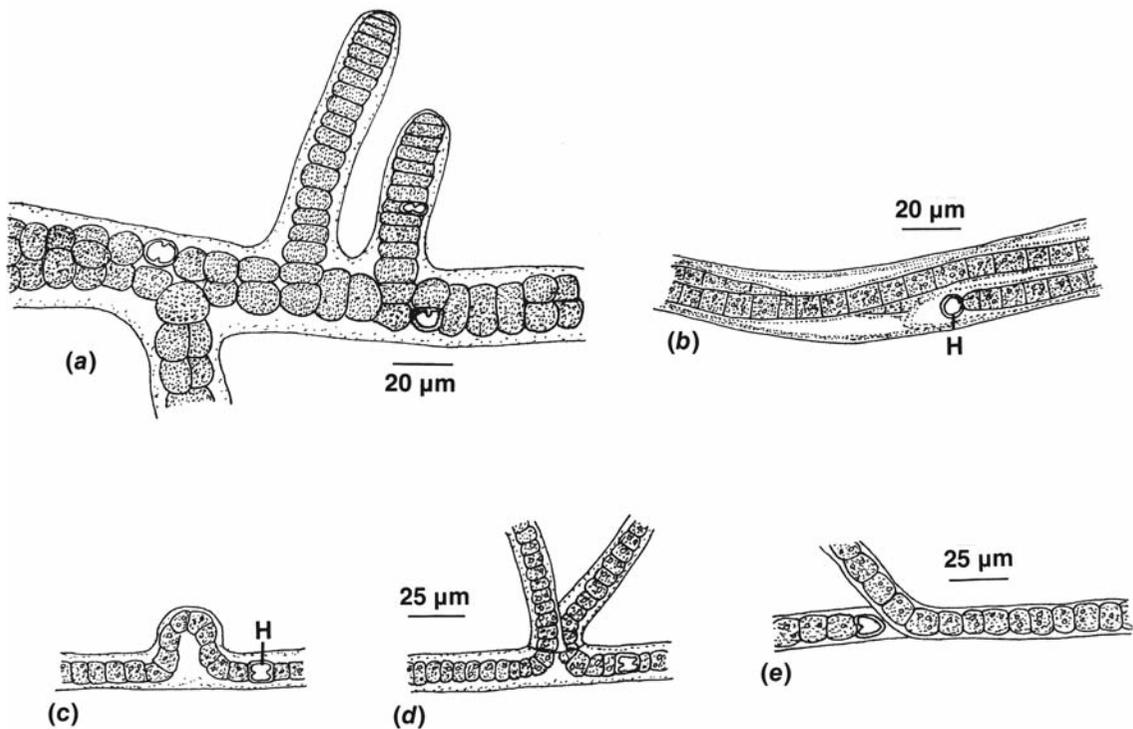
### Nostocales

The cyanobacteria in this order, such as *Nostoc* (Figs. 2.35, 2.46(b), (c), (d), 2.57(a)), *Anabaena* (Figs. 2.16, 2.18(d)), and *Aulosira* (Fig. 2.46(a)), have heterocysts. The most common method of reproduction is by

hormogonia. In some genera there is a polarity: heterocysts and/or akinetes are at the base, and a colorless hair is at the apex of the filament. A hair is a region of the trichome where the cells are narrow, elongated, highly vacuolated, and apparently colorless. *Calothrix* (Figs. 2.42(d), 2.57(e)) is common in the littoral zone of the ocean, where it is attached to rocks. *Rivularia* (Fig. 2.57(d)) forms colonies in which the sheaths of one filament are confluent with others. The sheaths are usually heavily encrusted with lime and are very firm. *Rivularia* grows in freshwater submerged on stones and plants. The polarity of the thallus occurs only under conditions of low-nitrogen concentration (Sinclair and Whitton, 1977). When these algae are grown in media containing nitrogenous compounds (e.g.,  $\text{NO}_3^-$ ,  $\text{NH}_4$ ) heterocysts are not formed, and the trichomes are not tapered and lack colorless hairs at the apex. These algae then resemble species of genera such as *Oscillatoria* (Figs. 2.17, 2.34(a), (b), 2.19(a), (b)). Hairs can also be



**Fig. 2.57** (a) *Nostoc linckia*. (b) *Anabaena circinalis*. (c) *Aulosira implexa*. (d) *Rivularia dura*. (e) *Calothrix fusca*. Bar = 10mm.



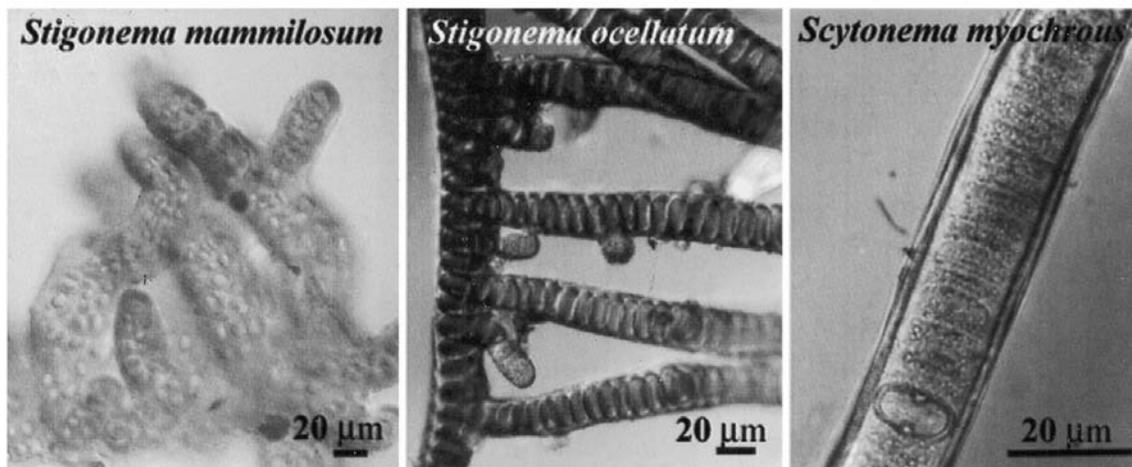
**Fig. 2.58** (a) *Stigonema turfaceum* showing true branching. (b) *Desmonema wrangelii* with a number of trichomes in a sheath. (c,d) *Scytonema arcangelii* illustrating formation of false branches. (e) *Tolypothrix tenuis* with a single false branch. (H) heterocyst. (After Smith, 1950.)

induced to form by growing the algae under conditions of phosphorus deficiency. Addition of phosphorus results in the loss of hairs and the formation of hormogonia (Livingstone and Whitton, 1983).

False branching occurs in some genera in this order. It results when the trichome lodges in the sheath, commonly in the area of a large heterocyst (Fig. 2.58(c), (d)). Committed cell division in the trichome results in (1) a rupture of the sheath and (2) a break in the trichome that gives the appearance of a branch. The first type of false branching results in only one false branch protruding through the sheath, as in *Tolypothrix* (Fig. 2.58). This type is due to the death of a cell or the formation of a separation disc or necridium (Fig. 2.34) (biconvex in shape because of pressure from adjacent cells, the necridia lyse as they mature). These weak points are normally next to a

heterocyst and eventually result in a break of the trichome, with one of the broken ends of the trichome protruding through the sheath as a false branch. The second type of false branching results in the formation of two false branches, as in *Scytonema* (Figs. 2.58(c), (d), 2.59). Here a loop is formed that protrudes through the sheath. Eventually the loop breaks in the middle as cell division continues, resulting in two false branches. It is probable that the large heterocysts lodging in the sheath cause the immovability of the trichome in the sheath. Experimental evidence that this is so comes from experiments with algae grown in high combined-nitrogen levels, which inhibit both heterocyst formation and false branching.

*Stigonema* has true branching with a tendency toward multiserial thalli. The branching axis of *Stigonema* is partly or wholly multiserial (Figs. 2.58, 2.59). *Stigonema* occurs on wet rocks and reproduces by hormogonia formed on the younger branches. The genus is the most morphologically complex in the cyanobacteria and is often differentiated into upright and prostrate filaments (heterotrichy).



**Fig. 2.59** Light micrographs of cyanobacteria in the Nostocales. (From Rascher et al., 2003.)



**Fig. 2.60 Jindong Zhao** Dr. Zhao received his Bachelor's degree in biology from Southwestern Normal University in 1982. He became a Ph.D. student in the Department of Botany, University of Texas at Austin in 1984 and received a Ph.D. degree in Biology in 1989. He spent the next three years as a postdoctoral fellow in Penn State University and the following two years in Applied Biosystems Inc. as a research scientist. Jindong Zhao returned to China and became an associate professor in the College of Life Sciences of Peking University in 1994. He was promoted to full professor in 1998. His laboratory focuses on studies of molecular mechanism of heterocyst differentiation and pattern formation of cyanobacteria.

## REFERENCES

- Aboulmagd, E., Oppermann-Sanio, F. B., and Steinbuechel, A. (2000). Molecular characterization of the cyanophycin synthetase from *Synechocystis* sp. strain PCC6308. *Arch. Microbiol.* 174:297–306.
- Adams, D. G. (2000). Heterocyst development in cyanobacteria. *Curr. Opin. Microbiol.* 3:618–24.
- Ahmadjian, V., and Hale, M. E. (1973). *The Lichens*. New York: Academic Press.
- Allen, M. M. (1984). Cyanobacterial cell inclusions. *Annu. Rev. Microbiol.* 38:1–25.
- Al-Thukair, A. A., and Golubic, S. (1991). New endolithic cyanobacteria from the Arabian Gulf. I. *Hyella immanis* sp. nov. *J. Phycol.* 27:766–80.
- Anderson, O. R. (1977). Fine structure of a marine ameba associated with a blue-green alga in the Sargasso Sea. *J. Protozool.* 24:370–6.
- Arment, A. R., and Carmichael, W. W. (1996). Evidence that microcystin is a thio template product. *J. Phycol.* 32:591–7.
- Arp, G., Reimer, A., and Reitner, J. (1999). Calcification in cyanobacterial biofilms of alkaline salt lakes. *Eur. J. Phycol.* 34:393–403.
- Awramik, S. M., and Vanyo, J. P. (1986). Heliotropism in modern stromatolites. *Science* 231:1279–81.
- Badger, M. R., Hanson, D., and Price, G. D. (2002). Evolution and diversity of CO<sub>2</sub> concentrating mechanisms in cyanobacteria. *Funct. Plant Biol.* 29:161–73.
- Barbeau, K., Rue, E. L., Trick, C. G., Bruland, K. W., and Butler, A. (2003). Photochemical reactivity of siderophores produced by marine heterotrophic bacteria and cyanobacteria based on characteristic Fe(III) binding groups. *Limnol. Oceanogr.* 48:1069–78.

- Belay, A., Kato, T., and Ota, Y. (1996). *Spirulina* (*Arthrospira*): potential application as an animal feed supplement. *J. Appl. Phycol.* 8:303–11.
- Bell, S. G., and Cobb, G. A. (1994). Cyanobacterial toxins and human health. *Rev. Med. Microbiol.* 5:256–64.
- Belnap, J., and Gardner, J. S. (1993). Soil substructure in soils of the Colorado Plateau: the role of the cyanobacterium *Microcoleus vaginatus*. *Great Basin Nat.* 53:40–7.
- Bergman, B., and Carpenter, E. J. (1991). Nitrogenase confined to randomly distributed trichomes in the marine cyanobacterium *Trichodesmium thiebautii*. *J. Phycol.* 27:158–65.
- Bergman, B., Gallon, J. R., Rai, A. N., and Stal, L. J. (1997). N<sub>2</sub> fixation by non-heterocystous cyanobacteria. *FEMS Microbiol. Rev.* 19:139–85.
- Berman-Frank, I., Lundgren, P., Chen, Y-B., et al. (2001). Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium *Trichodesmium*. *Science* 254:1534–7.
- Bhattacharjee, S. K. (1977). Unstable protein mediated ultraviolet light resistance in *Anacystis nidulans*. *Nature* 269:82–3.
- Bhaya, D. (2004). Light matters: phototaxis and signal transduction in unicellular cyanobacteria. *Mol. Microbiol.* 53:745–54.
- Bhaya, D., Watanabe, N., Ogawa, T., and Grossman, A. R. (1999). The role of an alternative sigma factor in motility and pilus formation in the cyanobacterium *Synechocystis* sp. strain PCC6803. *Proc. Natl. Acad. Sci., USA* 96:3188–93.
- Bittencourt-Oliveira, M., de Olivera, M. C., and Bolch, C. J. S. (2001). Genetic variability of Brazilian strains of the *Microcystis aeruginosa* complex (Cyanobacteria/Cyanophyceae) using phycocyanin intergenic spacer and flanking regions (*cpcBA*). *J. Phycol.* 37:810–18.
- Bolch, C. J. S., Orr, P. T., Jones, G. J., and Blackburn, S. J. (1999). Genetic, morphological and toxicological variation among globally distributed strains of *Nodularia* (Cyanobacteria). *J. Phycol.* 35:339–55.
- Booker, M. J., and Walsby, A. E. (1981). Bloom formation and stratification by a planktonic blue-green alga in an experimental water column. *Br. Phycol. J.* 16:411–21.
- Booth, W. E. (1941). Algae as pioneers in plant succession and their importance in erosion control. *Ecology* 22:38–46.
- Brasier, M. D., Green, O. R., Jephcoat, A. P., et al. (2002). Questioning the evidence for Earth's oldest fossils. *Nature* 416:76–81.
- Brock, T. D. (1973). Lower pH limit for the existence of blue-green algae: Evolutionary and ecological implications. *Science* 179:480–3.
- Buick, R. (1992). The antiquity of oxygenic photosynthesis: evidence from stromatolites in sulphate-deficient archaean lakes. *Science* 255:74.
- Caiola, M. G. (1975). A light and electron microscopic study of blue-green algae living in the coralloid roots of *Encephalartos altensteinii* and in culture. *Phycologia* 14:25–33.
- Canini, A., Bergman, B., Civitareale, P., Rotilla, G., and Caiola, M. G. (1992). Localization of iron-superoxide dismutase in the cyanobiont of *Azolla filiculoides* Lam. *Protoplasma* 169:1–8.
- Carmichael, W. W. (1992). Cyanobacteria secondary metabolites – the cyanotoxins. *J. Appl. Bacteriol.* 72:445–59.
- Carmichael, W. W., Drapeau, C., and Anderson, D. M. (2000). Harvesting of *Aphanizomenon flos-aquae* Ralfs ex Born. Flah. Var. *flos-aquae* (Cyanobacteria) from Klamath Lake for human dietary use. *J. Appl. Phycol.* 12:585–95.
- Carpenter, E. J., and Price, C. C. (1977). Nitrogen fixation, distribution, and production of *Oscillatoria* (*Trichodesmium*) spp. in the western Sargasso and Caribbean seas. *Limnol. Oceanogr.* 22:60–72.
- Carpenter, E. J., Capone, D. C., and Reuter, J. (eds.) (1992). *Marine Pelagic Cyanobacteria: Trichodesmium and other Diazotrophs*, Dordrecht: Kluwer Academic Publishers.
- Castenholz, R. W. (1973). Ecology of blue-green algae in hot-springs. In *The Biology of the Blue-Green Algae*, ed. N. G. Carr and B. A. Whitton, pp. 379–414. Berkeley: Univ. Calif. Press.
- Chauvat, F., Corre, B., Herdman, M., and Joset-Espardellier, F. (1982). Energetic and metabolic requirements for the germination of akinetes of the cyanobacterium *Nostoc* PCC7524. *Arch. Microbiol.* 133:44–9.
- Chen, H. M., Chien, C-Y., and Huang, T-C. (1996). Regulation and molecular structure of a circadian oscillating protein located in the cell membrane of the prokaryote *Synechococcus* RF-1. *Planta* 199:520–7.
- Clark, R. L., and Jensen, T. E. (1969). Ultrastructure of akinete development in a blue-green alga, *Cylindrospermum* sp. *Cytologia* 34:439–48.
- Cmiech, H. A., Leedale, G. F., and Reynolds, C. S. (1986). Morphological and ultrastructural variability of planktonic Cyanophyceae in relation to seasonal periodicity. II. *Anabaena solitaria*: Vegetative cells, heterocysts, akinetes. *Br. Phycol. J.* 21:81–92.

- Codd, G. A., Bell, S. G., Kaya, K., Ward, C. J., Beattie, K. A., and Metcalf, J. S. (1999). Cyanobacterial toxins, exposure routes and human health. *Eur. J. Phycol.* 34:405–15.
- Dalton, R. (2002). Microfossils: squaring up over ancient life. *Nature* 417:782–84.
- De Philippis, R., and Vincenzini, M. (1998). Extracellular polysaccharides from cyanobacteria and their possible applications. *FEMS Microbiol. Rev.* 22:151–72.
- Desikachary, T. V. (1959). *Cyanophyta*. New Delhi: Indian Council of Agricultural Research.
- Dill, R. F., Shinn, E. A., Jones, A. T., Kelly, K., and Steinen, R. P. (1986). Giant subtidal stromatolites forming in normal salinity waters. *Nature* 324:55–9.
- Dillon, J. G., and Castenholz, R. W. (1999). Scytonemin, a cyanobacterial sheath pigment, protects against UVC radiation: implications for early photosynthetic life. *J. Phycol.* 35:673–81.
- Drouet, F. (1937). The Brazilian Myxophyceae. I. *Am. J. Bot.* 24:598–608.
- Drouet, F. (1978). Revision of the Nostocaceae with constricted trichomes. *Beihefte Nova Hedwigia* 57:1–258.
- Dufresne, A., Salanoubat, M., Partensky, F., et al. (2003). Genome sequence of the cyanobacterium *Prochlorococcus marinus* SS120, a nearly minimal oxyphototrophic genome. *Proc. Natl. Acad. Sci., USA* 100:10020–5.
- El-Bestawy, E., Bellinger, E. G., and Sigee, D. C. (1996). Elemental composition of phytoplankton in a subtropical lake: X-ray microanalytical studies on the dominant algae *Spirulina platensis* (Cyanophyta) and *Cyclotella meneghiniana* (Bacillariophyceae). *Eur. J. Phycol.* 31:157–66.
- El-Shehawey, R. M., and Kleiner, D. (2003). Effect of controlled expression of *hetR* gene on heterocyst formation in the filamentous cyanobacterium *Anabaena* sp. PCC 7120. *Physiol. Plantarum* 119:44–8.
- Ferguson-Wood, E. J. (1965). *Marine Microbial Ecology*. London: Chapman and Hall.
- Ferjani, A., Mustardy, L., Sulpice, R., et al. (2003). Glucoglycerol, a compatible solute, sustains cell division under salt stress. *Plant Physiol.* 131:1628–37.
- Ferris, M. J., and Palenik, B. (1998). Niche adaptation in ocean cyanobacteria. *Nature* 396:226–8.
- Flores, E., and Wolk, C. P. (1986). Production, by filamentous, nitrogen-fixing cyanobacteria, of a bacteriocin and of other antibiotics that kill related strains. *Arch. Microbiol.* 145:215–19.
- Fogg, G. E. (1942). Studies on nitrogen fixation by bluegreen algae. I. Nitrogen fixation by *Anabaena cylindrica* Lemm. *J. Exp. Biol.* 19:78–87.
- Fogg, G. E. (1952). The production of extracellular nitrogenous substances by a blue-green alga. *Proc. R. Soc. London [B]* 139:372–9.
- Fogg, G. E. (1986). Light and ultraphytoplankton. *Nature* 319:96.
- Frederick, J. F. (1951). Preliminary studies on the synthesis of polysaccharides in the algae. *Physiol. Plant.* 4:621–6.
- Fredriksson, C., and Bergman, B. (1997). Ultrastructural characterization of cells specialized for nitrogen function in a non-heterocystous cyanobacterium, *Trichodesmium* spp. *Protoplasma* 197:76–85.
- Fulda, S., Mikkat, S., Schroder, W., and Hagemann, M. (1999). Isolation of salt-induced periplasmic proteins from *Synechocystis* sp. strain PCC 6803. *Arch. Microbiol.* 171:214–7.
- Gantt, E., and Conti, S. F. (1969). Ultrastructure of blue-green algae. *J. Bacteriol.* 97:1486–93.
- Gao, K., and Ye, C. (2003). Culture of the terrestrial cyanobacterium, *Nostoc flagelliforme* (Cyanophyceae), under aquatic conditions. *J. Phycol.* 39:617–22.
- Gates, J. E., Fisher, R. W., Goggins, T. W., and Azrolan, N. I. (1980). Antigenic differences between *Anabaena azollae* fresh from the *Azolla* fern leaf cavity and free-living cyanobacteria. *Arch. Microbiol.* 128:126–9.
- Gleason, F. K., and Paulson, J. L. (1984). Site of action of the natural algicide, cyanobacterin, in the blue-green alga, *Synechococcus* sp. *Arch. Microbiol.* 138:273–7.
- Glover, H. E., Keller, M. D., and Guillard, R. R. L. (1986). Light quality and oceanic ultraphytoplankton. *Nature* 319:142–3.
- Golden, S. S. (2003). Timekeeping in bacteria: the cyanobacterial circadian clock. *Curr. Opin. Microbiol.* 6:535–40.
- Golubić, S. (1973). The relationship between blue-green algae and carbonate deposits. In *The Biology of the Blue-Green Algae*, ed. N. G. Carr and B. A. Whitton, pp. 434–72. Berkeley: Univ. Calif. Press.
- Golubic, S., and Seong-Joo, L. (1999). Early cyanobacterial fossil record: preservation, palaeoenvironments and identification. *Eur. J. Phycol.* 34:339–48.
- Goodwin, T. W. (1974). Carotenoids and biliproteins. In *Algal Physiology and Biochemistry*, ed. W. D. P. Stewart, pp. 176–205. Berkeley: Univ. Calif. Press.
- Henson, B. J., Watson, L. E., and Barnum, S. R. (2004). The evolutionary history of nitrogen fixation, as assessed by *nifD*. *J. Molec. Evol.* 58:390–9.
- Herdman, M., Janvier, M., Ripplka, R., and Stanier, R. Y. (1979). Genome size of cyanobacteria. *J. Gen. Microbiol.* 111:73–85.

- Hill, D. J. (1975). The pattern of development of *Anabaena* in the *Azolla*-*Anabaena* symbiosis. *Planta* 122:179-84.
- Hockelmann, C., Moens, T., and Juttner, F. (2004). Odor compounds from cyanobacterial biofilms acting as attractants and repellents for free-living nematodes. *Limnol. Oceanogr.* 49:1809-19.
- Hoffman, L. (1999). Marine cyanobacteria in tropical regions: diversity and ecology. *Eur. J. Phycol.* 34:371-9.
- Hoiczky, E. (2000). Gliding motility in cyanobacteria: observations and possible explanations. *Arch. Microbiol.* 174:11-17.
- Hoiczky, E., and Baumeister, W. (1995). Envelope structure of four gliding filamentous cyanobacteria. *J. Bacteriol.* 177:2387-95.
- Hoiczky, E., and Baumeister, W. (1998). The junctional pore complex, a prokaryotic secretion organelle, is the molecular motor underlying gliding motility in cyanobacteria. *Curr. Biol.* 8:1161-8.
- Honda, D., Yokota, A., and Sugiyama, J. (1999). Detection of seven major evolutionary lineages in cyanobacteria based on the 16S rRNA gene sequence analysis with new sequences of five marine *Synechococcus* strains. *J. Mol. Evol.* 48:723-30.
- Hough, L., Jones, J. K. N., and Wadman, W. H. (1952). An investigation of the polysaccharide components of certain fresh-water algae. *J. Chem. Soc.* 3393-9.
- Jang, M.-H., Ha, K., Joo, G.-J., and Takamura, N. (2003). Toxin production of cyanobacteria is increased by exposure to zooplankton. *Freshwater Biol.* 48: 1540-5.
- Jarosch, R. (1962). Gliding. In *Physiology and Biochemistry of Algae*, ed. R. A. Lewin, pp. 573-81. New York and London: Academic Press.
- Jensen, T. E., and Clark, R. L. (1969). Cell wall and coat of the developing akinete of a *Cylindrospermum* species. *J. Bacteriol.* 97:1494-5.
- Jones, J. D., and Jost, M. (1970). Isolation and chemical characterization of gas vacuole membranes from *Microcystis aeruginosa* Kuetz. emend Elenkin. *Arch. Mikrobiol.* 70:43-64.
- Kapustka, L. A., and DuBois, J. D. (1987). Dinitrogen fixation by cyanobacteria and associative rhizosphere bacteria in the Arapaho prairie in the sand hills of Nebraska. *Am. J. Bot.* 74:107-13.
- Karsten, U. (1996). Growth and organic osmolytes of geographically different isolates of *Microcoleus chthonoplastes* (cyanobacteria) from benthic microbial mats: response to salinity changes. *J. Phycol.* 32:501-6.
- Konopka, A., Kromkamp, J., and Mur, L. R. (1987). Regulation of gas vesicle content and buoyancy in light- or phosphate-limited cultures of *Aphanizomenon flos-aquae* (Cyanophyta). *J. Phycol.* 23:70-8.
- Kotak, B. G., Lam, A. K-Y., Prepas, E. E., Kenefick, S. L., and Hurdey, S. E. (1995). Variability of the hepatotoxin microcystin-LR in hypertrophic drinking water lakes. *J. Phycol.* 31:248-63.
- Kulasooriya, S. A., Lang, N. J., and Fay, P. (1972). The heterocysts of blue-green algae. III. Differentiation and nitrogenase activity. *Proc. R. Soc. Lond. [B]* 181:199-209.
- Lang, N. J., and Fay, P. (1971). The heterocysts of blue-green algae. II. Details of ultrastructure. *Proc. R. Soc. Lond. [B]* 178:193-203.
- Lawry, N. H., and Simon, R. D. (1982). The normal and induced occurrence of cyanophycin bodies in several blue-green algae. *J. Phycol.* 18:391-9.
- Lesser, M. P., Mazel, C. H., Gorbunov, M. Y., and Falkowski, P. G. (2004). Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. *Science* 305:997-1000.
- Li, D-M., and Qi, Y-Z. (1997). *Spirulina* industry in China: present status and future prospects. *J. Appl. Phycol.* 9:25-8.
- Li, H., Sherman, D., Bao, S., and Sherman, L. A. (2001a). Pattern of cyanophycin accumulation in nitrogen-fixing and non-nitrogen-fixing cyanobacteria. *Arch. Microbiol.* 176:9-18.
- Li, R., Watanabe, M., and Watanabe, M. M. (1997). Akinete formation in planktonic *Anabaena* spp. (Cyanobacterium) by treatment with low temperature. *J. Phycol.* 33:576-84.
- Li, R., Carmichael, W. W., Britain, S., et al. (2001b). First report of the cyanotoxins cylindrospermopsin and deoxycylindrospermopsin from *Raphidiopsis curvava* (Cyanobacteria). *J. Phycol.* 37:1121-6.
- Little, M. G. (1973). The zonation of marine supralittoral blue-green algae. *Br. Phycol. J.* 8:47-50.
- Logan, B. W. (1961). Cryptozoon and associate stromatolites from the Recent, Shark Bay, Western Australia. *J. Geol.* 69:517-33.
- Lundgren, P., Soderbach, E., Singer, A., Carpenter, E. J., and Bergman, B. (2001). *Katagnymene*: characterization of a novel marine diazotroph. *J. Phycol.* 37:1052-62.
- Mague, T. H., and Holm-Hansen, O. (1975). Nitrogen fixation on a coral reef. *Phycologia* 14:87-92.
- Marquardt, J., Morschel, E., Rheil, E., and Westermann, M. (2000). Ultrastructure of *Acaryochloris marina*, an oxyphotobacterium containing chlorophyll *d*. *Arch. Microbiol.* 174:181-8.
- Mason, C. P., Edwards, K. R., Carlson, R. E., Pignatello, J., Gleason, F. K., and Woods, J. M. (1982). Isolation of

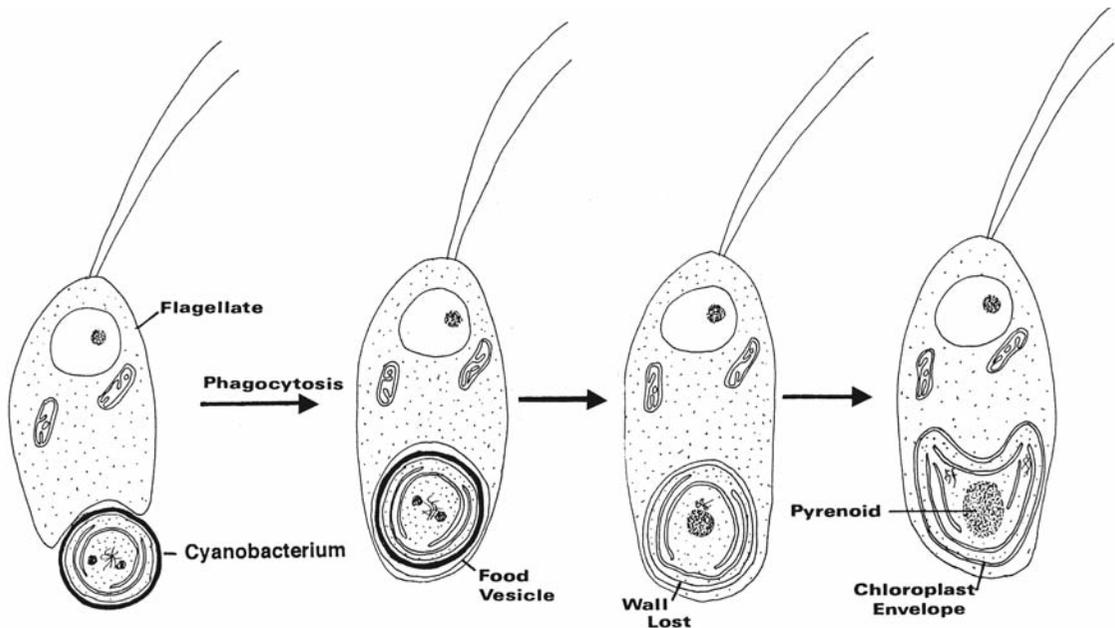
- chlorine-containing antibiotic from the freshwater cyanobacterium *Scytonema hofmanni*. *Science* 215:400–2.
- McCausland, M. A., Thompson, P. A., and Blackburn, S. L. (2005). Ecophysiological influence of light and mixing on *Anabaena circinalis* (Nostocales, Cyanobacteria). *Eur. J. Phycol.* 40:9–20.
- Meeks, J. C. (1998). Symbiosis between nitrogen-fixing cyanobacteria and plants. *BioScience* 48:266–76.
- Meeks, J. C., and Castenholz, R. W. (1971). Growth and photosynthesis in an extreme thermophile, *Synechococcus lividus* (Cyanophyta). *Arch. Mikrobiol.* 78:25–41.
- Meeks, J. C., and Elhai, J. (2002). Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. *Microbiol. Mol. Biol. Rev.* 66:94–121.
- Meeks, J. C., Campbell, E. L., Summers, M. L., and Wong, F. C. (2002). Cellular differentiation in the cyanobacterium *Nostoc punctiforme*. *Arch. Microbiol.* 178:395–403.
- Miller, S. R., Augustine, S., Olson, T. L., Blankenship, R. E., Selker, J., and Wood, A. M. (2005). Discovery of a free-living chlorophyll *d*-producing cyanobacterium with a hybrid proteobacterial/cyanobacterial small-subunit rRNA gene. *Proc. Natl. Acad. Sci., USA* 102:850–5.
- Mitsui, A., Kumazawa, S., Takashi, A., Ikemoto, H., Cao, S., and Arai, T. (1986). Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. *Nature* 323:720–2.
- Miyashita, H., Ikemoto, H., Kurano, N., Miyachi, S., and Chihara, M. (2003). *Acaryochloris marina* gen. et sp. nov. (cyanobacteria), an oxygenic photosynthetic prokaryote containing chl *d* as a major pigment. *J. Phycol.* 39:1247–53.
- Moffet, J. W., and Brand, L. E. (1996). Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. *Limnol. Oceanog.* 41:388–95.
- Montejano, G., and Leon-Tejera, H. (2002). Reproduction and baeocyte formation in two species of *Dermocarpella* (Cyanophyceae). *Eur. J. Phycol.* 37:323–7.
- Monty, C. L. V. (1967). Distribution and structure of recent stromatolite algal mats, eastern Andros Island, Bahamas. *Ann. Soc. Geol. Belg.* 90:55–99.
- Moore, D., McGregor, G. B., and Shaw, G. (2004). Morphological changes during akinete germination in *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria). *J. Phycol.* 40:1098–105.
- Mori, T., Saveliev, S. V., Xu, Y., et al. (2002). Circadian clock protein KaiC forms ATP-dependent hexameric rings and binds DNA. *Proc. Natl. Acad. Sci., USA* 99:17203–8.
- Nadeau, T.-L., and Castenholz, R. W. (2000). Characterization of psychrophilic oscillatorians (cyanobacteria) from Antarctic meltwater ponds. *J. Phycol.* 36:914–23.
- Nadeau, T.-L., Milbrandt, E. C., and Castenholz, R. W. (2001). Evolutionary relationships of cultivated Antarctic oscillatorians (cyanobacteria). *J. Phycol.* 37:650–4.
- Negri, A. P., Jones, G. J., Blackburn, S. I., Oshima, Y., and Hideyuki, O. (1997). Effect of culture bloom development and of sample storage on paralytic shellfish poisons in the cyanobacterium *Anabaena circinalis*. *J. Phycol.* 33:26–35.
- Nobles, D. R., Romanovicz, D. K., and Brown, R. M. (2001). Cellulose in cyanobacteria. Origin of plant cellulose synthase? *Plant Physiol.* 127:529–42.
- Oliver, R. L. (1994). Floating and sinking in gas-vacuolate cyanobacteria. *J. Phycol.* 30:161–73.
- Oliver, R. L., Thomas, R. H., Reynolds, C. S., and Walsby, A. E. (1985). The sedimentation of buoyant *Microcystis* colonies caused by precipitation with an iron-containing colloid. *Proc. R. Soc. Lond. [B]* 223:511–28.
- Otero, A., and Vincenzini, M. (2004). *Nostoc* (Cyanophyceae) goes nude: extracellular polysaccharides serve as a sink for reducing power under unbalanced C/N metabolism. *J. Phycol.* 40:74–81.
- Padan, E. (1979). Facultative anoxygenic photosynthesis in cyanobacteria. *Annu. Rev. Plant Physiol.* 30:27–40.
- Palenik, B., and Haselkorn, R. (1992). Multiple evolutionary origins of prochlorophytes, the chlorophyll *b*-containing prokaryotes. *Nature* 355:265–7.
- Pandey, D. C. (1965). A study of the algae from paddy soils of Ballia and Ghazipur districts of Uttar Pradesh, India. I. Cultural and ecological considerations. *Nova Hedwigia* 9:299–334.
- Partensky, F., Hess, W. R., and Vaulot, D. (1999). *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiol. Mol. Biol. Rev.* 63:106–27.
- Pascher, A. (1914). Über Symbiosen von Spaltpilzen und Flagellaten mit Blaualgen. *Ber. Dtsch. Bot. Ges.* 32:339–52.
- Pentecost, A. (1978). Blue-green algae and freshwater carbonate deposits. *Proc. R. Soc. Lond. [B]* 200:43–61.
- Peters, G. A., and Mayne, B. C. (1974). The *Azolla*, *Anabaena azollae* relationship. I. Initial characterization of the association. *Plant Physiol.* 53:813–19.
- Pignatello, J. J., Porwoll, J., Carlson, R. E., Xavier, A., Gleason, F. K., and Wood, J. M. (1983). Structure of the antibiotic cyanobacterin, a chlorine containing

- $\gamma$ -lactone from the freshwater cyanobacterium *Scytonema hofmanni*. *J. Org. Chem.* 48:4035–8.
- Postius, C., and Ernst, A. (1999). Mechanisms of dominance: coexistence of picocyanobacterial genotypes in a freshwater ecosystem. *Arch. Microbiol.* 172:69–75.
- Potts, M. (1996). The anhydrobiotic cyanobacterial cell. *Physiologia Plantarum* 97:788–94.
- Potts, M. (1999). Mechanisms of desiccation tolerance in cyanobacteria. *Eur. J. Phycol.* 34:319–28.
- Potts, M., Angeloni, S. V., Ebel, R. E., and Bassam, D. (1992). Myoglobin in a cyanobacterium. *Science* 256:1690–2.
- Prescott, G. W. (1962). *Algae of the Western Great Lakes Area*. Dubuque, Iowa: W. C. Brown.
- Qiu, B. S., Zhang, A. H., Liu, Z. L., and Gao, K. S. (2004). Studies on the photosynthesis of the terrestrial cyanobacterium *Nostoc flagelliforme* subjected to desiccation and subsequent rehydration. *Phycologia* 43:521–8.
- Raikow, D. F., Sarnelle, O., Wilson, A. E., and Hamilton, S. K. (2004). Dominance of the noxious cyanobacterium *Microcystis aeruginosa* on low-nutrient lakes associated with exotic zebra mussels. *Limnol. Oceanog.* 49:482–7.
- Rascher, U., Lakatos, M., Budel, B., and Luttge, U. (2003). Photosynthetic capacity of cyanobacteria of a tropical inselberg of the Guiana Highlands. *Eur. J. Phycol.* 38:247–56.
- Raymond, J., Siefert, J. L., Staples, C. R., and Blankenship, R.E. (2004). The natural history of nitrogen fixation. *Mol. Biol. Evol.* 21:541–54.
- Reid, R. P., Visscher, P. T., Decho, A. W., et al. (2000). The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. *Nature* 406:989–92.
- Rinehart, K. L., Namikoshi, M., and Choi, B. W. (1994). Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). *J. Appl. Phycol.* 6:159–76.
- Rippka, R., Waterbury, J., and Cohen-Bazire, G. (1974). A cyanobacterium which lacks thylakoids. *Arch. Mikrobiol.* 100:419–36.
- Romans, K. M., Carpenter, E. J., and Bergman, B. (1994). Buoyancy regulation in the colonial diazotrophic cyanobacterium *Trichodesmium tenue*, ultrastructure and storage of carbohydrate, polyphosphate, and nitrogen. *J. Phycol.* 30:935–42.
- Schutz, K., Happe, T., Troshina, O., et al. (2004). Cyanobacterial H<sub>2</sub> production – a comparative analysis. *Planta* 218:350–9.
- Sherman, D. M., Tucker, D., and Sherman, L. A. (2000). Heterocyst development and localization of cyanophycin in N<sub>2</sub>-fixing cultures of *Anabaena* sp. PCC 7120 (Cyanobacteria). *J. Phycol.* 36:932–41.
- Singh, R. N. (1961). *The Role of Blue-Green Algae in Nitrogen Economy of Indian Agriculture*. New Delhi: Indian Council for Agricultural Research.
- Smith, D. C., Muscatine, L., and Lewis, D. (1970). Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. *Biol. Rev.* 44:17–90.
- Smith, G. M. (1950). *Freshwater Algae of the United States*. New York: McGraw-Hill.
- Smith, G. M. (1955). *Cryptogamic Botany*. Vol. 1. New York: McGraw-Hill.
- Spencer, C. N., and King, D. L. (1985). Interactions between light, NH<sub>4</sub><sup>+</sup>, and CO<sub>2</sub> in buoyancy regulation of *Anabaena flos-aquae* (Cyanophyceae). *J. Phycol.* 21:194–9.
- Spratt, E. R. (1915). The root nodules of the Cycadaceae. *Ann. Bot.* 29:619–26.
- Staal, M., Meysman, F. J. R., and Stal, L. J. (2003). Temperature excludes N<sub>2</sub>-fixing heterocystous cyanobacteria in the tropical oceans. *Nature* 425:504–7.
- Stanier, R. Y. (1973). Autotrophy and heterotrophy in unicellular blue-green algae. In *The Biology of the Blue-Green Algae*, ed. N. G. Carr and B. A. Whitton, pp. 501–18. Berkeley: Univ. Calif. Press.
- Stewart, W. D. P., and Pearson, H. W. (1970). Effects of aerobic and anaerobic conditions on growth and metabolism of blue-green algae. *Proc. R. Soc. Lond. [B]* 175:293–311.
- Storey, W. B. (1968). Somatic reduction in cycads. *Science* 159:648–50.
- Sukenik, A., Eshkol, R., Livine, A., and Hadas, O. (2002). Inhibition of growth and photosynthesis of the dinoflagellate *Peridinium gatunense* by *Microcystis* sp. (cyanobacteria): a novel allelopathic mechanism. *Limnol. Ocean.* 47:1656–63.
- Suttle, C. A., and Chan, A. M. (1994). Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. *Appl. Env. Microbiol.* 60:3167–74.
- Swaminathan, M. S. (1984). *Rice. Sci. Am.* 250(1):80–93.
- Tandeau de Marsac, N. (1977). Occurrence and nature of chromatic adaptation in cyanobacteria. *J. Bacteriol.* 130:82–91.
- Tang, E. P. Y., Tremblay, R., and Vincent, W. F. (1997). Cyanobacterial dominance of polar freshwater ecosystems: are high-latitude mat-formers adapted to low temperature? *J. Phycol.* 33:171–81.
- Terauchi, K., and Ohmori, M. (2004). Blue light stimulates cyanobacterial motility via a cAMP signal transduction system. *Mol. Microbiol.* 52:303–9.

- Tischer, I. (1957). Untersuchungen über die granulären Eihenschlüsse und das Reduktions-Oxydations-Vermögen der Cyanophyceen. *Arch. Mikrobiol.* 27:400–28.
- Turner, S. (1997). Molecular systematics of oxygenic photosynthetic bacteria. *Pl. Syst. Evol. (Suppl.)* 11:13–52.
- Turpin, D. H., Miller, A. G., and Calvin, D. T. (1984). Carboxysome content of *Synechococcus leopoliensis* (Cyanophyta) in response to organic carbon. *J. Phycol.* 20:249–53.
- Urbach, E., Robertson, D. L., and Chisholm, S. W. (1992). Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. *Nature (Lond.)* 355:267–70.
- Vaishampayan, A., Sinha, R. P., Hader, D.-P., et al. (2001). Cyanobacterial biofertilizers in rice agriculture. *Bot. Rev.* 67:453–516.
- van Dok, W., and Hart, B. T. (1995). Akinete differentiation in *Anabaena circinalis* (Cyanophyta). *J. Phycol.* 32:557–65.
- Vanyo, J. P., and Awramik, S. M. (1985). Stromatolites and earth–sun–moon dynamics. *Precambrian Res.* 29:121–42.
- Wall, D., and Kaiser, D. (1999). Type IV pili and cell motility. *Mol. Microbiol.* 32:1–10.
- Walsby, A. E. (1974). The extracellular products of *Anabaena cylindrica* Lemm. I. Isolation of a macromolecular pigment–peptide complex and other components. *Br. Phycol. J.* 9:371–81.
- Walsby, A. E. (1978). The properties and buoyancy-providing role of gas vacuoles in *Trichodesmium* Ehrenberg. *Br. Phycol. J.* 13:103–16.
- Walsby, A. E. (1994). Gas vesicles. *Microbiol. Rev.* 58:94–144.
- Watanabe, A., and Kiyohara, T. (1963). Symbiotic blue-green algae of lichens, liverworts and cycads. In *Studies on Microalgae and Photosynthetic Bacteria*, ed. Japanese Soc. Plant Physiologists, pp. 189–96. Tokyo: University of Tokyo Press.
- Watanabe, M. F., Tsujimura, S., Oishi, S., Niki, T., and Namikoshi, M. (2003). Isolation and identification of homoanatoxin-a from a toxic strain of the cyanobacterium *Raphidiopsis mediterranea* Skuja isolated from Lake Biwa, Japan. *Phycologia* 42:364–9.
- Waterbury, J., and Stanier, R. (1977). Two unicellular bacteria which reproduce by budding. *Arch. Microbiol.* 115:249–57.
- Watson, S. B. (2003). Cyanobacterial and eukaryotic algal odour compounds: signals or by-products? A review of their biological activity. *Phycologia* 42:332–50.
- Weckesser, J., Broll, C., Adhikary, S. P., and Jürgens, U. J. (1987). 2-O-methyl-D-xylose containing sheath in the cyanobacterium *Gloeotheca* sp. PCC 6501. *Arch. Microbiol.* 147:300–3.
- Weller, D., Doemel, W., and Brock, T. D. (1975). Requirement of low oxidation-reduction potential for photosynthesis in a blue-green alga (*Phormidium* sp.). *Arch. Mikrobiol.* 104:7–13.
- Whale, G. F., and Walsby, A. E. (1984). Motility of the cyanobacterium *Microcoleus chthonoplastes* in mud. *Br. Phycol. J.* 19:117–23.
- Whitton, B. A., and Peat, A. (1969). On *Oscillatoria redekei* Van Goor. *Arch. Mikrobiol.* 68:362–76.
- Wildman, R. B., Loescher, J. H., and Winger, C. L. (1975). Development and germination of akinetes of *Aphanizomenon flos-aquae*. *J. Phycol.* 11:96–104.
- Wilhelm, S. W., Maxwell, D. P., and Trick, C. G. (1996). Growth, iron requirements, and siderophore production in iron-limited *Synechococcus* PCC 7002. *Limnol. Oceanog.* 41:89–97.
- Williams, S. B., Vakonakis, I., Golden, S. G., and LiWang, A. C. (2002). Structure and function from the circadian clock protein KaiA of *Synechococcus elongatus*: a potential clock input mechanism. *Proc. Nat. Acad. Sci., USA* 99:15357–62.
- Wilson, W. H., Carr, N. G., and Mann, N. H. (1996). The effect of phosphate status on the kinetics of cyanophage infection in the oceanic cyanobacterium *Synechococcus* sp. WH 7803. *J. Phycol.* 32:506–16.
- Winkenbach, F., and Wolk, C. P. (1973). Activities of enzymes of the oxidative and the reductive pentose phosphate pathways in heterocysts of a blue-green alga. *Plant Physiol., Lancaster* 52:480–3.
- Zhao, Y., Shi, Y., Zhao, W., et al. (2005). CcbP, a calcium-binding protein from *Anabaena* sp. PCC 7120, provides evidence that calcium ions regulate heterocyst differentiation. *Proc. Natl. Acad. Sci., USA* 16:5744–8.

# Part III

## Evolution of the chloroplast



**Fig. III.1** Diagrammatic representation of the uptake of a cyanobacterium by a protozoan into a food vesicle. This resulted in the establishment of an endosymbiosis between the cyanobacterium and the protozoan. Through evolution, the endosymbiotic cyanobacterium evolved into a chloroplast surrounded by two membranes of the chloroplast envelope.

The Rhodophyta (red algae) and Chlorophyta (green algae) form a natural group of algae in that they have chloroplasts surrounded by only the two membranes of the chloroplast envelope. The evolutionary event that led to the chloroplast occurred as follows (Fig. III.1). A phagocytotic protozoan took up a cyanobacterium into a food vesicle. Instead of being digested as a source of food, the cyanobacterium lived as an endosymbiont in the protozoan. This event benefited the protozoan because it received some of the photosynthate from the endosymbiotic alga, and it benefited the cyanobacterium because it received a protected stable environment. Through evolution the wall of the endosymbiotic

cyanobacterium was lost. A mutation in the endosymbiont which resulted in a loss of the wall would have been selected for in evolution because it would have facilitated the transfer of compounds between the host and the endosymbiont. The food vesicle membrane of the phagocytotic host became the outer membrane of the chloroplast envelope. The plasma membrane of the cyanobacterium symbiont became the inner membrane of the chloroplast envelope. Rearrangement of the thylakoid membranes and evolution of polyhedral bodies into a pyrenoid completed the transition to a true chloroplast such as occurs in extant green and red algae.

The endosymbiotic origin of chloroplasts was first proposed by the Russian biologist Konstantin Mereschkowsky (1855–1921) (Fig. III.2) with the fundamentals of the idea appearing in his 1905 work, *The Nature and Origins of Chromatophores in the Plant Kingdom* (see Martin and Kowallik, 1999, for English translation). Mereschkowsky worked on the symbiosis between algae and fungi in lichens around the city of Kazan and this led him to his theory on endosymbiosis. He likened plastids to “little green slaves” working for their host cells to produce food from sunshine.

Subsequent cytological and biochemical studies have reinforced Mereschkowsky’s theory of an endosymbiotic origin of plastids. Nucleotide sequencing (either directly from rRNA or from DNA encoding rRNAs) has shown the similarity between cyanobacteria and plastids. It has further shown that all plastids evolved from a single endosymbiotic event (McFadden, 2001; Keeling, 2004).



**Fig. III.2 Konstantin Mereschkowsky** Born August 4, 1855 in Warsaw, Poland (then part of Russia). Died by suicide in the Hotel des Familles, Geneva, Switzerland, on January 9, 1921. His father was a high official in the court of the Czar. He was the eldest son, having five brothers and three sisters. He entered the University of St. Petersburg in 1875, where he graduated with the diploma of *Kandidat* (with distinction) in 1880. In 1883 he became a *Privatdocent* at the same university. In 1883 he married Sultanova Olga Petronina by whom he had a son, Boris. From 1881 to 1898 he worked as a pomologist on fruit trees in the Crimea. In 1898 he left his wife and son to a life of poverty and traveled to the United States where he did research on unicellular algae (which he started when he was in the Crimea) at the University of California, Berkeley.

In 1902 he returned to Russia and took the position of curator of the zoology museum at Kazan University. In 1903 he passed his *Magister* with a dissertation "On the Morphology of Diatoms." At that time Mereschkowsky's research was influenced by Andrei Famintsyn, at the University of St. Petersburg, who was trying to cultivate chloroplasts isolated from zooxanthellae. Famintsyn was, however, not interested in having a younger competitor and denigrated Mereschkowsky's research, resulting in bad relations between the two. In 1905 Mereschkowsky published his famous paper in Russian and German "On the Nature and Origin of Chromatophores in the Plant Kingdom." In 1920, in Geneva, he published his most extensive paper on

the endosymbiotic origin of chloroplasts "La plant considérée comme un complexe symbiotique."

Mereschkowsky's personal life was a disaster. As a student he had revolutionary ideas. However, by the turn of the century he was a collaborator of the Czar's secret police. He was one of the organizers of the nationalistic, anti-Semitic organization "The Kazan Department of the Union of Russian People." Several of his colleagues were discharged after his denunciations. Mereschkowsky was forced to leave Russia in 1914, not because of his political views, but because of a sex scandal involving pedophilic activities between 1905 and 1914 with 26 young girls. The public scandal was nationwide. He fled to France in February 1918 and finally took refuge in Switzerland. Near the end of his life he lamented that his theory on the symbiotic origin of chloroplast "had made little headway" and that it was "often completely ignored." This was compounded by "finally the war, the revolution." After a particularly odious exchange with R. Chodat, Professor of Botany at the University of Geneva, who prevented Mereschkowsky from speaking, Mereschkowsky commented "One day history will remember me." On Sunday, January 9, 1921, having run out of money, and after paying all his hotel bills, he committed suicide by gassing himself with chloroform. Thus passed away at 65 years of age the most famous evolutionary botanist of the century. For a well written and researched biography of Mereschkowsky see Sapp et al. (2002).

## REFERENCES

- Keeling, P. J. (2004). Diversity and evolutionary history of plastids and their hosts. *Amer. J. Bot.* 91:1481–93.
- Martin, W., and Kowallik, K. V. (1999). Annotated English translation of Mereschkowsky's 1905 paper *Über Natur und Ursprung der Chromatophoren im Pflanzenreiche*. *Eur. J. Phycol.* 34:287–95.
- McFadden, G. I. (2001). Primary and secondary endosymbiosis and the origin of plastids. *J. Phycol.* 37:951–9.
- Mereschkowsky, C. (1905). *Über Natur und Ursprung der Chromatophoren im Pflanzenreiche*. *Biol. Zentralbl.* 25:593–604.
- Sapp, J., Carrapico, F., and Zolotonosov, M. (2002). Symbiogenesis: the hidden face of Constantin Merezhkowsky. *Hist. Phil. Life Sci.* 24:414–40.

# Glaucomphyta

The Glaucomphyta include those algae that have endosymbiotic cyanobacteria in the cytoplasm instead of chloroplasts. Because of the nature of their symbiotic association, they are thought to represent intermediates in the evolution of the chloroplast. The endosymbiotic theory of chloroplast evolution, first proposed by Mereschkowsky in 1905, is the one most widely accepted. According to this theory, a cyanobacterium was taken up by a phagocytic organism into a food vesicle. Normally the cyanobacterium would be digested by the flagellate, but by chance a mutation occurred, with the flagellate being unable to digest the cyanobacterium. This was probably a beneficial mutation because the cyanobacterium, by virtue of its lack of feedback inhibition, secreted considerable amounts of metabolites to the host flagellate. The flagellate in turn gave the cyanobacterium a protected environment, and the composite organism was probably able to live in an ecological niche where there were no photosynthetic organisms (i.e., a slightly acid body of water where free-living cyanobacteria do not grow; see Chapter 2). Pascher (1914) coined terms for this association; he called the endosymbiotic cyanobacteria **cyanelles**; the host, a **cyanome**; and the association between the two, a **syncyanosis**. In the original syncyanosis the cyanelle had a wall around it. Because the wall slowed the transfer of compounds from the cyanelle to the host and vice versa, any mutation that resulted in a loss of wall would have been beneficial and selected for in evolution. Most of the cyanelles in the

Glaucomphyta lack a wall and are surrounded by two membranes – the old food vesicle membrane of the cyanome and the plasma membrane of the cyanelle. As evolution progressed, these two membranes became the chloroplast envelope, the cyanome cytoplasm took over the formation of the storage product and the polyhedral bodies containing ribulose-1,5-bisphosphate carboxylase/oxygenase differentiated into the pyrenoid.

There are a number of similarities between cyanobacteria and chloroplasts that support the endosymbiotic theory: (1) they are about the same size; (2) they evolve oxygen in photosynthesis; (3) they have 70S ribosomes; (4) they contain circular prokaryotic DNA without basic proteins; (5) nucleotide sequencing of rRNA or of DNA encoding rRNAs have shown similarities; (6) they have chlorophyll *a* as the primary photosynthetic pigment.

The pigments of the Glaucomphyta are similar to those of the Cyanophyceae: both chlorophyll *a* and the phycobiliproteins are present; however, two of the cyanobacterial carotenoids, myxoxanthophyll and echinenone, are absent (Chapman, 1966).

Although similar to cyanobacteria, the cyanelles should be regarded as organelles rather than endosymbiotic cyanobacteria (Helmchen et al., 1995; McFadden, 2001). Cyanobacteria have over 3000 genes whereas cyanelles have about the same number of genes as plastids (about 200 genes). It is clear the cyanelles (and plastid) genomes have undergone substantial reduction

during endosymbiosis. Many of the missing genes eventually relocated to the nucleus, while other genes were lost – made redundant in the cyanelles' new role as an endosymbiont. For example, cyanobacteria have a respiratory electron-chain whereas plastids do not, the respiratory electron-chain is coded by the nucleus in eukaryotic algae.

The organisms in the Glaucophyta are very old; McFadden (2001) calls them the coelocanth of endosymbiosis. The Glaucophyta probably branched off the evolutionary tree before the divergence of red and green algae from one another (Keeling, 2004).

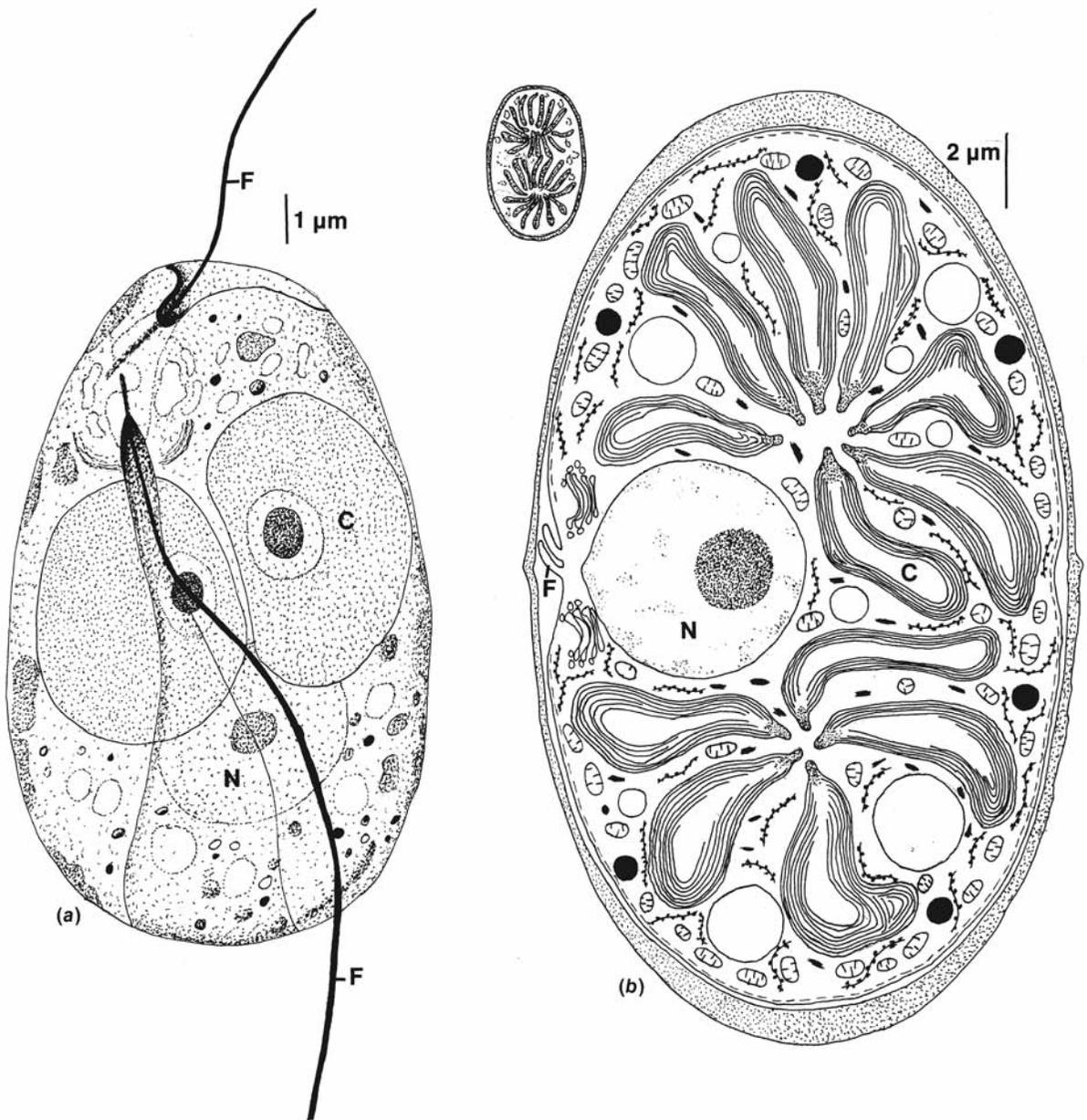
The fact that in such syncyanoses one is dealing with composite organisms that exhibit features altogether new and no longer characteristic of either partner alone, led Skuja in 1954 to establish the phylum Glaucophyta. It must be appreciated that the organisms in the phylum represent a very old group, and that, when evolving, they were very plastic and undergoing a great deal of change in the attempt to reach the relatively stable level of a cell with a chloroplast. Such a dynamic group was formed consisting of a large number of organisms not well suited to compete with their more highly developed progeny. Such a situation led to the demise of many of the original members of the Glaucophyta, resulting in the existence today of few extant members of the group.

*Cyanophora paradoxa* is a freshwater flagellate with two cyanelles in the protoplasm, each cyanelle with a central dense body (Fig. 3.1(a)). Nitrate reduction, photosynthesis, and respiration in the cyanelles of *Cyanophora paradoxa* are similar to the corresponding processes of chloroplasts and dissimilar to those of cyanobacteria (Floener and Bothe, 1982; Floener et al., 1982). This fact is cited as evidence that the cyanelles of *Cyanophora paradoxa* are close to chloroplasts in evolution. However, the cyanelles of *Cyanophora paradoxa* are primitive in regard to where ribulose-1,5-bisphosphate carboxylase/oxygenase is produced. Ribulose-1,5-bisphosphate carboxylase/oxygenase, the carbon dioxide-fixing

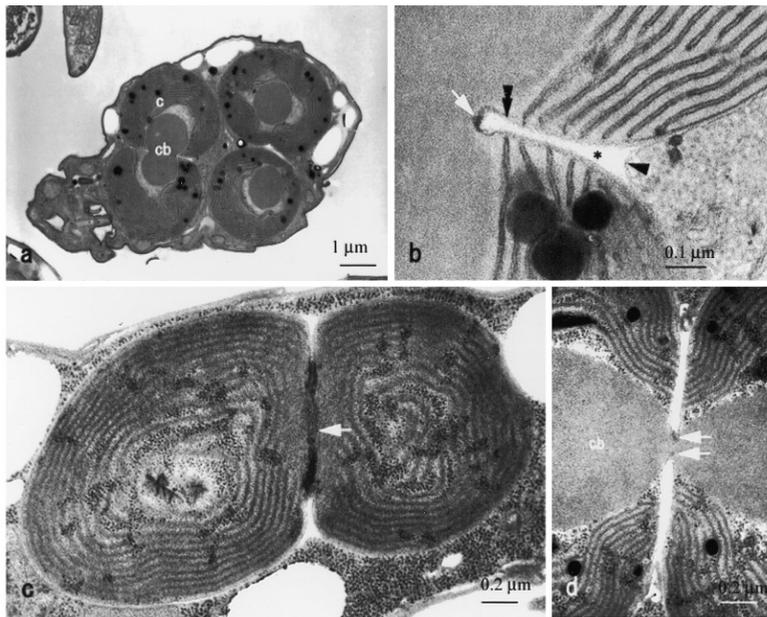
enzyme in photosynthesis, consists of 16 subunits, 8 large and 8 small. In higher plants the large subunits are encoded by DNA of the plastids, whereas the small subunits are encoded by nuclear DNA. In *Cyanophora paradoxa*, both sizes of subunits are encoded by cyanelle DNA. This non-cyanobacterial ribulose-1,5-bisphosphate carboxylase/oxygenase in cyanelles is now rationalized as lateral gene transfer or gene substitution from a mitochondrion or plastid (McFadden, 2001). The mechanism of division of cyanelles in *Cyanophora paradoxa* is intermediate between the division of cyanobacteria and that of plastids. Plastids have an inner and outer ring of electron-dense material in the area of the dividing organelle. In division of cyanelles of *Cyanophora paradoxa*, however, there is only an inner ring in the "stroma" inside the plasma membrane ("inner envelope") of the cyanelle. The outer ring, normally outside the outer chloroplast envelope, is missing (Fig. 3.2) (Iino and Hashimoto, 2003).

*Glaucocystis* is also a freshwater organism, found sparingly in soft-water lakes (lakes low in calcium). It has two groups of cyanelles, one on each side of the nucleus (Fig. 3.1(b)). The derivation of *Glaucocystis* from a biflagellate ancestor is evident from the two reduced flagella found inside the cell wall. Both of these organisms have starch formed in the cytoplasm, outside of the cyanelles, indicating that the host has accepted responsibility for the formation of the storage product.

There are other organisms that have endosymbiotic cyanobacteria that are not placed in the Glaucophyta because they represent evolutionary dead ends that did not lead to the evolution of chloroplasts. These organisms have cyanelles that still have a cell wall and are cytologically similar to cyanobacteria, such as the cyanelles of the fungus *Geosiphon* (Schnepf, 1964).



**Fig. 3.1** (a) *Cyanophora paradoxa* with two cyanelles (C), nucleus (N), and flagella (F). (b) Semidiagrammatic drawing of a cell of *Glaucocystis nostochinearum* showing two groups of cyanelles (C), reduced flagella (F), and a nucleus (N). ((a) after Mignot et al., 1969; (b) after Schnepf et al., 1966.)



**Fig. 3.2** Transmission electron micrographs of sections of dividing cells of *Cyanophora paradoxa*. (a) whole cell. (c) Cyanelle; (cb) central body. (b) A cross section of a division site with a septum (asterisk) dividing the inner envelope (double arrowhead) and the outer envelope (arrowhead). A cross section of an electron-dense cyanelle ring is seen at the leading edge of the inner envelope (arrow). (c) A moderately constricting cyanelle with a cyanelle ring (arrow) observed in a tangential section. (d) A deeply constricting division site. At the constricting neck, a pair of cross sections of a cyanelle ring is seen (arrow). (From Iino and Hashimoto, 2003.)

## REFERENCES

- Chapman, D. J. (1966). Pigments of the symbiotic algae (cyanomes) of *Cyanophora paradoxa* and *Glaucocystis nostochinearum* and two Rhodophyceae, *Porphyridium aeruginosa* and *Asterocystis ramosa*. *Arch. Mikrobiol.* 55:17–25.
- Floener, L., and Bothe, H. (1982). Metabolic activities in *Cyanophora paradoxa* and its cyanelles. II. Photosynthesis and respiration. *Planta* 156:78–83.
- Floener, L., Danneberg, G., and Bothe, H. (1982). Metabolic activities in *Cyanophora paradoxa* and its cyanelles. I. The enzymes of assimilatory nitrate reduction. *Planta* 156:70–7.
- Helmchen, T. A., Bhattacharya, D., and Melkonian, M. (1995). Analysis of ribosomal RNA sequences from glaucocystophyte organelles provide new insights into the evolutionary relationships of plastids. *J. Mol. Evol.* 41:203–10.
- Iino, M., and Hashimoto, H. (2003). Intermediate features of cyanelle division of *Cyanophora paradoxa* (Glaucocystophyta) between cyanobacterial and plastid division. *J. Phycol.* 39:561–9.
- Keeling, P. J. (2004). Diversity and evolutionary history of plastids and their hosts. *Amer. J. Bot.* 91:1481–93.
- McFadden, G. I. (2001). Primary and secondary endosymbiosis and the origin of plastids. *J. Phycol.* 37:951–9.
- Mereschkowsky, C. (1905). Ueber Natur und Ursprung den Chromatophoren in Pflanzenreich. *Biol. Zentralbl.* 25:593–604.
- Mignot, J. P., Joyon, L., and Pringsheim, E. G. (1969). Quelques particularités structurales de *Cyanophora paradoxa* Korsch., protozoaire flagellé. *J. Protozool.* 16:138–45.
- Pascher, A. (1914). Über Symbiosen von Spaltpilzen und Flagellaten. *Ber. Dtsch. Bot. Ges.* 32:339–52.
- Schnepf, E. (1964). Zur Feinstruktur von *Geosiphon pyriforme*. Ein Versuch zur Deutung cytoplasmatischer Membranen und Kompartimente. *Arch. Mikrobiol.* 49:112–31.
- Schnepf, E., Koch, W., and Deichgräber, G. (1966). Zur Cytologie und taxonomischen Einordnung von *Glaucocystis*. *Arch. Mikrobiol.* 55:149–74.
- Skuja, H. (1954). Glaucophyta. In *Syllabus der Pflanzenfamilien*, by A. Engler, ed. H. Melchoir, and E. Werdermann, Vol. 1, pp. 56–7. Berlin: Borntraeger.

# Rhodophyta

## RHODOPHYCEAE

The Rhodophyceae, or **red algae**, comprise the only class in the division Rhodophyta. The Rhodophyceae are probably one of the oldest groups of eukaryotic algae. The red algae are most likely directly descended from a cyanome in the Glaucophyta (see Chapter 3). It is likely that the first red alga evolved into an ecological niche that was unoccupied by cyanobacteria, the only extant photosynthetic alga that evolved oxygen. This ecological niche would have been in waters with a pH less than 5, which, for some unknown reason, cyanobacteria are not able to inhabit (Brock, 1973). Indeed, modern phylogenetic studies utilizing nucleic-acid sequencing have shown that *Cyanidium*, an alga that lives in acidic waters, is probably the oldest extant red alga (Oliveira and Bhattacharya, 2000).

The Rhodophyceae lack flagellated cells, have chlorophyll *a*, phycobiliproteins, floridean starch as a storage product, and thylakoids occurring singly in the chloroplast.

A majority of the seaweeds are red algae, and there are more Rhodophyceae (about 4000 species) than all of the other major seaweed groups combined. Although marine red algae occur at all latitudes, there is a marked shift in their abundance from the equator to colder seas. There are few species in polar and subpolar regions where brown and green algae predominate, but in temperate and tropical regions they far outnumber these groups. The average size of the plants also

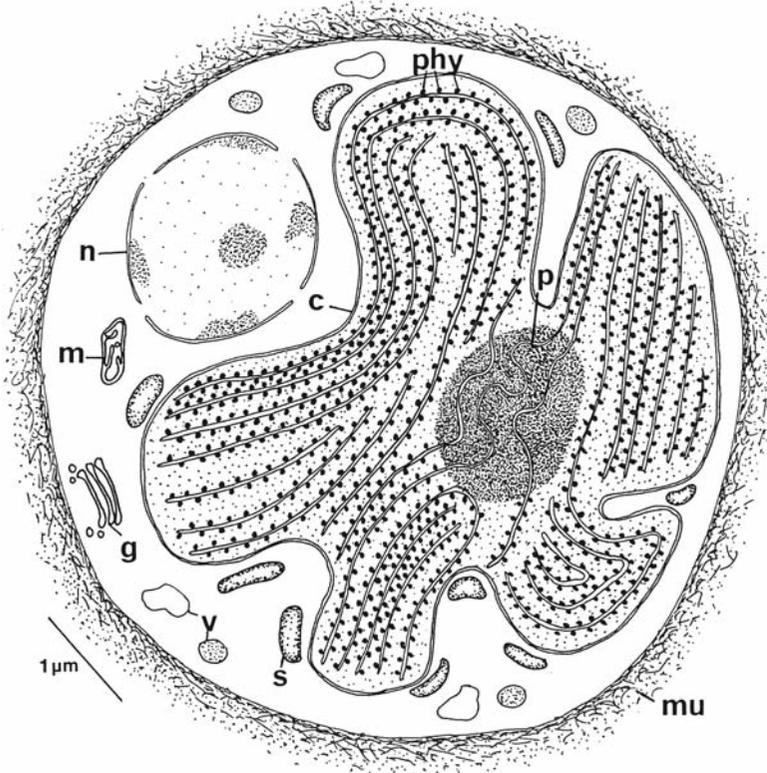
differs according to geographical region. The larger species of fleshy red algae occur in cool-temperate areas, whereas in tropical seas the Rhodophyceae (except for massive calcareous forms) are mostly small, filamentous plants. The Rhodophyceae also have the ability to live at greater depths in the ocean than do members of the other algal classes. They live at depths as great as 200 m, an ability related to the function of their accessory pigments in photosynthesis. About 200 species of Rhodophyceae are found in freshwater, where they do not reach as great a size as the red seaweeds (Skuja, 1938). The majority of freshwater red algae occur in running waters of small to mid-sized streams (Sheath and Hambrook, 1988). Few red algae occur at currents of less than  $30 \text{ cm s}^{-1}$ . This fast flow probably favors red algae because loosely attached competitors are washed out and because of a constant replenishment of nutrients and gases.

## Cell structure

The major features of a red algal cell (Fig. 4.1) are a chloroplast with one thylakoid per band and no chloroplast E.R., floridean starch grains in the cytoplasm outside the chloroplast, no flagella, pit connections between cells in filamentous genera, and a eukaryotic type of nucleus (Scott et al., 1980).

## Cell walls

Cellulose forms the microfibrillar framework in most rhodophycean cell walls, although in the



**Fig. 4.1** Semidiagrammatic drawing of a cell of *Porphyridium cruentum*. (c) Chloroplast; (g) Golgi; (m) mitochondrion; (mu) mucilage; (n) nucleus; (p) pyrenoid; (phy) phycobilisomes; (s) starch; (v) vesicle. (Adapted from Gantt and Conti, 1965.)

haploid phase of the Bangiales (*Bangia* and *Porphyra*) a  $\beta$ -1,3 linked xylan (polysaccharide composed of xylose residues) performs this function (Frei and Preston, 1964). Unicellular red algae have an amorphous matrix of sulfated polysaccharides without cellulose surrounding the cells (Arad et al., 1993). The amorphous polysaccharides or mucilages occur between the cellulose microfibrils in the rest of the red algae. The two largest groups of amorphous mucilages are the **agars** (Fig. 1.11) and the **carrageenans** (Fig. 4.15). These mucilages may constitute up to 70% of the dry weight of the cell wall. Cuticles, composed mostly of protein, can occur outside the cell wall (Craigie et al., 1992).

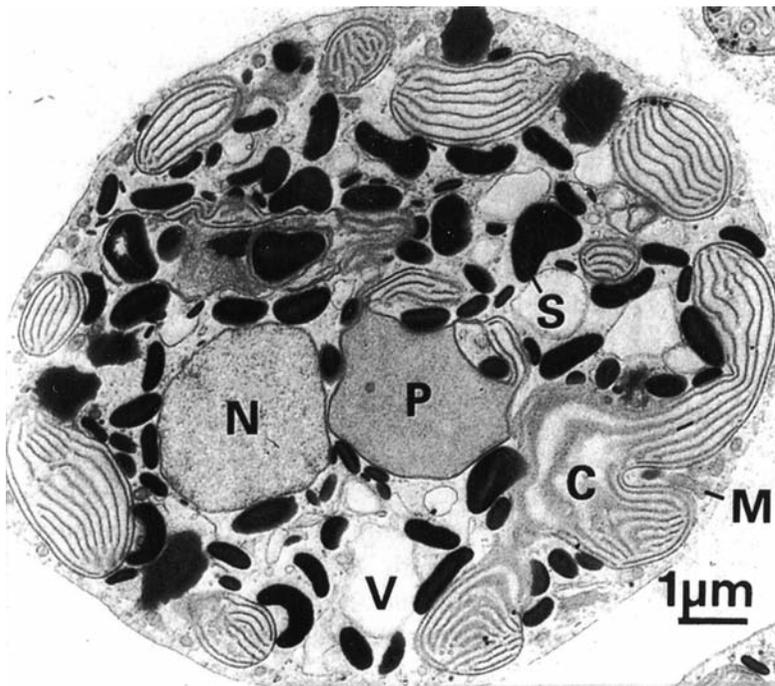
### Chloroplasts and storage products

Chloroplasts are usually stellate with a central pyrenoid in the morphologically simple Rhodophyceae (Fig. 4.1), whereas in the remainder of the Rhodophyceae they are commonly discoid (Fig. 4.3). In the Rhodophyceae with apical growth, the chloroplasts usually originate from small colorless proplastids with few thylakoids in the apical

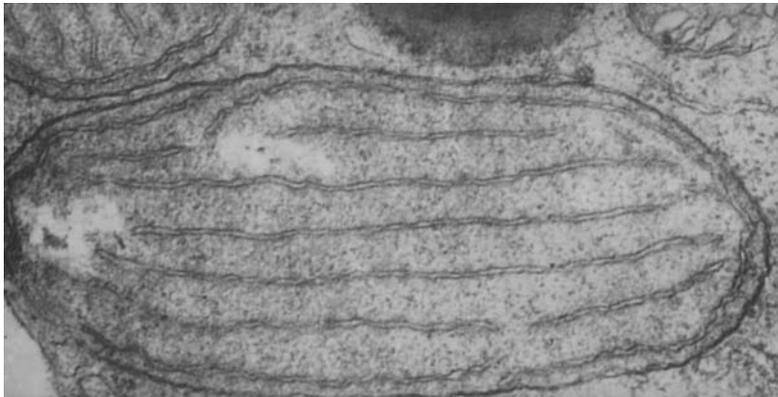
cell (Lichtlé and Giraud, 1969). Chloroplasts are surrounded by the two membranes of chloroplast envelope with no chloroplast endoplasmic reticulum present (Figs. 4.1, 4.2, 4.3). Thylakoids occur singly inside the chloroplasts. The phycobilin pigments are localized into phycobilisomes on the surface of the thylakoids, a situation similar to that in the cyanobacteria.

Chlorophyll *a* is in the chloroplasts. There have been erroneous reports of chlorophyll *d* occurring in the chloroplast. It has been shown that the chlorophyll *d* in these studies came from the cyanobacterium *Acaryochloris marina*, an epiphyte on red algae (Murakami et al., 2004).

The phycobiliproteins include R-phycoerythrin, allophycoerythrin, and three forms of phycoerythrin, the phycoerythrins being present in the greatest amount, giving the algae their pinkish color. B-phycoerythrin is present in the more primitive red algae and has been found in *Porphyridium* (Fig. 4.1), *Rhodosorus* (Fig. 4.24 (a)), *Rhodochorton* (Fig. 4.31) and *Smithora*. R-phycoerythrin occurs in most higher red algae, and C-phycoerythrin occurs in



**Fig. 4.2** Transmission electron micrograph of a section of a cell of *Rhodella violacea*. (C) Chloroplast; (M) mitochondrion; (N) nucleus; (P) pyrenoid; (S) starch; (V) vacuole. (From Marquardt et al., 1999.)

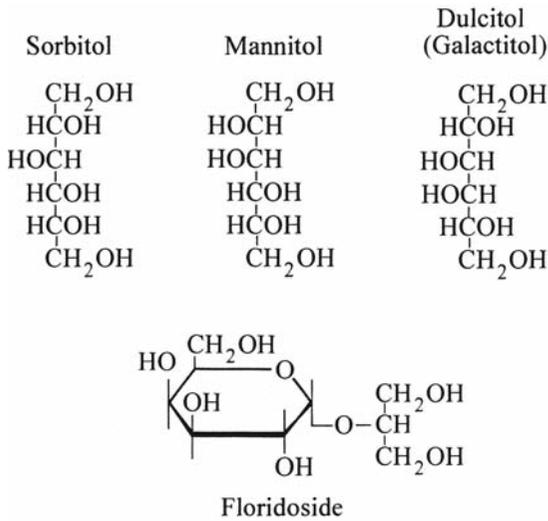


**Fig. 4.3** Chloroplast in a carpospore of *Polysiphonia*. (From Tripodi, 1974.)

*Porphyridium* (Fig. 4.1), *Porphyra* (Fig. 4.27), and *Polysiphonia* (Figs. 4.44, 4.45). The phycobiliproteins are in phycobilisomes on the surface of thylakoids (Fig. 4.1). The phycobilisomes are spherical if both phycoerythrin and phycocyanin are present. The phycobilisomes are discoid if only phycocyanin is present (Gantt, 1969).

**Complementary chromatic adaptation** occurs in the red algae. Orange and red light stimulate the production of long-wavelength absorbing phycocyanin, while green light stimulates the formation of short-wavelength absorbing phycoerythrin (Sagert and Schubert, 1995). The color will vary.

**Floridoside** (O- $\alpha$ -D-galactopyranosyl-(1,2)-glycerol) is the major product of photosynthesis in the red algae, although mannitol, sorbitol, digeneaside, and dulcitol also occur (Fig. 4.4) (Barrow et al., 1995; Karsten et al., 2003). The concentration of floridoside increases in red algal cells as the salinity of the medium increases (Reed, 1985). This change in floridoside concentration is thought to compensate, at least in part, for the changes in external osmolarity, thereby preventing water from leaving the algal cells as the salinity increases. The levels of floridoside can be as high as 10% of the tissue dry-weight in some



**Fig. 4.4** Chemical structure of low molecular polysaccharides that occur in the red algae.

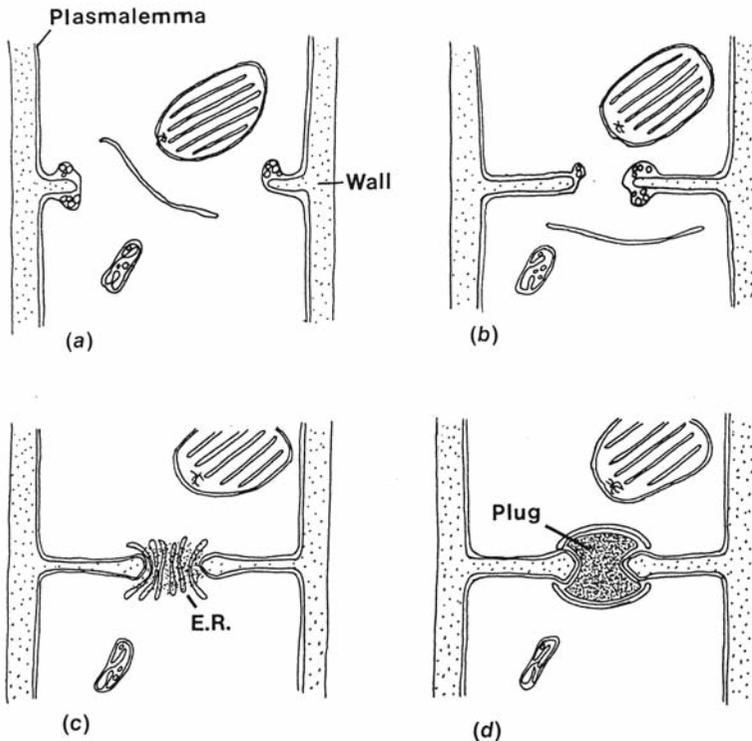
marine red-algal thalli. The first observable product of photosynthesis is phosphoglyceric acid, as is the case in higher plants. Floridoside appears after 30 seconds of illumination and after 2 hours floridoside is the major product of

photosynthesis. Floridoside apparently has the same function as sucrose, the common product of photosynthesis in green algae and higher plants.

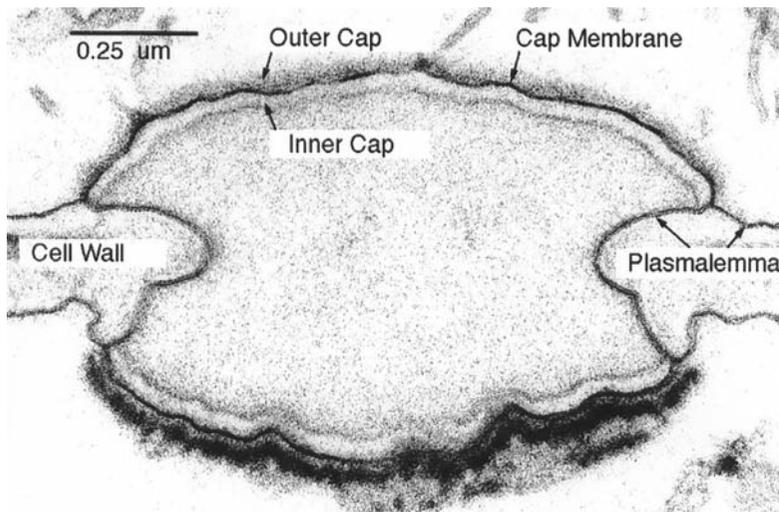
Floridean starch (Fig. 1.28) is the long-term storage product, occurring as grains in the cytoplasm outside of the chloroplast (Fig. 4.1). Floridean starch is similar to the amylopectin of higher plants, staining red-violet with iodine. In the more primitive Rhodophyceae the starch grains are clustered as a sheath around the pyrenoid of the chloroplast, whereas in the more advanced Rhodophyceae the starch grains are scattered in the cytoplasm (Hara, 1971; Lee, 1974).

### Pit connections

**Pit connections** occur between the cells in all of the orders except the Porphyridiales, and the haploid phase of the Bangiales. It has been pointed out that the term "pit connection" is inappropriate because the structure is neither a "pit" nor a "connection"; however, because the term has been used for so long, it is probably best to retain it. A pit connection consists of a proteinaceous plug core in between two thallus cells (Figs. 4.5, 4.6).



**Fig. 4.5** Semidiagrammatic drawing of the formation of a pit connection in a red alga. (a) The cross wall begins to furrow inward with the wall precursors found in vesicles derived from the cytoplasm; (b) the cross wall septum is complete, leaving an opening (aperture) in the center; (c) endoplasmic reticulum lies across the opening in the wall, and electron-dense material condenses in this area; (d) the pit connection is formed, consisting of a plug with the plasmalemma continuous from cell to cell. (Adapted from Ramus, 1969, 1971; Lee, 1971.)



**Fig. 4.6** A pit connection between cells of *Palmaria mollis*. The plasma membrane is continuous from cell to cell. The cap membrane is continuous with the plasma membrane. The inner and outer cap layers are on each side of the cap membrane. (From Pueschel, 1987.)

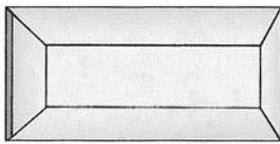
Cap membranes separate the plug core from the adjacent cytoplasm. The cap membrane is continuous with the plasma membrane, which in turn is continuous from one cell to the next. On the inside of the cap membrane can be an inner layer, while on the outside of the cap membrane can be an outer cap layer (Pueschel, 1987). The structure of the pit connection can vary. The more primitive red algae, such as *Rhodochaete* and *Compsopogon*, lack cap membranes and cap layers, with only a plug core present. It has been postulated that this represents the ancestral condition (Pueschel, 1989).

There are two types of pit connections. **Primary pit connections** are formed between two cells during cell division. **Secondary pit connections** result when two cells fuse. Both types of pit connections have the same structure (Kugrens and West, 1973). **Primary pit connections** are formed as follows (Fig. 4.5) (Ramus, 1969): soon after nuclear division, the cross wall grows inward from the lateral wall. When the cross wall is complete, there remains a hole (aperture) in the center through which the protoplasm of the two cells is continuous. A number of parallel vesicles traverse the hole, with electron-dense material condensing around the vesicles. Eventually the vesicles disappear, and the electron-dense material fills the hole. A membrane is formed around this material, producing a plug in the hole. The pit connection has been reported to contain proteins and polysaccharides (Pueschel

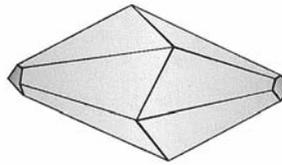
and Trick, 1991; Ramus, 1971). The pit connection may function as a site of structural strength on the thallus (Kugrens and West, 1973). In some algae the plugs of the pit connections become dislodged from between the cells of a developing gonimoblast, leaving the protoplasm continuous between the cells and allowing the passage of metabolites to the developing reproductive cells (Turner and Evans, 1978).

## Calcification

All members of the Corallinales and some of the Nemaliales (*Liagora* (Fig. 4.17 (a), (b)), *Galaxaura* (Fig. 4.34)) deposit  $\text{CaCO}_3$  extracellularly in the cell walls. Anhydrous calcium carbonate occurs in two crystalline forms, **calcite** (rhombohedral) and **aragonite** (orthorhombic) (Fig. 4.7). The two forms differ markedly in specific gravity, hardness, and solubility. The Corallinales deposit  $\text{CaCO}_3$  primarily as calcite, whereas the calcified members of the Nemaliales deposit  $\text{CaCO}_3$  primarily as aragonite. In *Liagora* (Fig. 4.17(a), (b)) (Nemaliales), the aragonite occurs as needle-like crystals in the wall, whereas in the Corallinales, the calcite occurs as massive deposits (Borowitzka et al., 1974). Calcified walls of living cells probably have a mucilaginous component that slows the loss of  $\text{Ca}^{2+}$  into the medium (Pearse, 1972). If a calcified thallus is killed, the dispersal of the calcified wall is greatly accelerated.



Aragonite



Calcite

**Fig. 4.7** The crystal structure of aragonite and calcite.

**Rhodoliths** are unattached biogenic (produced from living organisms) nodules composed at least partly of calcified red algae. A rhodolith begins as a central nucleus composed of a pebble or fragment of coral. Non-articulated coralline algae attach to the nucleus and grow. The shape of the rhodolith is determined by its environment, often being generally spherical because of frequent overturning due to water motion. Rhodoliths can reach 30 cm in diameter and be 500–800 years old. Sections of rhodoliths that reveal the banding of the coralline red algae can be used to determine the environment at the time of wall deposition (Halfar et al., 2000).

Skeletons of coralline algae are formed with little biological control, by impregnation of cell walls with magnesium and calcium at a ratio similar to the Mg/Ca in the water. Therefore, the Mg/Ca ratio in the cell walls reflects the Mg/Ca ratio in the water. Analysis of the Mg/Ca ratio in cell walls of fossil coralline red algae since the beginning of the Paleozoic Era have shown that there have been times of “aragonite seas” with relatively high Mg in seawater, resulting in coralline algae with cells walls contain high-Mg calcite and aragonite, and times of “calcite seas” with relatively low-Mg seawater, resulting in coralline algae with low-Mg calcite (Fig. 4.8) (Stanley et al., 2002). The differences in the Mg/Ca ratios in seawater are due to changes in the mid-ocean spreading rates.

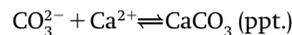
The coralline algae thrive in rock pools and on rocky shores exposed to very strong wave action and swift tidal currents. The red algae that have the highest rates of calcification also have the highest rates of photosynthesis and are usually found in waters less than 20 m deep (Goreau, 1963). Calcification of the thallus occurs about two to three times more rapidly in the light than in the dark, although significant calcification does occur in the dark (Okazaki et al., 1970). The above observations have led to the theory that

calcification may be linked to photosynthesis (Pearse, 1972). The most quoted theory on calcification is that calcium salts are precipitated from seawater by the alkalinity brought about by the extraction of carbon dioxide during photosynthesis, calcium carbonate being less soluble in alkaline waters than acid. The obvious and often mentioned drawback to this theory is that because all algae carry out photosynthesis, it is difficult to understand why they do not all calcify. Also the continued calcification of corallines in the dark is another argument against this theory.

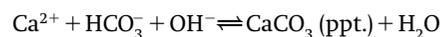
Seawater is more or less saturated with respect to calcium carbonate, and the addition of either calcium or carbonate will cause the carbonate to precipitate. The concentration of  $\text{CO}_3^{2-}$  is related through a complex series of equilibria (Digby, 1977a,b):



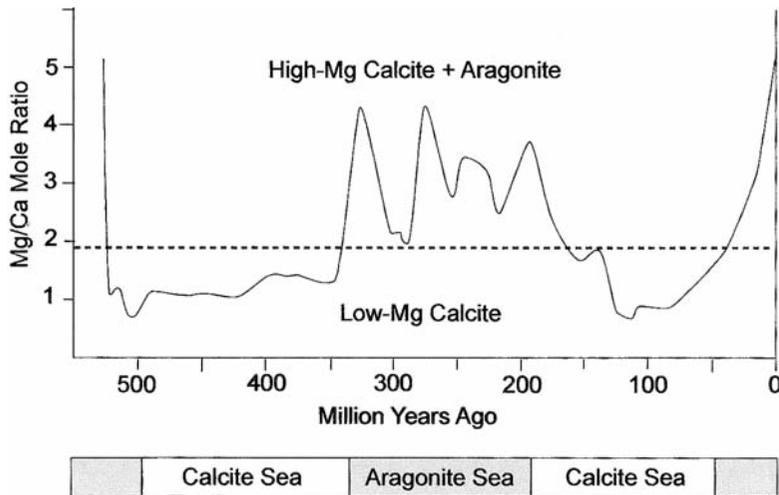
then



The addition of acid will drive the reactions to the left and cause carbonate to dissolve, whereas the addition of base will drive the reactions to the right and form more carbonate. At the pH of seawater (8.4), almost all of the  $\text{CO}_2$  in the water is in the form of bicarbonate ion,  $\text{HCO}_3^-$ . The addition of one equivalent of hydroxyl ions to seawater saturated with respect to calcium carbonate will precipitate one equivalent of calcium carbonate:

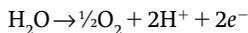


The fact that seawater is nearly saturated with calcium carbonate was demonstrated with seawater from the coast of Maine by Digby (1977a). By raising the pH of this seawater to 9.6, he caused precipitation of carbonates. Calcium carbonate precipitated first, being less soluble, followed by carbonate richer in magnesium.

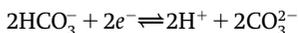


**Fig. 4.8** Effects of the Mg/Ca ratio of seawater on the mineralogy of carbonate deposition in coralline red algae. High Mg/Ca ratio in seawater results in the deposition of aragonite and calcite high in Mg, resulting in "aragonite seas." Low Mg/Ca ratio in seawater results in the deposition of calcite low in Mg and "calcite seas." (Modified from Stanley et al., 2002.)

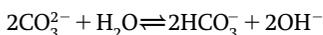
Digby (1977b) proposed a theory of calcification of red algae based on raising the pH of the seawater immediately outside the cells, causing precipitation of carbonates as outlined above. The first process is the normal photosynthetic splitting of water:



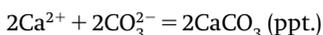
The oxygen then diffuses out of the cell. As mentioned above, in the sea most of the carbon dioxide is in the form of bicarbonate ions; these ions diffuse into the cells and receive the electrons freed initially by photosynthesis. The bicarbonate ions are then converted into carbonate ions and hydrogen according to the following reaction:



The carbonate ions diffuse out of the cell where they partially dissociate, forming bicarbonate and hydroxyl ions and thereby raising the pH:



When saturation with regard to calcium and carbonate is reached by a rise in pH outside the cells, calcium carbonate precipitates on the walls:



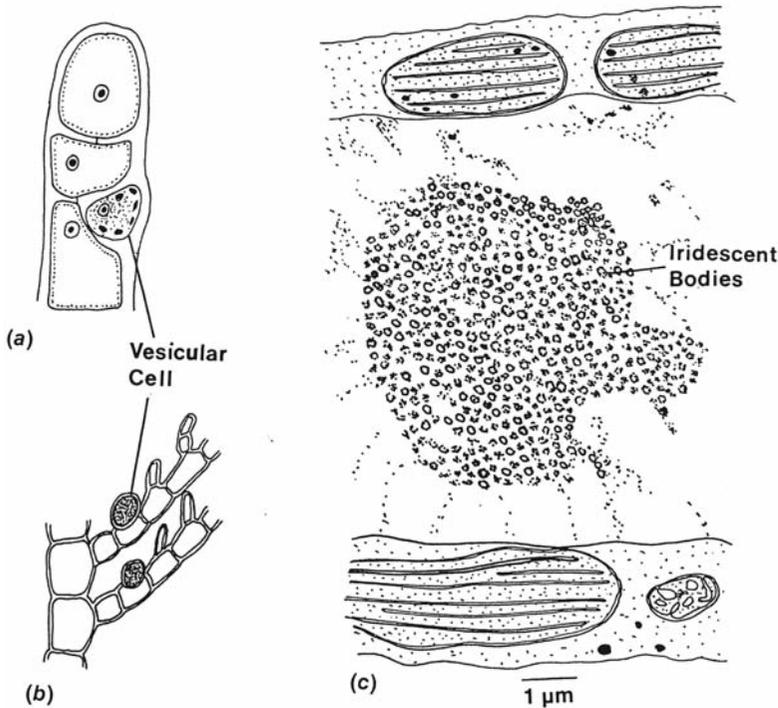
Continued precipitation of  $\text{CaCO}_3$  results in the calcified wall of the Rhodophyceae. Although the above theory explains the mechanism of calcification, it does not explain why calcification is specific to certain red algae.

It has been theorized that calcification of red algal thalli evolved as a protection against grazing by organisms such as limpets, although it has also been pointed out that grazing is beneficial to the coralline algae in that the grazers remove epiphytes from the red algal thallus (Pueschel and Miller, 1996).

## Secretory cells

Secretory cells (vesicular cells) occur in some Rhodophyceae (Fig. 4.9(a), (b)). These cells are colorless at maturity and commonly have a large central vacuole. The secretory cells in *Bonnemaisonia* are prominent and associated with high concentrations of iodine (Fig. 4.9(a)). The concentration of iodine can be high enough to produce a blue color in herbarium paper with starch as a filler (the chemical test for starch). Secretory cells are vestigial, lacking the large vacuole with its refractile contents, when these algae are grown in a medium without bromine (Wolk, 1968). Bromine can also occur as granular deposits in mucilage, such as in the thallus medulla of *Thysanocladia densa* (Pallaghy et al., 1983) or in the cuticle of *Polysiphonia nigrescens* (Pedersen et al., 1981).

Other types of secretory cells not associated with the accumulation of halogens occur. The cells are often called secretory cells, even though they are apparently not involved in secretion. In *Antithamnon* (Figs. 4.9(b), 4.10(a)), these cells



**Fig. 4.9** (a) Vesicular cell of the *Trilliella* stage of *Bonnemaisonia*. (b) Vesicular cells of *Antithamnion plumula*. (c) Iridescent bodies in the vacuole of a cell of *Chondria caerulea*. ((a) after Kylin, 1956; (b) after Kylin, 1930; (c) adapted from Feldmann, 1970a.)

have a large central vacuole containing sulfated acidic polysaccharide (Young and West, 1979). In *Opuntia californica*, there are “gland cells” with a large vacuole containing a homogeneous proteinaceous material (Young, 1979) (Fig. 4.10(b)). These “secretory cells” and “gland cells” may have compounds that act as deterrents to grazing, or they may accumulate special reserves for metabolic use.

## Iridescence

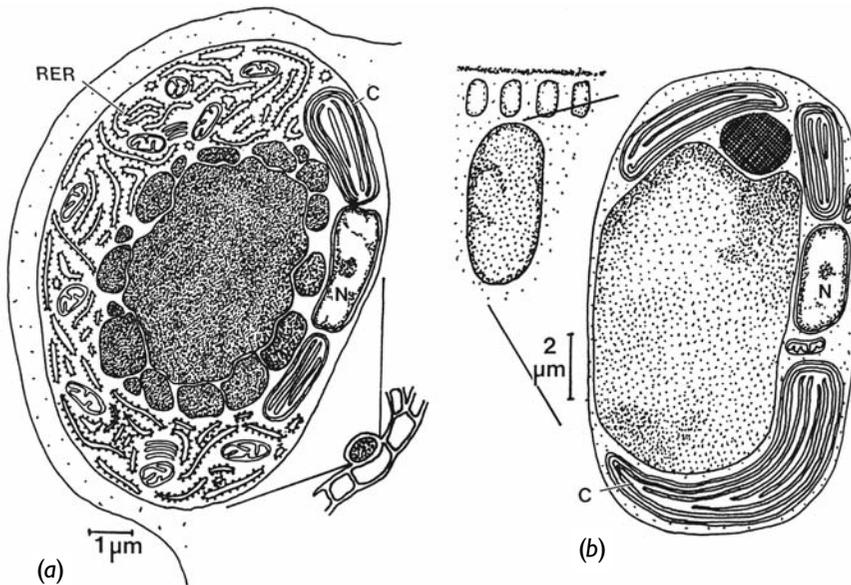
The thalli of some Rhodophyceae show a marked blue or green iridescence when observed in reflected light. **Iridescence** is solely a physical interference and is not related to any light-producing phenomena such as phosphorescence or bioluminescence (Gerwick and Lang, 1977). It results from the interference of light waves reflected from the surfaces of very thin multiple laminations separated by equally thin or thinner layers of material with a contrasting refractive index; the layers are uniform and produced by periodic secretion and deposition. Iridescence in the Rhodophyceae has been attributed to

different causes by different investigators. Feldmann (1970a,b) found iridescent bodies in *Chondria* (Fig. 4.9(c)) and *Gastroclonium*, whereas Gerwick and Lang (1977) attributed the iridescence in *Iridaea* to a multilayer cuticle.

## Epiphytes and parasites

Rhodophycean organisms range from autotrophic, independent plants to complete heterotrophic parasites. The spectrum includes non-obligate epiphytes (in the *Acrochaetium*–*Rhodochorton* complex), obligate epiphytes (*Polysiphonia lanosa* on *Ascophyllum* (Fig. 4.11)), semi parasites that have some photosynthetic pigments (*Choreocolax* (Fig. 4.13), *Gonimophyllum*), and parasites with no coloration (*Harveyella*, *Holmsella*).

The association between the obligate epiphyte red alga *P. lanosa* and its brown alga host *Ascophyllum* has been well studied. After the spore of *P. lanosa* germinates on the host, the red alga sends down a rhizoid that digests its way into the host tissue by means of enzymatic digestion of the host tissues. The enzymes are discharged from vesicles at the tip of the rhizoid. Once the rhizoid has

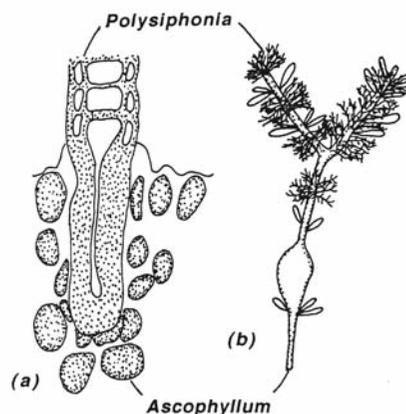


**Fig. 4.10** (a) Semidiagrammatic drawing of the fine structure of a vesicular cell of *Antithamnion*. The cell has a large central vacuole surrounded by protoplasm containing rough endoplasmic reticulum (RER), mitochondria, chloroplasts (C), and a nucleus (N). (b) Semidiagrammatic drawing of the fine structure of a gland cell of *Opuntiella californica*. The cell has a large central vacuole containing chloroplasts (C), a nucleus (N), and mitochondria. ((a) after Young and West, 1979; (b) after Young, 1979.)

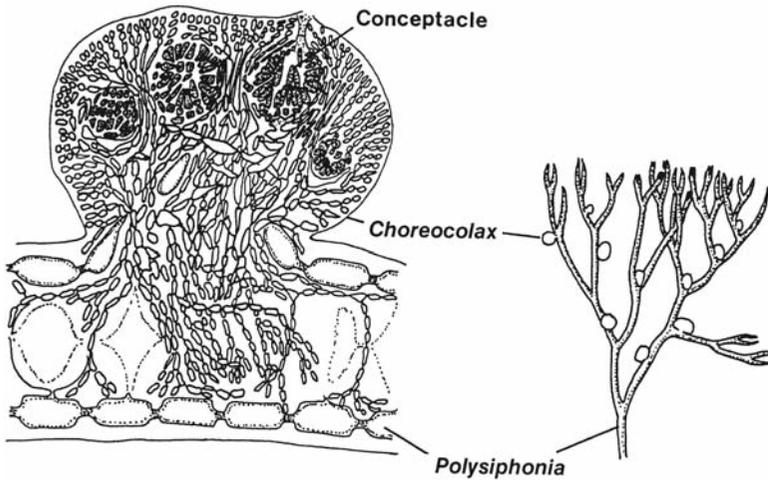
established itself, intrusive cells form the basal parietal cells of the thallus (Fig. 4.11) (Rawlence, 1972). Although *P. lanosa* is an obligate epiphyte, there is no transfer of metabolites from the host to the epiphyte, the epiphyte manufacturing all of its own requirements through photosynthesis (Harlin and Craigie, 1975; Turner and Evans, 1978).

Parasitic red algae can be either adelphoparasites (*adelpho* = brother) or alloparasites (*allo* = other). **Adelphoparasites** are closely related to, or belong to the same family as their hosts and constitute 90% of parasitic red algae (Goff et al., 1996). **Alloparasites** are not closely related to their hosts. The parasitic habit apparently has been adapted more easily when the host is closely related to the parasite (adelphoparasites) than when it is not (alloparasites), partially because it is easier for the parasite to establish secondary pit connections with the host (and therefore transfer nutrients) if the host and parasite are related.

*Choreocolax polysiphoniae* is an example of a rhodophycean parasite (Fig. 4.12). The alga is a complete parasite and is interesting in that it is parasitic on *Polysiphonia fastigata*, which is itself epiphytic on *Ascophyllum* (Fig. 4.11). Because *Choreocolax* is in the Gigartinales, and *Polysiphonia* is in the Ceramiales, this is a case of alloparasitism. *Choreocolax* consists of a more or less hemispherical white external portion made up of subdichotomously branched filaments enclosed and surrounded by gelatinous matter, and a mass of haustorial cells growing inside the host (Sturch,



**Fig. 4.11** (a) The rhizoid of *Polysiphonia lanosa* penetrating tissue of *Ascophyllum nodosum*. (b) *Polysiphonia* epiphytic on *Ascophyllum*. ((a) after Rawlence, 1972.)



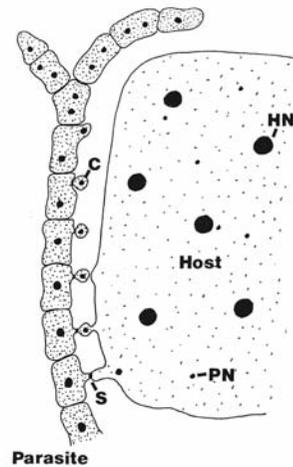
**Fig. 4.12** Drawing of a section of *Choreocolax polysiphoniae* on *Polysiphonia*. The parasitic *Choreocolax* has carposporophytes in various stages of development. (After Sturch, 1926.)

1926). When the haustorial cells have reached a certain distance from the original point of infection, they frequently give rise to a second external cushion. Secondary pit connections are established between the haustorial internal filaments of the parasite and the larger cells of the host. Apically dividing filaments of the parasite *Choreocolax* cells produce small conjuctor cells (Fig. 4.13) by the asymmetrical division of a cell. A conjuctor cell contains a highly condensed, small nucleus. The conjuctor cell fuses with an adjacent host *Polysiphonia* cell. The nucleus and cytoplasm of the conjuctor cell are incorporated into the host cell. The pit connection that originally connected the conjuctor cell and the sister *Choreocolax* cell now connects the host *Polysiphonia* cell and the parasite. This is often called a “secondary pit connection,” even though it is actually an ordinary pit connection.” Up to several hundred *Choreocolax* cells can fuse with a single host cell. The infected host *Polysiphonia* cell enlarges and the vacuole decreases in size with a concomitant increase in cytoplasmic contents (Goff and Coleman, 1984). The chloroplasts become scattered throughout the cytoplasm, instead of lying in the peripheral cytoplasm as they do in the non-infected cells. Although the parasite cells are colorless, they do contain very reduced plastids (Kugrens and West, 1973). In the association between the colorless parasite *Holmsella pachyderma* and the host *Gracilaria verrucosa*, both of which are Rhodophyceae, the main product of photosynthesis, floridoside, is transferred from

the host to the parasite where it is accumulated as floridoside, mannitol, and starch (Fig. 4.4) (Evans et al., 1973).

## Defense mechanisms of the red algae

Benthic marine red seaweeds are particularly susceptible to being overgrown by epiphytes because

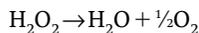


**Fig. 4.13** A drawing illustrating the progressive formation of conjuctor cells by a filament of the parasitic red alga *Choreocolax* living on a host *Polysiphonia* cell, leaving the pit connection of the conjuctor cell as a secondary pit connection. (C) Conjuctor cell; (HN) *Polysiphonia* host nucleus; (PN) *Choreocolax* parasite nucleus introduced into the host cell after fusion with the conjuctor cell of the parasite; (S) secondary pit connection. (After Goff and Coleman, 1984.)

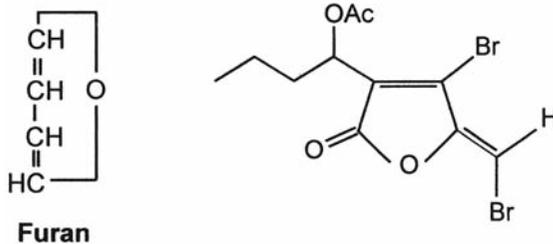
they are sessile and are restricted to the photic zone where conditions for fouling organisms are optimal. Epiphytes can significantly harm seaweeds by reducing the light, resulting in decreased photosynthesis and growth, by increasing drag and hence their susceptibility to breakage or being torn from the substrate, and by decreasing the reproductive output of the host.

Some red seaweeds secrete compounds that kill or retard the growth of epiphytes growing on them. *Delisea* secretes halogenated furones (Fig. 4.14) that affect the growth of epiphytes and keep the thallus clean (de Nys et al., 1995).

*Gracilaria conferta* (Fig. 4.42) has a defense mechanism that limits bacterial infection of the red alga. Invasive bacteria secrete agarases that break down the cell-wall agar of *Gracilaria* into shorter neoagarosehexaose oligosaccharides. *Gracilaria* cells respond to nanomolar concentrations of the oligosaccharides by increasing respiration and producing active oxygen species such as hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radicals ( $OH^-$ ) (Potin et al., 1999). The hydrogen peroxide is degraded to water and molecular oxygen:



Molecular oxygen is toxic and results in the elimination of 90% of the epiphytes within 15 minutes under experimental conditions (Weinberger and Friedlander, 2000). *Gracilaria conferta* has also been shown to release activated oxygen each morning after exposure to light. This short time release of activated oxygen, in addition to the defense related release of hydrogen peroxide,



**Fig. 4.14** Left: The chemical structure of furan, the basic building block of the furanones. Right: One of the halogenated furanones released by the red alga *Delisea pulchra*. Halogenated furanones inhibit the growth of epiphytes on this red alga.

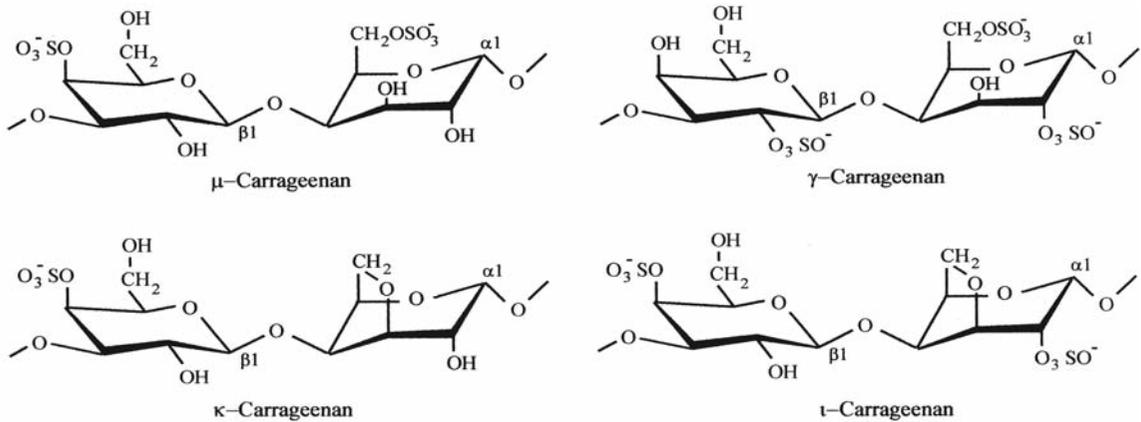
resulted in the selection of epiphytic bacteria that are relatively insensitive to these chemicals. *Corynebacterium-Arthrobacter* 1 bacteria were relatively resistant to hydrogen peroxide whereas *Vibrio* 1 and *Flavobacterium* 7 bacteria were sensitive (Bouarab et al., 1999; Potin, et al., 1999).

**Volatile halocarbons**, consisting of brominated, chlorinated or iodinated hydrocarbons, are also produced on exposure of *Gracilaria* cells to the oligosaccharide. These volatile hydrocarbons are electrophilic, attacking a variety of organic compounds and acting as natural **biocides** (pesticides). “White tip” disease of *Gracilaria* is due to the release of these biocides in response to bacterial infection. The bleaching of the tips of the alga is killing some of the algal cells while containing the pathogens at the site of attack (Largo et al., 1995).

## Commercial utilization of red algal mucilages

The two most important polysaccharides derived from the Rhodophyceae are agar and carrageenan. **Agar** is defined pharmaceutically as a phycocolloid of red algal origin that is insoluble in cold water but readily soluble in hot water; a 1.5% solution is clear and forms a solid and elastic gel on cooling to 32 to 39 °C, not dissolving again at a temperature below 85 °C. Agar is composed of two polysaccharides, agarose (Fig. 1.11) and agaropectin (Lahaye, 2001).

Agar is obtained commercially from species of *Gelidium* (Figs. 4.40, 4.41) and *Pterocladia* as well as from various other algae, such as *Acanthopeltis*, *Ahnfeltia*, and *Gracilaria* (Fig. 4.42) (Melo, 1998; Mollet et al., 1998). These algae are often loosely referred to as **agarophytes**. Commercial production of agar was a world monopoly of the Japanese for many years, and even in 1939 Japan was still the major producer. Wartime demands in areas deprived of Japanese agar led to the development of agar industries in many of the Allied countries, some of which have continued and prospered while others have declined or disappeared. The agarophytes are collected by diving, dragging, or raking them offshore at low tide. In the traditional processing procedure the



**Fig. 4.15** The chemical structures of the different types of carrageenans that occur in the red algae.

plants are then cleaned and bleached in the sun, with several washings in freshwater used to facilitate bleaching. The material is boiled for several hours, and the extract is acidified. This extract is then frozen and thawed. On thawing, water flows from the agar, carrying impurities with it. The agar that remains is dried and marketed as flakes or cakes. The more modern method extracts the agar under pressure in autoclaves. The agar is decolorized and deodorized with activated charcoal, filtered under pressure, and evaporated under reduced pressure. Further purification by freezing is then undertaken.

The greatest use of agar is in association with food preparation and technology, and in the pharmaceutical industry. It is used for gelling and thickening purposes, particularly in the canning of fish and meat, reducing the undesirable effects of the can and providing some protection against shaking of the product in transit. It is also used in the manufacture of processed cheese, mayonnaise, puddings, creams, and jellies. Pharmaceutically agar is used as a laxative, but more frequently it serves as an inert carrier for drug products where slow release of the drug is required, as a stabilizer for emulsions, and as a constituent of cosmetic skin preparations, ointments, and lotions. The use of agar as a stiffening agent for growth media in bacteriology and mycology, which was its main use almost a century ago, is still responsible for a very considerable part of the demand.

**Carrageenan** (Fig. 4.15) is a phycocolloid similar to agar but with a higher ash content and requiring higher concentrations to form gels. It is composed of varying amounts of the principal components,  $\kappa$ -carrageenan and  $\lambda$ -carrageenans, both negatively charged high-molecular-weight polymers (Chiovitti et al., 1995; Therkelsen, 1993).  $\kappa$ -Carrageenan is distributed throughout the wall while  $\lambda$ -carrageenan is localized to the cuticle (Vreeland et al., 1992).  $\kappa$ -carrageenan precipitates selectively from a cold, dilute solution in the presence of potassium ions. It forms a gel when heated and cooled with potassium ions and is therefore the gelling component.  $\lambda$ -Carrageenan is the non-gelling component and is not precipitated or gelled by potassium.  $\lambda$ -Carrageenan contains galactose-2,6-disulfate, whereas  $\kappa$ -carrageenan contains 3,6-anhydro-D-galactose. It has been shown in *Chondrus crispus* and *Gigartina stellata* that the proportion of  $\kappa$ - and  $\lambda$ -carrageenan in the cell wall varies according to the ploidy of the plant. In the tetrasporophyte the amount of  $\lambda$ -carrageenan present is high as compared with the amount of  $\kappa$ -carrageenan, whereas just the opposite is true in the gametophyte (Chen et al., 1973). Such results may prove valuable in determining the ploidy of Rhodophyceae that have unknown life cycles.

Carrageenan is usually obtained from wild populations of Irish moss, the name for a mixture of *Chondrus crispus* and the various species of *Gigartina*, particularly *G. stellata*. In the Philippines, *Euclima*, and in Vietnam and India, *Kappophycus* (Fig. 4.16) are extensively cultivated as a source of carrageenan (Reddy et al., 2003). Commercial extraction is similar to that for agar although



**Fig. 4.16** Cultivation of the carrageenan-containing *Kappophycus alvarezii* in an offshore area (left) and a shrimp pond (right) in Vietnam. (From Ohno et al., 1996.)

carrageenan cannot be purified by freezing. The dried alga is washed with freshwater to reduce the salt content and then boiled with 2 to 4 parts of alga to 100 parts of water. The soluble carrageenan is separated from the insoluble residue in a centrifuge. Following filtration and some evaporation under vacuum, the carrageenan is dried on a rotary drier.

Carrageenans are used extensively for many of the same purposes as agar; however, because of their lower gel strength, carrageenans are used less for stiffening purposes than is agar, although for stabilization of emulsions in paints, cosmetics, and other pharmaceutical preparations carrageenans are preferred to agar. Also, for the stiffening of milk and dairy products, such as ice cream, carrageenans have supplanted agar completely in recent years, and it is in this area that demands for these products are the greatest. One particular use is for instant puddings, sauces, and creams, made possible by the gelling action, which does not require refrigeration.

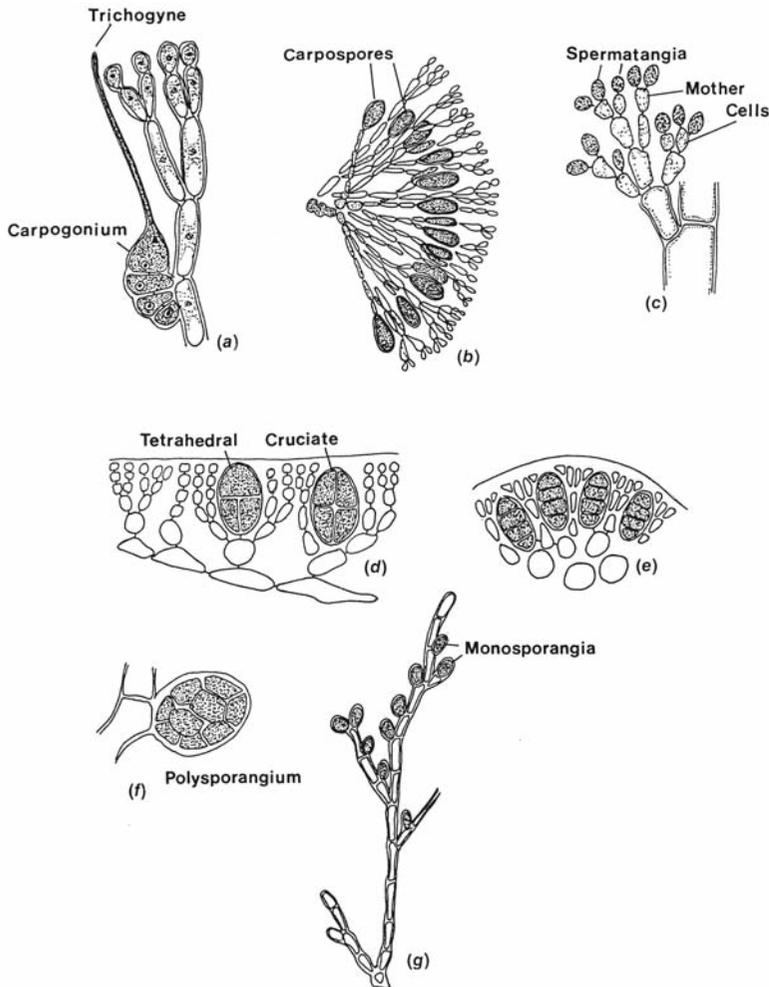
Carrageenans inhibit human immunodeficiency virus (HIV) replication and reverse transcriptionase *in vitro* (in the test tube) (Bourgougnon et al., 1996). Replication of the HIV virus depends on interaction of a glycoprotein on the HIV virus envelope with a receptor on the target cells in the human body. The sulfated carrageenans prevent attachment of the HIV virus to the target cells. This occurs by the stronger negative R-O-SO<sub>3</sub> groups on

the carrageenan binding to a loop on the HIV molecule. A carrageenan-based vaginal microbicide called Carraguard<sup>®</sup> has been shown to block HIV and other sexually transmitted diseases *in vitro*. Carraguard has entered clinical trials involving 600 non-pregnant, HIV-negative women in South Africa and Botswana (Spieler, 2002; Smit, 2004).

## Reproductive structures

The Rhodophyceae have no flagellated cells or cells with any vestigial structure of flagellation, such as basal bodies. In sexual reproduction, **spermatia** are produced which are carried passively by water currents to the female organ, the **carpogonium** (Figs. 4.17(a), 4.18). The fertilized carpogonium produces **gonimoblast filaments** that form **carposporangia** and diploid **carpospores** (Fig. 4.17(b)). The carpospores produce the diploid **tetrasporophyte** which subsequently gives rise to haploid **tetraspores**. Advanced red algae form chiefly tetrahedral tetrasporangia (Fig. 4.17(d)) with large spores, whereas less advanced groups generally form cruciate or zonate tetrasporangia (Fig. 4.17(d), (e)) with smaller spores (Ngan and Price, 1979). Tetraspores are generally larger than carpospores. The tetraspores complete the life cycle by germinating to form the gametophyte. Although this is the general life cycle of most Rhodophyceae, there are a number of modifications of it.

The postfertilization events vary from one order to another. The more advanced orders have **auxiliary cells** with which the fertilized



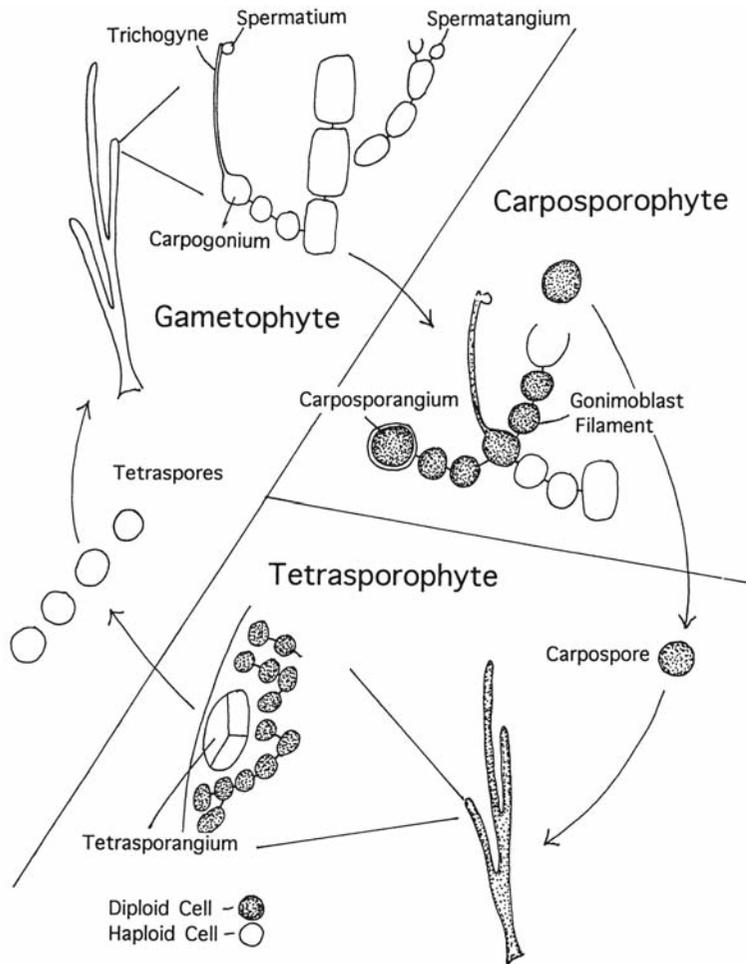
**Fig. 4.17** (a) A filament of *Liagora viscida* with a carpogonial branch. (b) Gonimoblast filaments of *L. viscida* with carpospores. (c) A spermatangial branch of *Acrochaetium corymbiferum* with spermatangia and spermatangial mother cells. (d) Tetrahedral and cruciate tetrasporangia of *Nematostoma laingii*. (e) Zonate tetrasporangia of *Hypnea musciformis*. (f) Polysporangium of *Pleonosporium vancouverianum*. (g) Monosporangia of *Kylinia rhipidandra*. ((a), (b), (c), (e) after Kylin, 1930; (f) after Kylin, 1924; (g) after Kylin, 1928.)

carpogonium fuses to form a multinucleate fusion cell. Papenfuss (1966) recognizes two types of auxiliary cells, nutritive and generative. **Nutritive auxiliary cells** provide nutrients for the developing carposporophyte, whereas **generative auxiliary cells** give rise to **gonimoblast filaments** (Figs. 4.18, 4.33, 4.44). The diploid tissue formed from the fertilized carpogonium forms the gonimoblast filaments. The gonimoblast filaments produce terminal carposporangia, which in turn form the carpospores. The carposporangia enlarge considerably during their maturation because of the development of the chloroplasts and the vesicles containing wall precursors. The pit connection between the carposporangium and the gonimoblast breaks before release of the carpospore. Also during the development of the goni-

moblast filaments, the pit connections between the older gonimoblast cells usually dissolve (Kugrens and West, 1972a, 1973, 1974).

### Carpogonium

The female organ, or carpogonium, consists of a dilated basal portion and a usually narrow gelatinous elongate tip, the **trichogyne**, which receives the male cells (Figs. 4.6(a), 4.10). Usually there are two nuclei in a carpogonium, one in the trichogyne, which degenerates soon after the carpogonium matures, and one in the basal part of the carpogonium, which functions as the female gamete nucleus. In most Rhodophyceae the carpogonium terminates a short, often branched, three- to four-celled lateral called the carpogonial branch. The cell from which the carpogonial



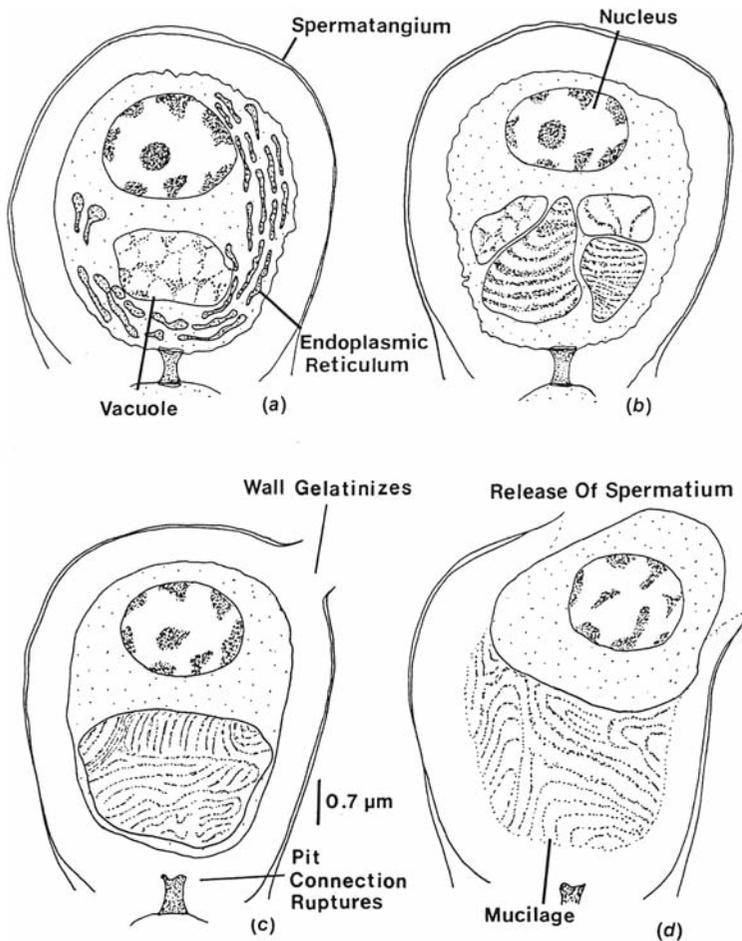
**Fig. 4.18** Simplified life cycle of a typical red alga.

branch arises is the **supporting cell**. The carpogonium and carpogonial branch are commonly colorless, although in some *Nemaliales* this is not true.

### Spermatium

The spermatia of the Rhodophyceae are spherical or oblong cells produced in **spermatangia**, a single spermatium being formed in a spermatangium and then released, leaving the empty sporangium (Fig. 4.19). The spermatangia (Fig. 4.22) are formed on spermatangial mother cells (Fig. 4.17(c)). The young spermatangia frequently have a pronounced polar orientation, with the nucleus in the apical portion and one or more vacuoles in the basal portion (Scott and Dixon, 1973a). As the spermatangium ages, vacuoles form in the basal area. These vacuoles contain fibrous material (probably mucopolysaccharides)

and make up half the volume of the spermatangium. Subsequently the vacuoles fuse to form one large vacuole. The spermatium is released by the gelatinization of the spermatangial wall near the apex and the concurrent release of the fibrous material in the basal vacuole. The fibrous material presumably swells and pushes the spermatium out of the spermatangium (Fig. 4.19). The fibrous material is sticky, and some of it adheres to the spermatium, thereby facilitating attachment to the trichogyne (Fig. 4.22(c)). During the development of the spermatium the pit connection with the spermatium mother cell is severed. The mature spermatium is uninucleate, and wall-less, but surrounded by mucilage, and may (Simon-Bichard-Bréaud, 1971; Peyrière, 1971) or may not (Kugrens and West, 1972a; Kugrens, 1974) contain functional chloroplasts.



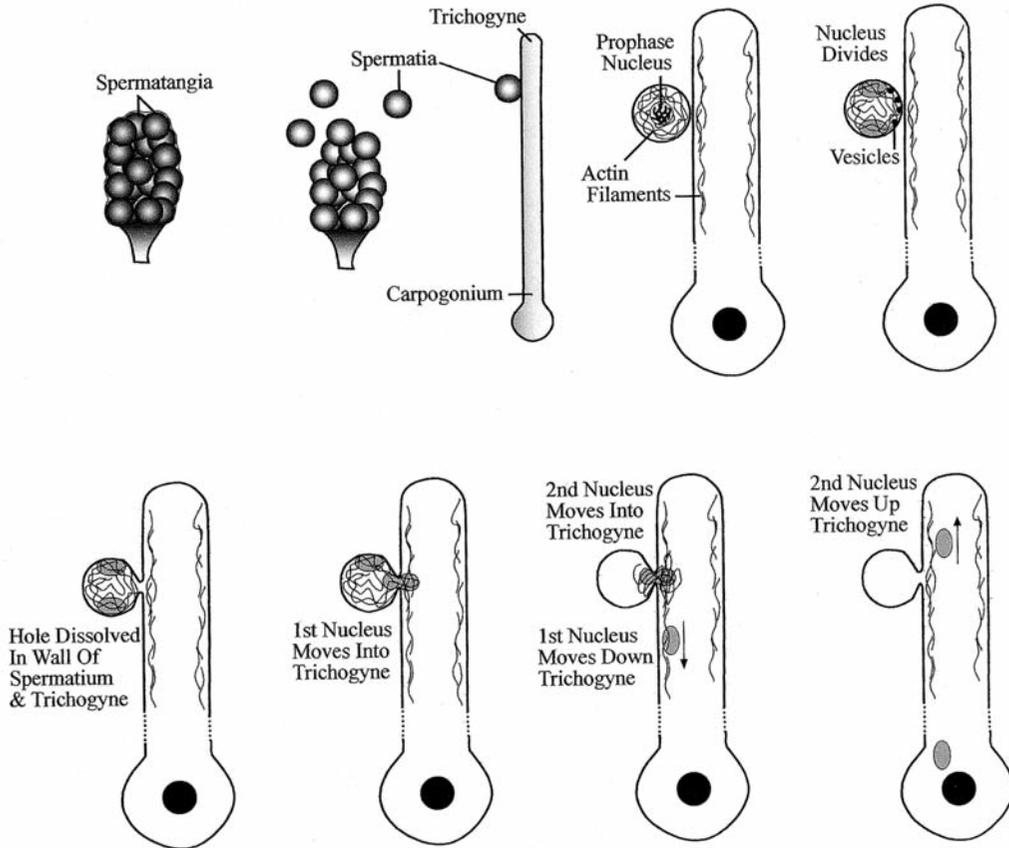
**Fig. 4.19** A semidiagrammatic drawing of the formation of the spermatium in the Rhodophyceae. (a) Young spermatium. (b) Formation of vacuoles containing fibrous material. (c) Fusion of vacuoles, breaking of the pit connection, and gelatinization of the wall of the spermatangium. (d) Extrusion of mucilage and release of the spermatium. (Adapted from Scott and Dixon, 1973a; Kugrens, 1974.)

## Fertilization

The spermatium usually is carried passively by water currents to the trichogyne of the carpogonium, although some spermata can glide in a manner similar to the gliding in *Porphyridium*. Actin filaments in the spermatium and carpogonium are involved in the subsequent steps (Fig. 4.20) (Kim and Kim, 1999; Pickett-Heaps et al., 2001). The wall of the spermatium and that of the carpogonium dissolve, the male nucleus divides, and the male nuclei move into the carpogonium. Fusion of one male nucleus and the carpogonial nucleus occurs in the basal portion of the carpogonium. Fertilization stimulates the production of polyamine spermine (Fig. 4.21) which steers the carpogonial branch toward the production of carpospores (Sacramento et al., 2004). The trichogyne will usually continue to grow until contact is made with a spermatium. After fertilization has

occurred, the trichogyne becomes separated at its base from the rest of the carpogonium by the progressive thickening of the cell wall.

There is a relatively low statistical probability that the non-motile spermata will be carried to receptive trichogynes of carpogonia. Some species have appendages on the spermata that extend the reach of the spermata five- to tenfold resulting in a better chance of attaching to a receptive carpogonium. The appendages are initially contained within vesicles within the spermangia and unfold when the spermata are released (Fig. 4.22). The appendages increase the surface area of the spermatium more than 30-fold, increasing the likelihood of interaction with a trichogyne of a carpogonium. Moieties of the sugar mannose cover the surface of the appendages. The surface of the trichogyne is covered with the lectin concanavalin A which binds the mannose



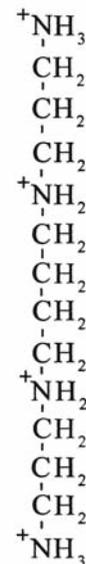
**Fig. 4.20** The behavior of the spermatium and its contents during fertilization in a typical red alga. (Modified from Wilson et al., 2003.)

moieties on the spermatangial appendages (Mine et al., 2003).

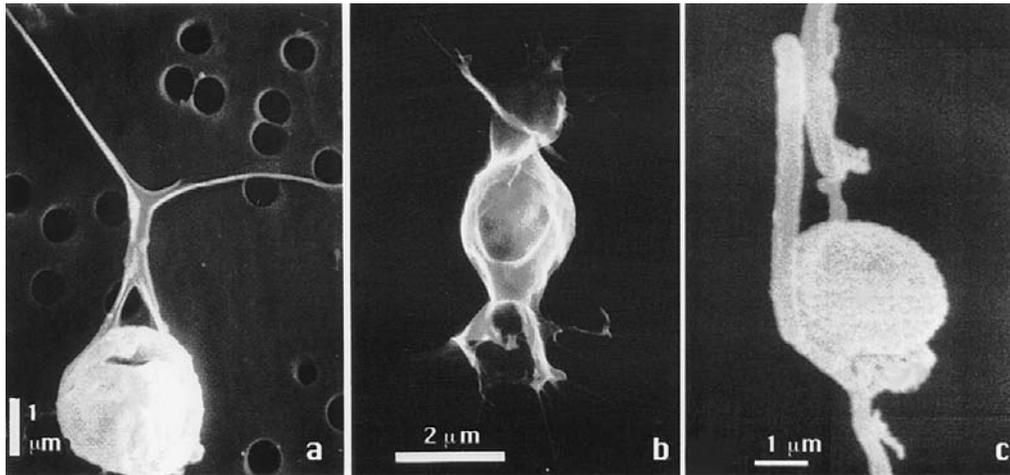
### Meiosporangia and meiospores

Tetrasporangia, polysporangia, and bisporangia formed on diploid plants are generally regarded as being the seat of meiosis although there are exceptions to this. The **tetrasporangia** (Figs. 4.17(e), 4.18) form four tetraspores either in a row (zonate), crosswise (cruciate), or most commonly in a tetrad (tetrahedral). In the formation of tetraspores a wall is laid down inside the tetrasporangia by the protoplast, which has prominent dictyosomes (each dictyosome associated with a mitochondrion as is common in the Rhodophyceae; Kugrens and West, 1972b; Scott and Dixon, 1973b). The tetraspores are not joined by pit connections.

### Spermine



**Fig. 4.21** The chemical structure of spermine.



**Fig. 4.22** Examples of spermatangial appendages. (a) Appendage of *Antithamnion nipponicum*. (b) Fimbriate cone-shaped appendages at each end of *Aglaothamnion neglectum*. (c) Fibrous strands attached to spermatia of *Tiffaniella snyderae*. ((a) from Kim and Fritz, 1993; (b) from Magruder, 1984; (c) from Fetter and Neushul, 1981.)

In some of the red algae it is possible to control tetrasporogenesis by varying the light period, but there is no general rule that can be applied to the response. In *Rhodochorton purpureum* (West, 1972; Dring and West, 1983) and *Acrochaetium asparagopsis* (Abdel-Rahman, 1982), tetrasporophytes produce tetrasporangia under short-day conditions. According to the physiological clock hypothesis, photoperiodism is controlled by an endogenous free-running circadian (approximately 24-hour) oscillation of some biochemical change. Each oscillation involves a regular alternation of two phases (each lasting approximately 12 hours) with a different sensitivity to light. The two phases are a **photophile** (light-loving) phase and a **skotophile** (dark-loving) phase. The initiation of a particular event depends on initiation or inhibition of metabolic changes of short-day or long-day organisms, respectively, by exposure to light at a particular point in the skotophile phase. In the case of *Rhodochorton purpureum* and *Acrochaetium asparagopsis*, light-breaks in the dark (skotophile) phase result in inhibition of tetrasporogenesis, whereas dark-breaks in the light (photophile) phase result in stimulation of tetrasporogenesis.

Tetrasporogenesis in these algae is therefore a short-day phenomenon (Abdel-Rahman, 1982).

**Polysporangia** contain more than four spores, usually in multiples of four (Fig. 4.17(f)). Polysporangia probably evolved from tetrasporangia because polysporangia occur predominantly in the most advanced order, the Ceramiales. **Bisporangia** are probably reduced tetrasporangia in which cell division has resulted in two spores after meiosis instead of four.

### Asexual spores

**Monosporangia** (one spore per sporangium) and **parasporangia** (more than one spore per sporangium) produce asexual spores that re-form the parent thallus. Monosporangia can be formed by the release of a vegetative cell from the thallus (*Goniotrichum*, Fig. 4.25(b), *Asterocystis*, Fig. 4.25(a)), or by the formation of sessile one-celled branches (*Kylinia*, Fig. 4.17(g)).

### Spore motility

Spores, whether monospores, tetraspores or carpospores, of almost all red algae are capable of motility by gliding. Some spores have smooth, directional and continuous gliding (e.g., *Batrachospermum* at  $2.2 \mu\text{m s}^{-1}$ ). In others, movement is non-continuous and unidirectional. Polysaccharide secretion appears to be responsible for the gliding (Pickett-Heaps et al., 2001).

## Classification

The Rhodophyta has a single class, the Rhodophyceae. In the past, the Rhodophyceae was divided into two subclasses, the Bangiophycidae and the Florideophycidae. The Bangiophycidae were supposed to lack pit connections, apical growth, and probably sexual reproduction, whereas the Florideophycidae had pit connections, apical growth, and sexual reproduction with a triphasic life cycle. The Bangiophycidae have since been found to have pit connections and apical growth in the *Conchocelis* filamentous stage of the Bangiaceae. Sexual reproduction also occurs in the Bangiaceae. In turn, the Florideophycidae do not necessarily have apical growth (intercalary growth occurs in the Corallinales (Dixon, 1973)), nor do they all have a triphasic life history (e.g., red algae in the Batrachospermales). For the above reasons, the two subclasses have been dropped in this treatment of the Rhodophyceae, as suggested by Gabrielson (Gabrielson, et al., 1985).

The classification of the more advanced orders of the red algae is based on complex characteristics of sexual reproduction. One of the more active fields of phycology in the last couple of decades has been in the application of nucleic-acid sequencing techniques in a delineation of the evolutionary relationships of these algae. While producing a more natural grouping of algae, these excellent studies have produced an even more complex classification system, which is difficult to present to a student taking a first course in phycology, to which this book is directed. In writing the current edition of the book, the author has spent some time trying to decide how to present the classification of the red algae, and has decided that a presentation of all of the more advanced orders would overwhelm the beginning student. As such, the author has selected those red algae that are commonly studied in phycology courses and/or are economically or ecologically important.

Order 1 **Cyanidiales:** unicells that inhabit volcanic areas with pH values ranging from 0.5 to 3.

Order 2 **Porphyridiales:** unicells, or multicellular algae that are held together by mucilage.

Order 3 **Bangiales:** plants having a filamentous phase with pit connections and a macroscopic phase without pit connections.

Order 4 **Acrochaetiales:** algae with a uniseriate filamentous gametophyte and tetrasporophyte (if both are present).

Order 5 **Batrachospermales:** uniaxial (one apical cell per branch); gonimoblast usually develops from the carpogonium or hypogenous cell.

Order 6 **Nemaliales:** multiaxial (more than one apical cell per branch); usually the gonimoblast develops from the carpogonium or the hypogenous cell.

Order 7 **Corallinales:** heavily calcified algae with the reproductive organs in conceptacles.

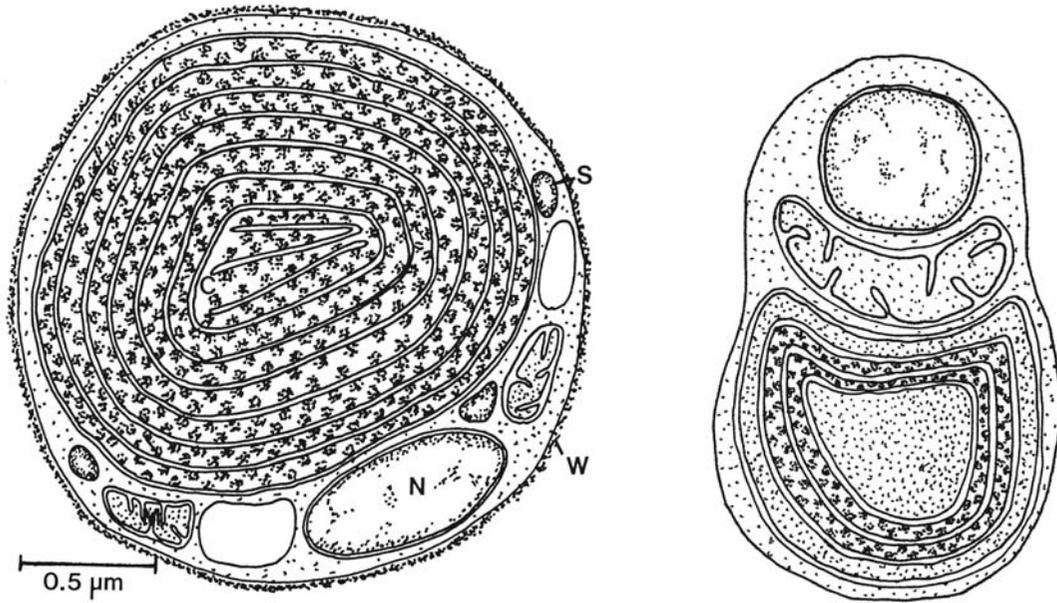
Order 8 **Gelidiales:** fleshy agarophytes, carpogonial branch consisting of a single cell, the carpogonium, no differentiated auxiliary cells.

Order 9 **Gracilariales:** fleshy agarophytes, two-celled carpogonial branch, no auxiliary cells, or connecting cells.

Order 10 **Ceramiales:** relatively delicate or filamentous forms with an auxiliary cell cut off after fertilization and borne on the supporting cell of a four-celled carpogonial filament.

Sequencing of nucleic acids have shown that the Cyanidiales and Bangiales represent separate natural groupings. The Porphyridiales are a grouping of three separate lines of unicells (Saunders and Hommersand, 2004). The Acrochaetiales, Batrachospermales, Nemaliales, and Corallinales are a natural grouping, as are the Gracilariales, Gelidiales, and Ceramiales (Harper and Saunders, 2001).

Using molecular data, it is estimated that the red algae diverged from other eukaryotes about 1400 million years ago (Yoon et al., 2004). The Cyanidiales diverged from the rest of the red algae soon after that, about 1370 million years ago. The Bangiales diverged from the remaining red algae



**Fig. 4.23** Left: *Cyanidium caldarum*. Right: *Cyanidioschyzon merolae*. (C) Chloroplast; (M) mitochondrion; (N) nucleus; (S) starch; (W) wall. (*Cyanidium* is after Seckbach and Ikan, 1972.)

about 1000 million years ago. The first fossil convincingly identified as a red alga is a 1200 million-year-old fossil similar to extant *Bangia* (Fig. 4.28) (Butterfield, 2000). Fossil coralline red algae have been recovered from the late Jurassic (160 million years ago) (Wray, 1977).

The relationship between freshwater and marine Rhodophyceae, as well as their evolution, was discussed in an interesting paper by Skuja (1938). He believed that the Rhodophyceae are a very old group (as is borne out by their fossil record) that originated in shallow coastal waters of primitive seas poor in salt. Living in shallow water, these plants had no need of a large quantity of phycoerythrins to absorb the blue-green light present at greater depths of water. Consequently these primitive Rhodophyceae were not pinkish-red but blue-green in color. These plants are represented by the freshwater Rhodophyceae of today, which are predominantly blue-green in color, and found primarily in the more primitive orders such as the Porphyridiales, Bangiales, Acrochaetales, and Nemaliales. Only later did the Rhodophyceae develop greater quantities of phycoerythrins, and a pinkish-red color, and pene-

trate into deeper waters where they attained their present state of development.

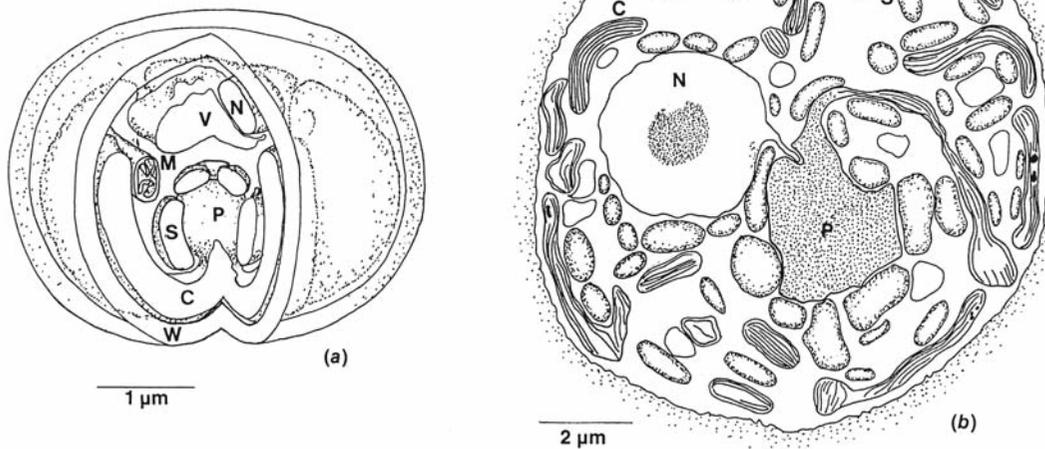
### Cyanidiales

This order contains three unicellular red algae: *Cyanidium caldarum*, *Cyanidioschyzon merolae*, and *Galderia sulphuraria* (Fig. 4.23). These algae inhabit volcanic areas with pH values ranging from 0.5 to 3 and temperatures up to 56°C (Gross et al., 2001).

*Cyanidium caldarum* and *Cyanidioschyzon merolae* are similar in that each of these unicells contains a single nucleus, mitochondrion, and plastid (Fig. 4.23). They differ in that *Cyanidium* is round, has a cell wall, and forms four endospores while *Cyanidioschyzon* is club shaped, has no cell wall, and divides by binary fission (Ohta et al., 1997). *Cyanidioschyzon* has the smallest genome size (16 520 305 base pairs and 5331 genes) so far recorded in eukaryotes and has had its genome sequence elucidated (Matsuzaki et al., 2004).

*Galderia sulphuraria* is morphologically similar to *Cyanidium caldarum*. *Galderia sulphuraria*, however, is able to grow heterotrophically while *Cyanidium caldarum* can not.

The algae in the Cyanidiales are probably the most primitive extant algae, evolving into an environment (acidic hot springs) that was an empty ecological niche at the time. The only other photosynthetic algae present at the time



**Fig. 4.24** (a) A diagrammatic drawing of a cell of *Rhodosorus marinus*. (b) A semidiagrammatic drawing of a section through a cell of *Rhodella maculata*. (C) Chloroplast; (M) mitochondrion; (N) nucleus; (P) pyrenoid; (S) starch grain; (V) vacuole; (W) wall. ((a) after Giraud, 1962; (b) adapted from Evans, 1970.)

were cyanobacteria. Cyanobacteria do not occur in ecological niches below a pH of 5 (Brock, 1973). It, therefore, makes sense that the first eukaryotic alga would have had an evolutionary advantage by evolving in an environment where there were no other photosynthetic algae to compete with.

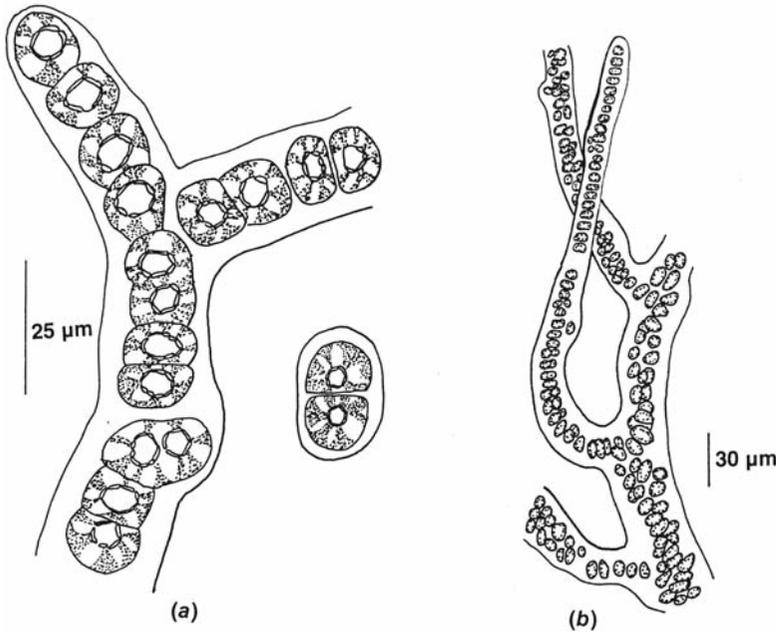
### Porphyridiales

These algae are either unicells or cells embedded in mucilage loosely organized into filaments. There are three evolutionary lines in the order (Oliveira and Bhattacharya, 2000; Karsten et al., 2003).

The unicells in the Porphyridiales are probably derived from monospores, carpospores or tetraspores of more evolutionarily advanced red algae (Ragan et al., 1994; Freshwater et al., 1994). These unicells are differentiated by cytological characteristics. Thus, *Porphyridium* (Fig. 4.1) has a single large stellate chloroplast with a central pyrenoid. *Rhodosorus* (Fig. 4.24(a)) has a lobed chloroplast with a basal pyrenoid, and *Rhodella* has a stellate chloroplast with a central pyrenoid, but with a

more dissected chloroplast than *Porphyridium* (Figs. 4.2, 4.24(b)).

*Porphyridium* is a common alga on soil and damp walls where it forms several-layered blood-red mucilaginous strata. Even though it is a soil alga, most species grow best in marine liquid media, indicating that it is probably of brackish or marine origin. *Porphyridium* has the ability to glide over a substrate it is in contact with. Overhead illumination results in random movement, whereas unilateral light causes movement toward the light source (Sommerfield and Nichols, 1970). The positively phototactic cells move by the extrusion of mucilage in vesicles in one direction, which results in the formation of a mucilage stalk behind the cells (Lin et al., 1975). *Porphyridium* releases different amounts of polysaccharides, depending on the environmental conditions it is living under (Ramus and Robins, 1975). During the log phase of growth, large Golgi bodies form polysaccharides, which are stored in vesicles under the cell membrane. During the stationary phase of growth in culture, the polysaccharide is secreted outside the cell, giving rise to a capsule. This behavior in culture can be related to the survival of the cells in nature. The rapid log phase of growth is equivalent to a soil environment that is moist with available nutrients. Here the polysaccharides are stored inside the cell, and there is



**Fig. 4.25** (a) *Asterocytis* sp. in the filamentous and bicellular form. (b) *Goniotrichum alsidii*. ((a) after Belcher and Swale, 1960; (b) after Taylor, 1957.)

only a thin mucilage layer around the cell. The stationary phase of growth is equivalent to a soil environment that is drying out with nutrients becoming limiting, thereby causing a cessation of cell growth. Here the polysaccharides are released to the outside of the cell, where they form a capsule that enables the cell to withstand the desiccation that follows.

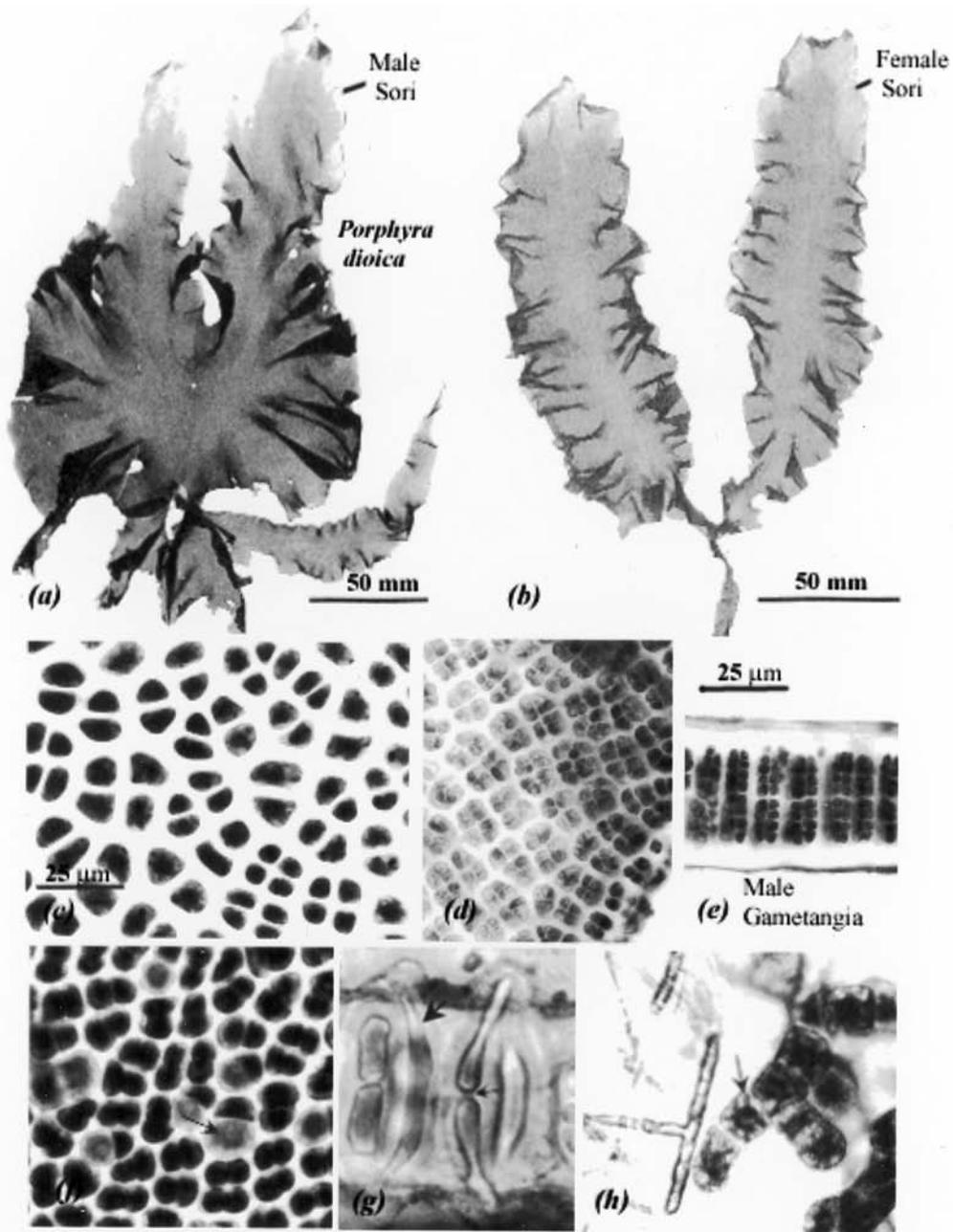
Also included in this order are algae that have cells joined together in thick mucilaginous filaments. *Goniotrichum* is a common marine epiphyte made up of branched mucilaginous filaments (Fig. 4.25(b)). *Goniotrichum* forms monospores simply by the release of a vegetative cell from a filament in photoperiods of over 12 hours of light (Fries, 1963). *Asterocytis* (Fig. 4.25(a)) exhibits what is probably an intermediate position in the evolution of a red unicell into a mucilaginous filamentous alga. In normal seawater *Asterocytis* forms branched filaments, whereas in seawater of one-fourth strength the organism forms unicells, which were previously classified in the genus *Chrootheca* (Lewin and Robertson, 1971).

### Bangiales

The algae in this order show alternation of a haploid thallus stage having no pit connections, with

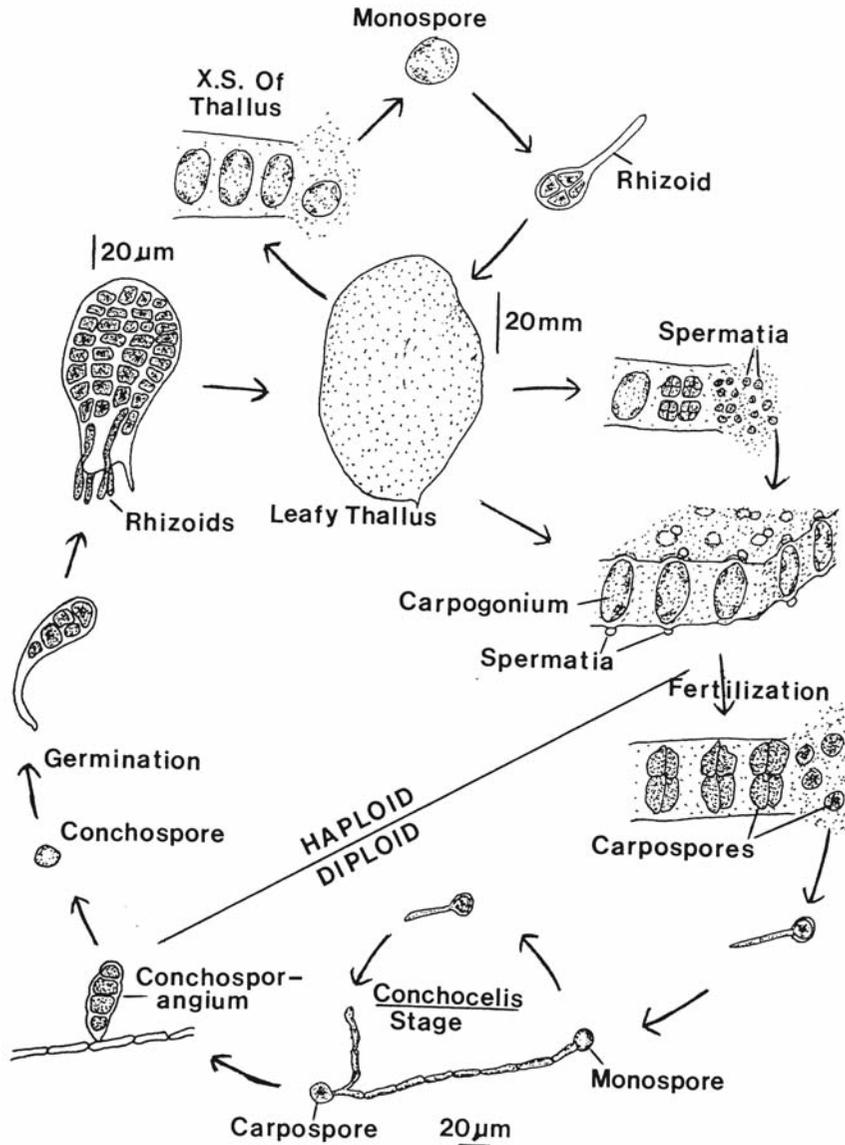
a diploid filamentous *Conchocelis* state that has pit connections (Lee and Fultz, 1970; Kornmann, 1994). The Bangiales is a monophyletic order and is a sister group to the higher red algae (Oliveira and Bhattacharya, 2000).

*Porphyra* (Figs. 4.26, 4.27) is an intertidal seaweed in the colder waters of the world. The thallus arises from a holdfast and is composed of a sheet of cells one to two layers thick. *Porphyra gardneri* (Fig. 4.27) is a foliose (leafy) monostromatic (single layer of cells) alga found growing epiphytically on several members of brown algae in the Laminariales. In British Columbia, Canada, the host *Laminaria setchellii* has its blade worn back almost to the stipe by November. During December, a new *Laminaria* blade is rapidly produced. The first thalli of *Porphyra gardneri* appear epiphytically on the *Laminaria* at the end of February. **Asexual reproduction** occurs soon after *Porphyra gardneri* appears in February. The margins of the thallus break down and release single-celled **monospores**. After 1 or 2 days, the monospores germinate by sending out long rhizoids that anchor the monospores in the host *Laminaria* tissue. From this, a new leafy thallus appears. Prolific monospore production results in a great increase of *Porphyra gardneri* during the spring months. **Sexual reproduction** begins



**Fig. 4.26** *Porphyra dioica*. (a) Male gametophyte with male gametangia in sori. (b) Female gametophyte with female gametangia in sori. (c) Surface view of vegetative cells in pairs. (d) Surface view of male gametangia. (e) Male gametangia in transverse section. (f) Gametangial mother cell (arrow) in surface view. (g) Carpogonium (large arrow) and first division of fertilized carpogonium (small arrow) in transverse section of thallus. (h) *Conchocelis* stage with conchosporangia (arrow). (From Holmes and Brodie, 2004.)

during late April. Spermatium mother cells in the thallus divide to form 64 spermata. The spermata contain a degenerate chloroplast with only a few thylakoids. Vesicles containing a fibrous material are discharged by the spermata just before spermata liberation. The released spermata are 3 to 5  $\mu\text{m}$  in diameter, have no starch granules, and are surrounded only by the fibrous



**Fig. 4.27** The life cycle of *Porphyra gardneri*. (Adapted from Hawkes, 1978.)

material from the released vesicles. The spermatia are carried to the carpogonia by water currents. Carpogonia (with a chromosome number of 4) differentiate from vegetative cells by the production of a swollen area of the cell wall, the **prototrichogyne**, directly above the carpogonium. In a monostromatic species, such as *Porphyra gardneri*, two prototrichogynes are produced by each carpogonium, one on each surface. In dis-

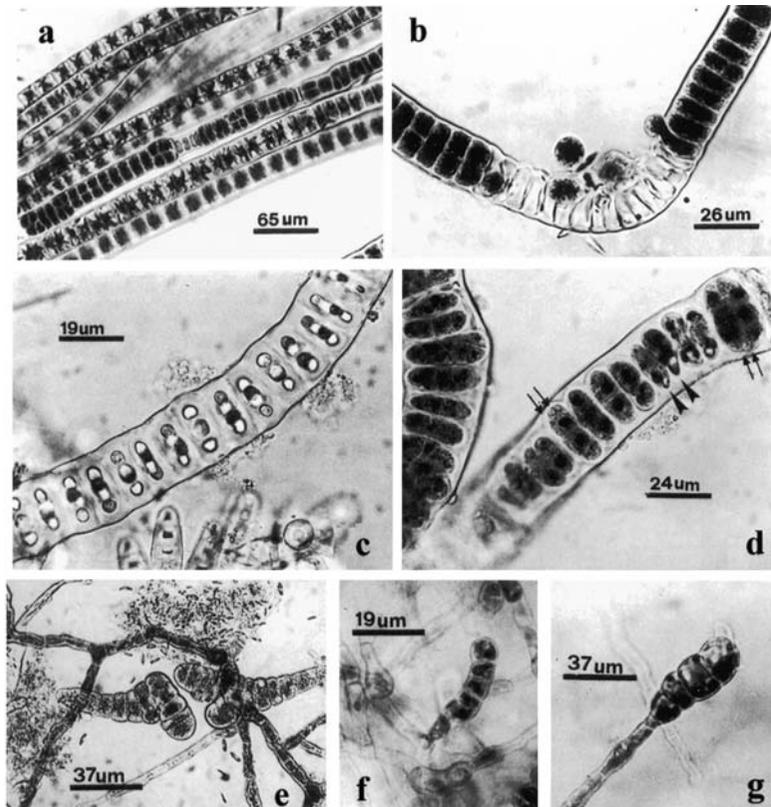
tromatic species, with two sheets of cells in the thallus, a single prototrichogyne is produced per carpogonium. A spermatium attaches to the prototrichogyne, a fertilization canal appears in the prototrichogyne, and the spermatial nucleus moves through the canal to fuse with the carpogonium. By the beginning of May, the fertilized carpogonia have divided to form two to four diploid carpospores (with a chromosome number of 8), 14 to 20 μm in diameter. Maximum carpospore production occurs during the months of June through August. The carpospores germi-

nate in 2 to 3 days to produce the diploid *Conchocelis* stage (Hawkes, 1978). The *Conchocelis* stage is filamentous and commonly lives in shells of dead marine animals. Under long-day conditions, the *Conchocelis* stage differentiates monospores, which re-form the *Conchocelis* stage (Dixon and Richardson, 1970). Under short days the *Conchocelis* stage forms conchosporangia (fertile cell rows), each cell of which produces a conchospore. Conchospores are released from the conchosporangia under low-temperature conditions (about 5°C) (Chen et al., 1970). The formation of the conchosporangia under short-day conditions is a true photoperiodic response because a light break in the middle of the dark period is inhibitory (Dring, 1967a). A functional phytochrome system is operative, with red light being the most effective in breaking the dark period (Dring, 1967b). This is one of the few demonstrations of a true photoperiodic response in the red algae, and it is unlikely that this phytochrome type of response occurs in sublittoral

Rhodophyceae because far-red light penetrates to less than 1 m of seawater and red light no deeper than 10 m (Dixon and Richardson, 1970). On release, the conchospores germinate in a bipolar manner, forming a germling that grows into the thallus phase, completing the life cycle.

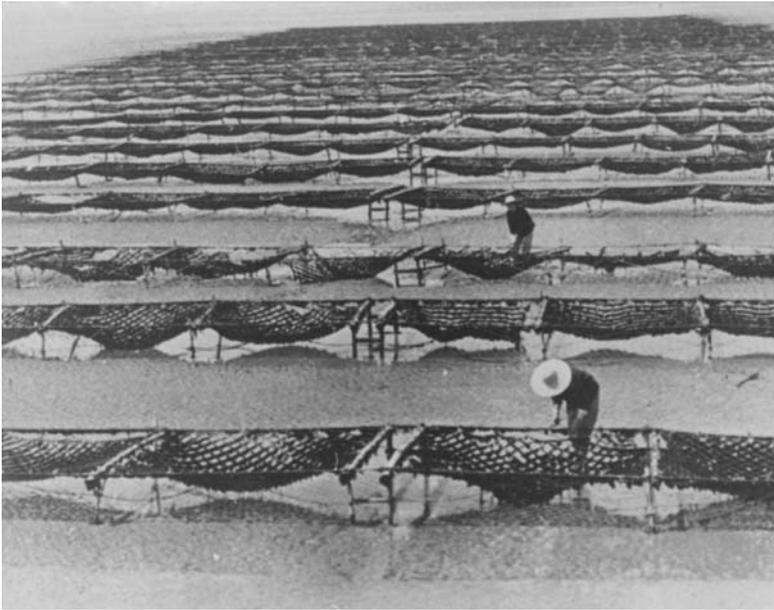
*Porphyra perforata* lives in the intertidal zone, where at low tide the plants are routinely exposed to air drying. As a result of evaporative water loss, the salt concentration of extracellular water can increase up to 10 times above normal levels. During desiccation at low tide, the alga can lose up to 90% of its fresh weight. Such desiccation results in inhibition of photosynthesis. Some of the inhibition of photosynthesis is probably due to a decrease in electron flow between water and photosystem II because of a reduced concentration of water in the cells (Satoh et al., 1983).

*Bangia* forms upright threads that are at first uniseriate, the cells subsequently undergoing longitudinal division to form a multiseriate filament



**Fig. 4.28** *Bangia atropurpurea*.

(a) Uniseriate filaments becoming multiseriate by successive division of cells. (b) Filament releasing monospores. (c) Male gametophytes with spermatia. (d) Female gametophytes. Arrowheads show a carpogonium with a single trichogyne. Arrows point to developing zygotes. (e) Conchosporangia. (f,g) Monosporangia on sporophytes. (From Gargiulo et al., 2001.)



**Fig. 4.29** Cultivation nets with *Porphyra* at low tide at Rudang, Jiangsu Province, People's Republic of China. (From Tseng, 1981.)

(Fig. 4.28). *Bangia* occurs in both marine and freshwater environments. *Bangia*-like fossils (*Bangiomorpha pubescens*) have been reported from the 1200 million-year-old Hunting formation on Somerset Island in Canada (Butterfield, 2000). It is possible to adapt freshwater *Bangia fuscopurpurea* to seawater by increasing the salinity by 10% of that of seawater every time the alga sporulates (den Hartog, 1971). If the thallus is moved directly from freshwater to seawater, the plant dies, illustrating that the spores have a better ability to adapt to changed salinity. Such an experiment shows the ease with which some of the smaller red algae can change from one habit to another. *Bangia* has a life cycle similar to that of *Porphyra* (Richardson, 1970; Sommerfeld and Nichols, 1973).

The diploid *Conchocelis* phase of *Porphyra* and *Bangia* differs chemically from the haploid thallus phase (Liu et al., 1996). The *Conchocelis* phase has cellulose in the wall of the cells, whereas in the thallus phase, cellulose is absent and, as the structural polysaccharide, is replaced by a xylan (polysaccharide composed of xylose residues) (Gretz et al., 1980; Mukai et al., 1981). The galactans in the *Conchocelis* phase are also different from those in the thallus phase (Gretz et al., 1983). These chemical differences are in addition to the structural differences, particularly the occurrence of

pit connections in the *Conchocelis* phase and their absence in the thallus phase.

The *Conchocelis* phase has been found as the fossil genus *Palaeoconchocelis starmachii* in the Upper Silurian of the Paleozoic (425 million years ago) (Campbell, 1980).

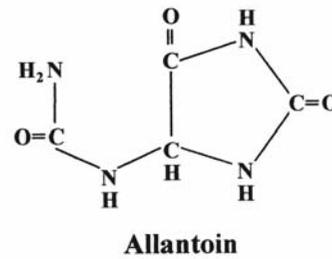
The leafy thallus phase of *Porphyra* is eaten as a vegetable in the Far East and Nova Scotia (Canada). In Japan, *Porphyra* is eaten as a vegetable called *nori*; in Nova Scotia, it is called *laver*. *Porphyra* is cultivated on farms in China (Fig. 4.29) and Japan. In Japan, most of the *Porphyra* comes from *Porphyra* farms in the shallow waters of such places as the Inland Sea and Tokyo Bay, although there is considerable collecting of plants from natural populations. *Porphyra* was first cultivated around 1700 in Tokyo Bay by placing bundles of bamboo or oak bushes (known as *hibi*) into the mud in early autumn, the usual procedure being to arrange the bundles in regular rows and at such a depth that the twigs were well covered by water at high tide. The modern method is to drive bamboo stakes into the mud in rows and then place netting between the stakes (Mumford and Miura, 1988). The *Conchocelis* stage growing in seashells releases the conchospores, which settle on the brush or nets and germinate to form the foliose *Porphyra* plant. From late November to March, the *Porphyra* plants (sometimes mixed with the green alga *Monostroma*)

are harvested by a person in a narrow boat who picks or scrapes the plants off by hand. The *Porphyra* is brought to the factory, where it is washed and chopped into small fragments. These fragments are stirred in a vat, from which measured amounts of the mixture are dipped by means of a small wooden container and poured over a stiff porous mat. As the liquid drains away, the nori fragments are spread evenly over the mat, which is hung on outdoor bamboo racks to dry. The thin film of dry nori is removed as a sheet from the mat, folded, and packaged for market. The food value of nori or laver lies in its high protein content (25% to 30% of the dry weight), vitamins, and mineral salts, especially iodine. The vitamin C content is about 1½ times that of oranges per unit weight, and it is also rich in vitamin B. Humans digest about 75% of the protein and carbohydrate, and in this respect it is much better than other seaweeds.

Prior to the discovery of the alternate *Conchocelis* phase of *Porphyra* by Drew, the yield of *Porphyra* fluctuated sharply from one year to the next. Up to this time, the number of *Porphyra* plants formed depended on the production of conchospores by the *Conchocelis* phase. These fluctuations in the production of spores have been overcome by the artificial cultivation of the *Conchocelis* phase, usually on shells. The shells containing the *Conchocelis* phase are attached to the nets, or the nets are dipped into baths to which crushed shells have been added. The best settlement of spores occurs in waters of high nitrogen, that is, near sewage outflows. Another way of seeding *Porphyra* is by monospores. Monosporangia have been induced in haploid thalli by three week exposure to allantoin (Fig. 4.30) followed by homogenization of the thalli. The resulting monospores reproduce the haploid thalli of *Porphyra* (Mizuta et al., 2003). Although the production of nori increased until the early 1960s, there has been no increase in production since then, mostly because of increasing pollution of the shallow waters in which *Porphyra* farming is carried out (Dixon, 1973).

### Acrochaetiales

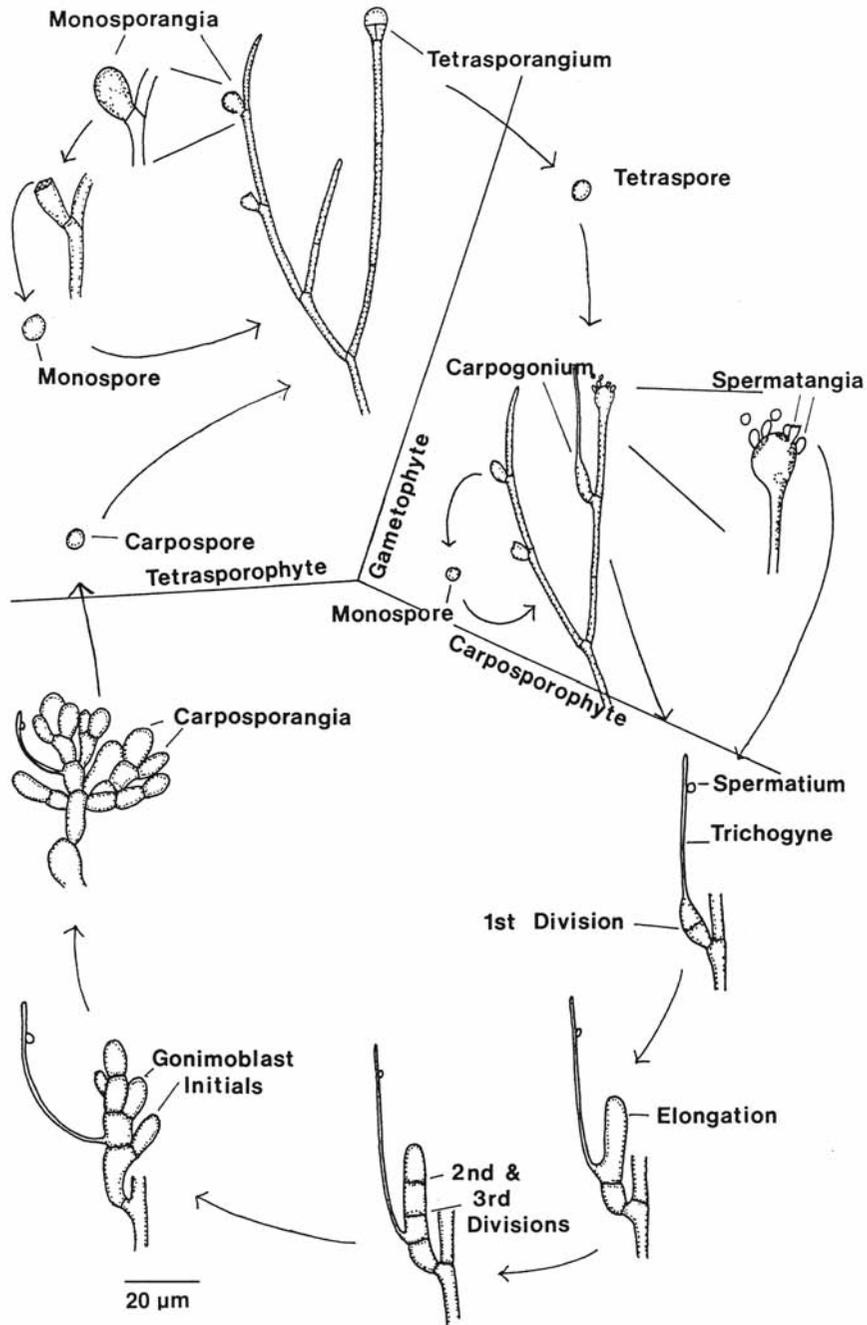
Algae with uniseriate filaments are in this order (Chemin, 1937; Feldman, 1953). Papenfuss (1945,



**Fig. 4.30** The chemical structure of allantoin, an inducer of monospore formation in *Porphyra*.

1947) recognizes four major genera in the order: (1) *Rhodochorton*, with each cell containing a few to many small discoid chloroplasts (Fig. 4.31); (2) *Acrochaetium*, with each cell having one parietal or laminate chloroplast (Fig. 4.17(c)); (3) *Audouinella*, with each cell having one or more spiral chloroplasts; (4) *Kylinia*, with each cell having one or more stellate chloroplasts (Fig. 4.17(g)).

Most of these algae are small epiphytes or endophytes. Some of them may still prove to be the alternate phase of more complex higher Rhodophyceae. *Rhodochorton investiens* can be used as an example of a triphasic life cycle (Fig. 4.31) (Swale and Belcher, 1963). Both the gametophyte and tetrasporophyte produce similar obovoid monospores in monosporangia arising just beneath a cross wall of the filament. The spore is liberated through the apex of the sporangial wall, which remains attached to the filament. After release, the monospores germinate without a resting phase to re-form the parent plant. The gametophyte is monoecious, with a filament ending in a cluster of spermatangia and a carpogonium being borne on the cell under the supporting cell of the spermatangia. The spermatangia occur in groups of four to six, emerging from the enlarged and flattened distal end of a terminal cell. The carpogonia are sessile and appear in the position of branch cells. At the distal end of the carpogonium is a narrow trichogyne. After fertilization, the carpogonium becomes divided by three cross walls into a row of four cells. The first transverse wall develops below the trichogyne, the upper cell then elongating and dividing into three cells. This results in the trichogyne emerging from the second cell of the row. Two-celled gonimoblast



**Fig. 4.31** The life cycle of *Rhodochorton investiens*. (Adapted from Swale and Belcher, 1963.)

filaments develop from each cell of the row, each gonimoblast filament producing two to three terminal carpospores. The carpospores germinate to form the tetrasporophyte, with larger cells of deeper color than those of the gametophyte.

Tetrangonia either are sessile or terminate a one-celled branch. The tetraspores germinate to produce the gametophyte, and thus complete the life cycle.

### Batrachospermales

This order includes the uniaxial (each filament with a single apical cell) freshwater Rhodophyceae.

The gonimoblasts usually arise from the fertilized carpogonium. No tetraspores are formed, and meiosis probably occurs when the diploid filamentous stage forms the thallus initials.

*Batrachospermum* (often called the “frog spawn” alga) is a freshwater alga that occurs in well-aerated, slow-moving streams. The gametophyte (Fig. 4.32) appears as delicate violet beads on a string. Each “bead” consists of whorls of branches arising at the cross walls of the elongated cells of the main axis. The gametophytes produce terminal carpogonia on short branches arising from the whorls of branches. Spherical spermatia are formed by small groups of antheridia at the tips of branches. The spermatia are carried to the carpogonium by water currents. After fertilization, the zygote cuts off gonimoblast initials which develop into gonimoblast filaments with terminal carposporangia. The carposporangia release diploid carpospores that germinate into filamentous prothalli. Monospores can be produced by the prothalli. The monospores germinate to re-form the parent plant. The prothalli also form erect filaments that elongate by apical growth. The apical cell of the erect filament cuts off three to five cells mitotically, then undergoes two meiotic divisions. The first meiotic division results in (1) a polar body and (2) a cell that divides again to form a second polar body and the apical cell of the haploid gametophyte. The macroscopic plant is thus composed of basal diploid cells on a haploid plant. The diploid portion of the plant has cells that are larger than those of the haploid portion (Hurdelbrink and Schwantes, 1972; von Stosch and Theil, 1979; Balakrishnan and Chaugle, 1980; Necchi, 2002).

## Nemaliales

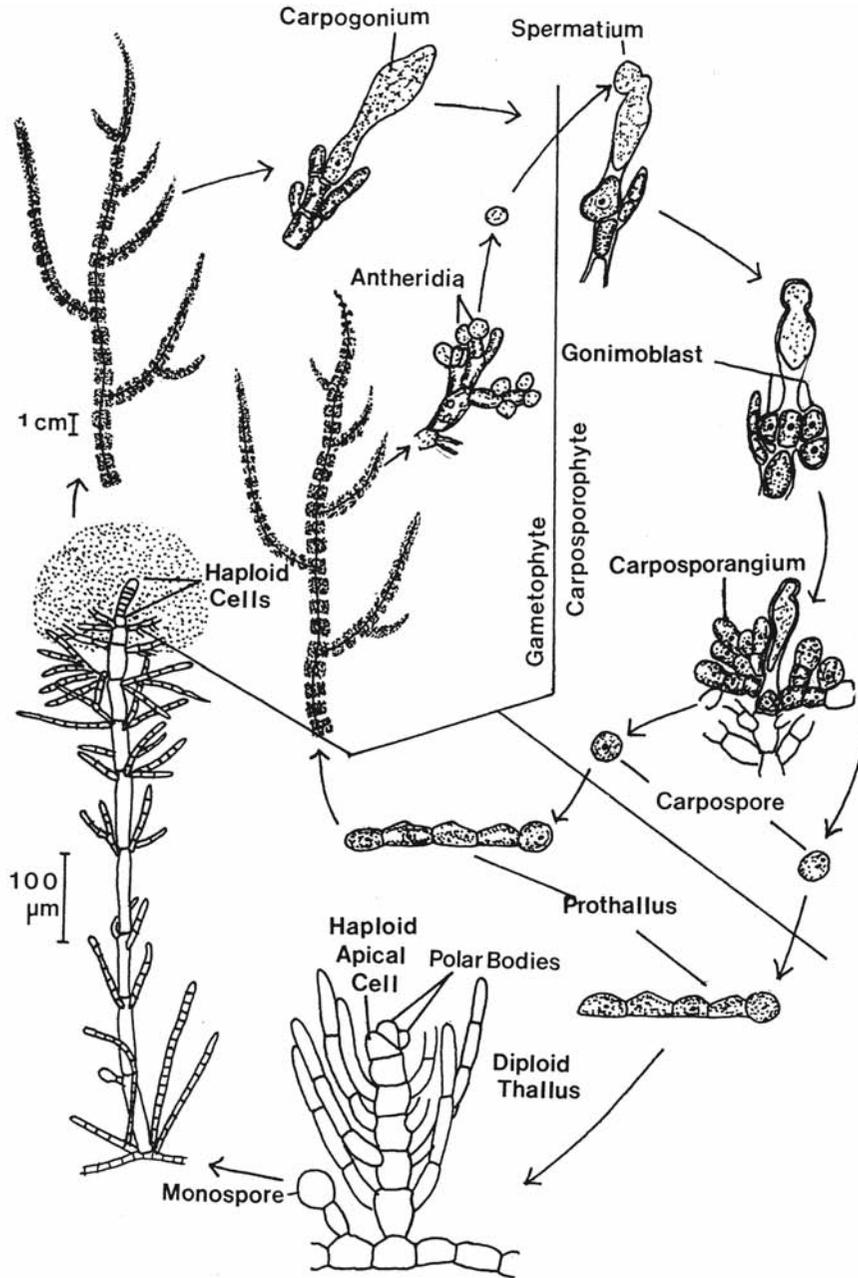
This order has multiaxial Rhodophyceae (with more than one apical cell), which usually have gonimoblasts developing from the carpogonium or hypogynous cell. There may be auxiliary cells present, but if there are, they are always nutritive auxiliary cells.

*Nemalion* is a common intertidal alga in north temperate seas. The thallus is a soft gelatinous cylinder reaching a length of 25 cm with a limited number of dichotomous branches (Fig. 4.33), being composed of a number of axial threads or filaments

in the center and richly branched laterals around the periphery. The laterals arise from the axial threads and grow out horizontally on all parts of the thallus except for the tip where they radiate out vertically. The laterals are all of about the same length, and their tips intercalate so as to give the thallus an even surface. The central axial cells are colorless, whereas the peripheral laterals usually have a stellate chloroplast with a central pyrenoid.

In *Nemalion*, the plants are homothallic. The carpogonial branch consists of an ordinary lateral of four to seven cells (Fig. 4.33). The elongate trichogyne projects slightly beyond the surface of the thallus. Spermatangial branches are produced from the terminal cells of the laterals, and at the tip of the two- to four-celled spermatangial branch are formed three to four spermatangia. Spermatangia produce spermatia that are released and pass to the trichogyne of the carpogonium where fertilization occurs. After fusion of the two gamete nuclei, the large zygote nucleus and the chloroplast divide into two. The carpogonium then divides transversely into two cells, the upper of which forms the gonimoblasts. The lower cell of the carpogonium gradually fuses with the **hypogynous cells** (those underneath the carpogonium), and eventually the upper carpogonial cell that has produced the gonimoblasts also fuses with these cells. These fusions probably have a nutritive function, providing the developing gonimoblasts and carposporangia with storage products. The gonimoblast threads hang downward, and each cell of the thread forms an upwardly curved two- to three-celled branchlet, the terminal cell of which enlarges to form the carposporangium. The carpospores give rise to a filamentous phase that produces tetraspores under short-day conditions (Cunningham and Guiry, 1989). The tetraspores produce filamentous gametophytes that form the erect axes under long-day conditions.

*Galaxaura* (Fig. 4.34) is a calcified alga that is widely distributed in the tropics. The calcification occurs as aragonite (Fig. 4.7) in the intercellular spaces of the cortex. *Galaxaura* has a gametophyte and tetrasporophyte that are similar in appearance. Male and female reproductive structures are borne in conceptacles deeply immersed in the medulla of the gametophytes, while tetraspores



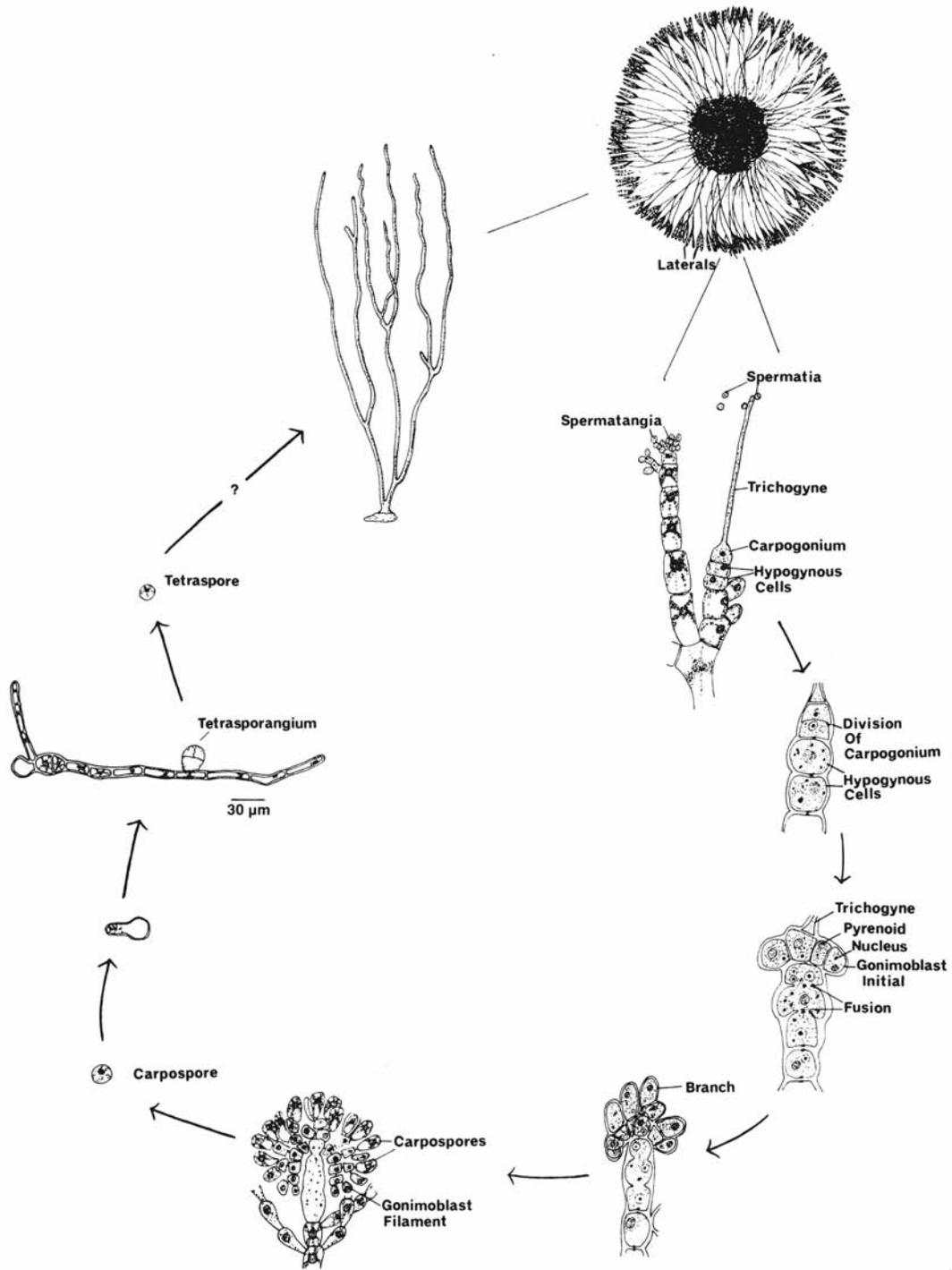
**Fig. 4.32** The life cycle of *Batrachospermum* sp. (Adapted from Balakrishnan and Chaugule, 1980; von Stosch and Thiel, 1979.)

occur scattered about the apical end of branches of the tetrasporophyte (Fig. 4.34). *Gymnocodium* and *Permocalculus* are two genera that arose in the Permian of the Paleozoic and became extinct during the Cretaceous of the Mesozoic (Johnson,

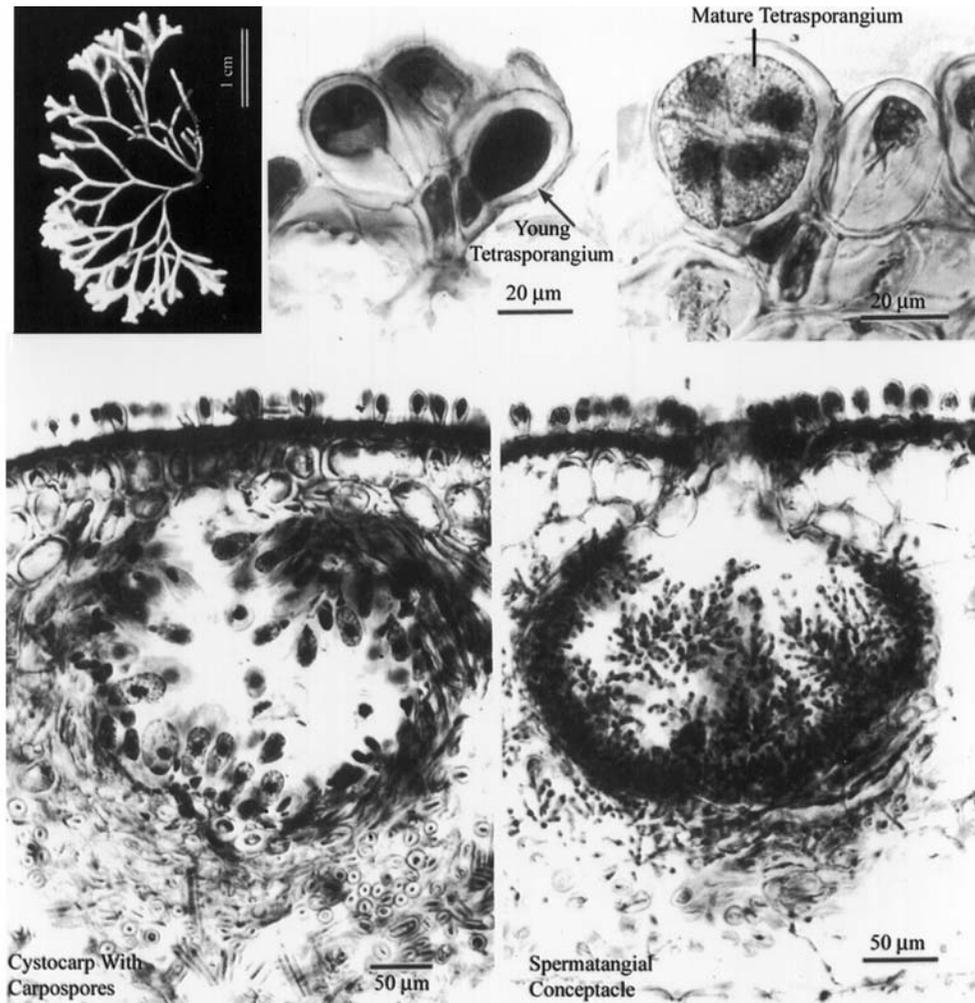
1961). The two genera were similar in morphology to *Galaxaura* with weak calcification restricted to an irregular outer zone.

### Corallinales

The Corallinales is an order of heavily calcified red algae (Figs. 4.35(a), 4.36(a), 4.37(a)) (Johansen, 1981; Silva and Johansen, 1986). Cytologically, the outer



**Fig. 4.33** The life cycle of *Nematium* sp. (Adapted from Oltmanns, 1904; Kylin, 1916; Fries, 1967; Umezaki, 1967.)

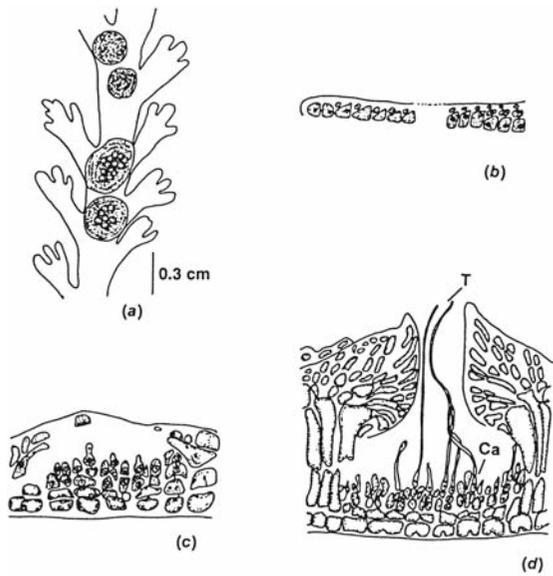


**Fig. 4.34** Photographs of vegetative and reproductive structures of *Galaxaura apiculata*. (From Kurihara et al., 2005.)

cap layer of the pit connections is large and dome-shaped (Pueschel and Trick, 1991). The order is characterized by having reproductive organs in **conceptacles** (cavities that open to the thallus surface) opening to the exterior by one or more pores (Figs. 4.35(d), 4.37(b)). In some genera, the tetrasporic conceptacles differ from sexual conceptacles in having numerous small pores in the roof rather than a single pore. The sexual plants are usually dioecious, with marked differences between male and female conceptacles. Both male and female organs are borne in **nemathecia** (wart-like elevations of the surface containing many reproductive organs), which develop on the

floor of the conceptacle. Spermatangia are formed abundantly from short filaments on the conceptacle floor. The female procarp consists of a two-celled carpogonial filament arising from a basal cell that functions as an auxiliary cell (Fig. 4.35(d)). The long trichogynes from the many carpogonia project through the conceptacular ostiole. After fertilization, a short ooblast from the carpogonium joins the auxiliary cell. All of the auxiliary cells of the conceptacle then fuse to form a large fusion or placental cell, from the margins of which issue the gonimoblast filaments with their carposporangia (Fig. 4.37(b)).

The thallus of the Corallinales is usually divided into two areas, the hypothallus and the perithallus. The **hypothallus** has relatively large cells and forms the basal part of the crustose



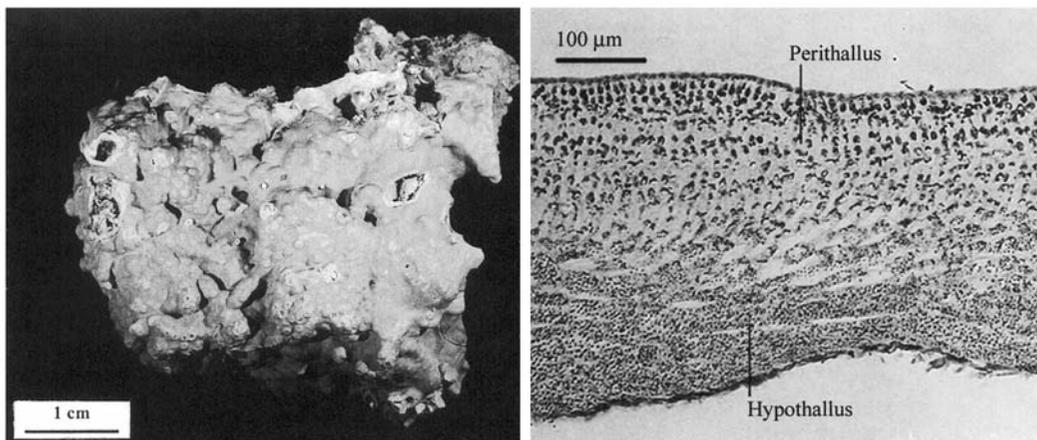
**Fig. 4.35** (a) *Melobesia marginata* epiphytic on *Laurencia spectabilis*. (b–d) *Melobesia lejolisii*: (b) section of sterile thallus showing cover cells and (c) section of immature fertile thallus. (d) *Melobesia limitata*: conceptacle with carpogonia (Ca) and trichogynes (T). ((a) after Smith, 1969; (b) after Suneson, 1937.)

plants (Figs. 4.36(b), 4.37(b)) and the central part of the erect branches. Also, in case of injury the scar tissue that develops is hypothallus tissue. The perithallus has smaller cells and is located above

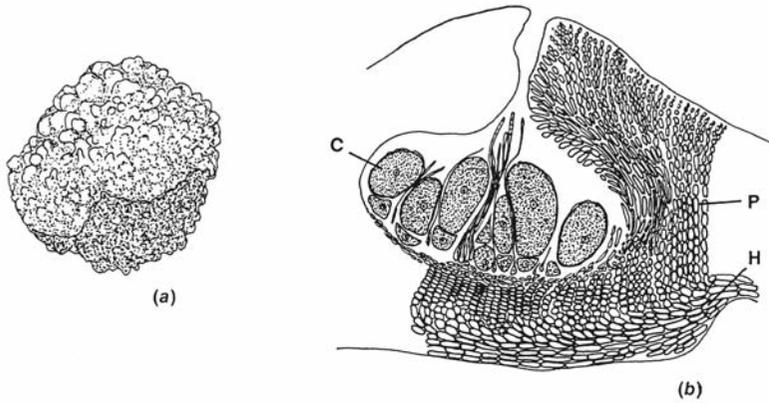
the hypothallus in crustose forms and outside of the medullar hypothallus in branched forms.

The Corallinaceae of the Corallinales has two subfamilies. The crustose and nodular forms (Figs. 4.35(a), 4.36(a), 4.37(a)) are in the Melobesoideae and the articulated or jointed forms (Fig. 4.38) are in the Corallinoideae (although recent investigations on gene sequences of rRNA have shown that these two subfamilies do not reflect the evolutionary history of the order (Bailey and Chapman, 1998)). In the Melobesoideae, the simplest type of thallus is in *Melobesia*, which has thin pink or red crusts that are widely distributed, especially as epiphytes on other algae and marine plants (Fig. 4.35(a)). The thallus consists of one to five layers of prostrate threads compacted to form a disc. A marker feature is the flat cover cells, which also occur in other Corallinales, forming the outer layer of cells (Fig. 4.35(b)). *Mesophyllum* and *Lithothamnion* are lithophytes that usually have considerably thicker crusts and sometimes nodules (Figs. 4.36, 4.37).

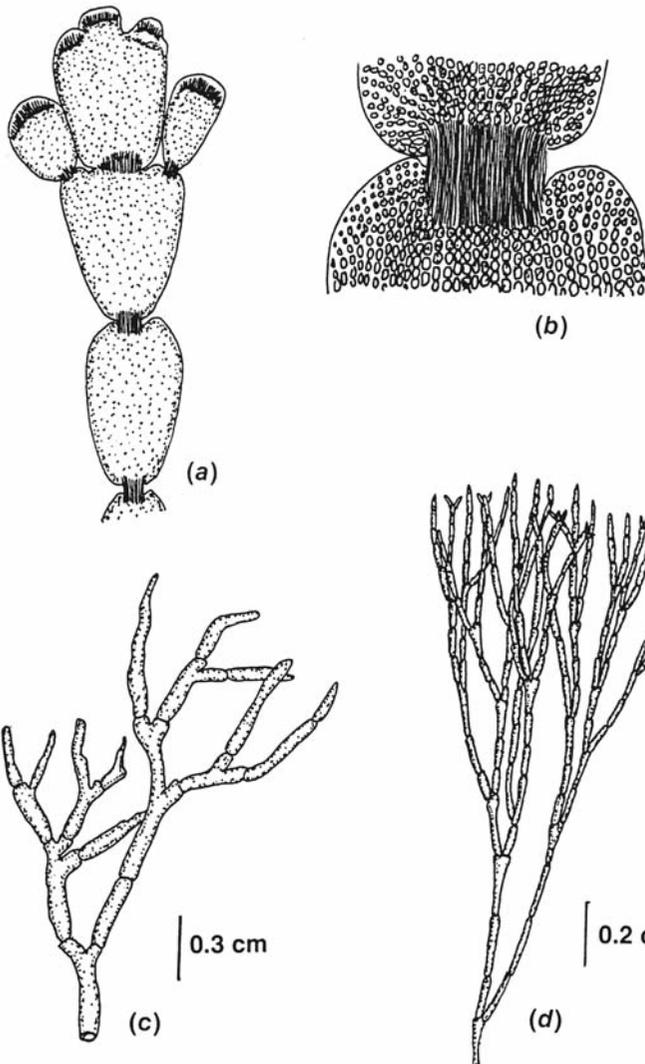
In the Corallinoideae the plants are multiaxial, having a medulla of elongated cells and a cortex of shorter cells (Fig. 4.38). Calcification normally occurs only in the cell walls of the cortical cells. The plants are composed of a number of calcified segments, each segment joined by a non-calcified joint. The segments consist of calcified cortical and non-calcified medullary



**Fig. 4.36** (Left) The coralline red alga *Mesophyllum alternans*. (Right) Section of the vegetative thallus showing the perithallus and hypothallus. (From Cabioch and Mendoza, 1998.)

**Fig. 4.37** (a) *Lithothamnion* sp.

(b) *L. lenormandi*: drawing of a section of thallus with a hypothallus (H) and perithallus (P). The section includes a mature conceptacle and carposporangia (C). ((a) after Oltmanns, 1904; (b) after Suneson, 1943.)

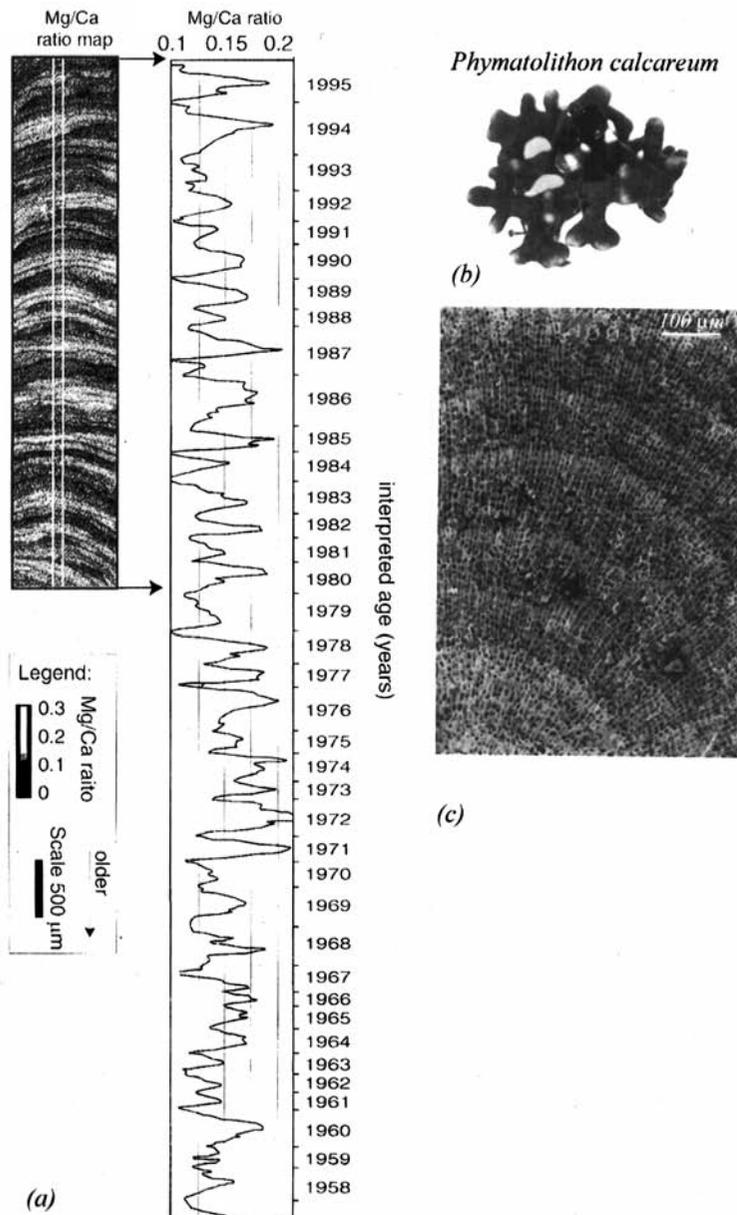
**Fig. 4.38** (a) *Corallina* sp. (b)

*Corallina* sp. showing non-calcified

joints and calcified segments. (c)

*Amphiroa rigida* var. *antillana*. (d)

*Jania rubens*. ((a),(b) after Oltmanns, 1904; (c),(d) after Taylor, 1957.)



**Fig. 4.39** (a) Left: A micrograph of a section of the thallus of *Lithothamnion glaciale* showing 16 yearly growth bands. Right: A 38-year record showing the cycles in the ratio of Mg/Ca cycles. Greater amounts of Mg (e.g., higher Mg/Ca ratios) occur during the summer months. (b) Photograph of the coralline red alga *Phymatolithon calcareum*. (c) Scanning electron micrograph of a longitudinal section of *Phymatolithon calcareum* showing banding. ((a) from Halfar et al., 2000; (b),(c) from Blake and Maggs, 2003.)

tissue, the arrangement of tissues giving the plants a certain amount of flexibility.

The crustose Corallinaceae occur in the intertidal zone, but only in areas that are not exposed to excessive drying, either on exposed rocks where they are kept moist by spray from breakers or in well-shaded areas. In some places they occur near the high-tide mark but are well covered by other algae. The sublittoral zone is a more favorable area for crustose algal growth, especially on reefs from

the low-tide mark to a depth of 25 to 30 m. The depth and agitation of the water have a considerable influence on the growth form of the coralline algae. Crustose types are present at all depths, but the highly ramified or branching forms occur only near the surface, where they are most plentiful down to 30 m. In the crustose forms, the thickest crusts are formed in shallow waters; the crusts become thinner with depth (as a result of thinner hypothalli and smaller cells), probably as a result

of slower growth. The crustose Corallinaceae are among the deepest-growing algae, down to 125 m in clear water. They are also among the longest-living, their life-span ranging from 10 to 50 years (Adey, 1970), probably as a result of their slow growth rate (0.3 to 3 mm year<sup>-1</sup>). The light saturation for photosynthesis for red crustose corallines was found by Adey (1970) to be between 700 and 1000 lux, which is considerably lower than the 4000- to 10000-lux saturation intensities found for other Rhodophyceae (Kanwisher, 1966; Brown and Richardson, 1968). These light saturation values are probably related to the great depth at which the crustose corallines are able to live.

Maërl is composed of shallow, subtidal deposits of calcareous red algae belonging to the Corallinales. Maërl has been obtained commercially for many years by dredging from the coast of Brittany in France, off Falmouth Harbor in England and Bantry Bay in Ireland. Maërl is placed on acidic soil to increase the pH of the soil for crops (Blunden et al., 1997).

The Corallinaceae form an important part of atolls and reefs (Dawson, 1966; Chisholm, 2003). The reefs are built up by the combined growth of coralline red algae (mostly species of *Porolithon*) and corals. When a reef first breaks the surface, the rigid, branched, brittle corals tend to break and fragment under severe surf action, whereas the massive coralline Rhodophyceae are unaffected by the pounding. In fact, the stronger the pounding, the faster they grow. The coralline reds thus grow into the breaking surf, developing upward and outward to form a rim slightly above sea level. While the corallines are forming the main framework of the reef, 90% of the reef comes to consist of sand and detritus cemented together by the plants and animals. Inside this rim of coralline Rhodophyceae, there is relative calm over the inner part of the reef. Within this inner area, carbonic acid, resulting from the solution of respiratory carbon dioxide produced by the plants and animals living there, tends to dissolve the solid calcareous materials. This dissolution of the inner part of the reef results in a central lagoon that stabilizes itself at a depth of 65 to 100 m.

The reef community is adapted to a low-stress environment characterized by the absence of significant seasonal change. The mean winter

temperature of the water where the reefs grow is between 27 and 29 °C, and the difference between the monthly mean temperatures is 3 °C or less. The water is clear (so the penetration of light is at a maximum), agitated, and of normal salinity. Even under these ideal circumstances many reef organisms (e.g., corals) do not grow at depths greater than 20 m.

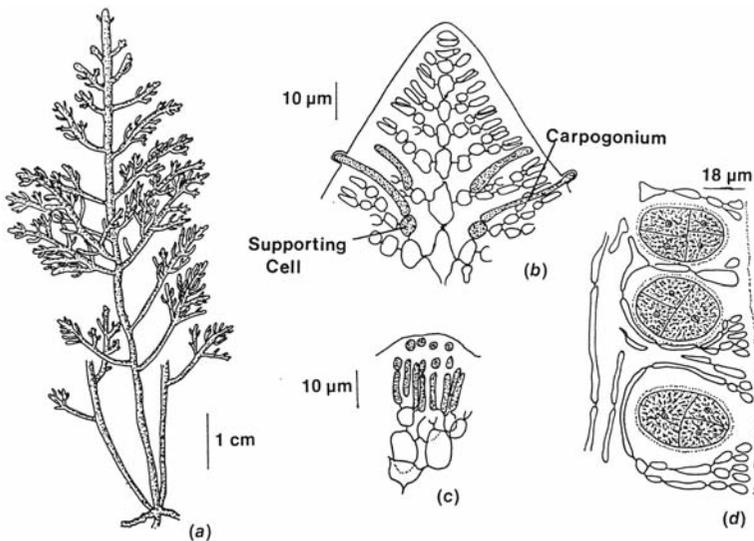
Non-articulated red algae grow at different rates during the year. This results in bands in the thallus (Fig. 4.39), similar to growth rings in trees. In some cases the banding is annual. Magnesium is present in greater quantities in warmer water, resulting in a higher concentration of magnesium in the portion of the band produced during the summer. The banding in coralline red algae can be an important **paleothermometer** since the algae can be up to 100 years old (Halfar et al., 2000; Blake and Maggs, 2003).

## Gelidiales

This is an order of uniaxial marine Rhodophyceae in which the carpogonial branch consists of a single cell, the carpogonium. After fertilization, the carpogonium may fuse with the supporting cell and/or nutritive filaments. The tetrasporophyte and the gametophyte are macroscopic plants, although not necessarily similar. The plants are commonly used in the production of agar, with almost half of the world supply coming from members of this order (Lewis and Hanisak, 1996).

No member of the order has had its life cycle completed in culture, but it is presumed from plants collected in the field that there is a triphasic life cycle of gametophyte, tetrasporophyte, and carposporophyte.

The gametophyte and tetrasporophyte of *Gelidium* have a dome-shaped apical cell that cuts off daughter cells basipetally (Fig. 4.40). The daughter cells divide to form a thallus that soon loses its uniaxial nature in the mature parts. The carpogonia are usually formed on special **ramuli** (branches) with a deep apical notch (Fig. 4.41(a)), behind which there is a depression on both surfaces (Figs. 4.40(b), 4.41(a), (b)). The carpogonia are produced in these depressions. The carpogonium is cut off from a cell beneath the surface of the thallus and has a long trichogyne that reaches to the outside of the thallus. The carpogonial branch



**Fig. 4.40** *Gelidium cartilagineum* var. *robustum*. (a) Whole plant. (b) Section of fertile apex of branch showing apical cell, carpegonia, and supporting cells. (c) Section of spermatangial area. (d) Section of tetrasporangial area. ((a) after Smith, 1969; (b),(c) after Fan, 1961; (d) after Smith, 1938.)

thus consists of a single cell. Nutritive filaments are cut off from the cells at the base of each of the laterals in the fertile area. After fertilization, the carpogonium may fuse with the supporting cell and/or nutritive filaments, with the gonimoblast filaments and carposporangia developing from this fusion cell. Male plants are similar in morphology to female plants, with the spermatangial areas forming irregular patches on the thalli. The cortical cells of a fertile area elongate, fade in color, and become transformed into spermatangial mother cells (Fig. 4.40(c)). The colorless spermatangia are formed by transverse division of the mother cell. The tetrasporangial mother cell is a cortical cell that is terminal on a lateral. It divides to produce four tetraspores in either a cruciate or a tetrahedral arrangement (Fig. 4.40(d)).

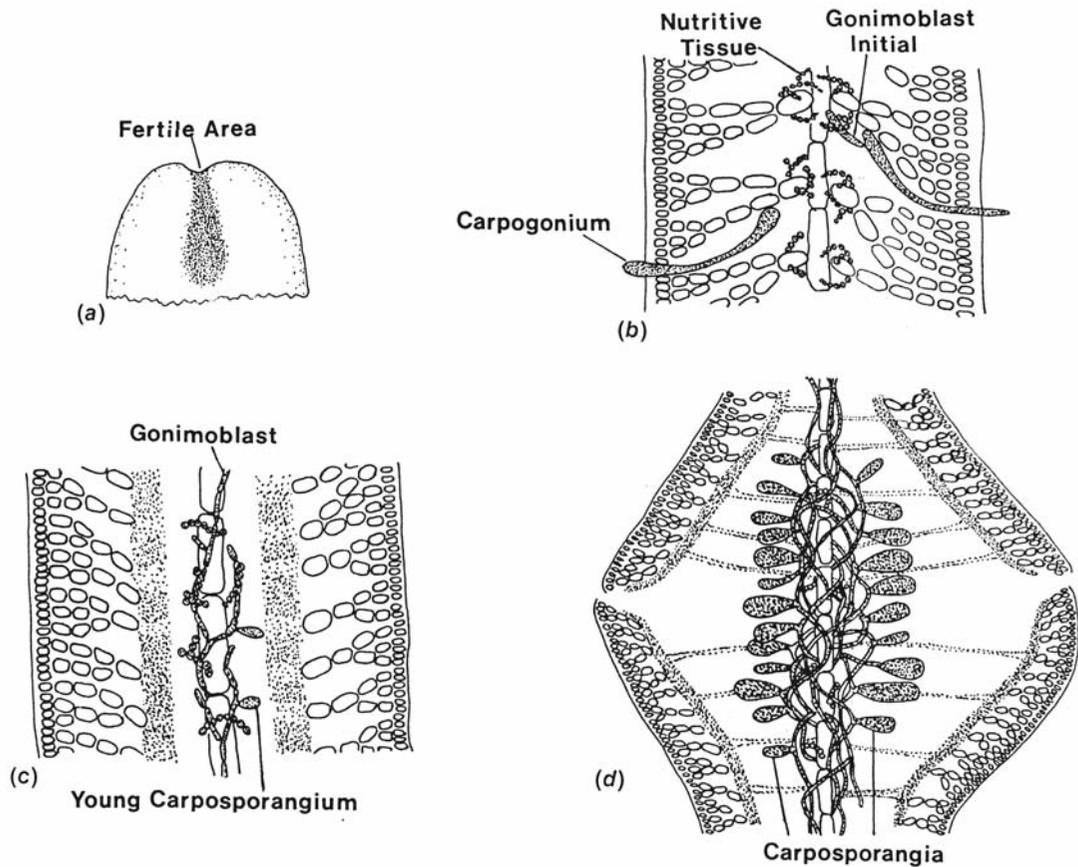
### Gracilariales

The Gracilariales are agarophytes that have a female reproductive system with a supporting cell of intercalary origin that bears a two-celled carpogonial branch flanked by two or more sterile branches (Fredericq and Hommersand, 1989).

The plants in the order are fleshy, having a tendency to be flattened or foliose with pseudo-parenchymatous tissues that lack filamentous cells in the mature vegetative thallus. The principal genus in the family is *Gracilaria*, a widely distributed northern lithophyte found at low-tide level and below, with about 100 species. The

dark-red thallus grows by means of a two-sided apical cell and has tapering branches (Fig. 4.42). There are large isodiametric cells in the medulla, with small cortical cells containing a number of ribbon-shaped chloroplasts. Unicellular hairs arise from enlarged peripheral cells that become multinucleate as they age.

The gametophytic plants are either male or female, and equal numbers of each are produced from tetraspores (Kain and Destombe, 1995). The male plants produce spermatia in antheridial pits over the surface of the thalli. The female plants form supporting cells from the outer layer of the large cells of the medulla (Kylin, 1930), the supporting cells producing the two-celled carpogonial branch and a number of laterals, a cell of which functions as the auxiliary cell. All of the cells of the procarp become multinucleate and develop into nutritive cells except for the carpogonium and the cell beneath it. After fertilization the carpogonium fuses with one of the nutritive cells that is acting as an auxiliary cell. Subsequently, this fusion cell fuses with the other multinucleate nutritive cells. At the same time, the cortical cells above the procarp divide to produce the cystocarp walls, the inner cells of which constitute nutritive cells. Gonimoblast initials are cut off from the fusion cell and develop into an inner sterile area that supports the outer carposporangia. The carposporangia ripen successively from the outside in. In some



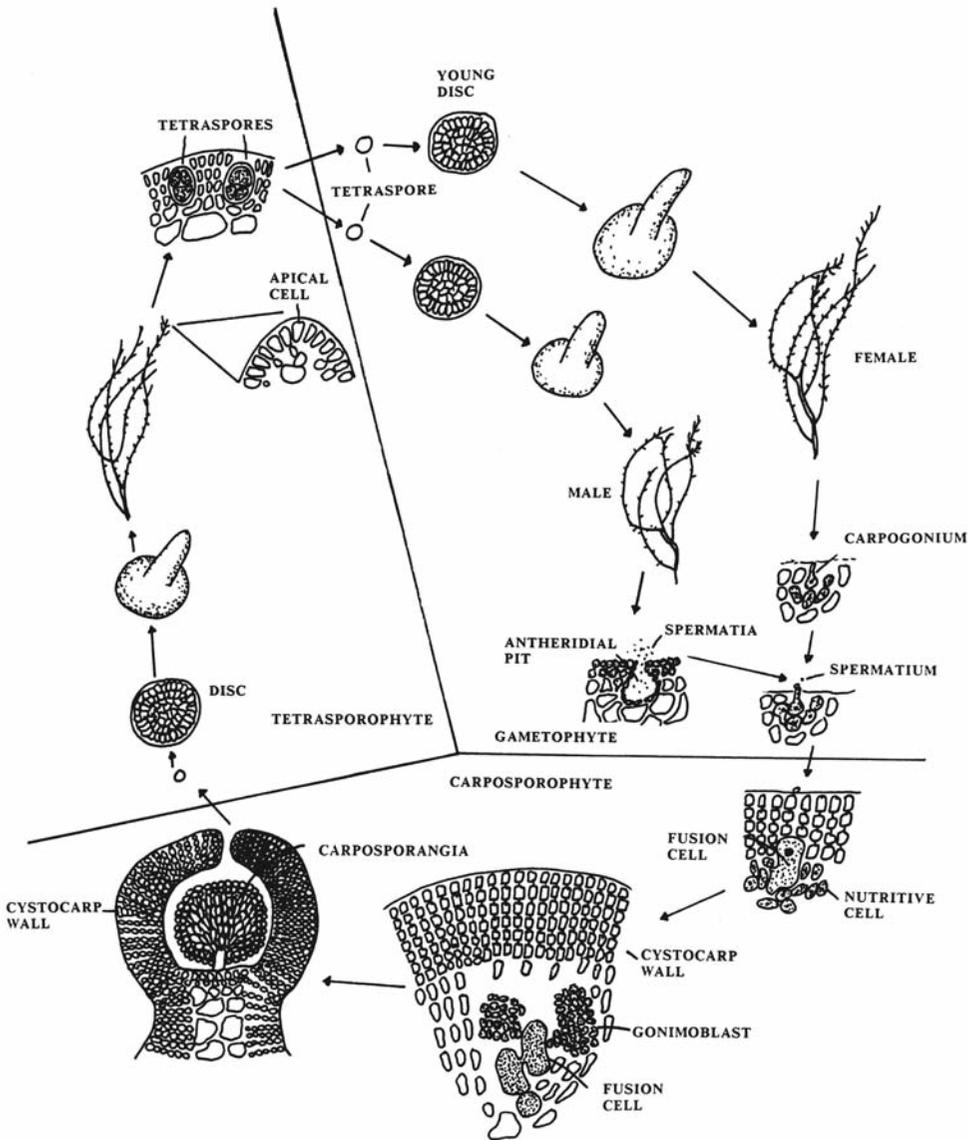
**Fig. 4.41** *Gelidium cartilagineum*. (a) Apex of fertile thallus. (b) Longitudinal section of thallus showing carpogonium. (c) Gonimoblast producing young carposporangia. (d) Carposporophyte with mature carposporangia. ((a) after Kylin, 1928; (b–d) after Smith, 1938.)

species, elongate cells radiate from the compact regions of the gonimoblast, penetrating the **pericarp** (cystocarp wall), and become connected with the cells of the pericarp.

The carpospores germinate to produce a parenchymatous disc that forms the tetrasporophyte as an erect protuberance. The tetrasporophyte is morphologically similar to the gametophyte and about the same size as the female gametophyte. Cruciate tetrasporangia are formed terminally on laterals in the cortex and are embedded in the thallus. The tetraspores germinate to form a parenchymatous disc that produces the gametophyte as an erect protuberance (Ogata et al., 1972).

*Gracilaria* is a major agarophyte, currently providing greater than half of the world's supply of agar. The cultivation of *Gracilaria*, both in the sea and in tanks, has been a principal factor in making this genus a source of agar-containing seaweeds (Lewis and Hanisak, 1996). In Taiwan, *Gracilaria* is farmed in brackish-water ponds as a main food source for the cultivation of the small abalone *Haliotis* (Lee, 1999).

Human consumption of species of the red alga *Gracilaria* has been linked to “**ogonori**” poisoning (Noguchi et al., 1994; Smit, 2004). The symptoms are hypotension (abnormally low blood pressure), vomiting, nausea, and death resulting from hypotensive shock. Ogonori poisoning is caused by prostaglandin  $E_2$  (Fig. 4.43). Soaking *Gracilaria* in freshwater results in the production of prostaglandin  $E_2$ . This is usually compounded by eating seafood which is rich in prostaglandin  $E_2$ .

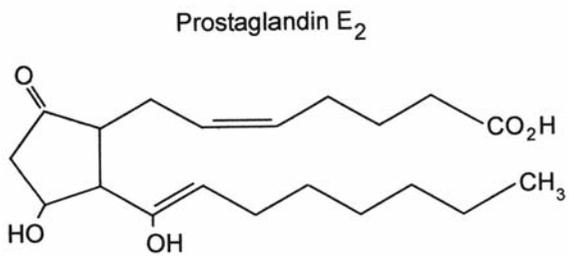


**Fig. 4.42** The life cycle of *Gracilaria* spp. (Adapted from Kylin, 1930; Ogata et al., 1972.)

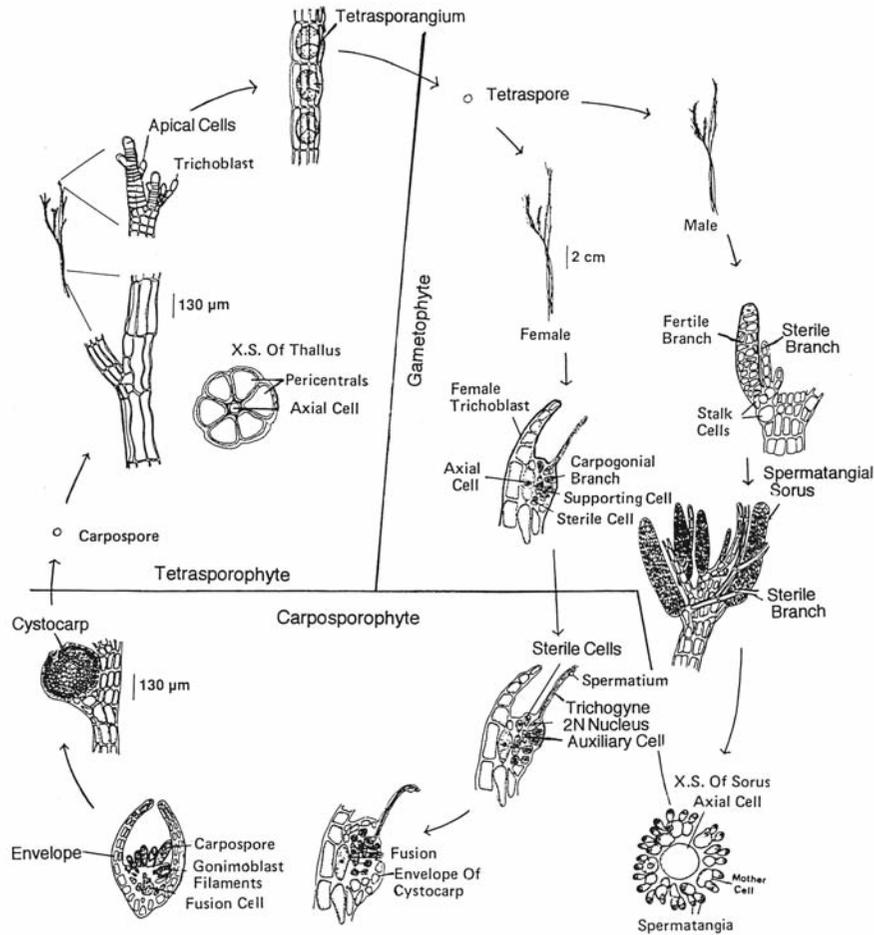
**Ceramiales**

These plants have the auxiliary cell cut off after fertilization and borne on the supporting cell of the four-celled carpo gonial filament. Most of the plants are relatively delicate filamentous or membranous forms.

In *Polysiphonia* the uninucleate, dome-shaped apical cell (Fig. 4.45(b)) is polyploid and contains 64 to 128 times the amount of DNA in most of the mature cells in the alga (Goff and Coleman, 1986).



**Fig. 4.43** The chemical structure of prostaglandin E<sub>2</sub>.

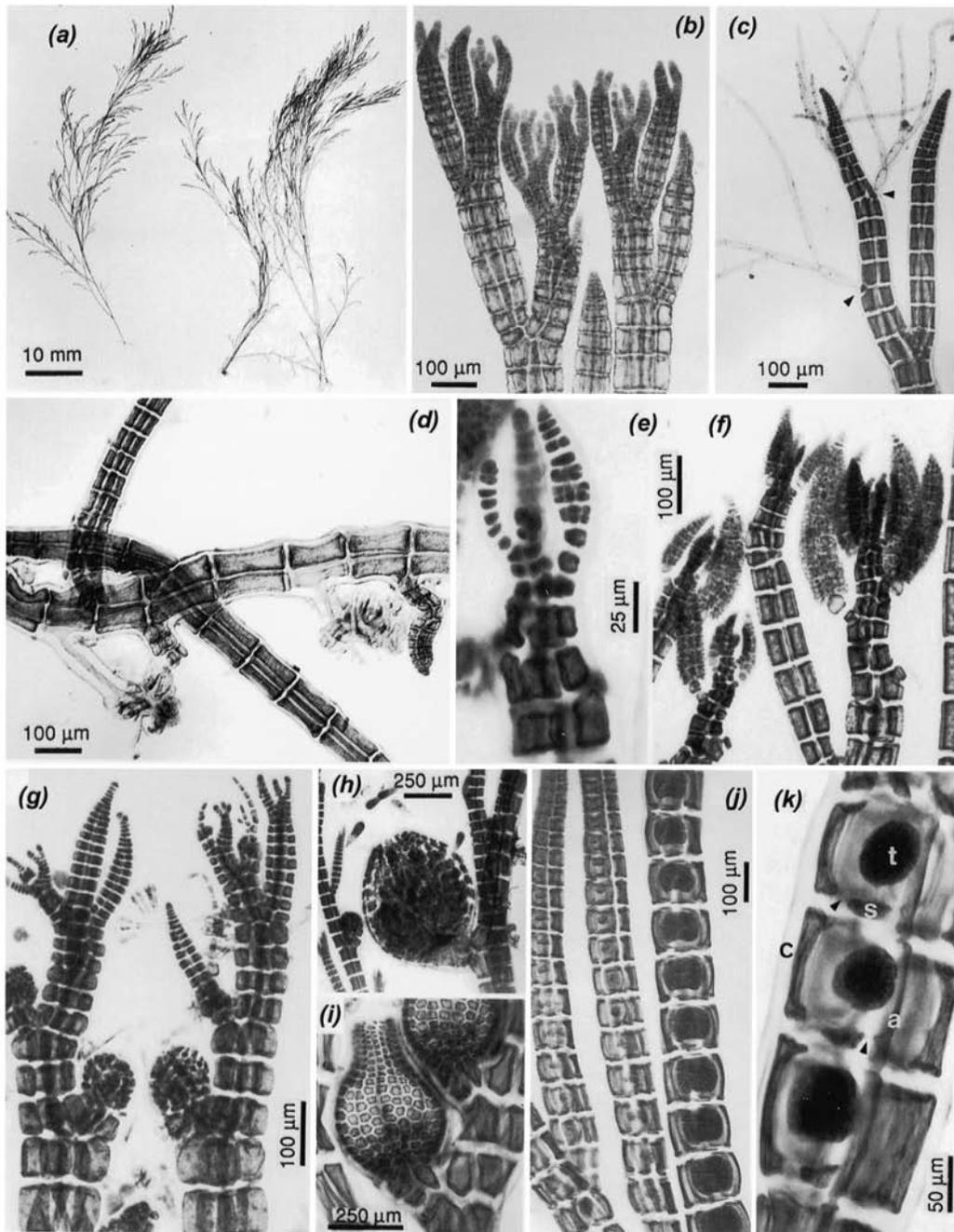


**Fig. 4.44** The life cycle of *Polysiphonia* sp. (Adapted from Kylin, 1923; Edwards, 1970a.)

Division of the cells derived from the apical cell is usually not accompanied by DNA replication; therefore, the farther the daughter cell is from the apical cell, the lower the ploidy of the cell, until the ploidy number stabilizes at  $1n$ . The apical cell forms daughter cells that produce lateral branches before dividing longitudinally into central and pericentral cells (Figs. 4.44, 4.45). The pericentrals are the same length as the axial cells. The lateral branches are of two kinds: the ordinary branches and the trichoblasts. The ordinary branches are polysiphonous with unlimited growth, similar to the main axis. The trichoblasts are uniseriate, usually colorless, and bear the sex organs (Fig. 4.45(c)). The trichoblasts progress

through a programmed cell death or **apoptosis** and drop off from the older parts of the thallus (Garbary and Clark, 2001).

*Polysiphonia* species occur either as lithophytes or as epiphytes on other algae. When the species grows on a solid substrate, some of the polysiphonous axes creep over the substratum to which they are firmly anchored by thick-walled, lobed rhizoids (Fig. 4.45(d)). When it grows as an epiphyte on another alga, the rhizoids penetrate that host tissue (Fig. 4.11). The procarys are produced near the base of a trichoblast (Figs. 4.44, 4.45(g)). The two basal cells of the trichoblast become polysiphonous, and a procarys develops from a pericentral of the upper of the two basal cells. The pericentral (supporting cell) produces in succession a lateral sterile cell, the four-celled carpogonial branch, and, last, the second sterile cell. The



**Fig. 4.45** Vegetative and reproductive structures of *Polysiphonia stricta*. (a) Habit of thalli from shallow subtidal bedrock. (b) Vegetative apices showing large apical cells with oblique branching. (c) Trichoblasts, composed of uniuucleate cells, attached by small scar cells (arrowheads). (d) Prostrate axis attached by unicellular rhizoids in open connection with pericentral cells. (e, f) Developing and mature spermatangial branches with sterile tips. (g) Tips

of female thallus with procarpus and early postfertilization cystocarps. (h) Mature globose cystocarp, with extruded pyriform carposporangia. (i) Mature cystocarp. (j) Developing and mature tetrasporangia in long straight rows. (k) Tetrasporangia (t), showing pit connections (arrowheads) between tetrasporangial stalk cells (s), central axial cells (a), and cover cells (c). (From Kim et al., 2000.)

sterile cells divide after fertilization and may have a nutritive role. The cells of the carpogonial branch (with the exception of the carpogonium) are commonly binucleate (Kylin, 1923). A large area of endoplasmic reticulum extends from one pit connection to the other pit connection in each cell of the carpogonial branch. This may be how the message of fertilization is transmitted down the carpogonial branch (Broadwater and Scott, 1982).

The male plants of *Polysiphonia* bear spermatangial sori on a trichoblast consisting of a two-celled stalk surmounted by the fertile regions (Figs. 4.44, 4.45(e), (f)). The upper stalk cell frequently bears a branch. Fertile regions become polysiphonous, and the two pericentrals divide copiously to form a compact layer of mother cells, each of which gives rise to two or three spermatangia (Kylin, 1923). After the spermatium fertilizes the trichogyne, the auxiliary cell is cut off from the supporting cell. The auxiliary cell then fuses with the carpogonium. The male nucleus fuses with the female in the carpogonium (Yamanouchi, 1906), and the diploid nucleus divides once. One of the diploid nuclei passes into the auxiliary cell, which subsequently fuses with the supporting cell. The fusion cell also fuses with the axial cell of the fertile segment. The gonimoblast initials are cut off from the fusion cell

and develop into a number of gonimoblast filaments. The terminal cells of these filaments develop into pear-shaped carposporangia. In the meantime, the fusion cell unites with the gonimoblast initial and the fertile cells. The sterile outer envelope of the cystocarp (Fig. 4.45(b)) originates from the other pericentrals of the axial cell that gave rise to the fertile pericentral that acted as the supporting cell. The young envelope consists of two lateral valves, composed of fused threads, which enclose the procarp like the shells of an oyster, the trichogyne alone projecting. After fertilization the two valves unite, and the envelope becomes two-layered. The carpospores form a tetrasporophyte, which is similar to the gametophyte, and which forms tetrahedrally arranged tetraspores (Fig. 4.45(j), (k)) in polysiphonous branches called **stichidia**. The tetraspores then germinate to form the gametophyte (Edwards, 1969).

*Polysiphonia denudata* completes its life history in 1.5 months in culture; thus the species probably has several life cycles each year (Edwards, 1970a). In some species of *Polysiphonia*, it is possible to influence stages of the life cycle by changing the photoperiod, but there appears to be no regularity among the different species (Edwards, 1970a,b).

In the marine environment, herbivorous damselfish exclude other fish from their territories, and maintain dense stands of filamentous algae. One damselfish, the dusky farmerfish (Fig. 4.46), is unique in that it maintains a monoculture of *Polysiphonia* in coral reefs by selective weeding of other indigestible algae (Hata and Kato, 2003). The farmerfish grazes on the *Polysiphonia* sp. The relationship between the farmerfish and *Polysiphonia* is an example of **mutualism** (a situation where two populations benefit equally).



**Fig. 4.46** The dusky farmerfish *Stegastes nigricans*, a cultivator of *Polysiphonia* in coral reefs.



**Fig. 4.47** Left: Heinrichs Leonhards Skuja. Right: Harald Kylin. (Photograph of Skuja from Wilen and Wingqvist, 1986; photograph of Kylin from *Die Gattung der Rhodophyceen*, CWK Gleerups Forlag, Lund, Sweden.)

**Heinrichs Leonhards Skuja** Born September 8, 1892 in Majori, Rigas-Jurmula, Latvia. Died July 19, 1972. During his youth, Dr. Skuja lived close to the Latvian coast and took an early interest in aquatic plants and animals. During World War I, he lived in the Caucasus, where he was engaged in floristic studies on the Apsheron Peninsula. In 1922, he began his academic studies at the faculty of natural sciences at the University of Latvia, passed a *mag. rer. nat.* examination in 1929, and became *dr. rer. nat.* in 1943. In the autumn of 1944, Dr. Skuja arrived in Sweden, where in 1947 he obtained a position as research professor (*laborator*) and in 1958 he was awarded the degree of *doctor honoris causa* by the University of Uppsala. Besides his extensive publications on the algae, Dr. Skuja also published in the areas of general botany, mycology, and lichenology.

**Harald Kylin** Born Johan Harald Olsson February 5, 1879 near Gothenburg, Sweden. Died 1949. He changed his name to Harald Kylin when he entered the gymnasium (high school) because his father, who was chairman of the parish council, thought Olsson was too common a name. He graduated from Uppsala University in 1901 with a M.Sc. degree and in 1905 with a Licentiate of Philosophy. In 1907 he defended his thesis on algae and became a docent of the University. In 1917 he obtained a chair in botany at the University of Lund and remained there until he retired in 1944. He wrote a large number of publications on the red algae. The last of these was his book *Die Gattung der Rhodophyceen*, which was finished by his wife after his death.



**Fig. 4.48** Gary W. Saunders (right in photograph, with Dr. Gerry Kraft) Born June 30, 1962 in Halifax, Nova Scotia. Dr. Saunders received his B.Sc. in 1985 and his M.Sc. in 1987 from Acadia University. He received his Ph.D. in 1991 from Simon Fraser University. In 1995 he joined the faculty in the Department of Biology at the University of New Brunswick in Canada where he is Professor and Director and Algal Curator of the Connell Memorial Herbarium. Dr. Saunders has been a leader in the field of molecular systematics of the red algae.

## REFERENCES

- Abdel-Rahman, M. H. (1982). The involvement of an endogenous circadian rhythm in photoperiodic timing in *Acrochaetium asparagopsis* (Rhodophyta, Acrochaetiales). *Br. Phycol. J.* 17:389–400.
- Adey, W. H. (1970). The effects of light and temperature on growth rates in boreal-subarctic crustose corallines. *J. Phycol.* 6:269–76.
- Arad, S., Dubinsky, O., and Simon, B. (1993). A modified cell wall mutant of the red microalga *Rhodella reticulata* resistant to the herbicide 2,6-dichlorobenzonitrile. *J. Phycol.* 29:309–13.
- Bailey, J. C., and Chapman, R. L. (1998). A phylogenetic study of the Corallinales (Rhodophyta) based on nuclear small-subunit rRNA gene sequences. *J. Phycol.* 34:692–705.
- Balakrishnan, M. S., and Chaugule, B. (1980). Cytology and life history of *Batrachospermum mahabaleshwarensis*. *Cryptogamie: Algologie* 1:83–97.
- Barrow, K. D., Karsten, U., King, R. J., and West, J. A. (1995). Floridoside in the genus *Laurencia* (Rhodomelaceae: Ceramiales) – a chemosystematic study. *Phycologia* 34:279–83.
- Belcher, J. H., and Swale, E. M. F. (1960). Some British freshwater material of *Asterocystis*. *Br. Phycol. Bull.* 2:33–5.
- Blake, C., and Maggs, C. A. (2003). Comparative growth rates and internal banding periodicity of maerl species (Corallinales, Rhodophyta) from northern Europe. *Phycologia* 42:606–12.
- Blunden, G., Campbell, S. A., Smith, J. R., Guiry, M. D., Hession, C. C., and Griffin, R. L. (1997). Chemical and physical characteristics of calcified red algal deposits known as maerl. *J. Applied Phycol.* 9:11–17.
- Borowitzka, M. A., Larkum, A. W. D., and Nockolds, C. E. (1974). A scanning electron microscope study of the structure and organization of the calcium carbonate deposits of algae. *Phycologia* 13:195–203.
- Bouarab, K., Potin, P., Correa, J., and Kloareg, B. (1999). Sulfated oligosaccharides mediate the interaction between a marine red alga and its green algal pathogenic endophyte. *Plant Cell* 11:1635–50.
- Bourgougnon, N., Lehay, M., Quemener, B., Chermann, J.-C., Rimbart, M., Cormaci, M., Furnari, G., and Kornprobat, J.-M. (1996). Annual variation in composition and *in vitro* anti-HIV-1 activity of the sulfated glucuronogalactan from *Schizymenia dubyi* (Rhodophyta, Gigartinales). *J. Applied Phycol.* 8:1155–61.
- Broadwater, S. T., and Scott, J. (1982). Ultrastructure of early development in the female reproductive system of *Polysiphonia harveyi* Bailey (Ceramiales, Rhodophyta). *J. Phycol.* 18:427–41.
- Brock, T. D. (1973). Lower pH limit for the existence of blue-green algae: evolutionary and ecological implications. *Science* 179:480–3.
- Brown, T. E., and Richardson, F. L. (1968). The effect of growth and environment on the physiology of the algae: Light intensity. *J. Phycol.* 4:38–54.
- Butterfield, N. J. (2000). *Bangiomorpha pubescens* n. gen., n. sp.: implications for the evolution of sex, multicellularity, the Mesoproterozoic/ Neoproterozoic radiation of eukaryotes. *Paleobiology* 26:386–404.
- Cabioch, J., and Mendoza, M. L. (1998). *Mesophyllum alternans* (Foslie) comb. nov. (Corallinales, Rhodophyta). A mediterraneo-atlantic species, and new considerations on the *Lithothamnion phillippi* Foslie complex. *Phycologia* 37:208–21.
- Campbell, S. E. (1980). *Palaeoconchocelis starmachii*, a carbonate boring micro-fossil from the Upper Silurian of Poland (425 million years old): implications for the evolution of the Bangiaceae (Rhodophyta). *Phycologia* 19:25–36.
- Chemin, E. (1937). Le développement des spores chez les Rhodophycées. *Rev. Gen. Bot.* 49:205–34, 300–27, 353–74, 424–48, 478–536.
- Chen, L. C.-M., Edelman, T., Ogata, E., and McLachlan, J. (1970). The life history of *Porphyra miniata*. *Can. J. Bot.* 48:385–9.
- Chen, L. C.-M., McLachlan, J., Neish, A. C., and Shacklock, P. F. (1973). The ratio of kappa- to lamda-carrageenan in nuclear phases of the Rhodophycean alga, *Chondrus crispus* and *Gigartina stellata*. *J. Mar. Biol. Assoc. UK* 53:11–16.
- Chiovitti, A., Liao, M.-L., Kraft, G. T., Munro, S. L. A., Craik, D. J., and Bacic, A. (1995). Cell wall polysaccharides from Australian red algae of the family Solieriaceae (Gigartinales, Rhodophyta): iota/kappa/beta-carrageenans from *Melanema dumosum*. *Phycologia* 34:522–7.
- Chisholm, J. R. M. (2003). Primary productivity of reef-building crustose coralline algae. *Limnol. Oceanogr.* 48:1376–87.
- Craigie, J. S., Correa, J. A., and Gordon, M. E. (1992). Cuticles from *Chondrus crispus*. *J. Phycol.* 28:777–86.
- Cunningham, E. M., and Guiry, M. D. (1989). A circadian rhythm in the long-day photoperiodic induction of erect axis development in the marine red alga *Nemalion helminthoides*. *J. Phycol.* 25:705–12.
- Dawson, E. Y. (1966). *Marine Botany*. New York: Holt, Rinehart and Winston.
- de Nys, R., Steinberg, P. D., Willemsen, P., Dworjanyan, S. A., Gabelish, C. L., and King, R. J. (1995). Broad

- spectrum effects of secondary metabolites from the red alga *Delisea pulchra* in antifouling assays. *Biofouling* 8:259–71.
- den Hartog, C. (1971). The effect of the salinity tolerance of algae on their distribution, as exemplified by *Bangia*. *Proc. 7th Int. Seaweed Symp.*, pp. 274–6.
- Digby, P. S. B. (1977a). Growth and calcification in the coralline algae, *Clathromorphum circumscriptum* and *Corallina officinalis*, and the significance of pH in relation to precipitation. *J. Mar. Biol. Assoc. UK* 57:1095–109.
- Digby, P. S. B. (1977b). Photosynthesis and respiration in the coralline algae, *Clathromorphum circumscriptum* and *Corallina officinalis* and the metabolic basis of calcification. *J. Mar. Biol. Assoc. UK* 57:1111–24.
- Dixon, P. S. (1973). *Biology of the Rhodophyta*, University Reviews in Biology No. 4. Edinburgh: Oliver and Boyd.
- Dixon, P. S., and Richardson, W. N. (1970). Growth and reproduction in red algae in relation to light and dark cycles. *Ann. N.Y. Acad. Sci.* 175:764–77.
- Dring, M. J. (1967a). Effects of daylength on growth and reproduction of the *Conchocelis*-phase of *Porphyra tenera*. *J. Mar. Biol. Assoc. UK* 47:501–10.
- Dring, M. J. (1967b). Phytochrome in red alga, *Porphyra tenera*. *Nature* 215:1411–12.
- Dring, M. J., and West, J. A. (1983). Photoperiodic control of tetrasporangium formation in the red alga *Rhodochorton purpureum*. *Planta* 159:143–50.
- Edwards, P. (1969). The life history of *Callithamnion byssoides* in culture. *J. Phycol.* 5:266–8.
- Edwards, P. (1970a). Field and cultural observations on the growth and reproduction of *Polysiphonia denudata* from Texas. *Br. Phycol. J.* 5:145–53.
- Edwards, P. (1970b). Field and cultural studies on the seasonal periodicity of growth and reproduction of selected Texas benthic marine algae. *Contrib. Mar. Sci. Univ. Texas* 14:59–114.
- Evans, L. V. (1970). Electron microscopical observations on a new red algal unicell, *Rhodella maculata* gen. nov., sp. nov., *Br. Phycol. J.* 5:1–13.
- Evans, L. V., Callow, J. A., and Callow, M. E. (1973). Structural and physiological studies on the parasitic red alga *Holmsella*. *New Phytol.* 72:393–402.
- Fan, K-C. (1961). Studies on *Hypneocolax*, with a discussion on the origin of parasitic red algae. *Nova Hedwigia* 3:119–28.
- Feldmann, G. (1970a). Sur l'ultrastructure des corps irisants des *Chondria* (Rhodophycées). *C. R. Séances Acad. Sci. Paris* 270:945–50.
- Feldmann, G. (1970b). Sur l'ultrastructure de l'appareil irisant du *Gastroclonium clavatum* (Roth.) Ardissonne (Rhodophyceae). *C. R. Séances Acad. Sci. Paris* 270:1244–6.
- Feldmann, J. (1953). L'évolution des organes femelles chez les Floridées. *Proc. 1st Int. Seaweed Symp.*, pp. 11–12.
- Fetter, R., and Neushul, M. (1981). Studies on developing and released spermatia in the red alga, *Tiffaniella snyderae* (Rhodophyta). *J. Phycol.* 17:141–59.
- Fredericq, S., and Hommersand, M. H. (1989). Proposal of the Gracilariales ord. nov. (Rhodophyta) based on an analysis of the reproductive development of *Gracilaria verrucosa*. *J. Phycol.* 25:213–19.
- Frei, E., and Preston, R. D. (1964). Non-cellulosic structural polysaccharides in algal cell walls. II. Association of xylan and mannan in *Porphyra umbilicalis*. *Proc. R. Soc. Lond. [B]* 160:314–27.
- Freshwater, D. W., Fredericq, S., Butler, B., Hommersand, M. H., and Chase, M. W. (1994). A gene phylogeny of the red algae (Rhodophyta) based on plastid *rbcL*. *Proc. Natl. Acad. Sci., USA* 91:7281–5.
- Fries, L. (1963). On the cultivation of axenic red algae. *Physiol. Plant.* 16:695–708.
- Fries, L. (1967). The sporophyte of *Nemalion multifidum* (Weber et J. Ag.). *Sven. Bot. Tidskr.* 61:457–62.
- Gabrielson, P. W., Garbary, D. J., and Scagel, R. F. (1985). The nature of the ancestral red alga: Inferences from a cladistic analysis. *BioSystems* 18:335–46.
- Gantt, E. (1969). Properties and ultrastructure of phycoerythrin from *Porphyridium cruentum*. *Plant Physiol.* 44:1629–38.
- Gantt, E., and Conti, S. F. (1965). The ultrastructure of *Porphyridium cruentum*. *J. Cell Biol.* 26:365–81.
- Garbary, D. J., and Clarke, B. (2001). Apoptosis in trichoblast development in *Polysiphonia harveyi* (Rhodophyta). *Phycologia* 40:324–9.
- Gargiulo, G. M., Genovese, M., Morabito, M., Culosa, F., and de Mase, E. (2001). Sexual and asexual reproduction in a freshwater population of *Bangia atropurpurea* (Bangiales, Rhodophyta) from eastern Sicily (Italy). *Phycologia* 40:88–96.
- Gerwick, W. H., and Lang, N. J. (1977). Structural, chemical and ecological studies on iridescence in *Iridaea* (Rhodophyta). *J. Phycol.* 13:121–7.
- Giraud, G. (1962). Les infrastructures de quelques algues et leur physiologie. *J. Microscopie* 1:251–64.
- Goff, L. J., and Coleman, A. W. (1984). Transfer of nuclei from a parasite to a host. *Proc. Natl. Acad. Sci. USA* 81:5420–4.

- Goff, L. J., and Coleman, A. W. (1986). A novel pattern of apical cell polyploidy, sequential polyploidy reduction and intercellular nuclear transfer in the red alga *Polysiphonia*. *Am. J. Bot.* 73:1109–30.
- Goff, L. J., Moon, D. A., Nyvall, P., Stache, B., Mangin, K., and Zuccarello, G. (1996). The evolution of parasitism in the red algae: molecular comparisons of adelphoparasites and their hosts. *J. Phycol.* 32:297–312.
- Goreau, T. F. (1963). Calcium carbonate deposition by coralline algae and coral in relation to their roles as reef builders. *Ann. N.Y. Acad. Sci.* 109:127–67.
- Gretz, M. R., Aronson, J. M., and Sommerfeld, M. R. (1980). Cellulose in the cell walls of the Bangiophyceae (Rhodophyta). *Science* 207:779–81.
- Gretz, M. R., McCandless, E. L., Aronson, J. M., and Sommerfeld, M. R. (1983). The galactan sulfates of the *Conchocelis* phases of *Porphyra leucostricta* and *Bangia atropurpurea* (Rhodophyta). *J. Exp. Bot.* 34:705–11.
- Gross, W., Heilmann, I., Lenze, D., and Schnarrenberger, C. (2001). Biogeography of the Cyanidiaceae (Rhodophyta) based on 18S ribosomal RNA sequence data. *Eur. J. Phycol.* 36:275–80.
- Halfar, J., Zack, T., Kronz, A., and Zachos, J. C. (2000). Growth and high-resolution paleoenvironmental signals of rhodoliths (coralline red algae): a new biogenic archive. *J. Geophys. Res.* 105:107–22.
- Hara, Y. (1971). An electron microscopic study on the chloroplasts of the Rhodophyta. *Proc. 7th Int. Seaweed Symp.*, pp. 153–8.
- Harlin, M. M., and Craigie, J. S. (1975). The distribution of photosynthate in *Ascophyllum nodosum* as it relates to epiphytic *Polysiphonia lanosa*. *J. Phycol.* 11:109–13.
- Harper, J. T., and Saunders, G. W. (2001). Molecular systematics of the Florideophycidae (Rhodophyta) using nuclear large and small subunit rDNA sequence data. *J. Phycol.* 32:1073–82.
- Hata, H., and Kato, M. (2003). Demise of monocultural algal farms by exclusion of territorial damselfish. *Mar. Ecol. Progr. Ser.* 263:159–167.
- Hawkes, M. W. (1978). Sexual reproduction in *Porphyra gardneri* (Smith et Hollenberg) Hawkes (Bangiales, Rhodophyta). *Phycologia* 17:329–53.
- Holmes, M. J., and Brodie, J. (2004). Morphology, seasonal phenology and observations on some aspects of the life history in culture of *Porphyra dioica* (Bangiales, Rhodophyta) from Devon, U.K. *Phycologia* 43:176–88.
- Hurdelbrink, L., and Schwantes, H. O. (1972). Sur le cycle de développement de *Batrachospermum*. *Mem. Soc. Bot. Fr.*, 269–74.
- Johansen, H. W. (1981). *Coralline Algae: A First Synthesis*. Boca Raton, Florida: CRC Press.
- Johnson, J. (1961). *Limestone-Building Algae and Algal Limestones*. Colorado School of Mines.
- Kain, J. M., and Destombe, C. (1995). A review of the life history, reproduction and phenology of *Gracilaria*. *J. Appl. Phycol.* 7:269–81.
- Kanwisher, J. W. (1966). Photosynthesis and respiration in some seaweeds. In *Some Contemporary Studies in Marine Science*, ed. R. Barnes, pp. 407–20. New York: Academic Press.
- Karsten, U., West, J. A., Zuccarello, G. C., et al. (2003). Low molecular weight carbohydrates of the Bangiophycidae (Rhodophyta). *J. Phycol.* 39:548–9.
- Kim, G. H., and Fritz, L. (1993). Ultrastructure and cytochemistry of early spermatangial development in *Antithamnion nipponicum* (Ceramiaceae, Rhodophyta). *J. Phycol.* 29:797–805.
- Kim, G. H., and Kim, S.-H. (1999). The role of F-actin during fertilization in the red alga *Aglaothamnion oosumense* (Rhodophyta). *J. Phycol.* 35:806–14.
- Kim, M.-S., Maggs, C. A., McIvor, L., and Guiry, M. D. (2000). Reappraisal of the type species of *Polysiphonia* (Rhodomelaceae, Rhodophyta). *Eur. J. Phycol.* 35:83–92.
- Kornmann, P. (1994). Life histories of monostomatic *Porphyra* species as a basis for taxonomy and classification. *Eur. J. Phycol.* 29:69–71.
- Kugrens, P. (1974). Light and electron microscope studies on the development and liberation of *Janczewskia gardneri* Setch. spermatia (Rhodophyta). *Phycologia* 13:295–306.
- Kugrens, P., and West, J. A. (1972a). Ultrastructure of spermatial development in the parasitic red alga *Levringiella gardneri* and *Erythrocytis saccata*. *J. Phycol.* 8:331–43.
- Kugrens, P., and West, J. A. (1972b). Ultrastructure of tetrasporogenesis in the parasitic red alga *Levringiella gardneri* (Setchell) Kylin. *J. Phycol.* 8:370–83.
- Kugrens, P., and West, J. A. (1973). The ultrastructure of an alloparasitic red alga *Choreocolax polysiphoniae*. *Phycologia* 12:175–86.
- Kugrens, P., and West, J. A. (1974). The ultrastructure of carposporogenesis in the marine hemiparasitic red alga *Erythrocytis saccata*. *J. Phycol.* 10:139–47.
- Kurihara, A., Arai, S., Shimada, S., and Masuda, M. (2005). The conspecificity of *Galaxura apiculata* and *G. hystix* (Nemaliales, Rhodophyta) inferred from

- comparative morphology and rbcL and ITS1 sequences. *Eur. J. Phycol.* 40:39–52.
- Kylin, H. (1916). Über *Spermothamnion roseolum* (Ag.) Pringsh. und *Trailiella intricata* Batters. *Bot. Not.*, 83–92.
- Kylin, H. (1923). Studien über die Entwicklungsgeschichte der Florideen. *Sven. Vet. Akad. Handl.* 63, No. 11.
- Kylin, H. (1924). Bemerkungen über einige *Ceramium*-Arten. *Bot. Not.*, 443–52.
- Kylin, H. (1928). Entwicklungsgeschichtliche Florideenstudien. *Lunds Univ. Arsskr. N.F. II* 24, No. 4.
- Kylin, H. (1930). Über die Entwicklungsgeschichtliche der Florideen. *Lunds Univ. Arsskr. N.F. II* 26, No. 6.
- Kylin, H. (1956). *Die Gattungen der Rhodophyceen*. Gleerups, Lund.
- Lahaye, M. (2001). Developments on gelling algal galatans, their structure and physico-chemistry. *J. Appl. Phycol.* 13:173–84.
- Largo, D. B., Fukami, K., Nishijima, T., and Ohno, M. (1995). Notes on thalli whitening called *ice-ice* in red algae, *Eucheuma/Kappaphycus* and *Gracilaria*. *Bull. Mar. Sci. Fish., Kochi Univ.* No. 15: 39–42.
- Lee, R. E. (1971). The pit connections of some lower red algae: Ultrastructure and phylogenetic significance. *Br. Phycol. J.* 6:29–38.
- Lee, R. E. (1974). Chloroplast structure and starch grain production as phylogenetic indicators in the lower Rhodophyceae. *Br. Phycol. J.* 9:291–5.
- Lee, R. E., and Fultz, S. A. (1970). The ultrastructure of the *Conchocelis* stage of the marine red alga *Porphyra leucosticta*. *J. Phycol.* 6:22–8.
- Lee, T.-M. (1999). Changes in activities and properties of biosynthetic enzymes in relation to high-temperature-induced proline accumulation in *Gracilaria tenuistipitata* (Gigartinales, Rhodophyta). *Phycologia* 37:433–8.
- Lewin, R. A., and Robertson, J. A. (1971). Influence of salinity on the form of *Asterocytis* in pure culture. *J. Phycol.* 7:236–8.
- Lewis, R. J., and Hanisak, M. D. (1996). Effects of phosphate and nitrate supply on productivity, agar content and physical properties of agar of *Gracilaria* strain G-16S. *J. Appl. Phycol.* 8:41–9.
- Lichtlé, C., and Giraud, G. (1969). Etude ultrastructurale de la zone apicale du thalle du *Polysiphonia elongata* (Harv.) Rhodophyceé, Floridée. Evolution des plastes. *J. Microscopie* 8:867–87.
- Lin, H., Sommerfeld, M. R., and Swafford, J. R. (1975). Light and electron microscope observations on motile cells of *Porphyridium purpureum* (Rhodophyta). *J. Phycol.* 11:452–7.
- Liu, Y. L., Ross, N., Lanthier, P., and Reith, M. (1996). A gametophyte cell wall protein of the red alga *Porphyra purpurea* (Rhodophyta) contains four apparent polysaccharide-binding domains. *J. Phycol.* 32:995–1003.
- Magruder, W. H. (1984). Specialized appendages on spermatia from the red alga *Aglaothamnion neglectum* (Ceramiales, Ceramiaceae) specifically bind with trichogynes. *J. Phycol.* 20:436–40.
- Marquardt, J., Schultze, A., Rosenkrau, V., and Wehrmeyer, W. (1999). Ultrastructure and photosynthetic apparatus of *Rhodella violaceae* (Porphyridiales, Rhodophyta) grown under iron-deficient conditions. *Phycologia* 38:418–27.
- Matsuzaki, M., and 41 other authors (2004). Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. *Nature* 428:653–7.
- Melo, R. A. (1998). *Gelidium* commercial exploitation: natural resources and cultivation. *J. Appl. Phycol.* 10:303–14.
- Mine, K., Kubouchi, Y., and Okuda, K. (2003). Fine structure of spermatial surface in the red alga *Antithamnion nipponicum* (Rhodophyta). *Phycol. Res.* 51:109–17.
- Mizuta, H., Yasui, H., and Saga, N. (2003). A simple method to mass produce monospores in the thallus of *Porphyra yezoensis* Ueda. *J. Appl. Phycol.* 15:345–9.
- Mollet, J. C., Rahaoui, A., and Lemoine, Y. (1998). Yields, chemical composition and gel strength of agarocolloids of *Gracilaria gracilis*, *Gracilariopsis longissima* and newly reported *Gracilaria* cf. *vermiculophylla* from Roscoff (Brittany, France). *J. Appl. Phycol.* 10:59–66.
- Mukai, L. S., Craigie, J. S., and Brown, R. G. (1981). Chemical composition and structure of the cell walls of the *Conchocelis* and thallus phases of *Porphyra tenera* (Rhodophyceae). *J. Phycol.* 17:192–8.
- Mumford, T. F., and Miura, A. (1988). *Porphyra* as food: cultivation and economics. In *Algae and Human Affairs*, ed. C. A. Lembi and J. P. Waaland, pp. 87–117. Cambridge: Cambridge University Press.
- Murakami, A., Miyashita, H., Iseki, M., Adachi, K., and Mimuro, M. (2004). Chlorophyll *d* in an epiphytic cyanobacterium of red algae. *Science* 303:1633.
- Necchi, O., and Jimenez, J. C. (2002). Somatic meiosis and development of the juvenile gametophyte in members of the Batrachospermales *sensu lato* (Rhodophyta). *Phycologia* 41:340–7.
- Ngan, Y., and Price, I. R. (1979). Systematic significance of spore size in the Florideophycidae (Rhodophyta). *Br. Phycol. J.* 14:285–303.

- Noguchi, T., Matsui, T., Miyazawa, K., et al. (1994). Poisoning by the red alga "ogonori" (*Gracilaria verrucosa*) on the Nojima Coast, Yokohama, Kanagawa Prefecture, Japan. *Toxicon* 32:1533–8.
- Ogata, E., Matsui, T., and Nakamura, H. (1972). The life cycle of *Gracilaria verrucosa* (Rhodophyceae, Gigartinales) in vitro. *Phycologia* 11:75–80.
- Ohno, M., Nang, H. Q., and Hirase, S. (1996). Cultivation and carrageenan yield and quality of *Kappaphycus alvarezii* in the waters of Vietnam. *J. Applied Phycol.* 8:431–7.
- Ohta, N., Sato, N., Ueda, K., and Kuroiwa, T. (1997). Analysis of a plastid gene cluster reveals a close relationship between *Cyanidioschyzon* and *Cyanidium*. *J. Plant Res.* 110:235–45.
- Okazaki, M., Ikawa, T., Furuya, K., Nisizawa, K., and Miwa, T. (1970). Studies on calcium carbonate deposition of a calcareous red alga *Serraticardia maxima*. *Bot. Mag. Tokyo* 83:193–201.
- Oliveira, M. C., and Bhattacharya, D. (2000). Phylogeny of the Bangiophycidae (Rhodophyta) and the secondary endosymbiotic origin of algal plastids. *Amer. J. Bot.* 87:482–92.
- Oltmanns, F. (1904). *Morphologie und Biologie der Algen*, Bd. I. Jena.
- Pallaghy, C. K., Minchinton, J., Kraft, G. T., and Wetherbee, R. (1983). Presence and distribution of bromine in *Thysanocladia densa* (Solieriaceae, Gigartinales), a marine red alga from the Great Barrier Reef. *J. Phycol.* 19:204–8.
- Papenfuss, G. F. (1945). Review of the *Acrochaetium*–*Rhodochorton* complex of the red algae. *Univ. Calif. Publ. Bot.* 18:299–334.
- Papenfuss, G. F. (1947). Further contributions toward an understanding of the *Acrochaetium*–*Rhodochorton* complex of the red algae. *Univ. Calif. Publ. Bot.* 18:433–47.
- Papenfuss, G. F. (1966). A review of the present system of classification of the Florideophycidae. *Phycologia* 5:247–55.
- Pearse, V. B. (1972). Radioisotopic study of calcification in the articulated coralline alga *Bossiella orbigniana*. *J. Phycol.* 8:88–97.
- Pedersen, M. E. E., Roomans, G. M., and v. Hofsten, A. (1981). Bromine in the cuticle of *Polysiphonia nigrescens*: Localization and content. *J. Phycol.* 17:105–8.
- Peyrière, M. (1971). Etude infrastructurale des spermatocystes du *Griffithsia flosculosa* (Rhodophycée). *C. R. Séances Acad. Sci. Paris* 273:2071–4.
- Pickett-Heaps, J. D., West, J. A., Wilson, S. M., and McBride, D. L. (2001). Time-lapse videomicroscopy of cell (spore) movement in red algae. *Eur. J. Phycol.* 36:9–22.
- Potin, P., Bouarab, K., Kupper, F., and Kloareg, B. (1999). Oligosaccharide recognition signals and defense reactions in marine plant-microbe interactions. *Curr. Opin. Microbiol.* 2:276–83.
- Pueschel, C. M. (1987). Absence of cap membrane as a characteristic of pit plugs in some red algal orders. *J. Phycol.* 23:150–6.
- Pueschel, C. M. (1989). An expanded survey of the ultrastructure of red algal pit plugs. *J. Phycol.* 25:625–36.
- Pueschel, C. M., and Miller, T. J. (1996). Reconsidering prey specializations in an algal-limpet grazing mutualism: Epithallial cell development in *Clathromorphum circumscriptum* (Rhodophyta, Corallinales). *J. Phycol.* 32:28–36.
- Pueschel, C. M., and Trick, H. N. (1991). Unusual morphological and cytochemical features of pit plugs in *Clathromorphum circumscriptum* (Rhodophyta; Corallinales). *Br. Phycol. J.* 26:335–42.
- Ragan, M. A., Bird, C. J., Rice, E. L., Gutell, R. B., Murphy, C. A., and Singh, R. K. (1994). A molecular phylogeny of the marine red algae (Rhodophyta) based on the nuclear small-subunit rRNA gene. *Proc. Natl. Acad. Sci., USA* 91:7276–80.
- Ramus, J. (1969). Pit connection formation in the red alga *Pseudogloiophloe*. *J. Phycol.* 5:57–63.
- Ramus, J. (1971). Properties of septal plugs from the red alga *Griffithsia pacifica*. *Phycologia* 10:99–103.
- Ramus, J., and Robins, D. M. (1975). The correlation of Golgi activity and polysaccharide secretion in *Porphyridium*. *J. Phycol.* 11:70–4.
- Rawlence, D. J. (1972). An ultrastructural study of the relationship between rhizoids of *Polysiphonia lanosa* (L.) Tandy (Rhodophyceae) and the tissue of *Ascophyllum nodosum* (L.) Le Jolis (Phaeophyceae). *Phycologia* 11:279–90.
- Reddy, C. R. K., Kumar, G. R. K., Siddhanta, A. K., Tewari, A., and Eswaran, K. (2003). *In vitro* somatic embryogenesis and regeneration of somatic embryos from pigmented callus of *Kappaphycus alvarezii* (Doty) Doty (Rhodophyta, Gigartinales). *J. Phycol.* 39:610–16.
- Reed, R. H. (1985). Osmoacclimation in *Bangia atropurpurea* (Rhodophyta, Bangiales): The osmotic role of floridoside. *Br. Phycol. J.* 20:211–18.
- Richardson, N. (1970). Studies on the photobiology of *Bangia fuscopurpurea*. *J. Phycol.* 6:215–19.
- Sacramento, A. T., Garcia-Jimenez, P., Alcazar, R., Tiburcio, A. F., and Robaina, R. R. (2004). Influence of polyamines on the sporulation of

- Grateloupia* (Halymeniaceae, Rhodophyta). *J. Phycol.* 40:887–94.
- Sagert, S., and Schubert, H. (1995). Acclimation of the photosynthetic apparatus of *Palmaria palmata* (Rhodophyta) to light qualities that preferentially excite photosystem I or photosystem II. *J. Phycol.* 31:547–54.
- Satoh, K., Smith, C. M., and Fork, D. C. (1983). Effects of salinity on primary processes of photosynthesis in the red alga *Porphyra perforata*. *Plant Physiol.* 73:643–7.
- Saunders, G. W., and Hommersand, M. H. (2004). Assessing red algal supraordinal diversity and taxonomy in the context of contemporary systematic data. *Amer. J. Bot.* 91:1494–507.
- Scott, J. L., and Dixon, P. S. (1973a). Ultrastructure of spermatium liberation in the marine red alga *Ptilota densa*. *J. Phycol.* 9:85–91.
- Scott, J. L., and Dixon, P. S. (1973b). Ultrastructure of tetrasporogenesis in the marine red alga *Ptilota hypnoides*. *J. Phycol.* 9:29–46.
- Scott, J., Bosco, C., Schornstein, K., and Thomas, J. (1980). Ultrastructure of cell division and reproductive differentiation of male plants in the Florideophycidae (Rhodophyta): Cell division in *Polysiphonia*. *J. Phycol.* 16:507–24.
- Seckbach, J., and Ikan, R. (1972). Sterols and chloroplast structure of *Cyanidium caldarium*. *Plant Physiol.* 49:457–9.
- Sheath, R. G., and Hambrook, J. A. (1988). Mechanical adaptation to flow in freshwater algae. *J. Phycol.* 24:106–11.
- Silva, P. C., and Johansen, H. W. (1986). A reappraisal of the order Corallinales (Rhodophyceae). *Br. Phycol. J.* 21:245–54.
- Simon-Bichard-Bréaud, J. (1971). Un appareil cinétique dans les gamétocystes mâles d'une Rhodophycée: *Bonnemaisonia hamifera* Hariot. *C. R. Séances Acad. Sci. Paris* 273: 1272–5.
- Skuja, H. (1938). Comments on fresh-water Rhodophyceae. *Bot. Rev.* 4:665–76.
- Smit, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: a review. *J. Appl. Phycol.* 16:245–62.
- Smith, G. M. (1938). *Cryptogamic Botany*, Vol. 1. New York: McGraw-Hill.
- Smith, G. M. (1969). *Marine Algae of the Monterey Peninsula, California*. 2nd edn. Stanford, Calif.: Stanford University Press.
- Sommerfeld, M. R., and Nichols, H. W. (1970). Comparative studies in the genus *Porphyridium* Naeg. *J. Phycol.* 6:67–78.
- Sommerfeld, M. R., and Nichols, H. W. (1973). The life cycle of *Bangia fuscopurpurea* in culture. I. Effects of temperature and photoperiod on the morphology and reproduction of the *Bangia* phase. *J. Phycol.* 9:205–10.
- Spieler, R. (2002). Seaweeds compound's anti-HIV efficacy will be tested in southern Africa. *Lancet* 359:1675.
- Stanley, S. M., Ries, J. B., and Hardie, L. A. (2002). Low-magnesium calcite produced by coralline algae in seawater of Late Cretaceous composition. *Proc. Natl. Acad. Sci., USA* 99:15323–6.
- Sturch, H. H. (1926). *Choreocolax Polysiphoniae* Reinsch. *Ann. Bot.* 40:585–605.
- Suneson, S. (1937). Studien über die Entwicklungsgeschichte der Corallinaceen. *Lunds Univ. Arsskr. N.F. II* 33, No. 2:1–132.
- Suneson, S. (1943). The structure, life-history and taxonomy of the Swedish Corallinaceae, *Lunds Univ. Arsskr. N.F. Aud.* 2, Bd. 39.
- Swale, E. M. F., and Belcher, J. H. (1963). Morphological observations on wild and cultured material of *Rhodochorton investiens* (Lenormand) nov. comb. (*Balbiana investiens* (Lenorm.) Sirodot). *Ann. Bot.* 27:281–90.
- Taylor, W. R. (1957). *Marine Algae of the Northeastern Coast of North America*. Ann Arbor: University of Michigan Press.
- Therkelsen, G. H. (1993). Carrageenan. In *Industrial Gums: Polysaccharides and Their Derivatives*. 3rd. edn., ed. R. L. Whistler, and J. N. BeMiller, pp. 145–80. San Diego: Academic Press.
- Tripodi, G. (1974). Ultrastructural changes during carpospore formation in the red alga *Polysiphonia*. *J. Submicrosc. Cytol.* 6:275–86.
- Tseng, C. K. (1981). Commercial cultivation. In *The Biology Of Seaweeds*, ed. C. S. Lobban, and M. J. Wynne. Berkeley and Los Angeles: Univ. Calif. Press.
- Turner, C. H. C., and Evans, L. V. (1978). Translocation of photoassimilated <sup>14</sup>C in the red alga *Polysiphonia lanosa*. *Br. Phycol. J.* 13:51–5.
- Umezaki, I. (1967). The tetrasporophyte of *Nemalion vermiculare* Suringar. *Rev. Algol.* 7:19–24.
- von Stosch, H. A., and Theil, G. (1979). A new mode of life history in the freshwater red algal genus *Batrachospermum*. *Am. J. Bot.* 66:105–7.
- Vreeland, V., Zablackis, E., and Laetsch, W. M. (1992). Monoclonal antibodies as molecular markers for the intracellular and cell wall distribution of -carrageenan epitopes in *Kappaphycus* (Rhodophyta) during tissue development. *J. Phycol.* 28:328–42.

- Weinberger, F., and Friedlander, M. (2000). Response of *Gracilaria conferta* (Rhodophyta) to oligoagars results in defense against agar-degrading epiphytes. *J. Phycol.* 36:1079–86.
- West, J. A. (1972). Environmental regulation of reproduction in *Rhodochorton purpureum*. In *Contributions to the Systematics of Benthic Marine Algae of the North Pacific*, ed. I. A. Abbott, and M. Kurogi, pp. 213–30. Kobe: Jpn. Soc. Phycol.
- Willen, T., and Wingqvist, E.-M. (1986). Svenska sjoar undersokta av H. Skuja med avseende pa vaxtplankton. *Svensk. Bot. Tidskr.* 80:198–208.
- Wilson, S. M., West, J. A., and Pickett-Heaps, J. D. (2003). Time-lapse videomicroscopy of fertilization and the actin cytoskeleton in *Murrayella pericladus* (Rhodomelaceae, Rhodophyta). *Phycologia* 42: 638–45.
- Wolk, C. P. (1968). Role of bromine in the formation of the refractile inclusions of the vesicle cells of the Bonnemaisoniaceae (Rhodophyta). *Planta* 78:371–8.
- Wray, J. L. (1977). *Calcareous Algae*. Amsterdam: Elsevier.
- Yamanouchi, S. (1906). The life history of *Polysiphonia violacea*. *Bot. Gaz.* 42:401–49.
- Yoon, H. S., Hackett, J. D., Ciniglia, C., Pinto, G., and Bhattacharya, D. (2004). A molecular timeline for the origin of photosynthetic eukaryotes. *Mol. Biol. Evol.* 21:809–18.
- Young, D. N. (1979). Fine structure of the “gland cells” of the red alga, *Opuntiella californica* (Solieriaceae, Gigartinales). *Phycologia* 18:288–95.
- Young, D. N., and West, J. A. (1979). Fine structure and histochemistry of vesicle cells of the red alga *Antithamnion defectum* (Ceramiaceae). *J. Phycol.* 15:49–57.

# Chlorophyta

The Chlorophyta, or **green algae**, have chlorophylls *a* and *b*, and form starch with the chloroplast, usually in association with a pyrenoid. The Chlorophyta thus differ from the rest of the eukaryotic algae in forming the storage product in the chloroplast instead of in the cytoplasm. No chloroplast endoplasmic reticulum occurs around the chloroplasts.

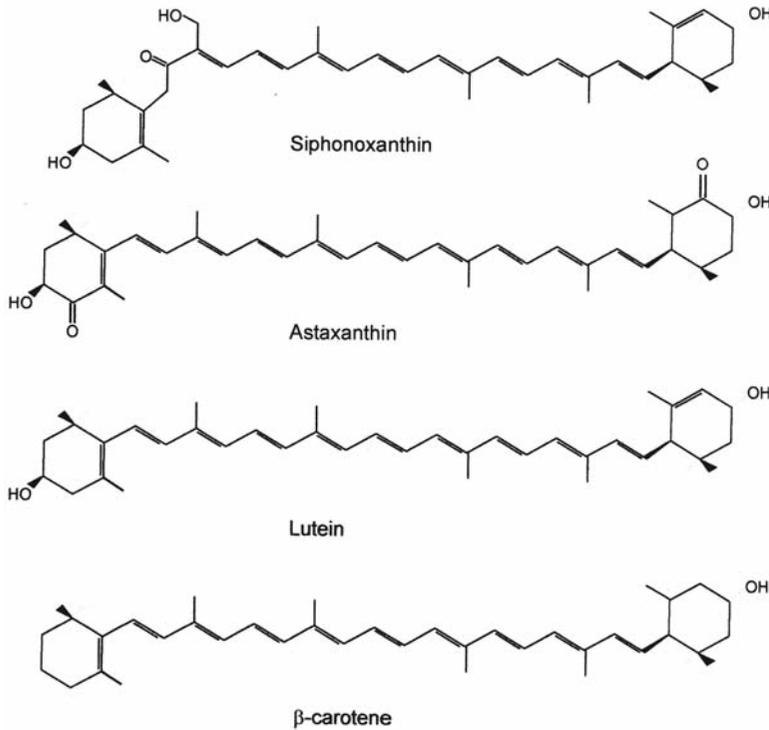
The Chlorophyta are primarily freshwater; only about 10% of the algae are marine, whereas 90% are freshwater (Smith, 1955). Some orders are predominantly marine (Caulerpales, Dasycladales, Siphonocladales), whereas others are predominantly freshwater (Ulotrichales, Coleochaetales) or exclusively freshwater (Oedogoniales, Zygnematales). The freshwater species have a cosmopolitan distribution, with few species endemic in a certain area. In the marine environment, the green algae in the warmer tropical and semi-tropical waters tend to be similar everywhere in the world. This is not true of the Chlorophyta in the colder marine waters; the waters of the Northern and Southern hemispheres have markedly different species. The warmer waters near the equator have acted as a geographical barrier for the evolution of new species and genera.

## Cell structure

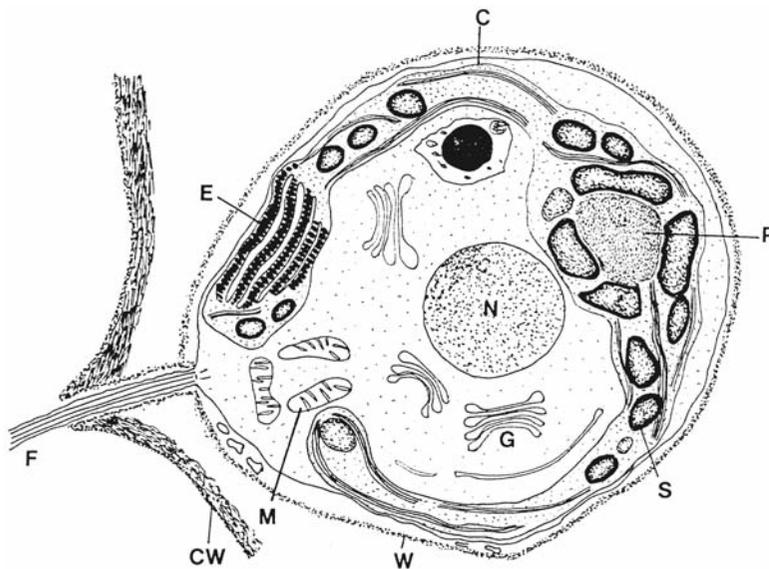
In the Chlorophyta, microtubular hairs do not occur on the flagella, although fibrillar hairs (*Chlamydomonas*, Fig. 1.7(b)) and Golgi-produced scales (*Pyramimonas* (Fig. 5.10), are present in some genera.

Cell walls usually have cellulose as the main structural polysaccharide, although **xylans** or **mannans** often replace cellulose in the Caulerpales (Huizing et al., 1979). The primitive algae in the Prasinophyceae have extracellular scales, or a wall derived from interlacing scales, composed of acidic polysaccharides (Becker et al., 1996). Algae in the Volvocales have walls composed of glycoproteins (Goodenough and Heuser, 1985). Chloroplast pigments are similar to those of higher plants; chlorophyll *a* and *b* are present. The main carotenoid is **lutein**. The siphonaceous genera, as well as the unicells *Tetraselmis* and *Mesostigma*, are the only green algae to have **siphonoxanthin** (Fig. 5.1) and its ester **siphonein** (Yoshi et al., 2003).

Accumulation of carotenoids occurs under conditions of nitrogen deficiency, high irradiance or high salinity. This is particularly true in *Dunaliella* (Figs. 5.62, 5.63) where  $\beta$ -carotene accumulates between thylakoids in the chloroplast, and *Haematococcus* (Fig. 5.63), where **astaxanthin** (Fig. 5.1) accumulates in lipid globules outside the chloroplast (Hagen et al., 2000; Wang et al., 2003). **Hematochrome** is a general term for these carotenoids. Accumulation of hematochromes color the cells orange or red, with hematochrome accumulating up to 8–12% of the cellular contents in *Dunaliella* (Orset and Young, 1999). Animals can not synthesize carotenoids and they acquire the pigments through the food chain from primary producers. Hematochromes are responsible for the coloring in fish, crustaceans and birds (such as the pink in flamingos).



**Fig. 5.1** The chemical structures of carotenoids of the Chlorophyta.



**Fig. 5.2** Semidiagrammatic drawing of a cell in a *Volvox* vegetative colony. The colony wall (CW) is distinct from the cell wall (W). (C) Chloroplast; (E) eyespot; (F) flagellum; (G) Golgi; (M) mitochondrion; (N) nucleus; (P) pyrenoid; (S) starch. (Adapted from Pickett-Heaps, 1970.)

Chloroplasts are surrounded only by the double-membrane chloroplast envelope, with no chloroplast endoplasmic reticulum (Fig. 5.2). The thylakoids are grouped into bands of three to five thylakoids without grana. In some of the siphonaceous genera (e.g., *Caulerpa*), amyloplasts

containing starch grains and a few thylakoids occur in the chloroplasts.

Starch is formed within the chloroplast, in association with a pyrenoid, if one is present (Fig. 5.2). The starch is similar to that of higher plants and is composed of amylose and amylopectin. The

photosynthetic pathways are similar to those of higher plants, many of these pathways first being worked out in green algae such as *Chlorella*.

Contractile vacuoles are present in vegetative cells of most Volvocales. Usually in biflagellate genera there are two contractile vacuoles at the base of the flagella. When there are two contractile vacuoles, they contract alternately with a rapid contraction and slow distention. The contractile vacuoles may control the water content of the cells where the protoplasm has a higher concentration of solutes than does the medium, leading to a total inflow of water that is compensated by the water pumped out by the contractile vacuoles. The contractile vacuoles may also function in removing wastes from the cells. Contractile vacuoles are sometimes called pulsating vacuoles because of their alternate filling and emptying action.

## Phototaxis and eyespots

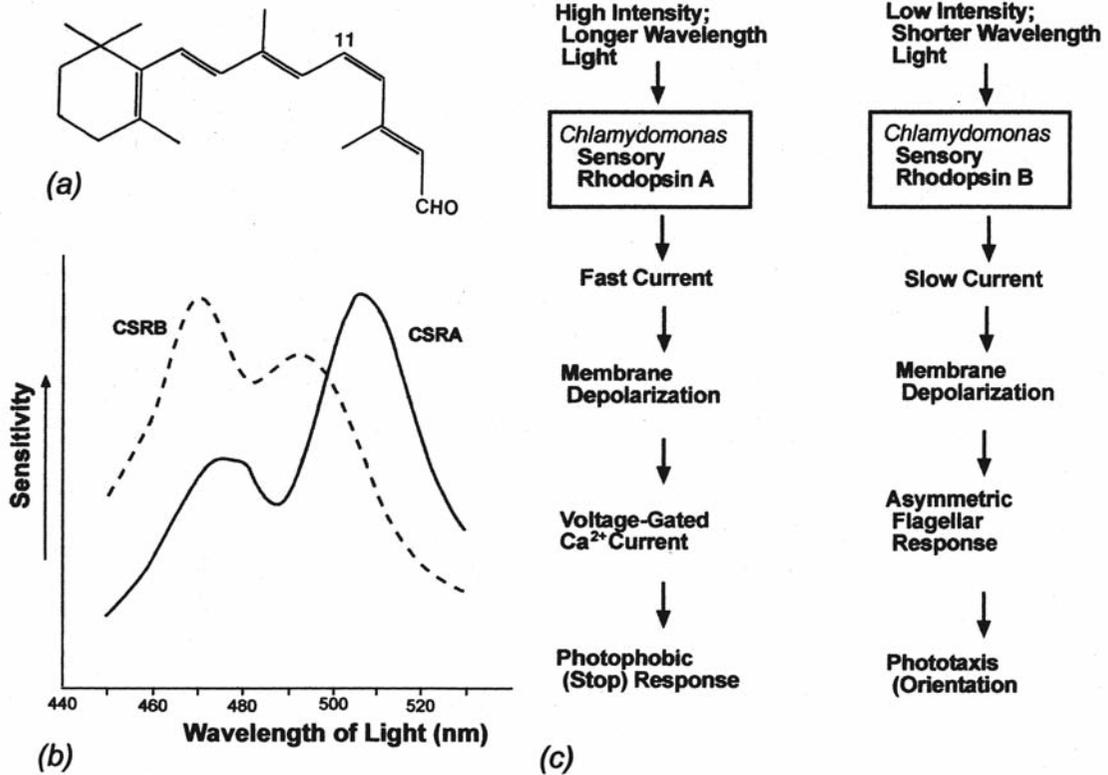
There are two types of phototactic movement in the Chlorophyta: movement by flagella and movement by the secretion of mucilage.

Most of the flagellated cells that show phototactic movement have an eyespot. In the Chlorophyta, the eyespot or stigma is always in the chloroplast, usually in the anterior portion near the flagella bases (Fig. 5.2). The eyespot consists of one to a number of layers of lipid droplets, usually in the stroma between the chloroplast envelope and the outermost band of thylakoids. The eyespot is usually colored orange-red from the carotenoids in the lipid droplets. The phototactic response varies with light intensity; Strasburger in 1878 (Bendix, 1960) observed that organisms with positive phototaxis at moderate light intensities exhibited negative phototaxis at very high light intensities. He also noted that at a given light intensity, temperature has an effect on phototaxis. *Haematococcus* zoospores (Fig. 5.63) at a given light intensity will be negatively phototactic at 4 °C, positively phototactic at 16–18 °C, and very strongly phototactic at 35 °C. Similar results were obtained with *Ulothrix* (Fig. 5.31) and *Ulva* (Fig. 5.33).

Green algae use a two-instant mechanism for perceiving light (i.e., successive measurements of

light are performed by one receptor as the cell changes its position in relation to the light source) (Boscof and Feinleib, 1979). Such a mechanism can operate only if the cell frequently changes its position with respect to the light source. The photoreceptor then compares the light intensity at two different time intervals. The photoreceptor in *Chlamydomonas* is in the plasma membrane above the eyespot (Melkonian and Robenek, 1980b) and consists of a **chromophore** (colored substance) linked to a protein (**opsin** or **apoprotein**) that is embedded in the plasma membrane. The chromophore is **11-cis-retinal** (the aldehyde of vitamin A) (Fig. 5.3). Light excitation causes isomerization of 11-cis-retinal into all-trans, triggering a conformational change that initiates the signaling process. The 11-cis-retinal is restored by an enzymatic process. The 11-cis-retinal is linked to a protein that varies from alga to alga. In *Chlamydomonas reinhardtii*, the protein is **chlamyopsin**, while in *Volvox carteri* the protein is **volvoxopsin** (Ebnet et al., 1999; Hegemann et al., 2001). Combined, the chromophore 11-cis-retinal and the protein produce a rhodopsin, a general class of compounds that absorb light maximally around wavelengths of 500 nm. *Chlamydomonas* has two rhodopsins: *Chlamydomonas* sensory rhodopsin A and *Chlamydomonas* sensory rhodopsin B (Sineshchekov et al., 2002). *Chlamydomonas* sensory rhodopsin A absorbs light maximally at 510 nm (Fig. 5.3), saturates at high light intensity, and mediates a fast photoreceptor current that is involved in the **photophobic response** (Fig. 5.3). The photophobic response causes the alga to stop and prevents the cell from crossing a light/dark border. *Chlamydomonas* sensory rhodopsin B absorbs light maximally at 470 nm, saturates at low light intensities, and generates a slow photoreceptor current that is involved in phototaxis.

The eyespot acts as an interference filter by reflecting blue and green light back onto the photoreceptor in the plasma membrane (Kriemer and Melkonian, 1990). Different amounts of light are reflected onto the photoreceptor as the alga swims through the medium. This results in changes in membrane potential involving rhodopsin. Entry of calcium into the cell is affected by the membrane potential of the plasma membrane, and, in turn, the concentration of calcium ions in the cytoplasm



**Fig. 5.3** (a) The structure of 11-*cis*-retinal, part of the photoreceptor molecule in *Chlamydomonas*. (b) Action spectra showing the sensitivity of the photoreceptors *Chlamydomonas* sensory rhodopsin A (CSRA) and *Chlamydomonas* sensory rhodopsin B (CSRB) to different wavelengths of light. (c) Scheme of light signal transduction initiated by the two sensory rhodopsin pigments in *Chlamydomonas*. ((b),(c) adapted from Sineshchekov et al., 2002.)

affects the rate of beating of the flagella. The swimming direction of the cell is affected by the rate of beating, because at one concentration of calcium ions, each flagellum beats differently (Kamiya and Witman, 1984). Therefore, changing the cytoplasmic calcium concentration differentially changes the beat of each flagellum, causing the cell to swim in a different direction. Melkonian and Robenek (1980b), using freeze-fracture replicas, have shown that the plasma membrane and outer membrane of the chloroplast envelope have more particles in the areas of the membranes over the eyespot than in other areas. The portion of the plasma membrane overlying the eyespot of *Chlorosarcinopsis gelatinosa* has 8200 protein particles  $\mu\text{m}^{-2}$  in

the half of the plasma membrane next to the cytoplasm (Melkonian and Robenek, 1980a). The plasma membrane not over the eyespot has only 2100 particles  $\mu\text{m}^{-2}$ . These protein particles in the membranes over the eyespot probably represent a part of the photoreceptor system because they disappear during flagellar retraction and before cell wall secretion, a time when the photoreceptor system would be of no use.

A second type of phototactic movement in the Chlorophyta uses secretion of mucilage. In 1848, Ralfs, in his monograph on desmids, described their movement to the surface of mud brought into the laboratory, and presumed this movement to be due to the stimulus of light. Braun (1851) noticed that young cells of *Penium curtum* quickly aligned their long axis and moved toward the light, accumulating on the lighted side of the vessel they were growing in. The movement in desmids is brought about by the extrusion of slime through cell wall pores in the apical part of the cell (Domozych et al., 1993; Nossag and Kasprick, 1993).

Green algae can also show geotactic responses to gravity. *Chlamydomonas* (Fig. 5.55) exhibits nega-

tive geotaxis by swimming against gravity. Such a feature would be selected for in evolution because when the algal cell (which is heavier than water) is confronted with darkness, it must move up to the surface in order to obtain light for growth and reproduction. In *Chlamydomonas*, negative geotaxis is an energy-dependent response that requires a horizontal swimming path of at least 200  $\mu\text{m}$  because the normal geotactic orientation maneuvers require long gradual turns. The rate of geotaxis is steady but slow relative to the average swimming speed (Bean, 1977).

## Asexual reproduction

There are a number of types of asexual reproduction, the simplest being **fragmentation** of colonies into two or more parts, each part becoming a new colony. **Zoosporogenesis** commonly occurs, usually induced by a change in the environment of the alga. In the Chlorophyta, **zoospores** are normally produced in vegetative cells (e.g., *Ulothrix*; Fig. 5.31), and only in a few cases are they formed in specialized sporangia (e.g., *Derbesia*; Fig. 5.42). Zoospores are usually formed in the younger parts of filaments, and the number of zoospores is generally a power of two in uninucleate genera. **Aplanospores** are non-flagellated and have a wall distinct from the parent cell wall (e.g., *Trebouxia*; Fig. 5.86(a)–(c)). Aplanospores are considered to be abortive zoospores and have the ability to form a new plant on germination. **Autospores** are aplanospores that have the same shape as the parent cell, and are common in the Chlorellales (e.g., *Chlorella*; Fig. 5.83). Autospores are usually formed in a multiple of two in the parent cell. **Coenobia** are colonies with a definite number of cells arranged in a specific manner (e.g., *Volvox*; Fig. 5.69). Genera with colonies arranged in coenobia form daughter colonies with a certain number of cells. In maturation of the daughter coenobia, there is enlargement but no division of vegetative cells in the coenobia.

## Sexual reproduction

Sexual reproduction in the Chlorophyceae may be isogamous, anisogamous, or oogamous, with the

general line of evolution occurring in the same direction. Usually gametes are specialized cells and not vegetative cells, although in the one-celled Volvocales the latter can occur. If the species is isogamous or anisogamous, the gametes are usually not formed in specialized cells although in the oogamous species, gametes are normally formed in specialized gametangia (e.g., *Coleochaete*, Fig. 5.25). Whereas most Chlorophyta form motile flagellated gametes (zoogametes), in the Zygnematales aplanogametes or amoeboid gametes are formed.

In some of the Chlorophyta, gametogenesis is induced by environmental changes, whereas in others the presence of two sexually different strains is necessary. In the latter, vegetative cells of one sex secrete a substance that initiates sexual differentiation in competent cells of the opposite sex. Such a situation is common in the Volvocales (Starr, 1972; Kirk and Kirk, 1986) and is considered in more detail later. In *Oedogonium* (Fig. 5.95), sex organs form without the complementary strain, but subsequent fertilization is under a complex hormonal control. In other genera, a chemotactic substance is sometimes produced by the egg that attracts the spermatozooids. This does not generally happen in isogamous species. In isogamous species, sexually different gametes meet at random and immediately adhere by means of an **agglutination** reaction. The agglutinative flagellar adhesion between gametes of different sex is designated as the **mating-type reaction**. Initially after mixing, the gametes of opposite sexes adhere by their flagella tips in clusters of up to 25 gametes. Soon the anterior ends of complementary gametes fuse, and the flagella free themselves. The motile zygote then swims for some time before settling and secreting a thick wall.

The mating-type substances (responsible for flagellar agglutination) are localized and function at the flagella tips. It is possible to isolate the mating-type substances that still have the ability to interact with the gametes of the opposite sex. When added to the opposite gamete type, they cause **isoagglutination** (male gametes will clump with each other when a female mating-type substance is added to the culture). The mating-type substances are discussed in more detail for *Chlamydomonas*, which has been most intensively studied.

**Table 5.1** Characteristics of the four classes of green algae

	Micromonadophyceae	Charophyceae	Ulvophyceae	Chlorophyceae
Position of flagella in cell		Lateral	Anterior	Anterior
Microtubular root		Large band with smaller band	Four, cruciately arranged	Four, cruciately arranged
Rhizoplast	May be present	No	Common	Common
Multilayered structure	May be present	Yes	No	No
Covering on motile cells	Scales	Scales	Scales	Theca
Interzonal spindle	Persistent	Persistent	Persistent	Collapsing
New cross wall formation		Phragmoplast	Cleavage furrow	Phycoplast
Cellulose terminal complex		Rosettes	Linear row	Linear row
Eyespot		None	Common	Common
Glycolate degradation	Glycolate dehydrogenase	Glycolate oxidase	Glycolate dehydrogenase	Glycolate dehydrogenase
Urea degradation		Urease	Urease	Urea amidolyase
Cu/Zn superoxide dismutase		Present	Absent	Absent

Soon after the gametes fuse (**syngamy**), meiosis is known to occur in the thick-walled zygotes of the Volvocales, Ulotrichales, Oedogoniales, Chlorellales, and Zygneatales.

## Classification

The four important classes in the Chlorophyta are the Prasinophyceae (Micromonadophyceae), Charophyceae, Ulvophyceae, and the Chlorophyceae (Table 5.1). The classes were originally formulated in the 1970s by Karl Mattox and Kenneth Stewart (Fig. 5.4) working at Miami University of Ohio, and by Jeremy Pickett-Heaps (photograph in Preface) working at the University of Colorado. Their classification scheme was based largely on ultrastructural characteristics. Later investigations utilizing molecular genetics have verified their work.

The Charophyceae are in the line that evolved into land plants (embryophytes). As such, there have been a number of proposals made over the years to include the Charophyceae and land plants into a supergroup (Sluiman, 1985). The latest fashion is the term Viridiplantae for this line of evolution (Cavalier-Smith, 1981). A splinter of this is to include the *Chara* and its relatives with

the land plants into the “Steptophyta,” casually ignoring the close relationship between *Chara* and the remainder of the lower green algae.

**Class 1 Prasinophyceae:** scaly or naked flagellates with interzonal spindles that are persistent during cytokinesis; primitive green algae, some of which gave rise to the other classes in the Chlorophyta.

**Class 2 Charophyceae:** motile cells asymmetrical; two flagella attached in a lateral position in the cell; flagellar root consisting of a broad band of microtubules and a second smaller microtubular root; multilayered structure (MLS) may be present; no rhizoplast; scales common outside of motile cells; persistent interzonal mitotic spindle in telophase; phragmoplast produces new cross walls after cell division; eyespots usually not present; glycolate broken down by glycolate oxidase; urea broken down by urease; predominantly freshwater; sexual reproduction involves the formation of a dormant zygote; meiosis occurs when the zygote germinates.



**Fig. 5.4** Kenneth Stewart (left) and Karl Mattox (right).

**Kenneth Stewart** Born September 4, 1932 in Moberly, Missouri. Dr. Stewart received his B.Sc. from Southern Illinois University at Carbondale, Illinois, in 1954. He served in the Army and worked in non-academic jobs until enrolling at the University of California, Davis, where he received his Ph.D. in 1968. In 1968 he joined the Department of Botany at Miami University, Oxford, Ohio.

**Karl Mattox** Born August 22, 1936 in Cincinnati, Ohio. He received his B.Sc. (1958) and M.A. (1960) from Miami University, Ohio, and his Ph.D. (1962) from the University of Texas. From 1962 to 1966, he was assistant professor in the Department of Botany at the University of Toronto. In 1966, he moved to the Department of Botany at Miami University.

**Class 3 *Ulvophyceae*:** flagella attached at anterior end of cell; motile cells have near-radial symmetry externally; flagella roots consist of four cruciately arranged microtubular roots and sometimes a rhizoplast; no multilayered structure (MLS); scales may be present on motile cells; persistent interzonal spindle in telophase; cleavage furrow produces the new cross wall in cell division; eyespots common; glycolate broken down by glycolate dehydrogenase; urea broken down by urease; predominantly marine; no dormant zygotes; alternation of generations common.

**Class 4 *Chlorophyceae*:** motile cells with radial or near-radial external symmetry; flagella attached at anterior end of cell; flagella roots consist of four cruciately arranged microtubular roots and sometimes a rhizoplast; no multilayered structure (MLS); theca common in motile cells; in telophase, the interzonal spindle collapses; phycoplast produces the new cross wall in cell division; eyespots common; glycolate breakdown by glycolate dehydrogenase; urea broken down by urea amidolyase; predominantly freshwater; zygote undergoes a dormant period; meiosis occurs when the zygote germinates.

#### Position of flagella in cells

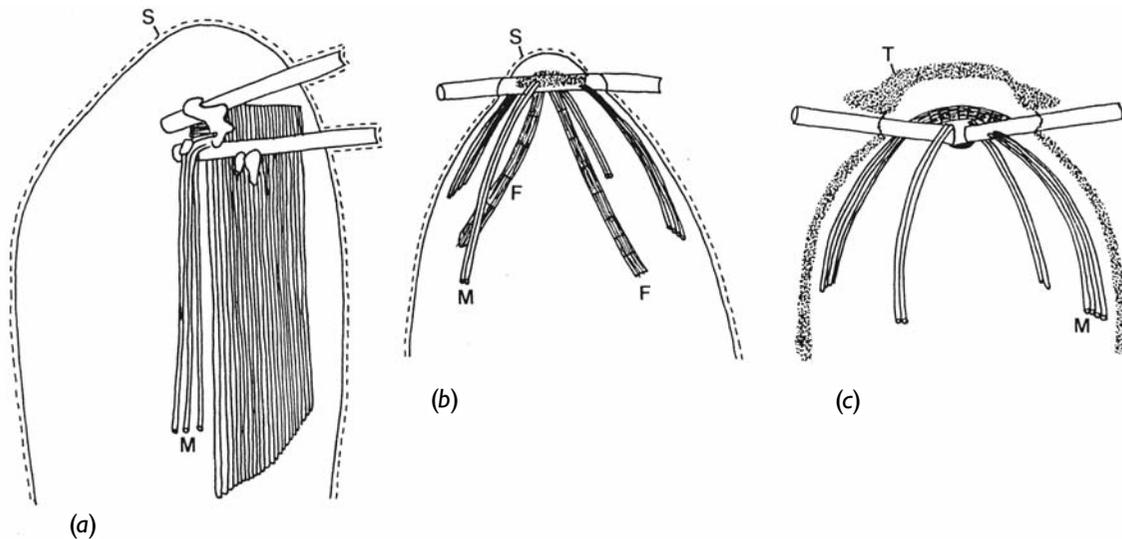
In the *Charophyceae*, the flagella are attached in a lateral position in the cell (Fig. 5.5). In the *Ulvophyceae* and *Chlorophyceae*, the flagella are attached at the anterior end.

#### Flagellar roots

Flagellar basal bodies are anchored in the protoplast by **microtubular roots** and/or **rhizoplasts** (Fig. 5.5) (Melkonian et al., 1988).

#### Microtubular roots

Microtubular roots consist of groups of 24-nm diameter microtubules that can have one of two basic configurations: (1) There can be a microtubular root consisting of a *large broad band of microtubules with a smaller second microtubular root* (*Charophyceae*), or (2) there can be *four groups of cruciately arranged microtubular roots* running from the basal bodies (*Ulvophyceae* and *Chlorophyceae*). The cruciately arranged microtubular roots have what is called an X-2-X-2 arrangement. This notation refers to the fact that two of the microtubular roots are usually composed of two microtubules, whereas the two other roots can have different numbers of microtubules in different organisms. Thus *Chlamydomonas moewusii* has a 4-2-4-2 arrangement, whereas motile cells of *Ulothrix* sp. have a 5-2-5-2 arrangement (Moestrup, 1978). One of the roots containing two microtubules is often linked to the outer membrane of



**Fig. 5.5** Schematic drawings of the side view of swimmers produced by three of the classes of green algae. (a)

**Charophyceae:** scaly cell with one large root (the MLS), one smaller root, and flagella extending at an angle from the point of insertion. (b) **Ulvophyceae:** four microtubular roots (two of each kind) in a cruciate arrangement, a pair of fibrous roots, and a scaly covering over the cell. (c)

**Chlorophyceae:** cruciate roots and the cell covered with a theca. (F) Fibrous roots; (M) microtubular root; (S) scales; (T) theca. (After Mattox and Stewart, 1984.)

the chloroplast envelope and is probably involved in phototaxis.

### Rhizoplasts (fibrous roots)

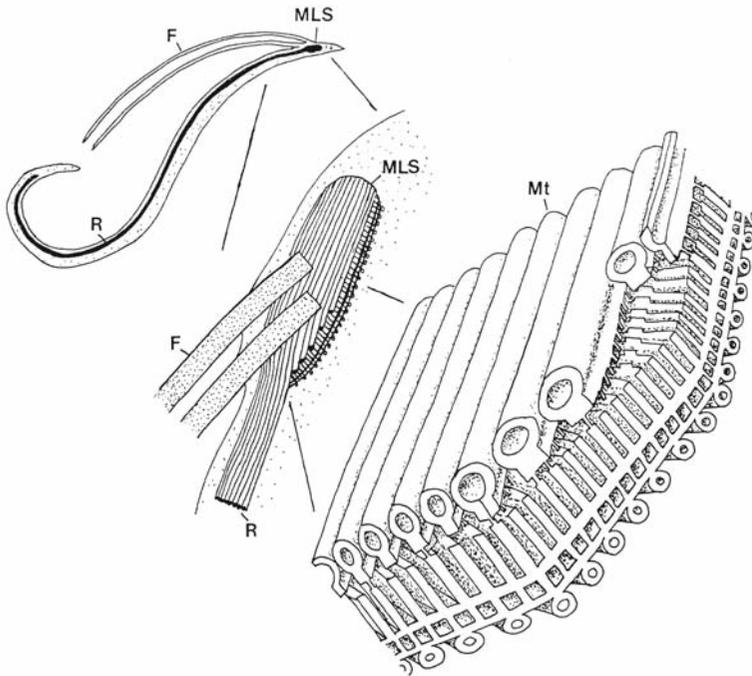
A rhizoplast is usually a cylinder containing 5- to 10-nm-diameter filaments interrupted at approximately 80-nm intervals by bands of electron-dense material (in the electron microscope). A rhizoplast runs from the basal bodies posteriorly toward the nucleus. Rhizoplasts are contractile (Salisbury and Floyd, 1978), and the distance between the bands in the rhizoplast varies depending on the state of contraction of the rhizoplast. The size of the filaments in the rhizoplast is similar to that of actin-myosin filaments in animal muscle cells. The method of contraction of the rhizoplast may be similar to that of muscle. Rhizoplasts may be present in the **Prasinophyceae**, **Chlorophyceae**, and **Ulvophyceae**, but are absent in the **Charophyceae**.

### Multilayered structure

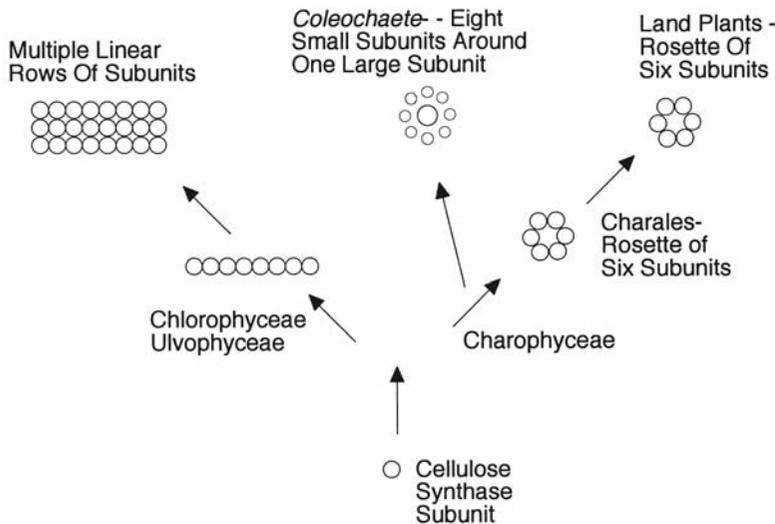
A multilayered structure (MLS) (Fig. 5.6) consists of a more or less rectangular body attached to the anterior end of the single broad band of microtubules in the **Charophyceae** and in the spermatozooids of lower land plants. The MLS lies directly beneath the basal bodies of the flagella. The MLS consists of four layers. The layer closest to the plasma membrane contains the microtubules of the root. Under this are two electron-dense layers. The bottom-most layer is composed of small microtubules. An MLS may be present in the **Prasinophyceae** and **Charophyceae**, but is absent in the **Chlorophyceae** and **Ulvophyceae**.

### Occurrence of scales or a wall on the motile cells

Motile cells covered with scales may occur in the **Prasinophyceae**, **Charophyceae**, and **Ulvophyceae**. The presence of scales on the motile cells is probably the primitive condition. As evolution progressed, the scales became interweaved along their edges so a coherent cell covering was formed, as in the genus *Tetraselmis* (Fig. 5.12) (Domozych et al., 1981; Mattox and Stewart, 1984). The end result of this evolution was the **theca** that covers the motile cells in the **Chlorophyceae**. This theca has a crystalline substructure and is composed of hydroxyproline-rich glycoproteins associated with various polysaccharides (Roberts, 1974;



**Fig. 5.6** Drawing of a green algal cell containing a multilayered structure. (F) Flagellum; (Mt) microtubule; (MLS) multilayered structure; (R) microtubular root. (Adapted from Carothers and Kreitner, 1967.)



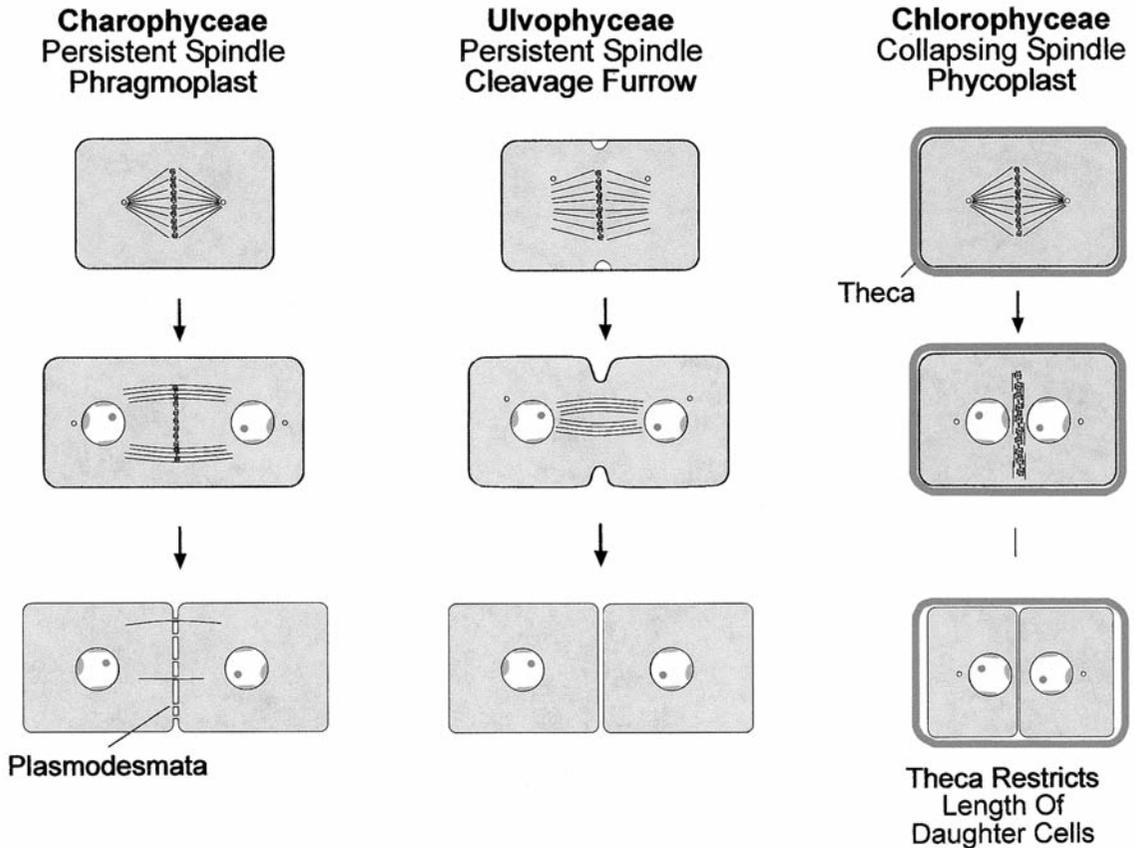
**Fig. 5.7** Proteins of cellulose synthetase in the plasma membrane have aggregated into terminal complexes along two phylogenetic lines in the Chlorophyta. Rosettes of cellulose synthetase proteins have evolved in the Charophyceae, while aggregations of linear rows have evolved in the Chlorophyceae and Ulvophyceae. (Adapted from Okuda and Brown, 1992.)

Miller, 1978; Deason, 1983). The theca in motile cells of the Chlorophyceae is thus not to be confused with the cell walls of non-motile stages of the more advanced Chlorophyta, which have **cellulose** as the main skeletal molecule.

Cellulose is produced by the enzyme **cellulose synthetase** that occurs as proteins embedded in the plasma membrane of the cell. Six to ten cellulose synthetase molecules are grouped into a single subunit. The subunits are, in turn, aggre-

gated into **terminal complexes**. In the Chlorophyta, there are two different types of terminal complexes (Fig. 5.7) (Okuda et al., 1994; Tsekos, 1999):

- 1 In the **Charophyceae**, terminal complexes have subunits aggregated into rosettes.
- 2 In the **Chlorophyceae** and **Ulvophyceae**, terminal complexes consist of linear rows of subunits.



**Fig. 5.8** Schematic drawing of the type of cell division in the Charophyceae, Ulvophyceae, and Chlorophyceae.

### Cell division

Two types of interzonal spindles occur in telophase cells of the Chlorophyta: the **persistent type** and the **collapsing type**. The new cell wall can be formed between the daughter cells by means of a **phragmoplast**, a **cleavage furrow**, or a **phycoplast**. Three of the classes in the Chlorophyta are characterized by the type of spindle in the class and the way the new cross wall is formed (Fig. 5.8).

#### Persistent interzonal spindle and phragmoplast (Charophyceae)

In the Charophyceae, the **microtubular spindle persists** even after the daughter nuclei have separated in telophase. The daughter nuclei are separated by the length of the persistent spindle while a new cross wall is formed by a **phragmoplast** in the more advanced members. Wall formation by a

phragmoplast initially involves the production of vesicles by the dictyosomes. The vesicles contain the components of the new cross walls. The persistent spindle microtubules may function in guiding the vesicles to the area of the new cross wall which will separate the two daughter cells. The vesicles fuse, releasing their contents which form the new cross wall. Plasmodesmata are formed in the cross wall between the daughter cells where the persistent spindle microtubules traverse the cross wall. In some of the primitive Charophyceae, the new cross wall is formed by a phragmoplast in association with an infurrowing of the plasma membrane. In the more advanced Charophyceae (those in the Coleochaetales and Charales), the cross wall is formed only by a phragmoplast.

#### Persistent interzonal spindle and a cleavage furrow (Ulvophyceae)

In the Ulvophyceae, the **interzonal spindle persists** during telophase, holding the daughter

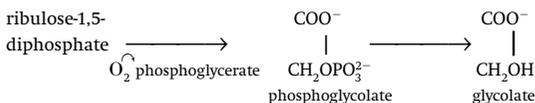
cells apart while the new cross wall is formed by an **infurrowing of the plasma membrane**. As the plasma membrane furrows inward, dictyosome vesicles fuse with the plasma membrane, behind the infurrowing, producing the new cross wall.

**Collapsing interzonal spindle and a cleavage furrow (Chlorophyceae)**

In the **Chlorophyceae**, the **mitotic spindle collapses** after nuclear division. This results in the two daughter nuclei coming close together in telophase because there is no longer a persistent interzonal spindle to hold the nuclei apart. The position of the new cross walls becomes outlined by microtubules of the **pycoplast** that arise perpendicular to the former position of the spindle microtubules. Dictyosome vesicles fuse between the pycoplast microtubules, forming the new cross wall. The Chlorophyceae have motile cells with a cell wall (theca) and thus differ from the Ulvophyceae and Charophyceae. The pycoplast evolved in conjunction with the production of cell walls in these algae (Mattox and Stewart, 1977). During cell division in cells with a persistent spindle (in the Prasinophyceae, Charophyceae, and Ulvophyceae), there is extensive elongation of the cell during anaphase. This cell elongation presents no problem in naked cells or cells covered with scales. However, in the Chlorophyceae the cells are not easily able to elongate in response to the anaphase elongation of the persistent spindle. The reason is that the Chlorophyceae have walls around the motile cells. Therefore, evolution of the pycoplast and collapsing spindle, which does not involve rapid elongation of the daughter cells, presents an evolutionary advantage.

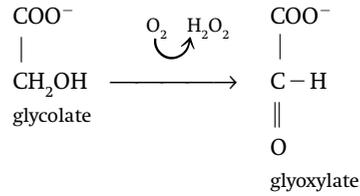
**Glycolate degradation**

Glycolate, the major substrate of photorespiration, is derived from phosphoglycolate, which is formed by the oxygenation of ribulose-1,5-diphosphate:



The glycolate is metabolized in microbodies called **peroxisomes**. In the peroxisomes, glycolate

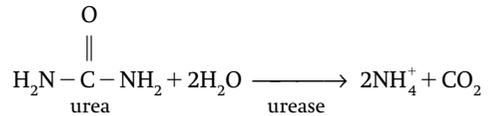
is oxidized to glyoxylate. The  $\text{H}_2\text{O}_2$  produced in the reaction is cleaved by the enzyme **catalase** to  $\text{H}_2\text{O}$  and  $\text{O}_2$ :



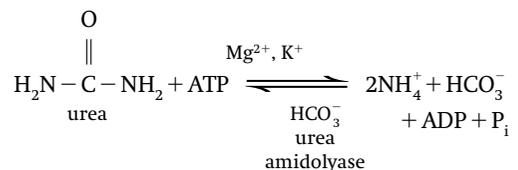
The above oxidation of glycolate can be catalyzed by the enzyme **glycolate dehydrogenase** or the enzyme **glycolate oxidase** (Gruber et al., 1974). In the **Charophyceae**, the reaction is catalyzed by **glycolate oxidase**, whereas in the **Chlorophyceae** and the **Ulvophyceae**, the reaction is catalyzed by **glycolate dehydrogenase** (Suzuki et al., 1991). Glycolate oxidase probably represents the primitive condition since *Cyanophora paradoxa* in the Glaucophyta catalyzes the reaction with this enzyme (Betsche et al., 1992).

**Urea degradation**

In the **Charophyceae**, **Ulvophyceae**, and **higher plants**, urea is broken down by the enzyme **urease**:



In the **Chlorophyceae**, urea is broken down by the enzyme **urea amidolyase** (Syrett and Al-Houty, 1984):



**Superoxide dismutase**

Superoxide dismutases (SOD) are a group of enzymes that catalyze the reaction:



Superoxide dismutases are important because they take highly reactive, potentially damaging

oxygen radicals ( $O_2^-$ ) and other toxic oxygen species (OH, singlet oxygen) and convert them to less toxic moieties.

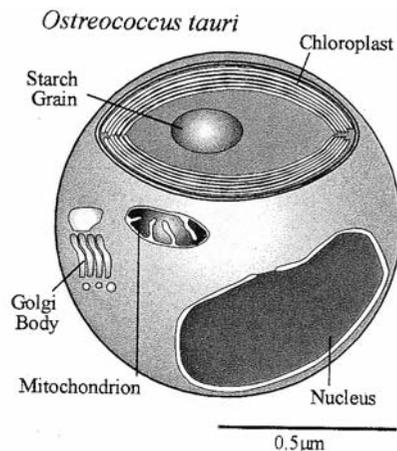
There are three forms of superoxide dismutases, named for the metal in their reaction centers: **iron SOD** (FeSOD), **manganese SOD** (MnSOD), and **copper-zinc SOD** (Cu/Zn SOD). The amino acid sequences and three-dimensional protein structure of FeSOD and MnSOD are very similar, suggesting a close evolutionary relationship, whereas those of Cu/Zn SOD are quite different, indicating very little, if any, evolutionary relationship to the other two (deJesus et al., 1989).

FeSOD and MnSOD occur in all classes of green algae and most land plants. Cu/Zn SOD, however, occurs only in the Charophyceae and land plants, reinforcing the concept that organisms similar to those in the Charophyceae evolved into land plants.

Cu/Zn SOD is the only superoxide dismutase that occurs in the cytosol. MnSOD occurs in the mitochondrion while FeSOD occurs in the chloroplast. DeJesus et al. (1989) argue that green algae evolving to land plants would need superoxide dismutase in the cytosol to counteract oxygen diffusing in from the atmosphere, which would produce damaging radicals in the cytosol. The charophycean lineage of green algae is the only one to have superoxide dismutase in the cytosol and is a major reason for the successful colonization of the land by descendants of the Charophyceae. The other lineages of green algae were unable to make the transition to the land because they had superoxide dismutase restricted to mitochondria, chloroplasts, and peroxisomes and were not able to counteract the production of damaging radicals formed by oxygen diffusing in from the atmosphere.

## Prasinophyceae

The Prasinophyceae (Micromonadophyceae) contain primitive green flagellates with scales composed of acidic polysaccharides (Wustman et al., 2004). The class is a catch-all for those algae that “exhibit primitive characteristics for many of the features used to define other classes” (Mattox and Stewart, 1984; Fawley et al., 2000).



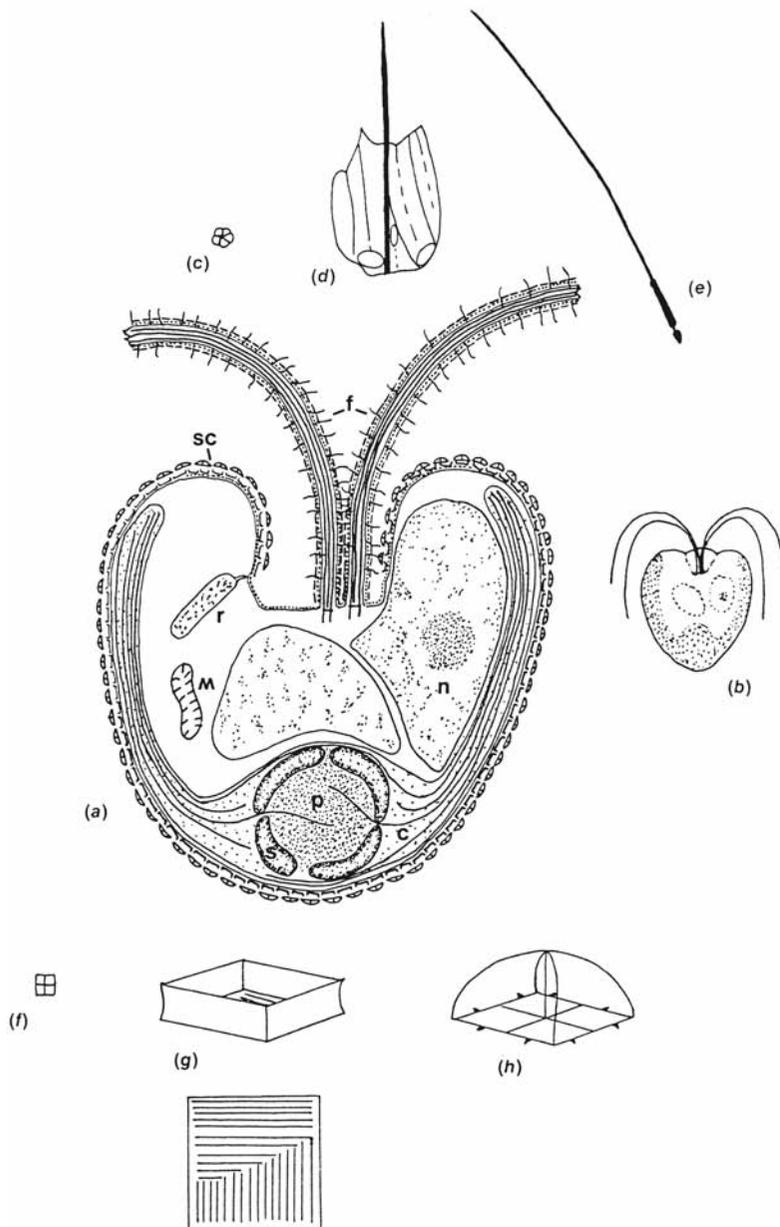
**Fig. 5.9** The general organization of *Ostreococcus tauri*.

*Ostreococcus tauri* (Fig. 5.9) is less than 1 μm in diameter and is the smallest eukaryotic alga known. The alga has a relatively large nucleus, a single chloroplast with a starch granule, a mitochondrion, and a Golgi apparatus. Unlike other algae in the group, there are no scales outside the cell wall (Courties et al., 1998).

*Pyramimonas obovata* has heart-shaped (cordate) cells, with four flagella arising from an anterior flat-bottomed depression (Fig. 5.10). There are three different layers of scales on the body and two layers on the flagellum (Belcher et al., 1974). The scales are formed in dictyosomes, from which they are transported to the scale reservoir and then to the surface of the cell in vesicles.

Species of *Pyramimonas* have adapted to tide-pools by means of a settling rhythm. Cells in tide-pools anticipate the incoming tide and move down into the sand where they attach to sand grains by their flagella, before the first waves cover the tide pool. After the tide pool is exposed by the receding tide, the *Pyramimonas* cells move upward into the tide pool. The settling is controlled by an endogenous circadian oscillator and will continue in the laboratory for a few days after the cells are removed from the wild (Griffin and Aken, 1993).

*Pterosperma* is a genus that is closely related to *Pyramimonas*. *Pterosperma* produces walled cysts called phycmata (Fig. 5.11) that are similar to microfossils such as *Tasmanites* and *Cymatiosphaera* that have been reported from the Precambrian up to the Holocene (Inouye et al., 1990).

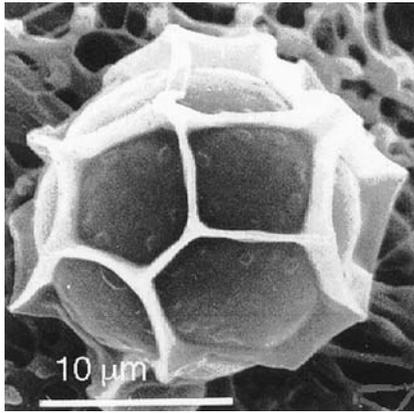
**Fig. 5.10** *Pyramimonas obovata*.

(a) Semidiagrammatic drawing of a cell showing the organelles, two of the four flagella, and the layers of scales on the body and the flagella. (b) Whole cell. (c) Inner flagella scale. (d) Outer flagellar scale. (e) Flagellar hair. (f) Inner body scale. (g) Intermediate body scale, top and side view. (h) Outer body scale. (c) Chloroplast; (f) flagellum; (m) mitochondrion; (n) nucleus; (p) pyrenoid; (r) scale reservoir; (s) starch; (sc) scales. (After Belcher et al., 1974.)

*Tetraselmis* (Fig. 5.12) is commonly found in marine waters. The cells are oval-shaped and surrounded by a theca that is formed by the coalescence of many small stellate scales that are produced in the Golgi apparatus. Four flagella are inserted in an apical pit in the cell. The flagella are covered with hairs and scales, and the flagella emerge from an opening in the theca. There is a cup-shaped chloroplast with a basal pyrenoid. A

nucleus occurs in the center of the cell, and Golgi bodies are found anterior to the nucleus.

*Tetraselmis* produces stellate scales in the Golgi apparatus which fuse extracellularly to yield the theca (Manton and Parke, 1960). The theca is composed of neutral and acidic polysaccharides associated with certain amino acids (Becker et al., 1996), similar to the glycoprotein walls in volvocean flagellates (those green algae in the



**Fig. 5.11** A scanning electron micrograph of a phycoma of *Pterosperma cristatum*. Square, pentagonal or hexagonal compartments are formed by wing-like protrusions of the cell wall. (From Inouye et al., 1990.)

Volvocales) (Roberts, 1974) but unlike the cellulose cell walls in the Charophyceae. The typical volvocan flagellates and eventually the rest of the higher Chlorophyceae probably arose from a flagellate such as *Tetraselmis* or *Mantionella* (Fig. 5.54) (Domozych et al., 1981).

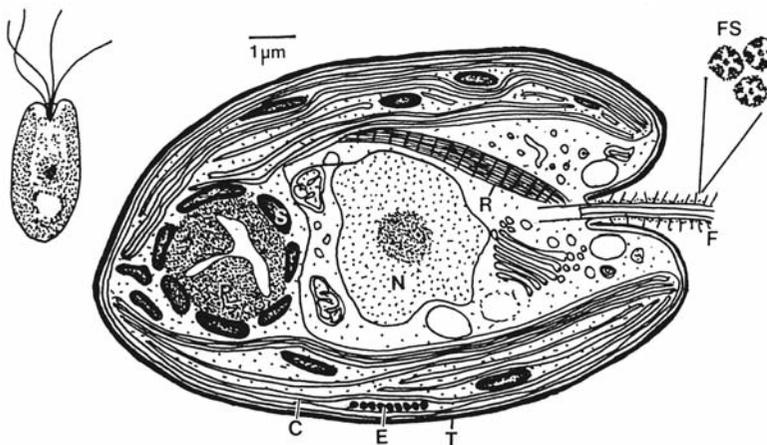
The genus *Tetraselmis* was previously divided into a number of genera including *Tetraselmis*, *Platymonas*, and *Prasinocladus* before it was recognized that all of the cells were similar. All of the organisms are now recognized as the single genus *Tetraselmis* with subgenera (Hori et al., 1982a,b):

Subgenus *Tetraselmis*: spherical pyrenoid into which narrow channels of cytoplasm extend.

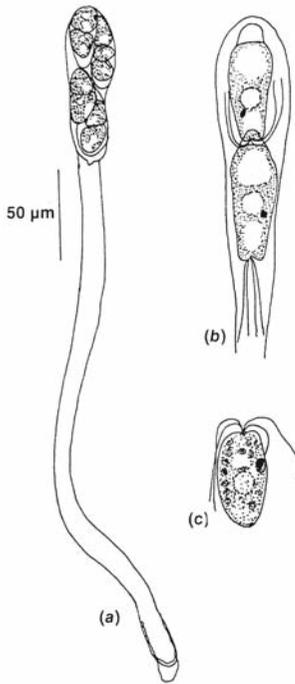
Subgenus *Prasinocladia*: pyrenoid not penetrated by cytoplasm but by a single lobe of the nucleus so that the pyrenoid appears cup-shaped in the microscope.

Algae in the subgenus *Prasinocladia* grow as dense yellow-green tufts on rocks or shells in small tide-pools near the high-tide mark (Fig. 5.13) (Chihara, 1963). The plants occur on most temperate coasts. In the mature attached vegetative state, the two to four protoplasts are at the apical area of the wall. Protoplasts are released as zoospores by escaping through a break in the apical area of the wall. The quadriflagellate zoospores swim actively for a while, settle, lose their flagella, and secrete a wall. Germination occurs immediately after settling and first involves the secretion of an outer wall. The protoplast elongates with the deposition of an inner wall, causing a break in the outer wall; and, still surrounded by the elongating inner wall, the protoplast secretes additional wall material and positions itself at the top of the resulting tube. The protoplasts can divide into two cells to form new tubes. Any protoplast is capable of forming flagella and becoming a zoospore.

*Convoluta* is a small flatworm, several millimeters long (Fig. 5.14(a)), that lives on sandy marine shores (Russell and Yonge, 1963). It occurs in large colonies forming green patches on the yellow sand, appearing from beneath the sand immediately after the tide has left it and disappearing just before the tide returns. The green color of the flatworm is due to the presence in the animal of vast numbers of algae. The algae are not present

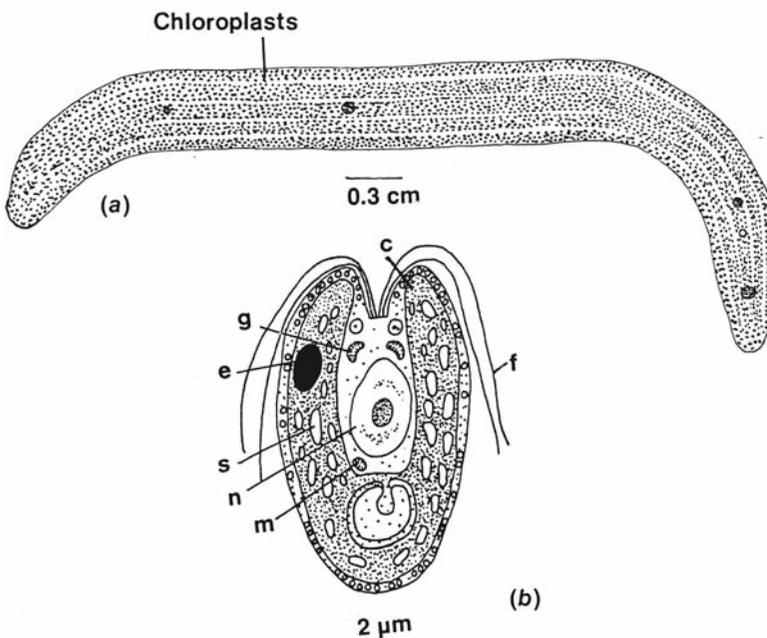


**Fig. 5.12** Drawing of a cell of *Tetraselmis*. (C) Chloroplast; (E) eyespot; (F) flagellum; (FS) flagellar scales; (N) nucleus; (P) pyrenoid; (R) rhizoplast (only one of two is shown); (S) starch; (T) theca.

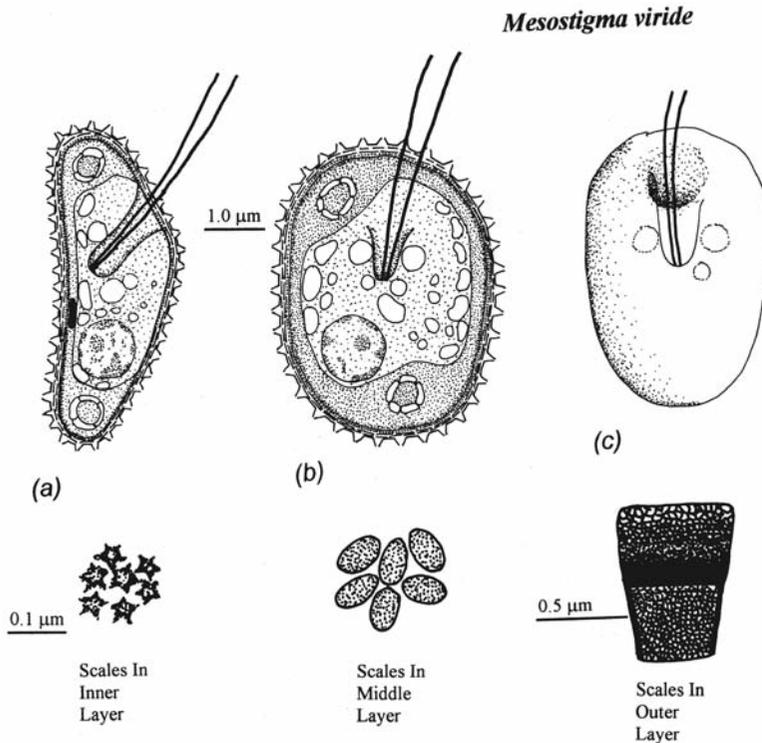


**Fig. 5.13** *Tetraselmis* subgenus *Prasinocladia*. (a) Vegetative cells on a stalk. (b) Zoospores within a stalk. (c) Zoospore. (After Proskauer, 1950.)

in the egg, but there are algae adhering to the egg case that are ingested by the emerging worms. The alga that infects *Convoluta roscoffensis* is *Tetraselmis* (Fig. 5.14(b)) (Oschman, 1966). These algae lie in the extracellular spaces between adjacent cells of the animal. The infecting algae undergo morphological alterations within the worms, losing their flagella, theca, and eyespot in that order. The *Tetraselmis* theca does not contain cellulose (Lewin, 1958), a possible explanation for the relative ease with which the worm can dissolve the theca and set up the symbiosis. Upon loss of the theca, the alga assumes an irregularly shaped form, with finger-like processes of the algal cells penetrating between adjacent animal cells. *Convoluta* flatworms may contain algal cells of either the *Tetraselmis* or *Prasinocladia* subgenera, although one animal will usually contain cells of only one subgenus. In the United Kingdom, the flatworms having cells of the *Tetraselmis* subgenus are longer, are more likely to contain gametes, and lay more egg capsules containing more embryos than those containing cells of the *Prasinocladia* subgenus (Douglas, 1985). If the *Convoluta* eggs are not infected by *Tetraselmis*, they fail to develop properly and soon die. In early life, *Convoluta*, like other flatworms, feeds on smaller



**Fig. 5.14** (a) *Convoluta roscoffensis* flatworm with symbiotic green algae. (b) *Tetraselmis convolutae*, the algal symbiont of *Convoluta*. (c) Chloroplast; (e) eyespot; (f) flagella; (g) Golgi body; (m) mitochondrion; (n) nucleus; (s) starch. ((a) after Russell and Yonge, 1963; (b) after Parke and Manton, 1967.)

**Fig. 5.15** *Mesostigma viride*.

Cytology of the cell from the lateral (a) and medial (b) side. (c) Whole cell showing flagellar pit. Scales covering the body are also shown. The inner scales also cover the flagella. (Adapted from Manton and Ettl, 1965; Marin and Melkonian, 1999.)

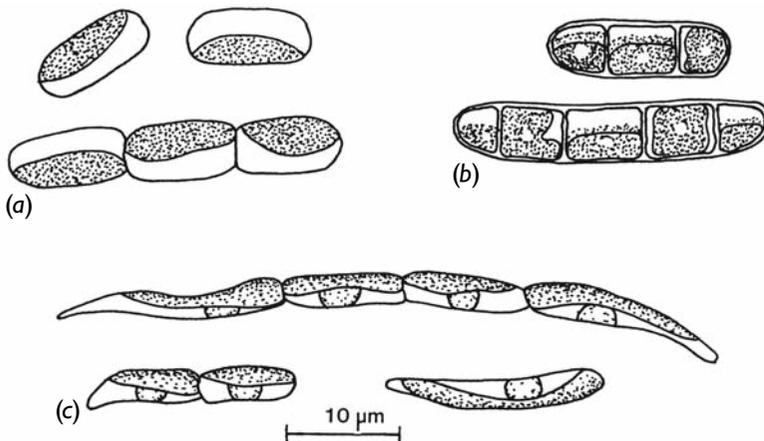
animals. As the flatworm gets older, it relies on photosynthate from the internal algae, and its digestive organs degenerate so that it is not able to feed like a normal animal. The *Convoluta*, however, needs a more varied diet than just that of algal photosynthate and begins after a time to feed upon the algae – to kill the geese that laid the golden eggs – so that the algae gradually disappear, the flatworm presenting the strange appearance of a green head and a white tail. Finally the flatworm dies of starvation, but not before it has laid a large number of eggs.

## Charophyceae

This is the line of algal evolution that led to the development of land plants. The motile cells of the advanced members of the class are similar to the flagellated male gametes of the bryophytes and vascular cryptogams. The *motile cells* of the Charophyceae are *asymmetrical and have two laterally or subapically inserted flagella*. The *microtubular root system contains a multilayered structure that is associated with a broad microtubular root and a second,*

*smaller, microtubular root*. Rhizoplasts are not present. The mitotic spindle is persistent during cytokinesis, and cell division occurs by means of a phragmoplast. No eyespots occur. Sexual reproduction results in the formation of a dormant zygote. Meiosis occurs when the zygote germinates. Glycolate is broken down by glycolate oxidase, whereas urea is broken down by urease. The algae in the class are predominantly freshwater algae.

The unicellular alga *Mesostigma* is thought to be the closest extant relative to the “ancestral green flagellate” and represents the most primitive alga in the Charophyceae (Marin and Melkonian, 1999; Lemieux et al., 2000). *Mesostigma viride* (Fig. 5.15) is a freshwater biflagellate alga with flagella arising from a depression in the cell (Manton and Ettl, 1965). Each flagella is anchored by two microtubular roots, with one root having an associated multilayered structure (Melkonian, 1989). The single chloroplast contains pyrenoids, an eyespot, and unique pigments (Yoshii et al., 2003). The cell is covered by three layers of scales. An inner layer of five-sided scales, a middle layer of oval-shaped scales, and an outer layer of basket-shaped scales.



**Fig. 5.16** (a) *Stichococcus bacillaris*. (b) *Klebsormidium* sp. (c) *Raphidonema nivale*. ((c) after Hoham, 1973.)

## Classification

Within the Charophyceae are four important orders:

- Order 1 **Klebsormidiales**: unbranched filaments without holdfasts; plasmodesmata absent; zoospores naked and released through a pore in the wall.
- Order 2 **Zygnematales**: sexual reproduction by conjugation; unicells or unbranched filaments without holdfasts; plasmodesmata absent in filamentous forms; flagellated cells not produced.
- Order 3 **Coleochaetales**: oogamous sexual reproduction; motile cells have a covering of scales, sheathed setae present; unicells, branched filaments or discoid thalli.
- Order 4 **Charales**: oogamous sexual reproduction; sterile cells surround antheridia and oogonia; male gametes covered with scales; zoospores not produced; complex plant body with apical growth and differentiation into nodes and internodes; plasmodesmata present.

### Klebsormidiales

The algae in this order are terrestrial or freshwater algae with unbranched filaments that do not have holdfasts. Each cell contains a single parietal chloroplast. There are no plasmodesmata between cells. The zoospores are naked and are

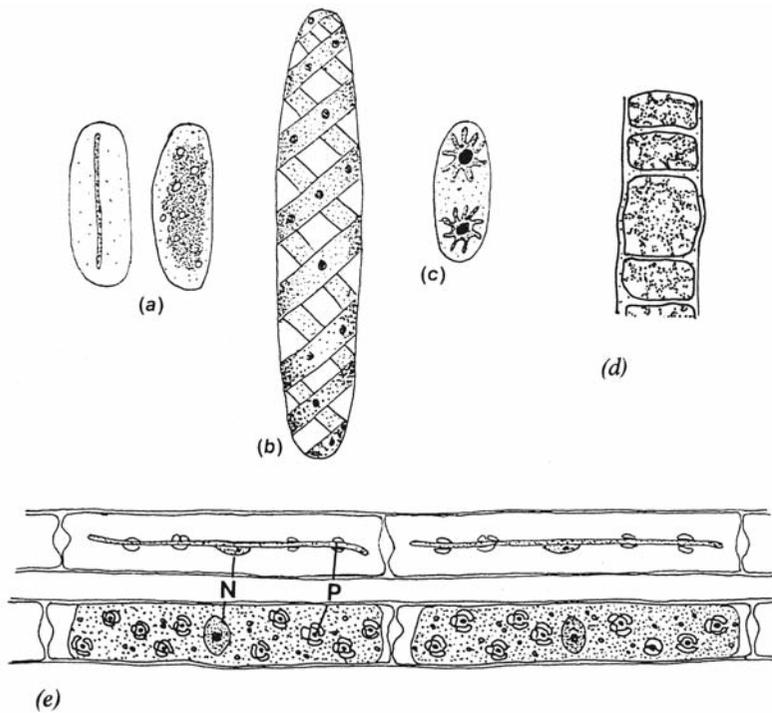
released through a pore in the wall. Algae definitely placed in this order on the basis of their cytology include *Klebsormidium* (Fig. 5.16(b)) (Pickett-Heaps, 1974), *Stichococcus* (Fig. 5.16(a)) (Floyd et al., 1972), and *Raphidonema* (Fig. 5.16(c)) (Pickett-Heaps, 1976).

Sexual reproduction is isogamous by biflagellate gametes. Reproduction and morphology of the algae in this order are similar to those in species of *Ulothrix*, with which some of the members of the Klebsormidiales were originally classified before their ultrastructural differences became apparent.

### Zygnematales

The Zygnematales are a closely related group (McCourt et al., 2000; Gontcharov et al., 2002) of freshwater algae that are unique among the Chlorophyta in having sexual reproduction by isogamous conjugation in which the gametes are non-flagellated. The union of the two gametes can be through a conjugation tube formed by the parent cells, or the gametes can move from their parent cells into the medium and fuse. The zygote or zygospore forms a cell wall and goes through a resting period before germinating meiotically. The life cycle is therefore primarily haploid, with the zygote representing the diploid generation. Zygnematalean algae, particularly *Mougeotia* (Fig. 5.17(e)), dominate freshwaters affected by acid precipitation (Graham et al., 1996).

There are basically three types of chloroplasts in the order: (1) spirally twisted bands extending



**Fig. 5.17** (a) *Mesotaenium de greyi*, high- and low-intensity orientations of the chloroplast. (b) *Spirotaenia condensata*. (c) *Cylindrocystis brebissonii*. (d) *Zygonium* sp. (e) *Mougeotia scalaris*, above-profile and below-surface views of the chloroplast. (N) Nucleus; (P) pyrenoid.

the length of the cell as in *Spirogyra* (Fig. 5.18) and *Spirotaenia* (Fig. 5.17(b)); (2) an axial plate extending the length of the cell as in *Mougeotia* (Fig. 5.17(e)) and *Mesotaenium* (Fig. 5.17(a)); and (3) two stellate chloroplasts next to each other as in *Cylindrocystis* (Fig. 5.17(c)). In those cells with flat axial chloroplasts, there is a marked chloroplast orientation in response to light intensity. In *Mesotaenium* and *Mougeotia* (Fig. 5.17), the chloroplast presents a surface view to the light under low intensities. When irradiated with high-intensity light, or red light at low intensity, the chloroplast rotates to present an edge view. Actin microfilaments attached to the chloroplasts are directly responsible for movement of the chloroplast (Mineyuki et al., 1995) with a phytochrome system directing actin functioning.

Within the Zygnematales there are three families (some phycologists group the last two families into one family).

Family 1 Zygnemataceae: cylindrical cells united permanently into unbranched filaments; cell wall without pores.

Family 2 Mesotaeniaceae: basically non-filamentous; cell walls without pores;

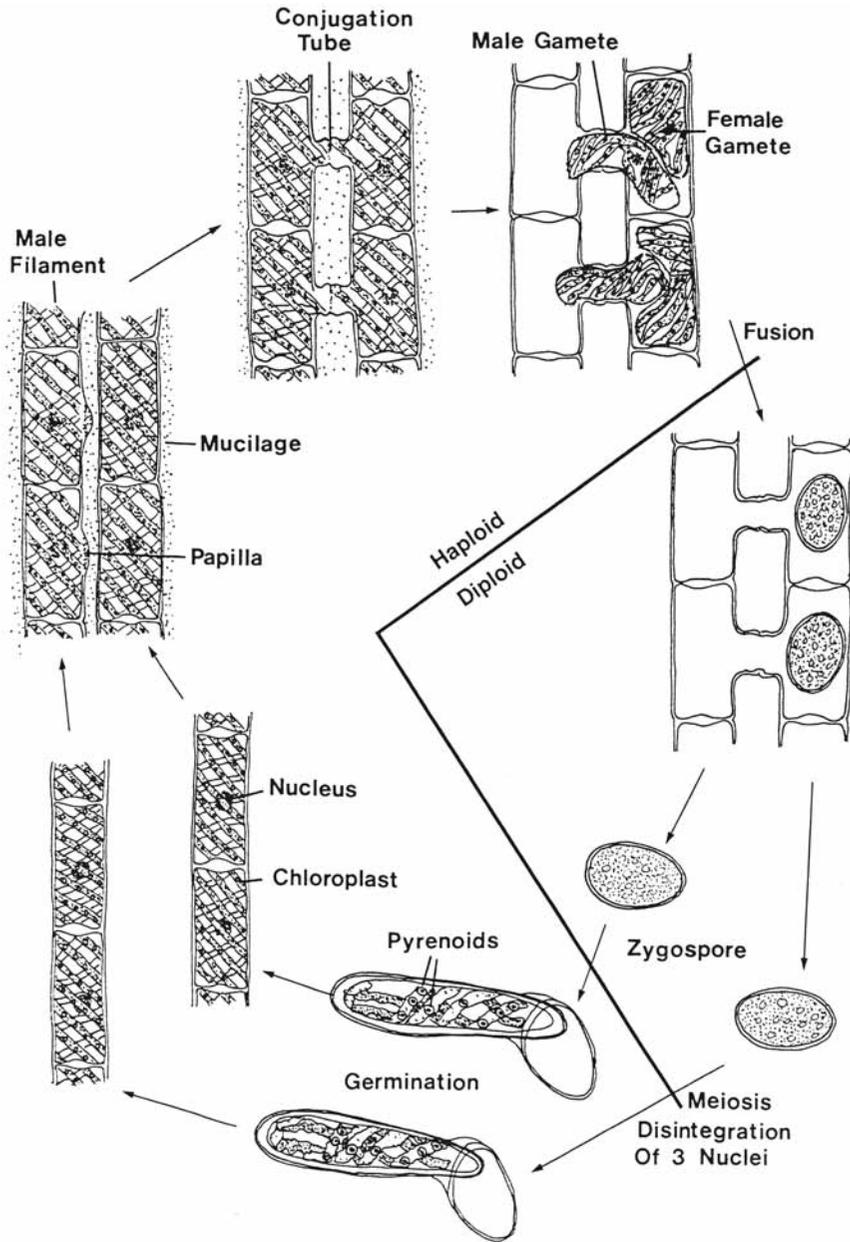
no new semicell formed in cell division.

Family 3 Desmidiaceae: basically non-filamentous; cell walls with pores; new semicell formed in cell division.

### Zygnemataceae

The cells of the Zygnemataceae are permanently united into unbranched filaments, and the cell walls lack pores. Union of the two aplanogametes is usually by the establishment of a conjugation tube between two cells.

Members of the Zygnemataceae are among the most common filamentous freshwater algae, favoring small stagnant bodies of water, but with a few found attached in the littoral zone of lakes (*Spirogyra adnata*) and in flowing water (Berry and Lembi, 2000). They are especially abundant in the spring months, generally occurring as bright green free-floating masses, with some type of attaching organ present in young stages of several genera. Planktonic species of *Spirogyra* or *Mougeotia* often have twisted or spirally coiled threads. *Zygonium* (Fig. 5.17(d)), which lives next to thermal springs with acidic waters, is responsible for the dense purple mats that occur in the

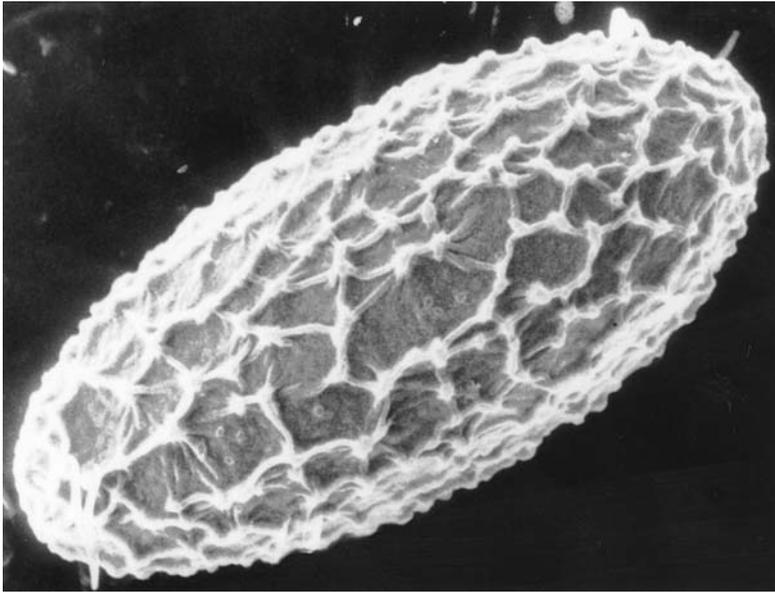


**Fig. 5.18** The life cycle of *Spirogyra*.

hot springs of the Yellowstone National Park, where it lives at temperatures of 21 to 30 °C and a pH of 2.4 to 3.1; the optimum temperature for photosynthesis is 25 °C, and the pH optimum is 1 to 5 (Lynn and Brock, 1969).

*Spirogyra* occurs primarily in the springtime because it tolerates high light intensities in cool

water (Graham et al., 1995). *Spirogyra* has ribbon-shaped chloroplasts with a number of pyrenoids along the length of the chloroplast (Fig. 5.18). A nucleus is suspended in the center of the cell. Every cell in the filament except the basal one is capable of cell division. Asexual reproduction occurs by fragmentation of the filaments, whereas sexual reproduction is by conjugation, which is initiated by two filaments coming to lie



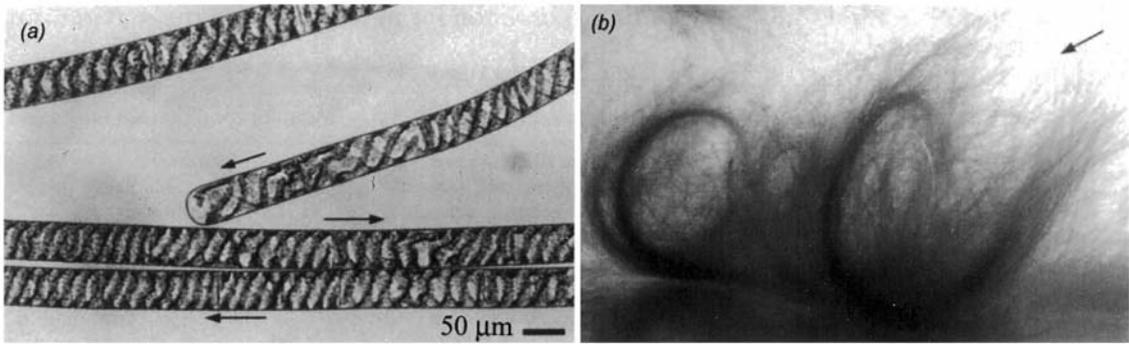
**Fig. 5.19** Scanning electron micrograph of a zygote of *Spirogyra acanthopora* showing the sculpturing of the wall. (From Simons et al., 1982.)

next to one another, and being bound together in a layer of mucilage. The cells of one filament put out papillae toward the cells of the opposite filament. Subsequently, papillae arise from opposite and corresponding points on the other filament so that the two papillae are in contact from the beginning of their formation. The two threads are then pushed apart as the papillae elongate. A hole is dissolved at the tips of the papillae so that the conjugation tube is continuous from one cell to the next. The male gamete (the protoplast that moves through the conjugation tube) contracts by the bursting of small contractile vacuoles within the cell membrane, which is followed by a similar contraction of the female gamete as the male gamete moves through the conjugation tube. The male protoplast fuses with the female inside the parent wall of the female. The zygote (zygospore) secretes a three-layered wall around itself. The three layers of the wall from the outside to the inside are the **exospore**, **mesospore**, and **endospore**. The **exospore** is sometimes sculptured (Fig. 5.19) and contains cellulose and/or pectin. The **mesospore** is sometimes colored and contains sporopollenin. The **endospore** is thin and colorless, and contains cellulose and pectin (Simons et al., 1982). Ripening of the zygote is accompanied by the disappearance of chlorophyll and the conversion of the starch into a

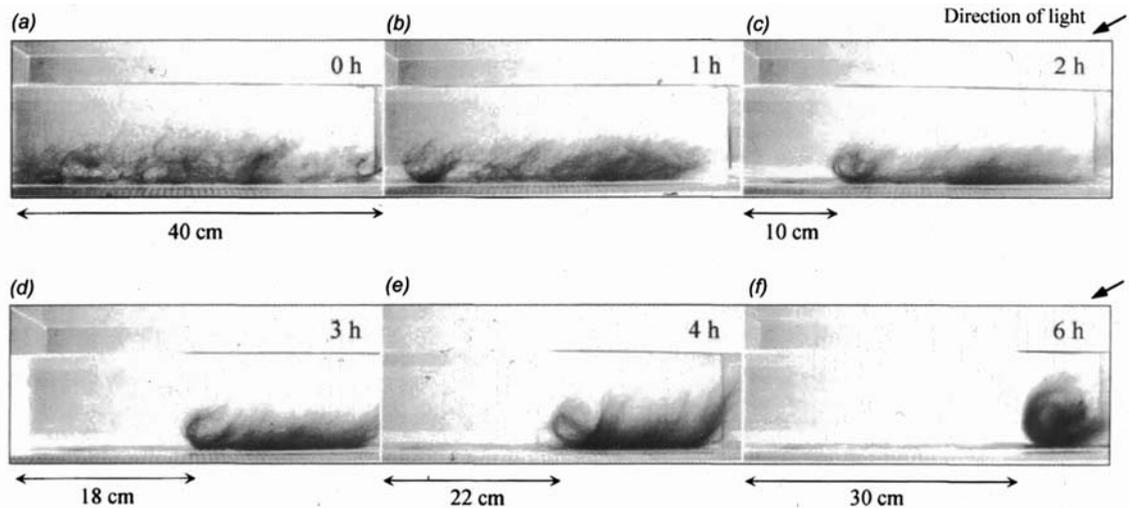
yellowish oil. During the resting period of the zygote (in nature until the following spring), the nucleus divides meiotically to form four haploid nuclei, three of which disintegrate. The zygote germinates by sending out a tubular growth that ruptures the outer two wall layers while the inner wall extends to accommodate the growth. The outgrowth then undergoes transverse divisions to form the first cells of the filament. The life cycle is thus primarily haploid, with the zygote being the only diploid cell. Conjugation is referred to as “physiologically anisogamous” because of the different behavior of morphologically similar gametes.

The above type of conjugation, which is called **scalariform** conjugation, occurs between two separate filaments. Another type of conjugation is **lateral** conjugation, which occurs between cells of the same filament. Here a conjugation tube is formed between adjacent cells, or in some cases the cross wall between adjacent cells simply dissolves. The members of this family would seem to be excellent tools for the study of fertilization, but this is not so because of the difficulty of inducing sexual reproduction at will.

*Spirogyra* mats show an unusual phototactic movement toward blue light (optimal at 470 nm), but not red light (Figs. 5.20, 5.21) (Kim et al., 2005). Directing blue light from an angle toward



**Fig. 5.20** Phototactic movement of *Spirogyra*. (a) Gliding movement between filaments. Arrows indicate the direction of filament movement. (b) Phototropic curvature of filaments toward light. Arrow shows the direction of light. (From Kim et al., 2005.)



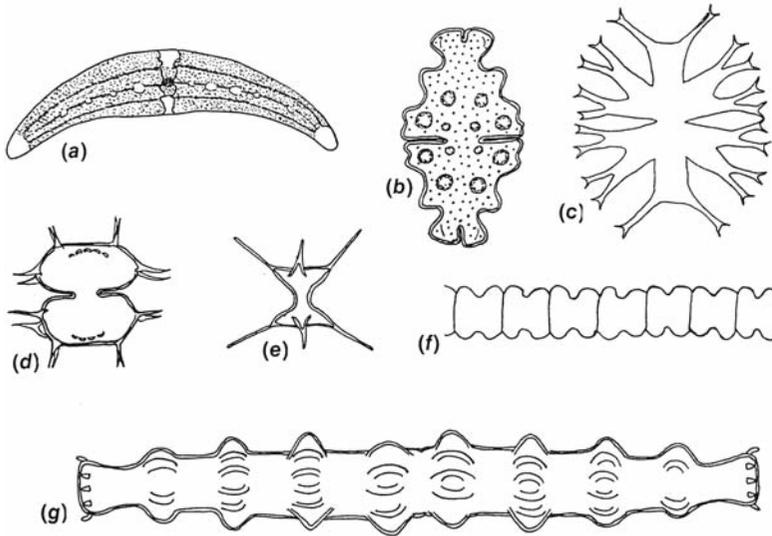
**Fig. 5.21** Sequential photographs of *Spirogyra* movement toward the light source. Arrows on the right show the direction of light. (From Kim et al., 2005.)

scattered *Spirogyra* filaments cause the filaments to align toward the light within one hour. The filaments bind with other filaments to form thicker parallel bundles that curve and form an open-loop shape. The bundle of filaments move toward the light source at  $10 \mu\text{m s}^{-1}$  by two different movements, the gliding movement between filaments and curvature of filaments. The bundles of filaments join and form a larger mat when they meet. The coordination of filaments is essential for the phototactic movement. The filaments always form

a bundle before they start moving toward light. A single filament repeatedly curls, straightens, and bends, but there is no phototactic movement. Filaments will only glide on other filaments. The exact mechanism involved is not known, but it may be that filaments oriented toward blue light prolong movement and shorten their movement when they are not oriented toward the light.

#### Mesotaeniaceae

This family has organisms that are basically unicellular, even though in some cases they are joined together to form filaments, and the cells taper to their ends. The walls do not have pores.



**Fig. 5.22** (a) *Closterium moniliforme*. (b) *Euastrum* affine. (c) *Micrasterias radiata*. (d) *Xanthidium antilopaeum*. (e) *Staurastrum curvatum*. (f) *Spondylosium moniliforme*. (g) *Pleurotaenium nodosum*. (After Smith, 1950.)

The Mesotaeniaceae are called the saccoderm desmids; unlike the placoderm desmids of the following family, they do not have a new semicell formed after cell division. The nucleus is central in the cell, and there are three types of chloroplast structure, which are similar to those in the Zygnemataceae. *Mesotaenium* (Fig. 5.17(a)), *Cylindrocystis* (Fig. 5.17(c)), and *Spirotaenia* (Fig. 5.17(b)) are in the family. Sexual reproduction takes place by conjugation and is similar to that in the other two families of the order.

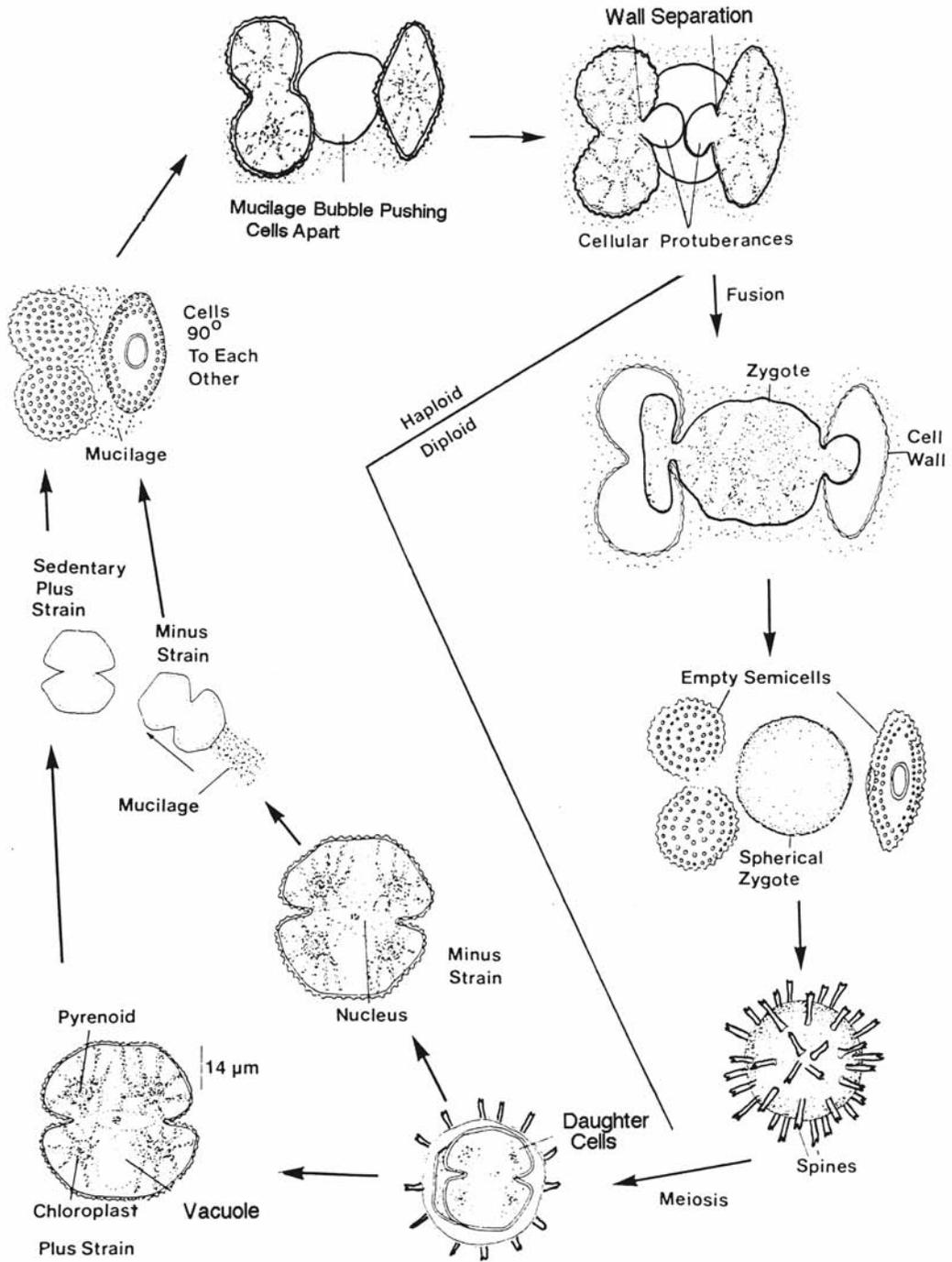
These organisms are indicative of water low in magnesium and calcium and therefore usually unpolluted water. They are common in upland pools and peat bogs; *Mesotaenium* and *Cylindrocystis* are found in wet soil.

### Desmidaceae

These are the placoderm desmids. They have two distinct halves or semicells separated by a median constriction, the **sinus**, and joined by a connection zone, the **isthmus**. The cell walls have pores in them (Gerrath, 1969). The cells can be solitary, joined end to end in filamentous colonies, or united in amorphous colonies. Some of these plants have a relatively complex and attractive shape (Fig. 5.22). The taxonomy of many of these desmids is complicated by **polymorphism** (different forms within the same species), which often makes organisms of the same species appear as different species (Sormus and Bicudo, 1974).

*Cosmarium botrytis* has a central nucleus and two chloroplasts in each semicell, each with a central pyrenoid (Fig. 5.23). At the tip of each semicell are crystals of barium carbonate that move irregularly (Brook, 1989). Mixing cells of opposite strains ( $mt^+$  and  $mt^-$ ) results in the cells moving about, with each strain indifferent to the other. During this period  $mt^-$  cells release a proteinaceous pheromone that causes  $mt^+$  cells to form gametes; likewise  $mt^-$  cells are similarly induced to produce gametes by a pheromone (Fukumoto et al., 2003).

After pairing, the cells move around each other and twist about until their broadest faces are in contact, and their longitudinal axes are at right angles. A sphere of mucilage is secreted around the conjugants and also between them, forcing the cells apart. The protoplast escapes from the gametes by the separation of the wall in the region of the isthmus (Starr, 1954). Within 4 to 7 minutes after making contact, the protoplasts fuse, forming an irregularly shaped zygote. The zygote then rounds off and forms a smooth-walled cell. The chromatophores arrange themselves at the periphery of the cytoplasm, and the ornamentation of the wall begins to appear about 1 hour after the fusion of the gametes. Spines develop on the wall, with the tips commonly divided into two or three parts. The zygote germinates meiotically, completing the life cycle.



**Fig. 5.23** The life cycle of *Cosmarium botrytis*.

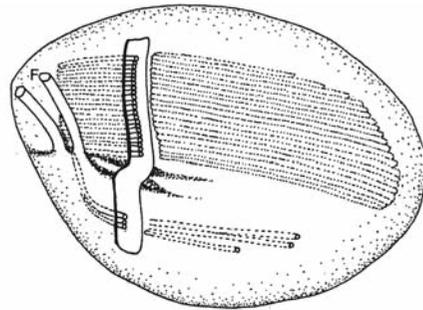
The desmids are usually indicators of relatively unpolluted water, low in calcium and magnesium, with a slightly acidic pH. Normally a large number of species are present in such waters, without a single species comprising most of the population.

### Coleochaetales

The algae in the Coleochaetales are characterized by the presence of sheathed setae and by oogamous sexual reproduction. Small-subunit ribosomal RNA sequencing (Wilcox et al., 1993; Kranz et al., 1995) has indicated that the green algae in this order evolved into the bryophytes and lycophods. Like the bryophytes and lycophods, the algae in the Coleochaetales have asymmetrical motile cells covered with scales, microtubular roots consisting of a large and small band (Sluiman, 1983), a persistent interzonal spindle, and a phragmoplast at cytokinesis; glycolate is degraded by glycolate oxidase (Tolbert, 1976). In addition some of the species in the Coleochaetales have evolved a protective sheath around the oogonium, which is an advance toward the protected oogonia in the Bryophyta.

Most of *Coleochaete* (Fig. 5.25) occur as epiphytes or endophytes in the shallow littoral zone of fresh-water lakes (Ciminio and Delwiche, 2002) and may be observed by microscopic examination of macrophyte leaves or inorganic substrates such as beer cans, soda bottles, or plastic bags discarded by fishermen (Graham, 1984). The thallus is composed of branching filaments, which are free in some species, whereas in others the branches are laterally opposed to form a pseudoparenchymatous disc. All members of the genus have sheathed hairs called **setae** (*Coleochaete* comes from the Greek *koleos* sheath + *chaite* long hair). The base of the setae is covered with a gelatinous material. The setae probably function as an antiherbivore defense, since broken setae exude a substance that repels potential predators (Marchant, 1977).

Zoospores and spermatozoids of *Coleochaete* (Fig. 5.24) are asymmetrical, are covered with scales, and possess multilayered structures and microtubular roots similar to those of lower land plants (Graham and McBride, 1979; Sluiman, 1983). The flagella arise subapically and project to the side of the cell (Fig. 5.24). The flagella are anchored by a microtubular root system composed of a single

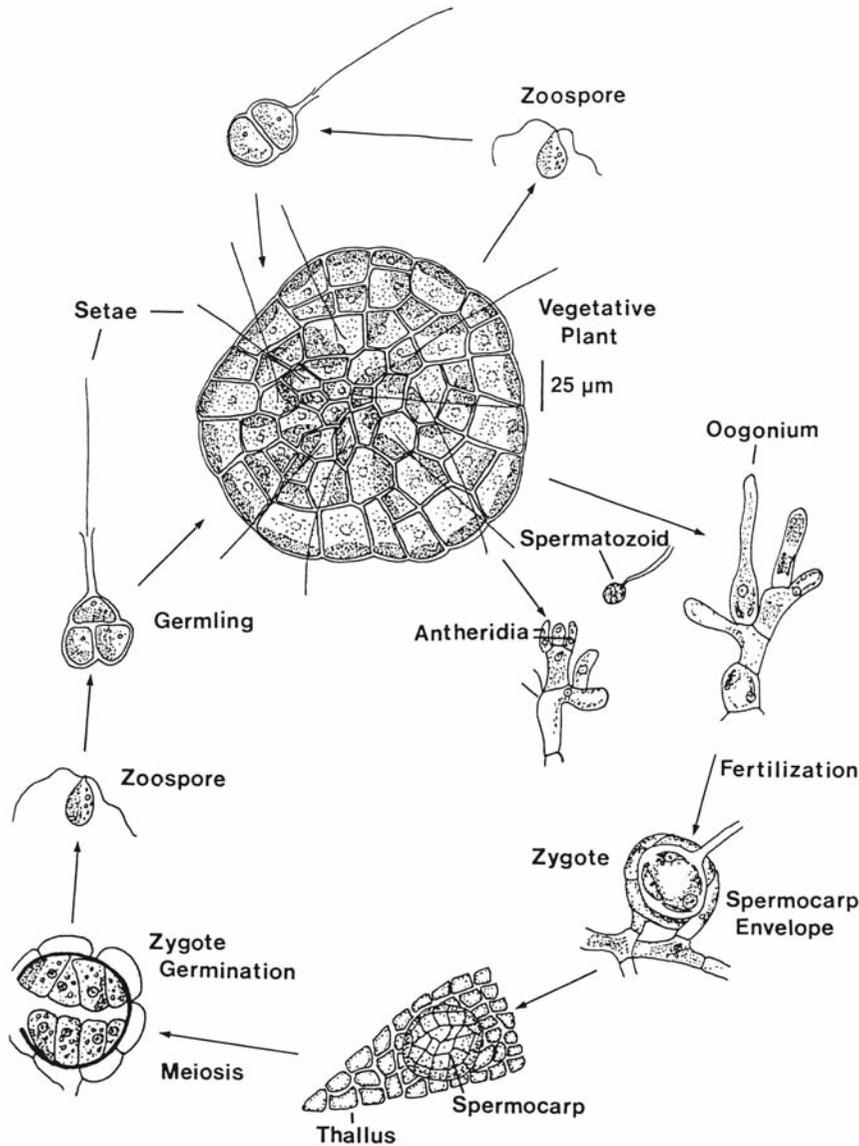


**Fig. 5.24** Drawing showing the subapical flagella (F) in a zoospore of *Coleochaete*. The flagella are anchored in the cytoplasm by two microtubular roots. A broad microtubular root is associated with a multilayered structure. There is a small microtubular root composed of three microtubules. (After Sluiman, 1983.)

broad band of microtubules associated with a multilayer structure and a second microtubular root.

Asexual reproduction is by biflagellate zoospores which are formed singly in a cell, most frequently in the spring. Initiation of zoosporogenesis is induced primarily by temperature. Northern temperate species undergo zoosporogenesis when brought to 20 °C for a few days (Graham and Krantzfelder, 1986). Day length and irradiance have little effect on zoosporogenesis.

Sexual reproduction in *Coleochaete* is oogamous (Fig. 5.25) and the plants can be homothallic or heterothallic, depending on the species. Antheridia are borne at the tips of the branches, and each antheridium forms a single spermatozoid. Oogonia are modified one-celled branches, and an oogonium is a flask-shaped structure with a long colorless neck, the trichogyne. At maturity the tip of the neck breaks down, with some colorless protoplasm being extruded and the basal protoplast rounding off to form a single egg. The spermatozoid swims into the oogonium and fertilizes the egg. The zygote remains in the oogonium, secretes a thick wall, and greatly increases in size. At the same time there is an upgrowth of branches from the cell below the oogonium and from neighboring cells to form a pseudoparenchymatous layer enclosing the oogonium. The oogonium, with its enclosing sheath layer of cells, becomes reddish and is termed a **spermocarp**; the spermocarps remain dormant over the winter.



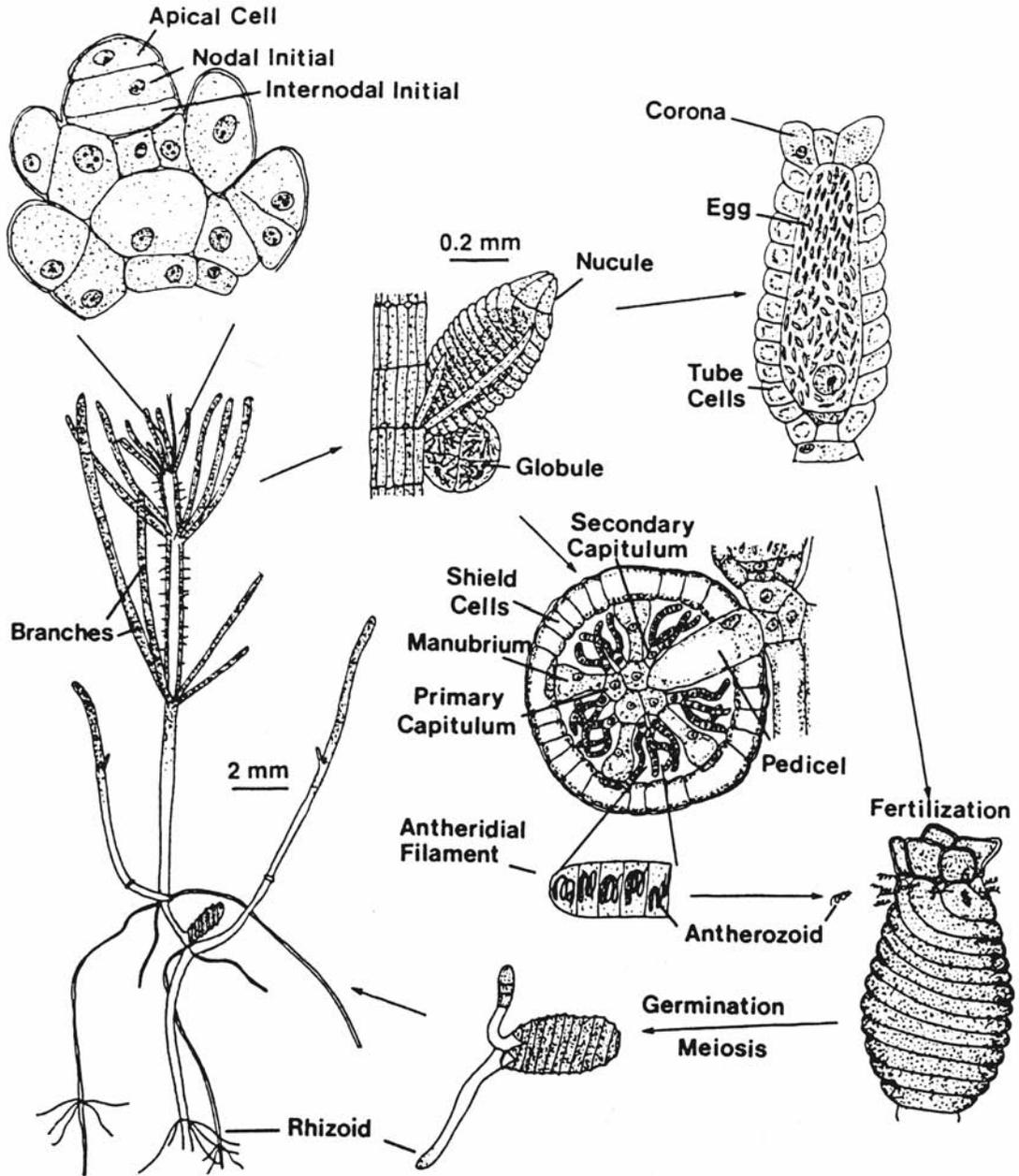
**Fig. 5.25** The life cycle of *Coleochaete scutata*. (After Oltmanns, 1898; Smith, 1950.)

The zygote germinates meiotically to form 8 to 32 biflagellate zoospores, which are liberated through a break in the spermocarp and zygote walls, and swim for a short time before settling and developing new thalli.

**Charales**

The green algae in the Charales have a complex plant body with apical growth and differentiation

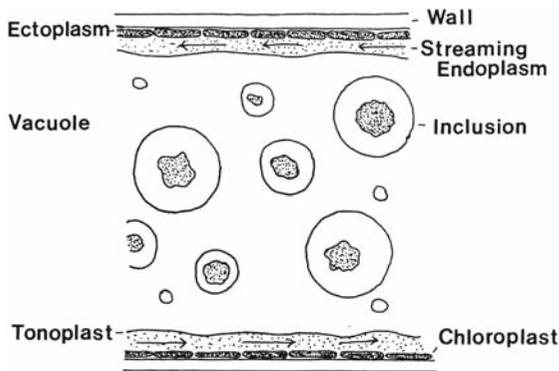
of the body into **nodes** and **internodes**. Reproduction is oogamous, with sterile cells surrounding the antheridia and oogonia. The male gametes have a cell covering of scales. No zoospores are formed. A phragmoplast develops during cell division, resulting in the formation of a cross wall with plasmodesmata. Land plants (embryophytes) probably evolved from algae similar to those in the Charales (Karol et al., 2001). As such, the Charales are sometimes separated from the rest of the green algae and grouped with embryophytes as **streptophytes** (Lewis and McCourt, 2004).



**Fig. 5.26** The life cycle of *Chara* sp. (Adapted from Smith, 1955; Scagel et al., 1965.)

The algae in the Charales are primarily freshwater, with only a few species occurring in brackish water. They are most common at the bottom of clear lakes, usually forming extensive growths. Many of the Charales are heavily calcified, with concentrations of plants on the bottom of lakes

leading to the formation of marl ( $\text{CaCO}_3$  and  $\text{MgCO}_3$  deposits) and hence the common name of the Charales, **stoneworts**. Calcification in *Chara* and *Nitella* results from the precipitation of calcium carbonate ( $\text{CaCO}_3$ ) in water high in  $\text{Ca}^{2+}$ . The localized  $\text{OH}^-$  efflux at certain areas of the internodal cell raises the local  $\text{CO}_3^{2-}$  ion concentration, leading to  $\text{CaCO}_3$  supersaturation and precipitation (Lucas, 1979). In *Chara corallina*,



**Fig. 5.27** Schematic view of part of an internodal cell of *Nitella*. The chloroplasts are stationary in the ectoderm, whereas the endoderm moves by cytoplasmic streaming. (After Kamitsubo, 1980.)

rectangular crystals of calcite are deposited in bands on the surface of the cell wall of the cylindrical internodal cell. These bands correspond to localized alkaline (pH 9.5 to 10.5) regions on the cells. There is no organic material associated with the crystals, and they are formed entirely outside the cell wall (Borowitzka, 1982; Wray, 1977).

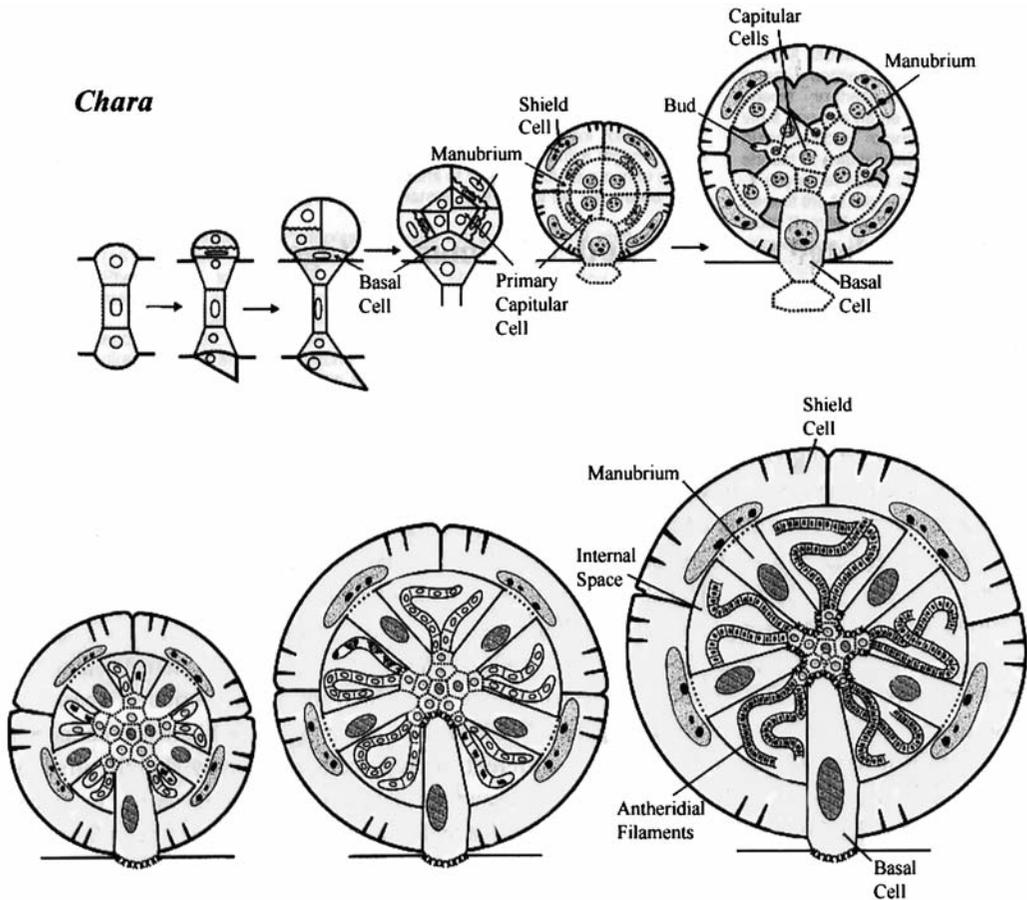
The algae in the Charales have an axis divided into nodes and internodes (Fig. 5.26). Each **node** bears a whorl of branches composed of a number of cells that cease to grow after they have reached a certain length. The **internode** consists of a single long cell in *Nitella*. In some genera, such as *Chara*, the internodal cell is ensheathed (corticated) by a layer of vertically elongated cells of a much smaller diameter originating at the nodes (Fig. 5.26). Growth occurs by a dome-shaped apical cell, each derivative of the apical cell dividing transversely into two daughter cells. The upper daughter cell is the nodal initial, and the lower is the internodal initial. The **nodal initial** matures into the cells of the node, and the **internodal initial** matures into the single long cell of the internode (Smith, 1955). The axis is attached to the substratum by uniseriately branched rhizoids. The rhizoids are positively geotropic and grow downward. Near the rhizoid tip are a group of **statoliths** consisting of vacuoles containing crystals of barium sulfate that may be associated with the response of the rhizoids to gravity (Braun and Richter, 1999).

The uninucleate cells have many small ellipsoidal chloroplasts in longitudinal, spirally twisted, parallel rows. In the center of the cells is a single large vacuole. The large size of the internodal cell and its vacuole has made the cell a favorite tool of physiologists interested in the control of ion uptake in plants. The pH of the cell sap of *Chara* is controlled at about 5 as a result of a dynamic equilibrium between active influx and passive efflux of  $H^+$  across the tonoplast. The  $H^+$  transport system involves two proton ( $H^+$ ) translocating systems in the tonoplast (Shimmen and MacRobbie, 1987). **Cytoplasmic streaming** is particularly evident in these large internodal cells. The cytoplasm is divided into an inner **endoplasm** next to the vacuole, and an outer **ectoplasm** containing the chloroplasts (Fig. 5.27). Streaming endoplasm follows the orientation of the spirally arranged stationary chloroplasts in the ectoplasm at a rate of  $60 \mu\text{m s}^{-1}$  at  $20^\circ\text{C}$ . Streaming is due to the interaction of actin microfilaments with cytoplasmic microtubules (Collings et al., 1996).

The Charophyta do not form zoospores, but there are specialized sexual bodies. Asexual reproduction may be effected by (1) star-shaped aggregates of cells developed from the lower nodes, called **amylum stars** because they are filled with starch; (2) **bulbils** developed on rhizoids; and (3) protonema-like outgrowths from a node.

The sexual organs are the **globule** (male) and **nucule** (female) (Fig. 5.28), the terms antheridium and oogonium not being appropriate because the sexual reproductive structures include both a sex organ and a multicellular sheath derived from cells beneath the sex organ (Smith, 1955). Globules and nucules are borne on nodes, usually on the same plant. In *Chara*, the nucule is above the globule.

The globule develops from haploid nodal cells that occur at lateral branches (Kwiatkowska, 2003). Following the first mitotic division of the initial cell, the lower cell develops into a basal cell and does not divide further, while the upper one transforms into the mother cell of the globule (Fig. 5.28). The initial cell of the globule divides three times, longitudinally and transversely, to produce eight cells with their apices joined in the center of the sphere. Successive mitotic divisions produce three cells within each octant yielding a sphere composed of 24 cells. Outer cells become

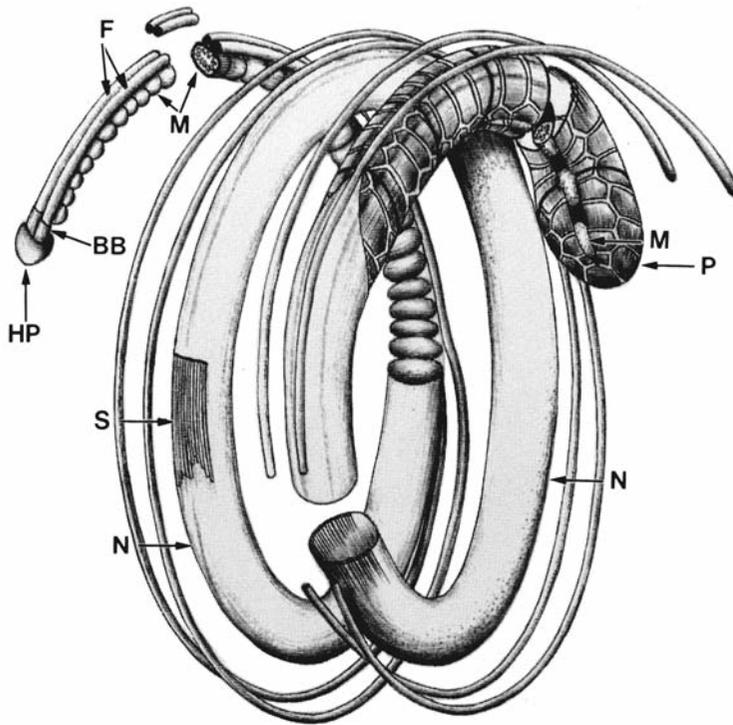


**Fig. 5.28** The development of a globule of *Chara*. (From Kwiatkowska, 2003.)

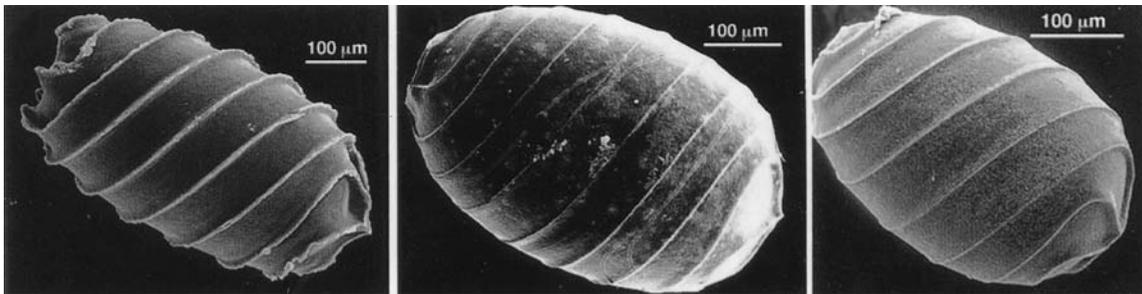
transformed into shield cells that form the colorful cover of the globule due to chromoplasts near the inner wall of the cells. The color changes from pale yellow to orange as the globules mature. The median cells develop into manubria and eight inner cells are transformed into primary capitular cells which give rise to the antheridial filaments. Each cell of an antheridial filament is an antheridium whose protoplast matures into a single antherozoid. The antherozoid is coiled in a compact helix of two and a half turns in the antheridium (Fig. 5.29). When the antherozoids are mature, the shield cells of the globule separate from one another and expose the antheridial filaments. Soon after the antheridial filaments are exposed, and antherozoids emerge backward through a pore in the cell wall.

Liberation of antherozoids generally takes place in the morning, and their swarming may continue until evening (Smith, 1955). The sperm have two somewhat unequal flagella attached subterminally near the anterior end of the cell (Fig. 5.29). There are three different regions within the body of the sperm: (1) the head region, consisting of a microtubular sheath over the mitochondria taking up one-fourth of the body; (2) the middle region, with microtubules covering the nucleus and taking up half of the body; and (3) the tail region, where the microtubules ensheath the plastids. Scales formed by the Golgi are on the outside of the flagella.

The nucle is supported by a pedicel cell, and in the center is the oogonium with its single egg. Five spirally twisted tube cells cover the oogonium except at the tip, where there are five corona cells. When the nucle is mature, the spirally twisted tube cells separate from one another just below



**Fig. 5.29** Reconstruction of the mature spermatozoid of *Chara vulgaris*. (BB) Basal bodies; (F) flagella; (HP) head piece; (M) mitochondrion; (N) nucleus; (P) plastid; (S) spline of microtubules. (From Duncan et al., 1997.)



**Fig. 5.30** Scanning electron micrographs of zygotes of *Chara muelleri* (left) and *Chara fibrosa* (middle, right). (From Casanova, 1997.)

the corona. Antherozoids swim through these openings to the oogonium, where they penetrate its gelatinized wall. The zygote secretes a thick wall, and the inner wall of the tube cells becomes thickened (Fig. 5.30). Other portions of the walls of the sheath decay, leaving the persisting portions of walls of the tube cells projecting like the threads of a screw. The zygote, with the surrounding remains of the sheath, falls to the bottom of the pool and there germinates after a period of weeks or months (Smith, 1955).

The zygote can be carried considerable distances attached to water fowl (Proctor, 1962). The zygote will not germinate in darkness and needs white or red light (Takatori and Imahori, 1971). Meiosis occurs in the first division of the zygote; thus the thallus is haploid, and the zygote is the only diploid part of the life history.

Because calcification occurs in most genera, the group is well represented as fossils, especially by the female fructification, which when calcified is termed a **gyrogonite**. The earliest fossils of Charales occur in the Uppermost Silurian. All of the extant Charales are placed in the Characeae, which date back to the Upper Carboniferous and which have the enveloping cells of the female

fructification in a left-hand spiral. An extinct family, the Trochiliscaceae, also had a spirally twisted envelope, but it was twisted to the right.

## Ulvophyceae

The motile cells of the Ulvophyceae have *apically attached flagella, near-radial symmetry externally, and a cruciate microtubular-root system that is not associated with a multilayered structure*. These characteristics differ from those of the Charophyceae but are similar to those of the Chlorophyceae. The Ulvophyceae, however, differ from the Chlorophyceae in having (1) *a persistent interzonal spindle that does not collapse at telophase, and (2) motile cells without a cell wall*.

The algae in the Ulvophyceae are predominantly marine although there are a number of freshwater species. All filamentous marine green algae or larger green seaweeds studied ultrastructurally have been shown to belong to the Ulvophyceae. The life cycle usually involves the alternation of a haploid thallus with a diploid thallus. The wide occurrence of alternation of generations in the Ulvophyceae might be due to the more stable marine environment fostering the evolution of a longer life cycle. Dormant zygotes are not known in the class. A number of genera in the Ulvophyceae produce swimmers with scales (Mattox and Stewart, 1973), indicating that the class had a scaly ancestor.

## Classification

The following orders are placed in the Ulvophyceae. Cladistic analysis of nuclear-encoded rRNA sequence data have shown that the Ulotrichales and Ulvales form one group in the class, while the Caulerpales, Siphonocladales, and Dasycladales form a second group (Watanabe et al., 2001).

Order 1 **Ulotrichales**: uninucleate filamentous algae with a parietal chloroplast.

Order 2 **Ulvales**: uninucleate cells with a parietal chloroplast; thallus is a hollow cylinder or a sheet, one or two cells thick.

Order 3 **Cladophorales**: multinucleate filamentous algae with a parietal perforate or reticulate chloroplast.

Order 4 **Dasycladales**: thallus has radial symmetry composed of an erect axis bearing branches; thallus uninucleate but multinucleate just before reproduction; gametes formed in operculate cysts.

Order 5 **Caulerpales**: coenocytic algae lacking cellulose in the walls; siphonoxanthin and siphonein usually present.

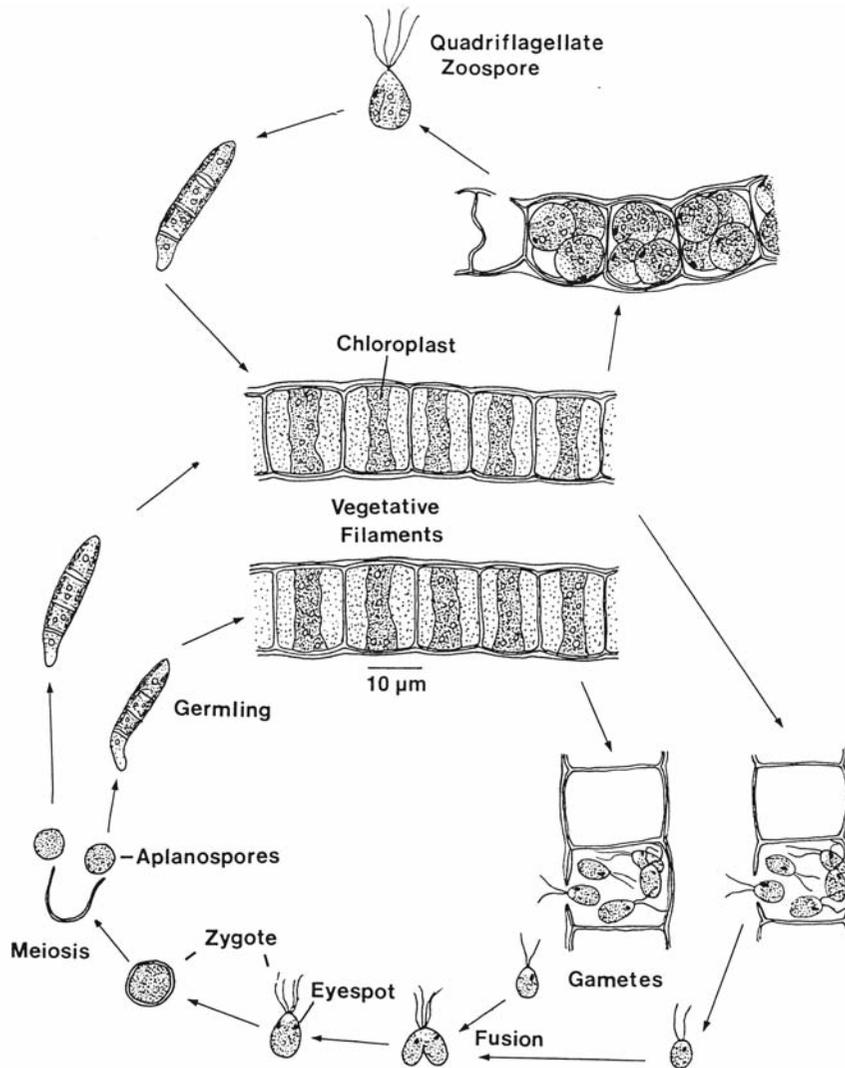
Order 6 **Siphonocladales**: algae with segregative cell division; siphonoxanthin present.

## Ulotrichales

Uninucleate filamentous green algae with a parietal chloroplast constitute this order.

*Ulothrix* (Fig. 5.31) is found in quiet or running freshwater and occasionally on wet rocks or soil. The thallus consists of unbranched filaments of indefinite length that are adfixed to the substratum by a special basal cell. All of the cells except the basal one are capable of cell division and forming zoospores or gametes. Species with narrow cells form 1, 2, or 4 quadriflagellate zoospores per cell, whereas those with broad cells form 2, 4, 8, 16, or 32 zoospores per cell. The zoospores have a conspicuous eyespot and are liberated through a pore in the side of the parent wall. Zoospores from species with narrow filaments are the same size, whereas those from broad-celled species form macro- and microzoospores that differ from each other in size, position of the eyespot, and length of the swarming period. Zoospores that are not discharged from the parent may secrete a wall and become thin-walled aplanospores. These later germinate to form a new filament.

Gametes of *Ulothrix* are formed in the same way as zoospores but are biflagellate. The gametes are of the same size, with fusion occurring only between gametes from different filaments; and there is never any parthenogenetic development of unfused gametes. The zygote remains for a while, settles, secretes a thick wall, and undergoes a resting period during which it accumulates a large amount of storage material. The first division of the zygote is meiotic, with the zygote



**Fig. 5.31** The life cycle of *Ulothrix zonata*. (Adapted from Smith, 1955.)

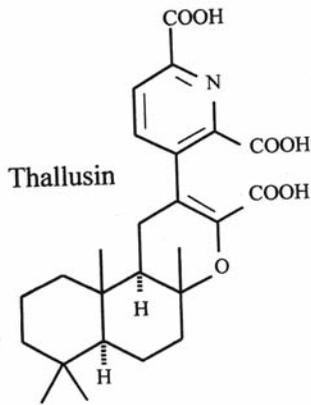
forming 4 to 16 zoospores or aplanospores (Berger-Perrot et al., 1993).

In many northern lakes in the United States and Canada, *Ulothrix zonata* grows abundantly in early spring in shallow waters along rocky shorelines. *Ulothrix zonata* is dominant until the water temperature reaches 10 °C, when it disappears owing to massive conversion of the thallus to zoospores. At this time, *Cladophora glomerata* becomes the dominant attached alga. In culture, formation of the quadriflagellate zoospores of *Ulothrix zonata* occurs around 20 °C at relatively

high light levels ( $520 \mu\text{E m}^{-2} \text{s}^{-1}$ ) and photoperiods of either short-day (8 hours light:16 hours dark) or long-day cycles (16 hours light:8 hours dark). Zoospore formation is minimal at 5 °C, low irradiance ( $32.5 \mu\text{E m}^{-2} \text{s}^{-1}$ ), and neutral day length (12 hours light:12 hours dark) (Graham and Krantzfelder, 1986).

### Ulvales

In nature, these plants have a thallus that is either an expanded sheet one (*Monostroma*) or two cells (*Ulva*; Fig. 5.33) thick. *Ulva* can also occur as a hollow cylinder. The genus *Enteromorpha* was previously erected for those *Ulva* in a hollow cylinder. These thalli composed of hollow cylinders are



**Fig. 5.32** The structure of thallusin, a morphogenetic inducer in *Ulva*. Thallusin is produced by bacteria living on the surface of *Ulva*.

now recognized as species of *Ulva* (Hayden et al., 2003).

The normal morphology of these algae is lost if the plants are grown without bacteria. Under these conditions, *Ulva* develops into a pincushion-like colony, whereas *Monostroma* grows as a group of round cells with rhizoids (Provasoli and Pinter, 1980). Bacteria of the Cytophaga-Flavobacterium-Bacteroides group grow on the surface of the algae and produce a morphogenetic factor called **thallusin** (Fig. 5.32) (Matsuo et al., 2005). The alga absorbs thallusin, resulting in the familiar morphology of the thallus.

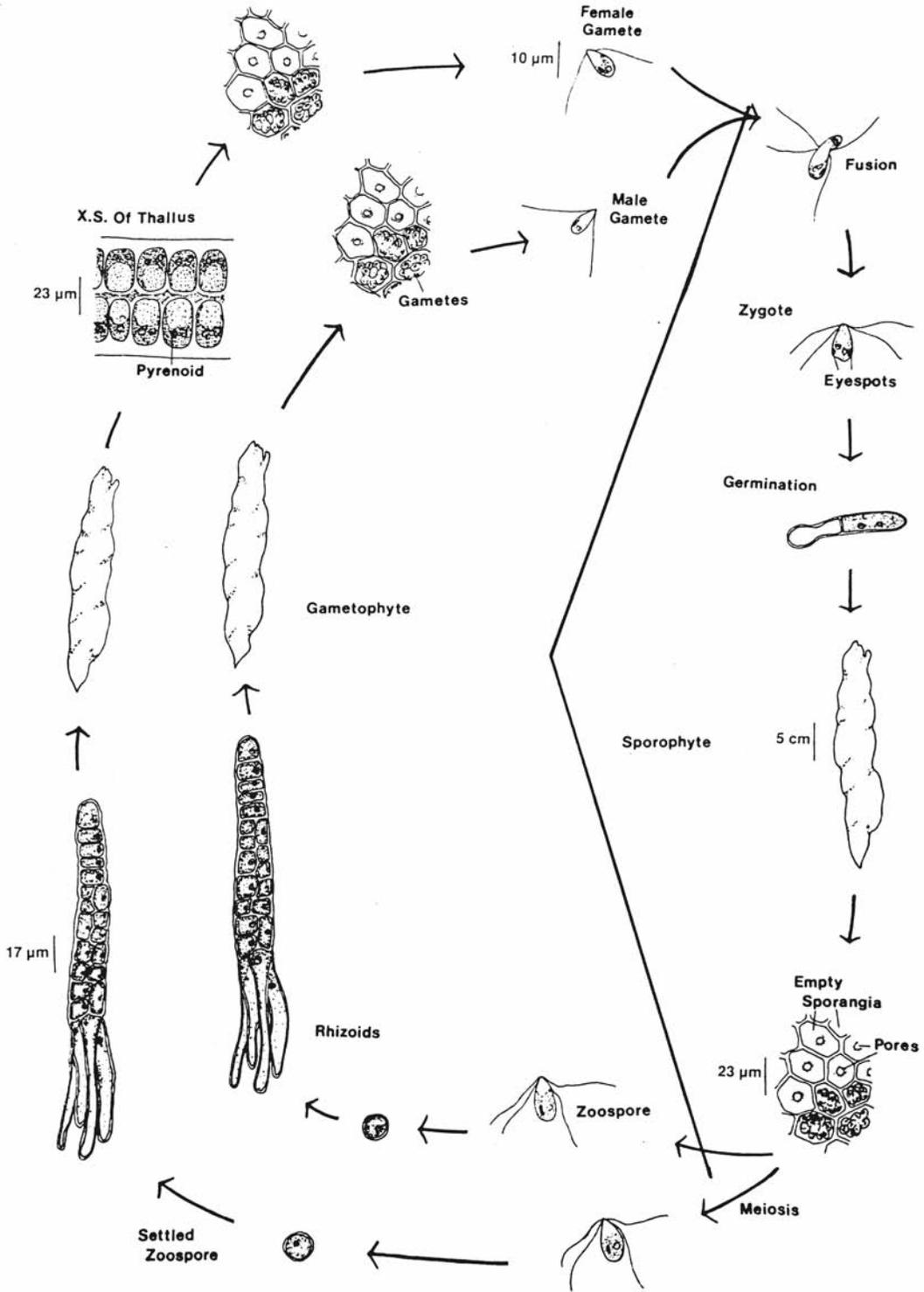
*Ulva* fronds are composed of two layers of cells, with each cell having a large cup-shaped chloroplast toward the exterior of the cell (Fig. 5.33). The holdfast is formed by the cells of the thallus, sending down long slender filaments that coalesce to form the holdfast. The holdfast portion is perennial and proliferates new blades each spring. Cell division may occur anywhere in the thallus, but all divisions are in a plane perpendicular to the thallus surface.

The vegetative state of *Ulva mutabilis* is maintained by the blade cells excreting regulatory factors into the cell walls and into the environment. The production of one of these regulatory factors, a glycoprotein, decreases as the thallus matures. Eventually, the level of this regulatory factor decreases to the point where the regulatory factor is too low to maintain the vegetative

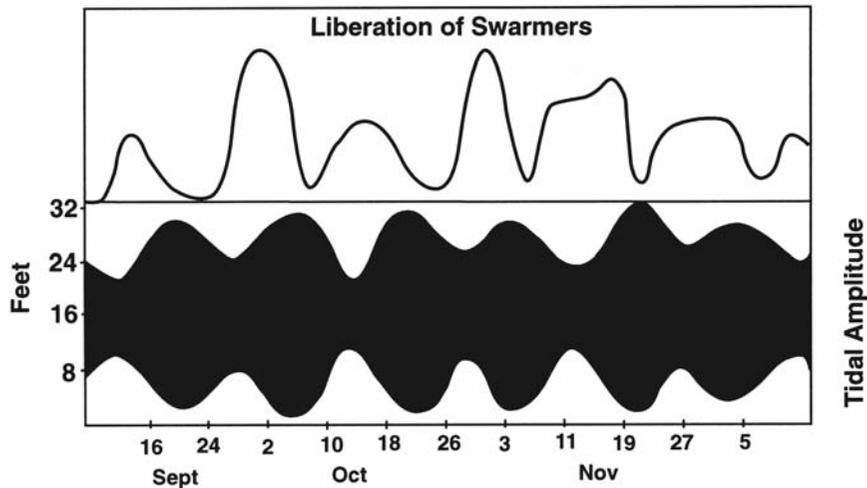
state, and gametogenesis begins (Stratmann et al., 1996).

*Ulva* (Fig. 5.33) has an isomorphic alternation of generations, with the gametophyte forming biflagellate gametes and the sporophyte producing quadriflagellate zoospores. There is a periodic fruiting pattern in the genus, which is controlled by the lunar cycle. Gametes are released a few days before zoospores, with fruiting occurring during a series of neap tides (tide of minimum range occurring at the first and third quarters of the moon) in *U. pertusa* in Japan (Sawada, 1972) and during a series of spring tides (tide of maximum range during the new and full moon) in *U. lobata* on the Pacific Coast of North America (Smith, 1947). *Ulva (Enteromorpha) intestinalis* also shows a similar fortnightly periodicity at the beginning of a series of spring tides in England, except that here both gametes and zoospores are released at the same time (Fig. 5.34) (Christie and Evans, 1962). Although it is possible to correlate fruiting in the genus with tidal patterns, it is not the tidal movements that induce fruiting and release of swimmers because the plants will exhibit a regular periodicity even when submerged. It is probably the amount of moonlight that the plants receive that gives the initial impetus to fruiting, although this may be timed by the tide as is the case with *Dictyota*.

Reproductive areas form near the margins of *Ulva* fronds (Fig. 5.33), with the fertile portions changing from green to olive-green to brownish green due to the accumulation of  $\gamma$ -carotene (Hiraoka et al., 1998). Gametogenesis and sporogenesis (Melkonian, 1980a,b) are similar and marked by a sharp reduction in photosynthetic capacity (Gulliksen et al., 1982). A cell divides to form 8 to 32 motile cells, with meiosis occurring in the formation of zoospores but not in the formation of gametes. Prior to the development of the motile cells, the mother cell forms a beak-like outgrowth extending to the thallus surface, through which the swimmers escape. The swimmers are released when the thallus is wet by the water of the incoming tide. The positively phototactic biflagellate gametes have a parietal chloroplast with a pyrenoid and eyespot. Haploid fronds are unisexual, so gametes from the same frond will not fuse. Gametes can be the same size, or one can be



**Fig. 5.33** The life cycle of *Ulva arasaki*. (Adapted from Chihara, 1969.)



**Fig. 5.34** Relationship between liberation of swimmers (gametes and zoospores) by *Ulva* (*Enteromorpha*) *intestinalis* and tidal amplitude. Maximum liberation of swimmers occurs 3 to 5 days before the highest tide of each lunar period. (After Christie and Evans, 1962.)

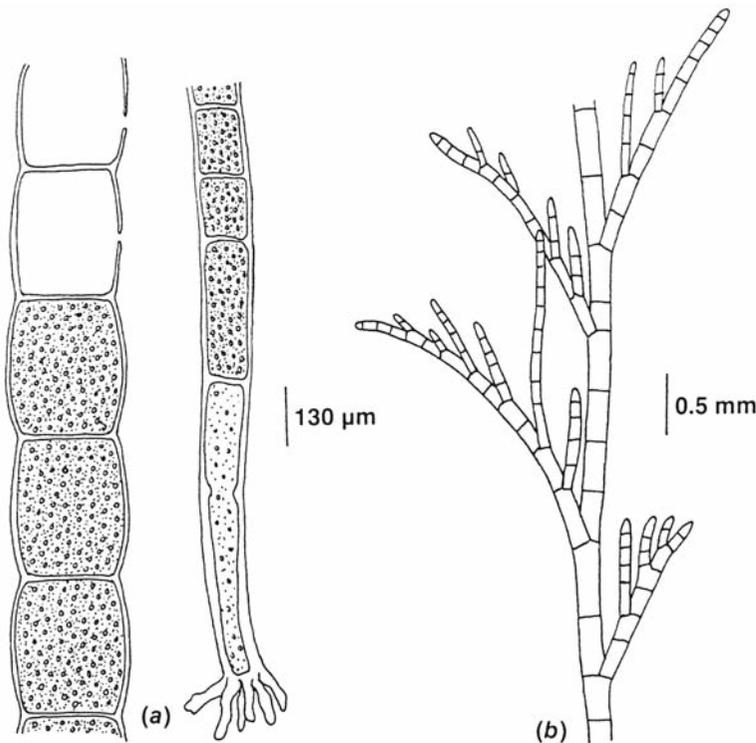
slightly larger than the other. The mixing of gametes of different strains results in the formation of cell clusters, joined by the tips of their flagella (Miyamura et al., 2003; Miyamura, 2004). Clusters separate almost immediately into mating pairs held together by their flagella (Bråten, 1971). The anterior ends of the gametes fuse within a few seconds, and the flagella separate, with the cells becoming negatively phototactic and swimming away from the light source. Within 3 minutes the cells jackknife and fuse laterally, with the quadriflagellate zygote remaining motile for a couple of minutes. The zygote settles, attaches to the substrate by its anterior flagellated end, and absorbs the flagella into the protoplasm. The zygote secretes a wall as soon as it settles, and nuclear fusion has occurred 30 minutes after the onset of copulation. The chloroplast from the plus gamete disintegrates (Bråten, 1973). In many ways the above process is similar to that in *Chlamydomonas* except that it occurs much more quickly.

Within a few days the zygote germinates, with mitotic division of the nucleus. After the first cell division one cell develops into a rhizoid, whereas the other eventually forms the blade. In some species it is possible to get a parthenogenetic development of gametes into a new plant.

Zoospores of *Ulva* are usually negatively phototactic, whereas gametes are positively phototactic. Upon fusion of gametes, phototaxis is reversed and the partially fused gamete pairs swim away from the light source (Miyamura et al., 2003). The total number of particles in the outer chloroplast envelope membrane over the large eyespot of *Ulva* zoospores is 11 300, whereas over the smaller eyespots of the female and male gametes there are only 5500 and 4900 particles, respectively. The lower number of particles in the gametes may be related to their positive phototaxis, especially because on fusion of the gametes, the total number of particles becomes 10 400, close to that of zoospores, with the fused gametes becoming negatively phototactic (Melkonian, 1980b).

*Ulva* is normally a marine genus although it can be found in brackish waters, particularly in estuaries. It normally grows on rocks in the middle to low intertidal zone, although the fronds are not situated at the same level throughout the year. During the colder months the plants grow mainly in the middle intertidal zone, covering wide vertical areas. In the warmer months the *Ulva* is lower in the intertidal zone and in a narrower band. Here the fronds are less exposed and subjected to less desiccation, which is more damaging to the plants in the high summer temperatures.

*Ulva* is an opportunistic alga, capable of rapid colonization and growth when conditions are favorable. This occurs primarily because of a rapid growth rate and the ability to take up and store nutrients available in pulsed supply. Because of



**Fig. 5.35** (a) *Chaetomorpha aerea*.  
(b) *Cladophora microcladioides*. (After Smith, 1969.)

its ability to quickly respond to enhanced nutrient supply, *Ulva* has proliferated in many areas that have received anthropogenic (related to man) nutrient enrichment. A feature of nuisance growths of *Ulva* in enclosed and semienclosed waters is that *Ulva* comprises a large proportion of drift plants, which may smother other benthic communities or be cast ashore where they decompose, causing considerable aesthetic nuisance (Blomster et al., 2002; Nelson et al., 2003; Kim et al., 2004).

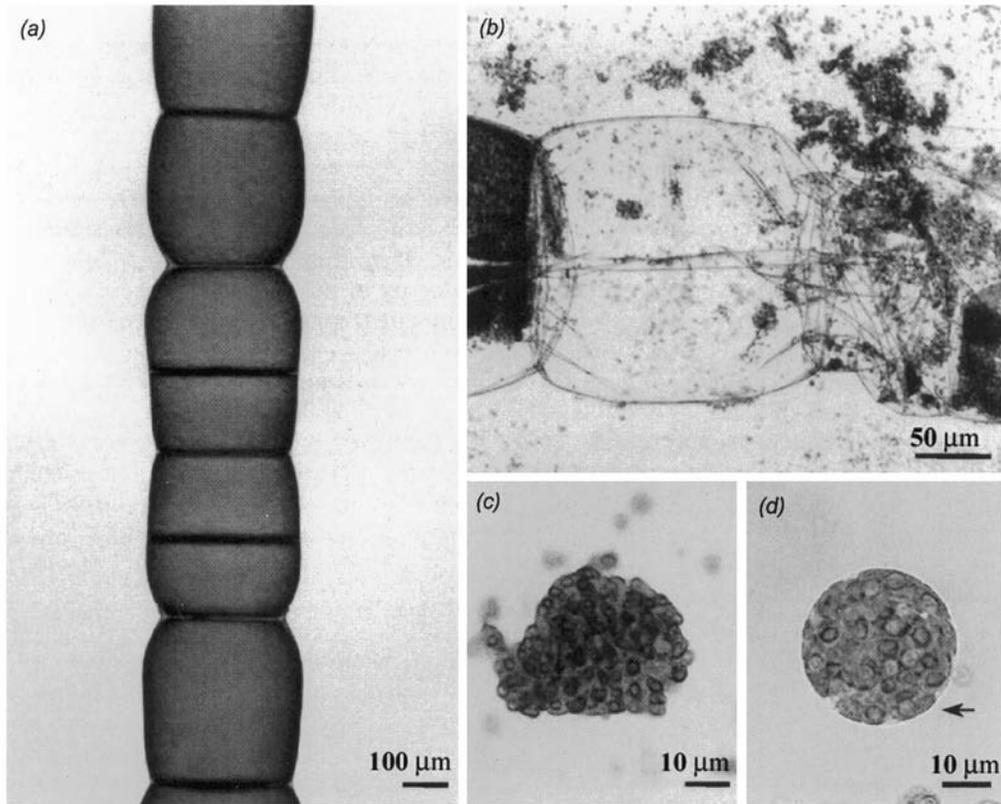
*Ulva* is commonly known as the sea lettuce or green laver, and has been eaten as a salad or used in soups (Chen, 1998). The chemical composition of dried *U. latuca* is 15% protein, 50% sugar and starch, less than 1% fat, and 11% water, making it usable as roughage in the human digestive system. In World War I, Phillipsen prepared a salad with *U. latuca*, and *Monostroma*, which he flavored with salad cream, vinegar, lemon, pepper, onions, and oil, and which he described as “wonderfully nice, slightly piquant and not inferior to the best garden salad” although one wonders after adding all of his condiments whether

he was able to appreciate anything about seaweeds. The eminent French algologist Savaugau prepared such a salad without condiments and said that “it was leathery and waxy in taste, and in spite of a good digestion I thought I would be ill” (Chapman, 1970).

### Cladophorales

The filamentous genera in this order have multinucleate cells, usually with a parietal or reticulate chloroplast. The filaments may be branched or unbranched. The reticulate chloroplast has pyrenoids at the intersections of the reticulum.

*Cladophora* (Fig. 5.35(b)) and *Chaetomorpha* (Figs. 5.35(a), 5.36), each with an isomorphic alternation of generations, are common members of this order. *Cladophora*, found in freshwater and marine habitats, may be the most ubiquitous macroalga in freshwaters worldwide (Dodds and Gudder, 1992). This filamentous alga can reach nuisance levels as a result of cultural eutrophication. *Cladophora* is predominantly benthic, and is often found in the region of unidirectional flow or in periodic wave action. In freshwater, it is a mid- to late-succession



**Fig. 5.36** *Chaetomorpha aerea* showing the process by which a new filament is produced from cell contents after disruption of the filament. (a) Vegetative filament. (b) Protoplasm is expelled from damaged cells and spreads in seawater. (c) Extruded cell organelles aggregate in seawater. (d) Regenerated protoplast forms an envelope within 10 minutes after wounding. (From Klotchkova et al., 2003.)

species. *Cladophora* is colonized by a wide variety of epiphytes because it offers a substrate that is anchored against flow disturbance.

### Dasycladales

The plants in this order are all tropical and subtropical marine plants, most of them calcified. The members of the order are a clearly defined group having the following characteristics: (1) radial symmetry with an erect axis bearing branches; (2) uninucleate vegetative thallus, with a multinucleate condition developing just before reproduction; (3) gametes formed in operculate cysts within specialized gametangia.

The coenocytic algae in the Dasycladales and Caulerpales respond to injury by rapidly forming gel-like wound plugs, thereby preventing loss of cytoplasm. The wound plugs are formed from extruded cytoplasm that forms the plug through interaction of carbohydrates and lectins (Ross et al., 2005). A new cell wall is formed under the gelatinous plug.

The Dasycladales has a paleontological record that extends back to the Precambrian–Cambrian boundary (ca. 570 million years ago) (Berger and Kaefer, 1992). Of the 175 known fossil genera, only 11 are extant. The Dasycladales are in fact “living fossils.” As defined by Stanley (1979), **living fossils** are organisms that include extant clades that have survived for long intervals of geological time at low numerical diversity and exhibit primitive morphological characteristics that have undergone little evolutionary change.

There are two families within the order, the first of which is extinct:

- Family 1 Receptaculitaceae: laterals produced spirally on erect axis; relatively large plants; all extinct.
- Family 2 Dasycladaceae: laterals produced in whorls on erect axis; relatively small plants; extinct and extant.

### Receptaculitaceae

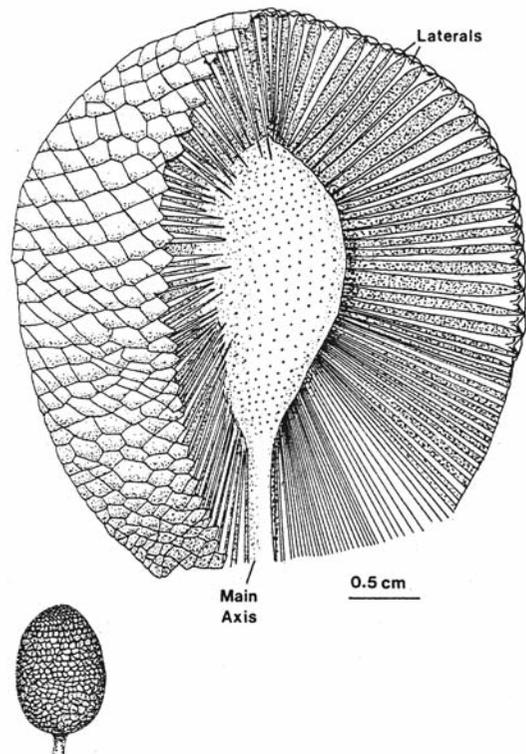
The plants in this order existed from the Lower Ordovician to the Permo-Carboniferous Period. They were non-septate marine dasycladaceous algae shaped like a light bulb, with the upper portion consisting of a large number of spiral laterals attached to the main axis. They were calcified, with calcification being heavier around the periphery of the thallus. *Ischadites abbottae* was a Silurian alga that grew in shallow reef water. The thallus was globose, formed by spirally attached laterals with the largest laterals in the mid latitudes (Fig. 5.37). The upper laterals gradually became wider toward the periphery of the plant, then suddenly expanded into heads at the periphery. The main axis and laterals were calcified (Nitecki, 1971).

### Dasycladaceae

This family is characterized by a thallus having laterals arranged in whorls on the main axis. There are a number of different algae in the family (Zechman, 2003), with *Acetabularia* (Figs. 5.38, 5.40, 5.41) being the best known (Mandoli, 1998). At maturity *Acetabularia* has a naked axis with a single gametangial disc at the apex. In contrast, the axes of *Dasycladus* (Fig. 5.39(a)) and *Neomeris* (Fig. 5.39(b)) are enclosed in whorls of laterals that form a fairly solid cortication.

The first fossils of this family appeared about the Middle Silurian, and some of the extant genera are well represented in the fossil record, *Neomeris* going back to the Cretaceous and *Acetabularia* to the Tertiary (Johnson, 1961).

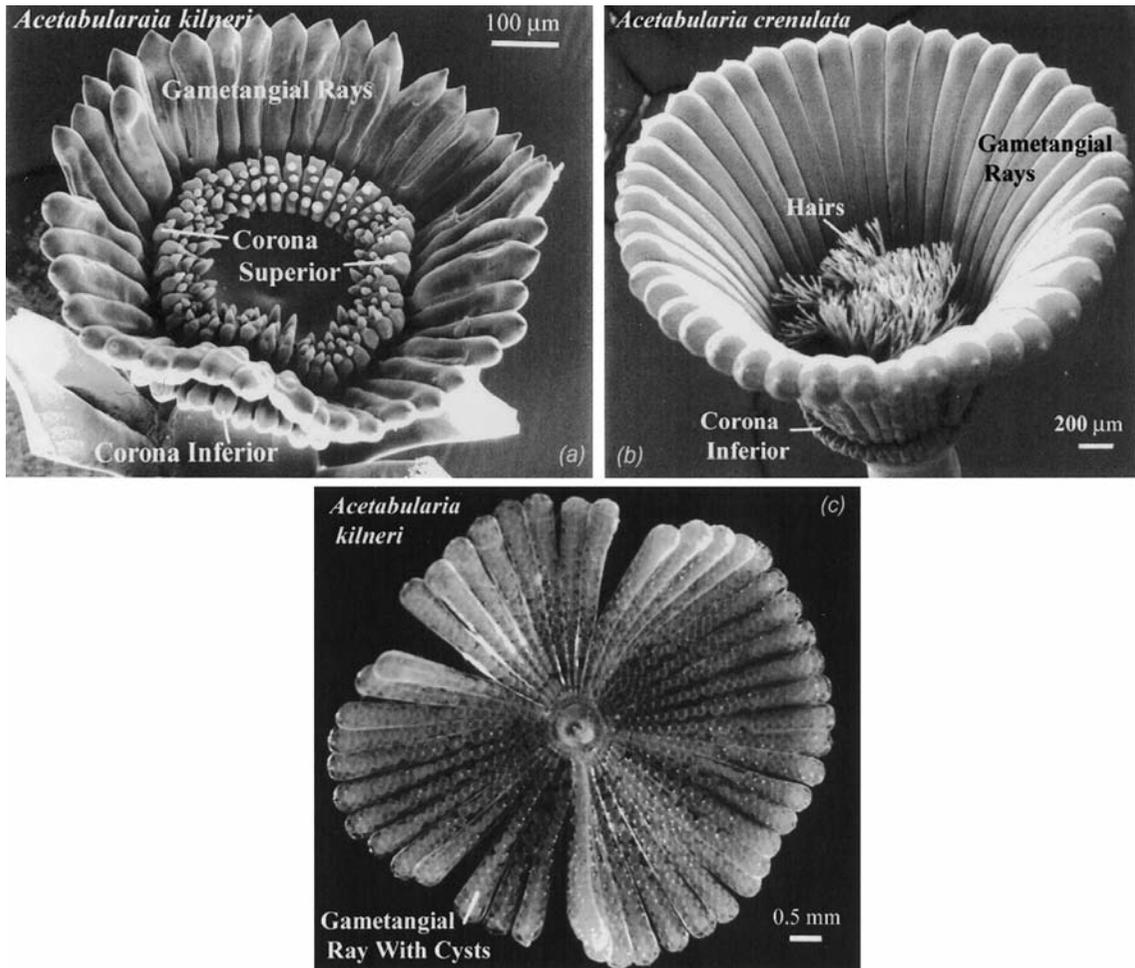
The life cycle of *Acetabularia* (Fig. 5.41) is similar to other plants in the order. *Acetabularia* (mermaid's wineglass) is a warm-water alga found in shallow protected lagoons and on the borders of mangrove swamps, growing on shells, coral fragments, and other algae. The thallus is calcified (Kingsley et al., 2003), with less calcification occurring in warm stagnant water. The young



**Fig. 5.37** Reconstruction of *Ischadites*, whole plant and cutaway drawing of the upper portion showing main axis, laterals, and lateral scars. (After Nitecki, 1971.)

single-celled *Acetabularia* plant has two growing apices, one giving rise to the rhizoidal system that attaches the plants to the substrate, with the other growing apex becoming the erect thallus. As the apex of the axis grows, whorls of sterile hairs are produced just beneath the apex. Each of these whorls is eventually shed, leaving whorls of scars to mark their former attachment positions. During the vegetative growth of the thallus, the nucleus remains in one of the rhizoids.

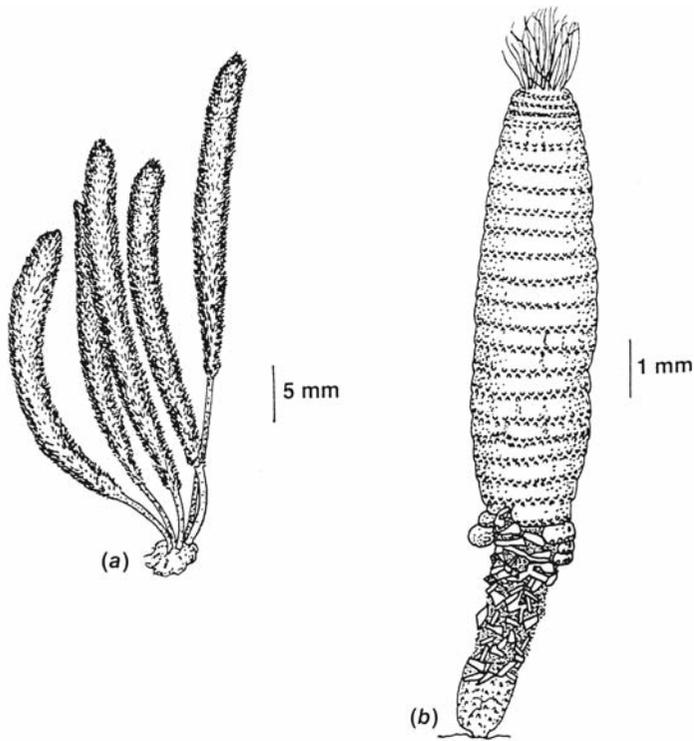
A mature thallus forms a number of gametangial rays at the apex of the thallus, which can be joined or free from each other, depending on the species. Near the base of each gametangial ray, at the tip of the thallus, is a coronal knob, which commonly bears sterile hairs. The coronal knobs together comprise the corona superior. Some species also have a corona inferior beneath the gametangial rays. Once the gametangial rays have reached full size, the primary nucleus in one of



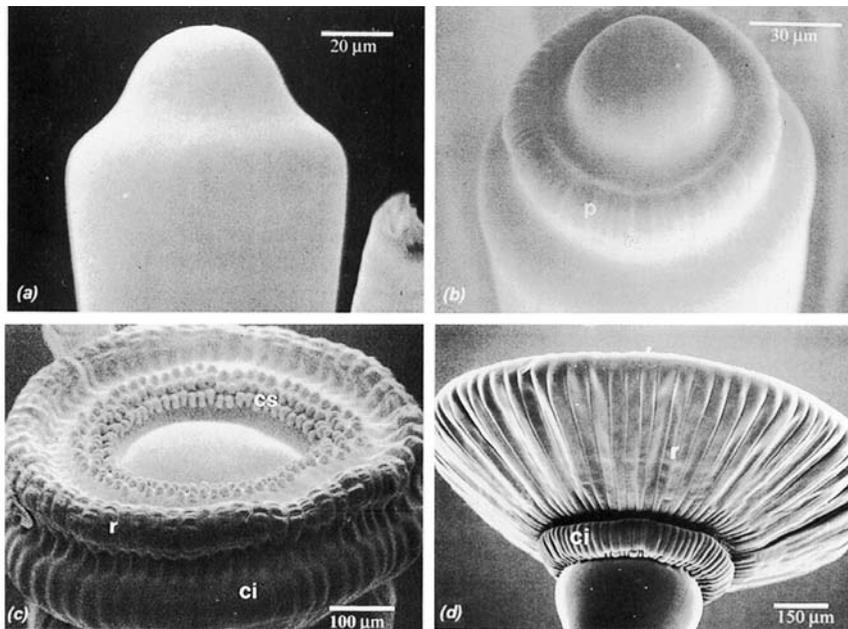
**Fig. 5.38** Structure of the thallus of *Acetabularia*. (a) and (b) are scanning electron micrographs; (c) is a light micrograph. (From Berger et al., 2003.)

the rhizoids enlarges to about 20 times its original diameter. This nucleus divides by meiosis (Koop, 1975a) into a large number of small secondary nuclei (Woodcock and Miller, 1973a,b), which are carried by cytoplasmic streaming along microtubules into the gametangial rays (Menzel, 1986). In the rays, each nucleus is held in place, a certain distance from other nuclei, by microtubules (Woodcock, 1971). The cytoplasm contracts around each nucleus, and a wall is formed, producing a resistant resting cyst. The cysts enlarge to many times their original size, a process that is accompanied by a number of nuclear divisions. The gametangial rays fall off, with a

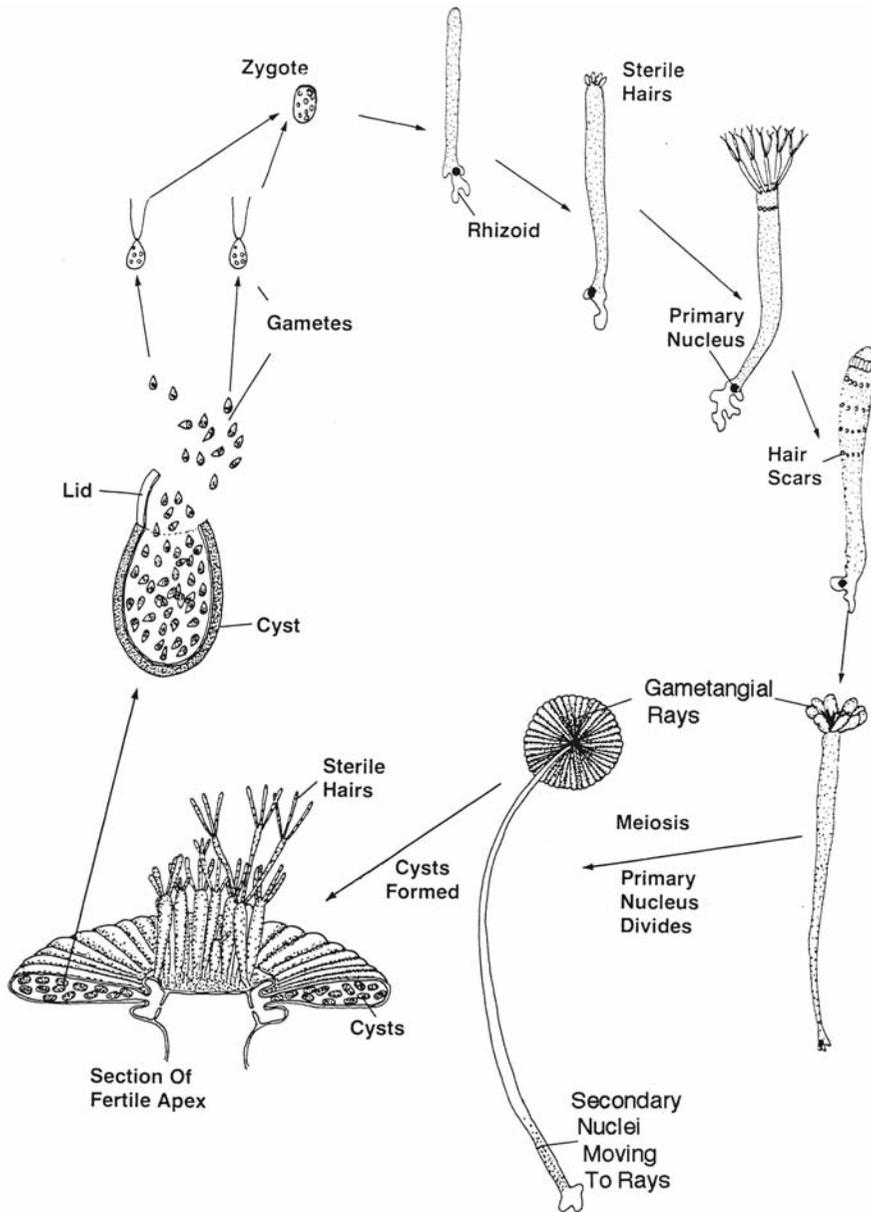
plug sealing the supporting part of the thallus (Menzel, 1980). The cysts of *Acetabularia mediterranea* are usually formed in the summer, are strongly calcified, and do not germinate until the following spring, requiring a resting period of 12 to 15 weeks (Koop, 1975b). At germination of the cyst (Cooper and Mandoli, 1999), the protoplasm divides into a thousand or more pyriform biflagellate isogametes. The gametes are released through a lid in the cell wall. Gametes produced by different cysts are morphologically similar, with gametes from a single cyst probably of the same sex. Parthenogenetic development of gametes has been noted. The zygote can germinate soon after fusing of gametes. In *Acetabularia calyculus*, gametes are of two sizes. After fusion to form a zygote, the chloroplasts of the smaller, male gametes are preferentially destroyed,



**Fig. 5.39** (a) *Dasycladus vermicularis*. (b) *Neomeris annulata*. (After Taylor, 1960.)



**Fig. 5.40** Scanning electron micrographs of cap initiation in *Acetabularia acetabulum*. (ci) Corona inferior; (cs) corona superior; (r) ray cap; (p) primodia. (From Sawitzky et al., 1998.)



**Fig. 5.41** The life cycle of *Acetabularia mediterranea*.  
(Adapted from Egerod, 1952; Smith, 1955.)

leading to maternal inheritance of chloroplast genes (Kuroiwa, 1985).

### Caulerpales

This order contains the coenocytic or siphonaceous Chlorophyta. The non-septate thallus thus resembles a garden hose without any cross walls

separating the usually large thallus, except during reproduction. The cells have numerous lens-shaped or fusiform-shaped chloroplasts and, in some cases, amyloplasts. Two carotenoids, **siphonoxanthin** (Fig. 5.1) and **siphonein**, not normally found in the Chlorophyta, occur in this order (with the exception of *Dichotomosiphon*, which has only siphonein; Kleinig, 1969). Cellulose is usually not a wall component and is replaced by a  $\beta$ -1,3 linked xylan or a  $\beta$ -1,4 linked mannan (Parker, 1970). The

Caulerpales are marine algae and occur as seaweeds in the warmer oceans.

The most important families in the Caulerpales (or Siphonales or Codiiales) are as follows:

- Family 1 Derbesiaceae: stephanokontic zoospores; no amyloplasts; no oogamous reproduction.
- Family 2 Codiaceae: only biflagellate swimmers; thallus basically filamentous; amyloplasts may be present; no oogamous reproduction.
- Family 3 Caulerpaceae: only biflagellate swimmers; thallus composed of a stem bearing blades; amyloplasts present; no oogamous reproduction.
- Family 4 Dichotomosiphonaceae: oogamous reproduction.

### Derbesiaceae

*Derbesia* (Fig. 5.42) is a filamentous alga found in tropical and temperate waters growing on stones near the low-tide line or on larger algae. The life cycle of *Derbesia* (Feldmann, 1950) involves the alternation of this filamentous sporophyte with a bulbous vesicular gametophyte, the *Halicystis* stage, which was originally described as an independent plant. The *Halicystis* stage is found in deep water, usually epiphytic on a coralline alga. The filamentous *D. tenuissima* sporophyte (Page and Kingsbury, 1968) has an interwoven basal portion that supports erect branched filaments, which are occasionally divided by plug-like septa.

This filamentous sporophyte forms ellipsoidal sporangia as short lateral branches cut off by a septum from the main axis. The sporangia form zoospores meiotically (Neumann, 1969) with a whorl of flagella at one end. The zoospores swim for a while, settle, and germinate to produce a filament that forms the vesicular *Halicystis* gametophyte (Fig. 5.43). The *Halicystis* plants have nuclei arranged in an outer layer under the cell wall, with the chloroplasts in an inner layer next to the large central vacuole. The first visible stage of gamete formation is the migration of protoplasm to the plant apex; this protoplasm becomes separated by a membrane and is now the gametangium. The nuclei in the gametangium undergo synchronous divisions, with the

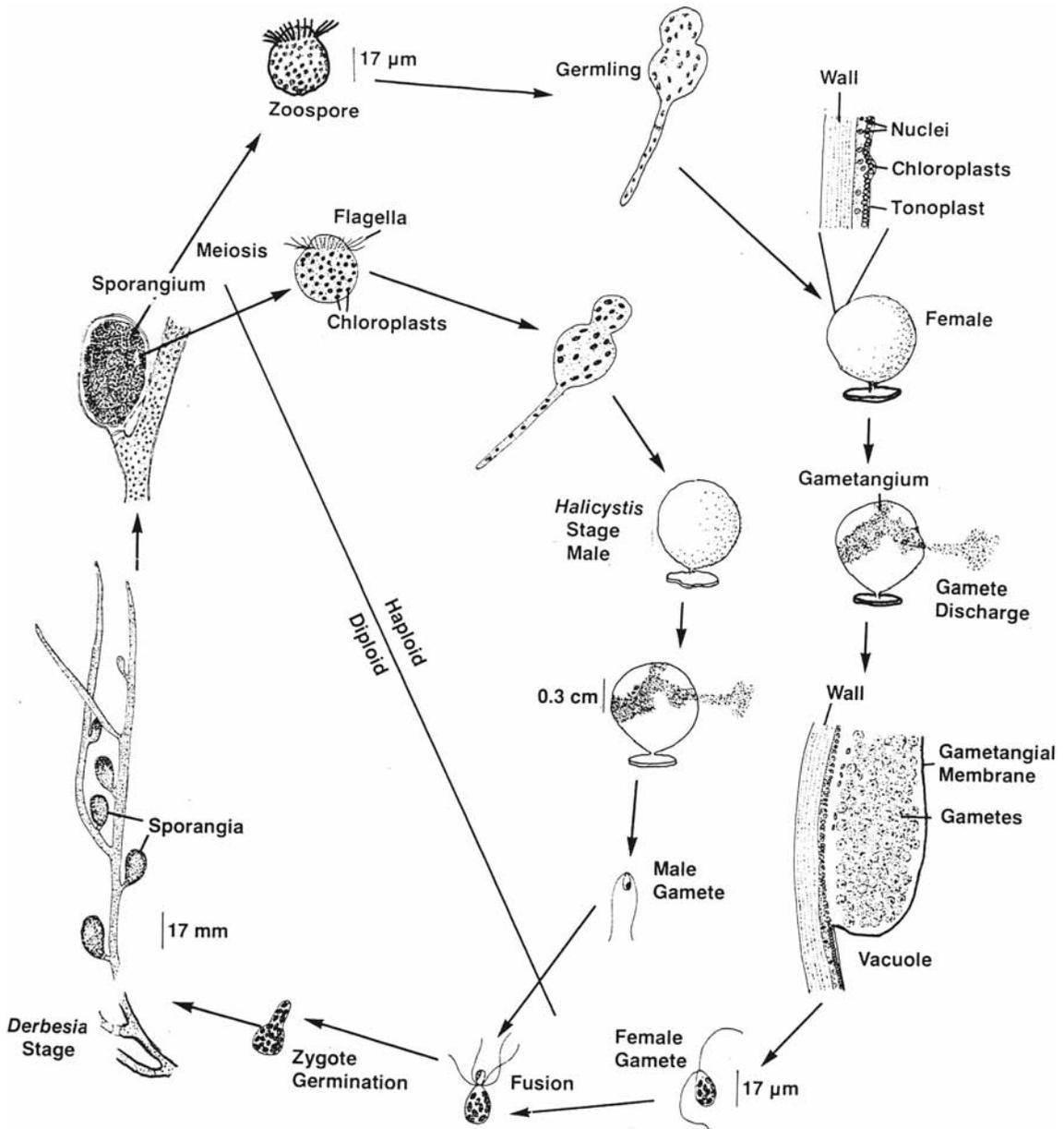
protoplasm cleaving to form the uninucleate gametes.

Plants are heterothallic, producing only one type of gamete. The male plants have olive-green gametangia, whereas those of the female are blackish. Release of the gametes is induced by light, which causes an instantaneous increase in turgor pressure, rupturing the weakened pore area of the wall, and causing a forcible expulsion of the gametes. Following release, the pore is sealed by the gametangial membrane (Wheeler and Page, 1974). Within 12 hours the cell is uniformly green, and it is capable of forming a second gametangium after 24 hours. The biflagellate gametes have one chloroplast in the male and 8 to 12 in the larger female (Roberts et al., 1981). The female gametes are less active than the male. Immediately after mixing, the male gametes surround the female. One of the males begins to fuse with the female, and the whole group of gametes sinks. The zygote germinates to form the filamentous sporophyte. There is a debate as to whether the nuclei from the male and female gametes fuse after plasmogamy of the male and female gametes, or whether karyogamy occurs in the sporangia of the filamentous *Derbesia* phase (Lee et al., 2000; Schnetter and Eckhardt, 2000).

An endogenous rhythm controls gamete formation in the *Halicystis* stage of *D. tenuissima* (Page and Kingsbury, 1968). Gametogenesis has a basic period of 4 to 5 days in the laboratory, but in nature usually occurs in multiples of this figure, and is evidently timed by the tides. The rhythm is unaffected by changes in temperature or light intensity, indicating that it is an endogenous rhythm not directly linked to metabolic processes, such as photosynthesis. After induction of gametogenesis, about 7 hours of dark is necessary for maturation of the gametes, after which light causes their immediate release.

*Bryopsis* (Fig. 5.44(d)) is a common alga in quiet water of tide pools and other sheltered locations. The genus has a main axis that supports lateral upright branches.

The cell walls of the gametophytic phases of *Derbesia tenuissima* and *Bryopsis plumosa* contain large amounts of xylans, whereas the walls of the sporophytes contain large amounts of mannans (Huizing et al., 1979).



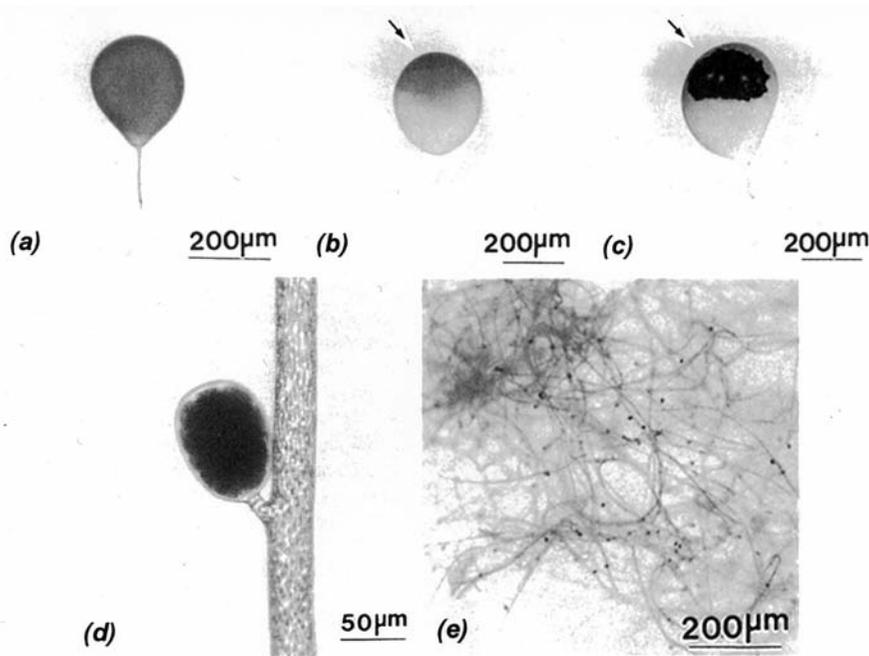
**Fig. 5.42** The life cycle of *Derbesia*. (Adapted from Smith, 1955; Zeigler and Kingsbury, 1964.)

### Codiaceae

These algae differ from those in the Derbesiaceae in having biflagellate swimmers. The structure of the thallus is basically filamentous. The family can constitute a significant proportion of seaweed populations in tropical waters, including some attractive forms such as “mermaid’s fan”

(*Udotea*, Fig. 5.44(f)) and “Neptune’s shaving brush” (*Penicillus*, Fig. 5.44(e)).

*Codium* (Fig. 5.45) occurs from the low-tide mark up to 70 m depth in tropical and temperate marine waters. The genus was originally absent from much of the East Coast of North America. In 1957, *C. fragile* was found along the central Atlantic Coast (Bouck and Morgan, 1957), and it has subsequently spread as far north as Maine. It is probable that the alga was introduced on



**Fig. 5.43** *Derbesia tenuissima*. (a) Vegetative *Halicystis* gametophyte. (b) Mature male gametophyte (arrow points to gametangium). (c) Mature female gametophyte (arrow points to gametangium). (d) Sporangium with fully cleaved protoplasm. (e) Mature sporophyte. (From Lee et al., 2000.)

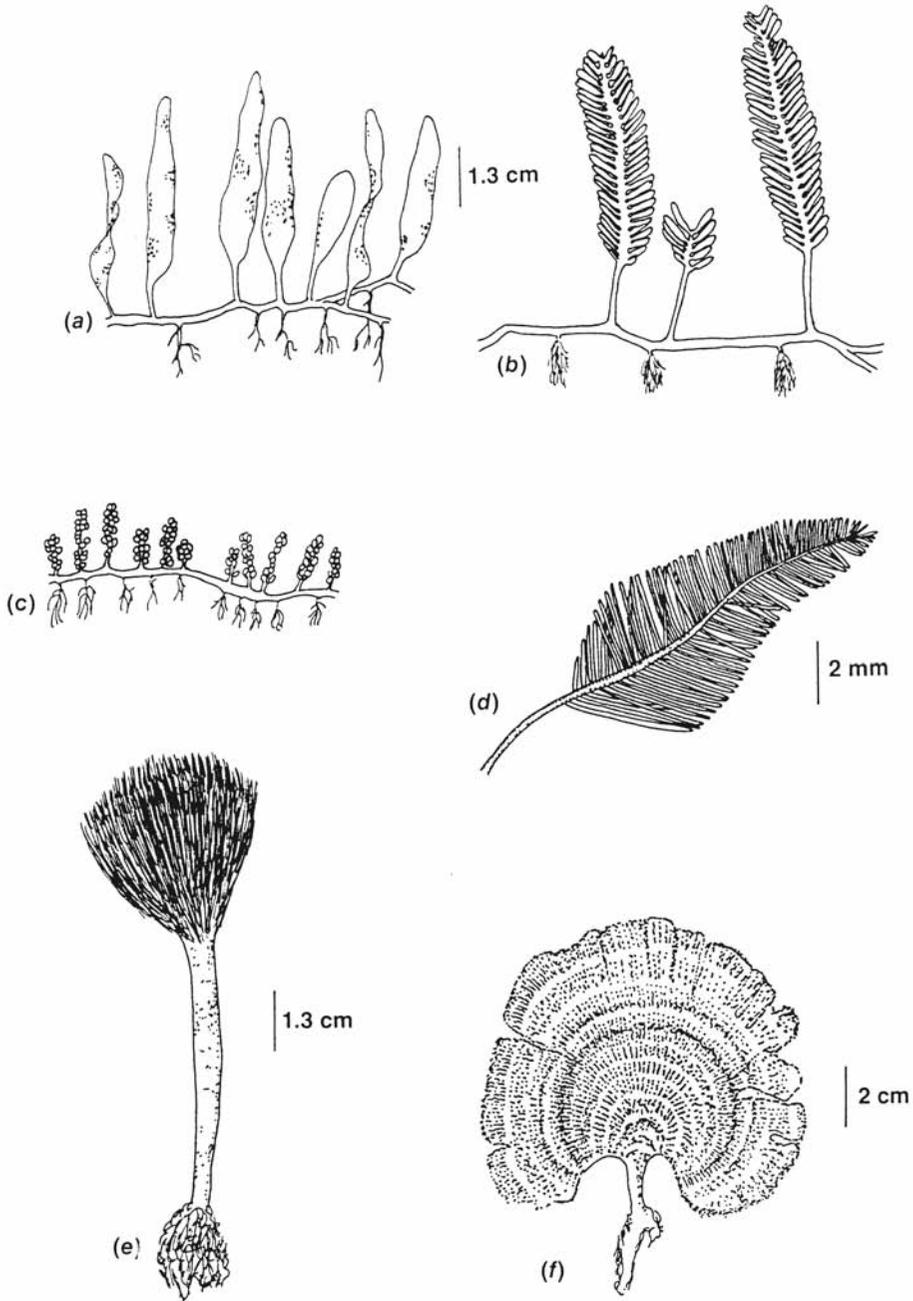
oysters transplanted from Europe. Since its introduction, *Codium* has become a pest in oyster beds, attaching to oysters and causing them to be cast adrift during heavy storms (Fig. 5.46).

The thallus has a crustose prostrate portion that bears several cylindrical dichotomously branched shoots. The shoots have a central medulla composed of interwoven colorless filaments that give rise to inflated branchlets, the **utricles**, which surround the medulla. The utricles have a thick peripheral layer of cytoplasm around a large central vacuole. The discoid chloroplasts are in the outer part of the cytoplasm and the small nuclei in the interior. The colorless filaments of the medulla are divided in places by walls, especially near the base of the utricles. Dark-green female and brown male gametangia are produced from the utricles of the diploid thallus. The gametes are formed meiotically and are released when the lid-like apical portion of a gametangium ruptures, extruding a

gelatinous mass with a central canal through which the gametes move. The gametes initially lack flagella and are carried passively. After flagella extrusion, the gametes swim away, with the male gamete fusing with the side of a larger female gamete. The flagella from the male gamete are lost, and the flagella of the female gamete propel the zygote. After settling and flagella retraction, the zygote germinates immediately into a new *Codium* thallus. The gamete thus constitutes the only haploid structure in the life cycle.

Whole plants of *C. fragile* are able to fix nitrogen, owing to an association between the alga and a nitrogen-fixing bacterium (*Azotobacter*) on the surface of the alga (Head and Carpenter, 1975). The alga secretes 0.7 to 1.3 mg glucose per gram of dry weight of the alga per hour, or 16% to 31% of the carbon assimilated to the outside of the thallus. The bacterium uses the secreted glucose and in turn fixes the nitrogen. The nitrogen fixation occurs only under conditions of nitrogen deficiency and is probably an important factor in the growth of *Codium* in shallow bays under oligotrophic conditions.

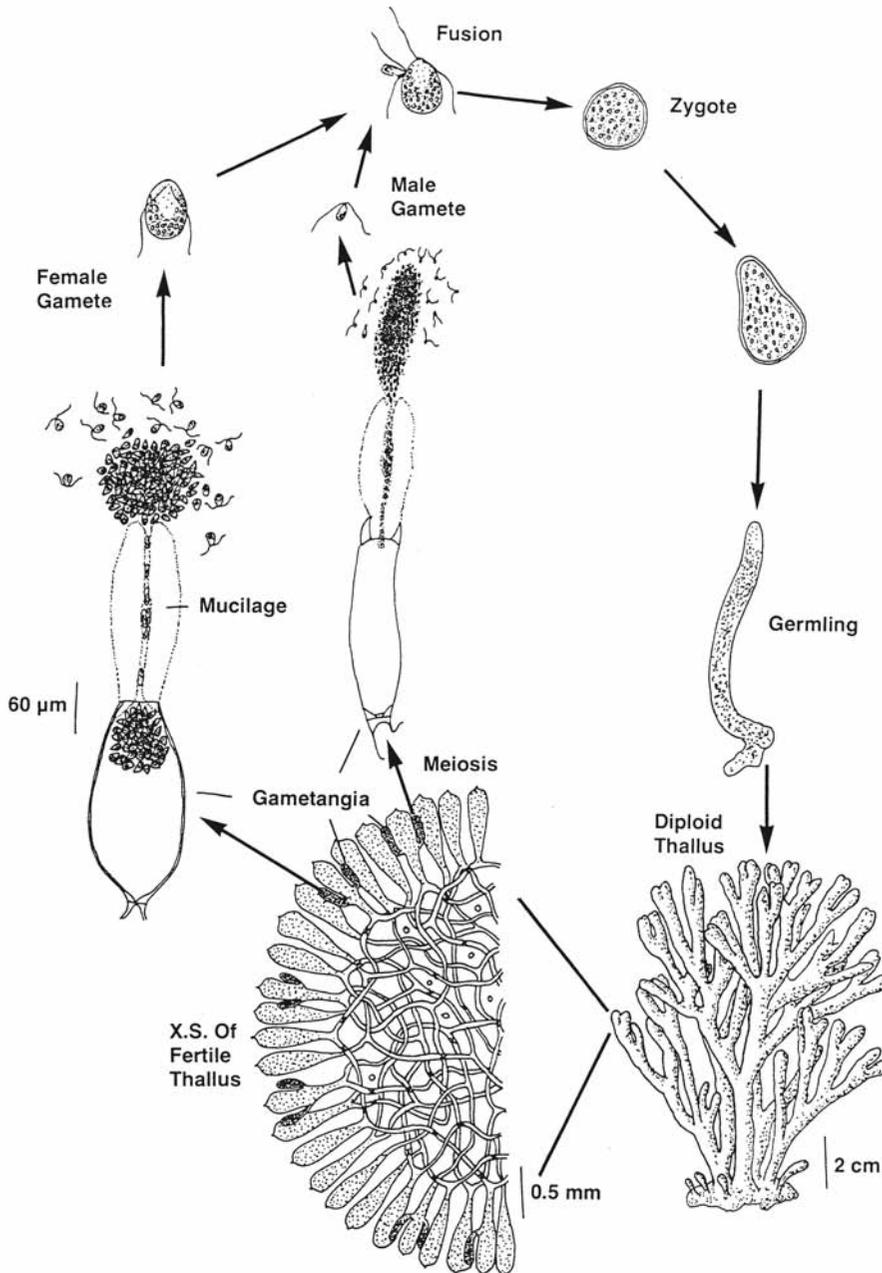
*Codium fragile* shows a number of adaptations to its habitat. During winter months when the



**Fig. 5.44** (a) *Caulerpa prolifera*. (b) *Caulerpa floridana*.  
 (c) *Caulerpa microphysa*. (d) *Bryopsis plumosa*. (e) *Penicillus capitatus*. (f) *Udoatea conglutinata*. (After Taylor, 1960.)

availability of dissolved inorganic nitrogen is at its highest in the water, *C. fragile* accumulates reserves of nitrogen which are utilized in times of relative nitrogen deficiency (Hanisak, 1979). The

period of maximum carbon fixation, pigment content, and chloroplast size occurs during the early winter when competition from other algae is minimal and variation in tidal amplitude is decreased (Benson et al., 1983). In the summer, the physical environment is more extreme, owing to increased drying in the intertidal zone and



**Fig. 5.45** The life cycle of *Codium* sp.

increased competition from other algae. *Codium fragile* effectively retires from much of this competition by undergoing reproduction in the summer. This is accompanied by the development of frond hairs which may increase nutrient uptake.

Symbiotic associations between a number of molluscs and flatworms with chloroplasts of the Codiales are fairly common. Some molluscs (*Elysia*, *Tridachia*, *Placobranchus*) normally feed on siphonaceous Chlorophyceae such as *Codium* and *Caulerpa* by puncturing the cells and sucking out the contents. The chloroplasts are not always digested, and many chloroplasts lodge in the body



**Fig. 5.46** *Codium* sp. on a scallop.

of the animal and actively photosynthesize (Trench et al., 1969, 1973a,b). In *E. viridis*, the chloroplasts can remain functional for at least 3 months when the animals are starved in the light. The rates of photosynthesis of chloroplasts intact in *Codium* and of chloroplasts in *Elysia* are of the same order (Trench et al., 1973b). Chloroplasts isolated from *Codium* release only 2% of their fixed carbon into the medium, mainly as glycolic acid. Isolated chloroplasts in animal homogenate release up to 40% of the fixed carbon, mostly as glucose with some glycolic acid. The animal cells obviously cause the symbiotic chloroplasts to release a large amount of their photosynthate, calculated to be at least 36% of the fixed carbon (Trench et al., 1973b).

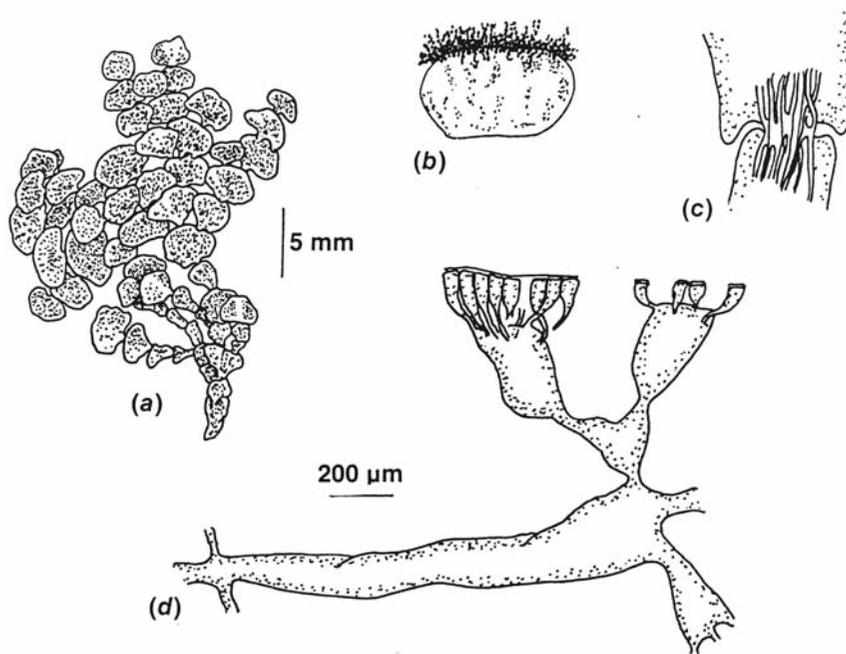
The plants of *Halimeda* (Fig. 5.47) consist of calcified segments separated by more or less flexible little calcified nodes. Coenocytic filaments make up the thallus. At the surface of each segment the

filaments are inflated to form utricles which are closely appressed to one another in mature plants and form an unbroken surface separating the intercellular spaces from contact with the outside. The cell walls of the utricles are calcified. Occasionally, *Halimeda* plants bear clusters of bead-like reproductive structures on branched stalks arising from the surface of the segments. These form biflagellate swimmers, but it is not known whether they are gametes or zoospores.

Two types of plastids exist in *Halimeda* – amyloplasts and chloroplasts. Both types develop from proplastids (Borowitzka and Larkum, 1974). Actively growing plants of *Halimeda* may add a segment a day, the newly formed segment being non-calcified and white, containing only amyloplasts (Wilbur et al., 1969). At this stage the utricles have not completely closed, leaving intercellular spaces continuous with the outside medium. After the segment is 36 to 48 hours old, the utricles have closed with one another, and aragonite  $\text{CaCO}_3$  crystals begin to appear on fibrous material outside the walls of the utricles. By the time that calcification has begun, the segment contains well-developed chloroplasts and is green. The older the segment becomes, the fewer amyloplasts and the more chloroplasts there are present.

Incorporation of radioactive  $^{45}\text{Ca}$  is stimulated by light, with calcium incorporation into the thallus showing a diurnal rhythm, being greater during the day than during the night (Stark et al., 1969), even under constant illumination. This difference in calcification rates is reflected in the movement of chloroplasts to the periphery of the segments during the day and away from the periphery during the night. The calcification process appears to be a two-step process: first the ions are bound to the wall, with the consequent increase in their concentration, and then the  $\text{CaCO}_3$  is precipitated. *Halimeda* and *Penicillus* (Fig. 5.44(e)) have lower rates of  $\text{CaCO}_3$  deposition than the calcified red algae (Goreau, 1963).

*Halimeda* is particularly successful at colonizing bottom habitats where ambient light intensities are from 10 to 20 times less than at the surface. The *Halimedas* are exceptional among the calcareous Chlorophyta in that they tend to be more heavily calcified in deep than in shallow



**Fig. 5.47** (a) *Halimeda tuna*. (b) Fertile segment of *Halimeda*. (c) Nodal region of *Halimeda opuntia*. (d) Central filament of *Halimeda discoidea* with lateral branches forming outer utricles. ((a) after Taylor, 1960; (c),(d) after Egerod, 1952.)

water. Although this difference may be due to a decreased amount of organic matter rather than an increased calcification, the overall effect is opposite to that of other calcareous green algae such as *Penicillus* (Fig. 5.44(e)), *Udotea* (Fig. 5.44(d)), and *Rhipocephalus*, which are invariably less calcified in deep water. *Halimeda* often performs the major role in calcification in lagoons. Hoskin (1963) examined the sand of Alacran Reef, Mexico, and found that it consisted of 35% *Halimeda*, 29% coral, 8% other coralline algae, 8% mollusks, 6% foraminifera, 1% miscellaneous skeletal grains, 9% fecal pellets, and 4% aggregates by volume.

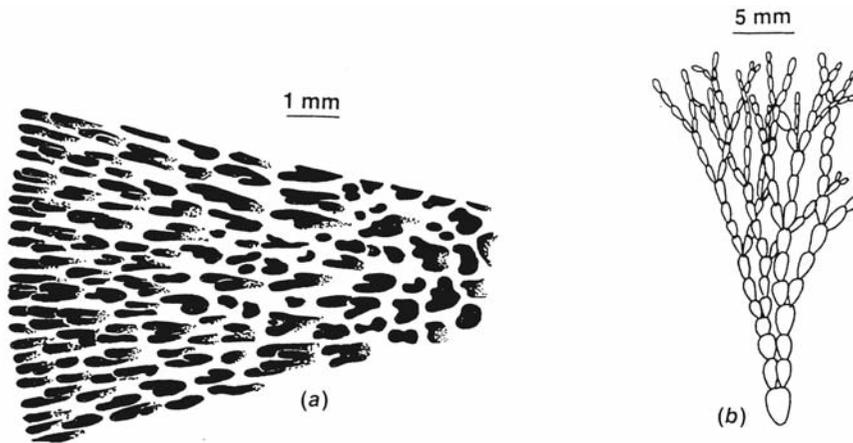
The family Codiaceae is one of the most important groups of rock-building algae, and, in the course of its long history, has been represented by a large number of genera (Johnson, 1961). In extant (living) plants the calcified genera usually have the outer portion calcified, whereas the inner portion is not. This type of calcification

exists in fossil forms, resulting in good preservation in the outer part of the fossil, but with the structural features gradually fading toward the center. A section of *Paleocodium* from the lower Carboniferous (Fig. 5.48(a)) shows a structure similar to *Codium* (Fig. 5.46). Restoration of a branch of *Ovulites margaritula* from the Eocene (Fig. 5.48(b)) results in a structure similar to *Halimeda*.

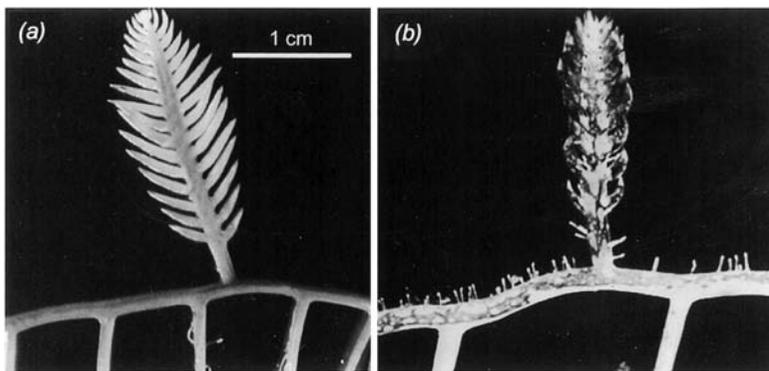
### Caulerpaceae

The coenocytic plants of this family have two types of plastids – chloroplasts and amyloplasts. *Caulerpa* (Fig. 5.49) is the only genus in the family and is a common inhabitant of intertidal and infratidal tropical and semitropical marine waters. The plants have a creeping green rhizome with root-like colorless rhizoids and frond-like erect shoots. The erect shoots exhibit a considerable variation in morphology, many times resembling the blades of higher plants, after which some of the species are named. The thallus derives support from turgor pressure and from wall ingrowths, the **trabeculae**. The walls have a  $\beta$ -1,3 linked xylan as the main structural component.

Chloroplasts are prominent in the leaves and rhizome but totally lacking in the rhizoids and the extreme apex of the growing rhizome tip



**Fig. 5.48** (a) Diagrammatic sketch of a section of the thallus of *Paleocodium* from the Lower Carboniferous, showing arrangement of branching filaments. (b) Restoration of a branch of *Ovulites margaritula* from the Eocene. ((a) after Johnson, 1961; (b) after Munier-Chalmas in Johnson, 1961.)



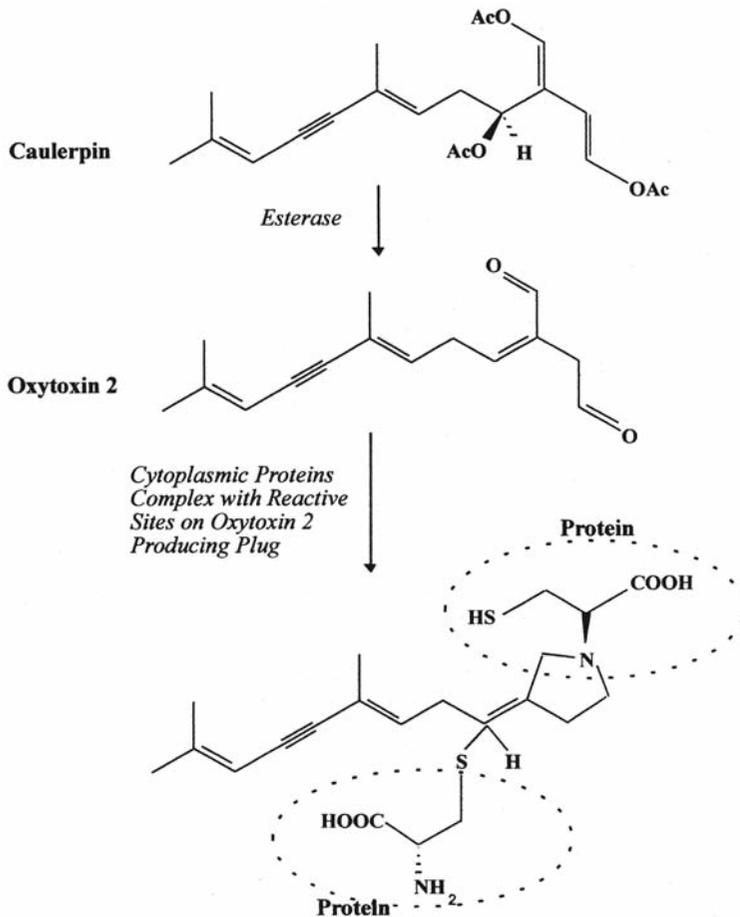
**Fig. 5.49** *Caulerpa taxifolia*, non-fertile (a) and fertile (b) thalli. (From Zuljevic and Antollic, 2000.)

and growing blade. Amyloplast distribution is the reverse of chloroplast distribution, large numbers of amyloplasts being present in the rhizoids and blade tip with few amyloplasts in the rhizome and blades. There is a large central vacuole except at the growing tips.

Mature regions of *Caulerpa* have two systems of protoplasmic streaming: large longitudinal streams in the vacuole and smaller streams oriented  $45^\circ$  to the blade axis in the peripheral cytoplasm. Bundles of microtubules are associated with the cytoplasmic streaming (Sabnis and Jacobs, 1967). The rate of streaming is relatively slow,  $3\text{--}5 \mu\text{m s}^{-1}$ , as compared to  $60 \mu\text{m s}^{-1}$  for *Nitella*.

In sexual reproduction, male and female gametangia are formed on the same frond by migration of the cytoplasm from the rhizomes into the fronds and subsequent cleavage to yield gametes (Fig. 5.49(b)) (Goldstein and Morral, 1970). The large female gametes and smaller males are released in a greenish viscous fluid. After a few minutes the gametes agglutinate in groups of up to 50, followed by separation of pairs and the formation of zygotes. The development of *Caulerpa* has not been followed beyond zygote formation.

The development of aquaculture, aquariums and international shipping has led to worldwide exposure of marine environments to



**Fig. 5.50** Wounding the coenocytic thallus of *Caulerpa taxifolia* results in conversion of cytoplasmic caulerpin into oxytoxin 2 by an esterase followed by a dehydration. The reactive sites on oxytoxin 2 immediately cross link cytoplasmic proteins, resulting in the formation of a plug that excludes water from the thallus. The whole process is complete within seconds.

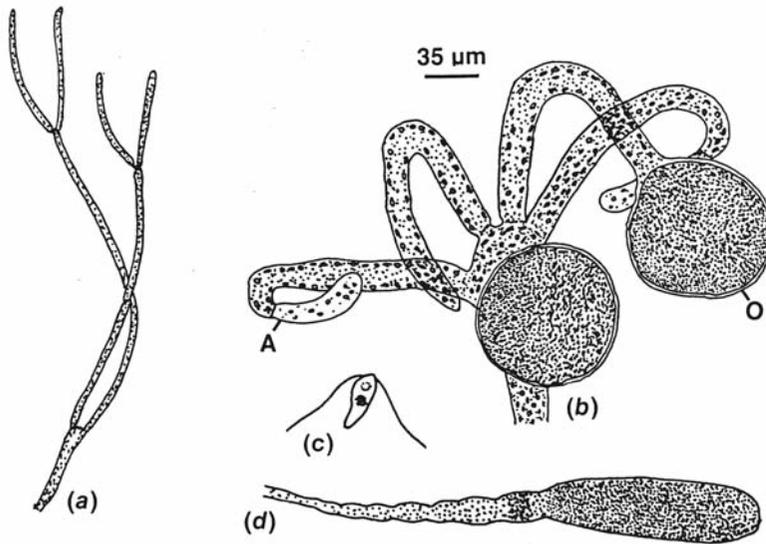
non-indigenous species of algae. More than 60 macroalgal species have been introduced into the Mediterranean Sea (Piazzi et al., 2001; Philips and Price, 2002; Verlaque et al., 2003). Species of *Caulerpa* are the more aggressive of these introduced algae, quickly overgrowing native algae by the rapid elongation of its stolons (2 cm d<sup>-1</sup>). The spread of *Caulerpa* is also due to a unique method of asexual reproduction. No zoospores are formed; instead breakage of the thallus results in new viable plants. On breakage of the coenocytic thallus, the thallus fragments are sealed within seconds by a gelatinous external wound plug that prevents the cytoplasm from coming into contact with seawater (Adolph et al., 2005). The cytoplasm of *Caulerpa* contains caulerpenyne, which on wounding of the thallus is converted by an esterase into oxytoxin 2 (Fig. 5.50). Oxytoxin 2 is a very reactive 1,4 dialdehyde which

cross links cytoplasmic proteins, producing the wound plug.

These *Caulerpa* spp. in the Mediterranean Sea may be Red Sea migrants through the Suez Canal or **Lessepsian species**. The term refers to Ferdinand de Lesseps who designed and led the team that built the Suez Canal. Curiously, there is no similar term for the Panama Canal (which de Lesseps also initially designed). The Panama Canal has a series of higher freshwater lakes which effectively prevent marine species from traveling from one side to the other.

#### Dichotomosiphonaceae

*Dichotomosiphon* differs from the rest of the Caulerpales in having oogamous sexual reproduction. The thallus is a dichotomously branched tubular coenocyte bearing colorless rhizoids. Both lens-shaped chloroplasts and amyloplasts are present



**Fig. 5.51** *Dichotomosiphon tuberosus*. (a) Vegetative thallus. (b) Fertile plants with oogonia (O) and antheridia (A). (c) Spermatozoid. (d) Portion of thallus with akinetes. ((b) after Ernst, 1902; (c) after Moestrup and Hoffman, 1975; (d) after Smith, 1955.)

in the cytoplasm (Moestrup and Hoffman, 1973). Siphonein is present, but the related pigment siphonoxanthin is absent (Kleinig, 1969). The cell wall has a  $\beta$ -1,3 linked xylan as the main structural wall component (Maeda et al., 1966).

There are two species of *Dichotomosiphon*: the marine *D. pusillus* and the freshwater *D. tuberosus* (Fig. 5.51). The latter grows in lakes with an organic silty bottom, most of the thallus buried, and only the tips of the branches above the silt. In water deeper than 2 m only asexual reproduction occurs, by the formation of akinetes in series at the ends of branches. The akinetes germinate directly to form new thalli (Ernst, 1902). In shallow water, reproduction is sexual, homothallic, and oogamous. Conical antheridia are produced at the tips of branches and separated from the rest of the thallus by a septum. The antheridia burst open explosively at the apex, releasing the biflagellate spermatozoids, which have a single reduced chloroplast but lack an eyespot (Moestrup and Hoffman, 1975). An oogonium is spherical, and just before fertilization develops a small beak-like opening at its apex.

### Siphonocladales

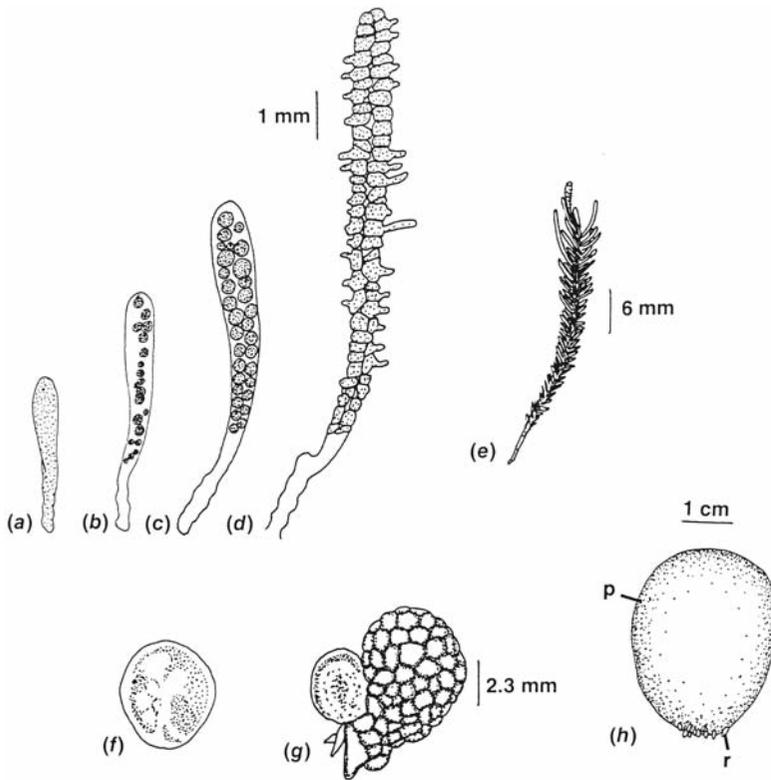
These organisms have multicellular thalli, are wholly marine, and are usually tropical. The cells are multinucleate, with reticulate chloroplasts, and divide in a distinct manner known as

**segregative cell division.** Most of the organisms have siphonoxanthin (Fig. 5.1) (except *Dictyosphaeria*) in addition to the normal pigments of the Chlorophyta (Kleinig, 1969). Sexual reproduction appears to be isogamous in most cases.

*Siphonocladus tropicus* initially has an undivided single-celled primary vesicle (Fig. 5.52). In segregative cell division, the continuous protoplast of the primary vesicle breaks into spherical masses of varying size that soon become surrounded by a wall and enlarge to fill the area within the expanding parent vesicle. After each segment has become firmly pressed against adjacent ones, it sends out a lateral protuberance, which constitutes a branch initial. The mature thallus consists of an erect axis with lateral branches (Egerod, 1952).

A young plant of *Valonia* (Fig. 5.52(h)) consists of a bladder-like multinucleate primary cell. Small holdfast cells are formed by segregative cell division. A mature cell has a conspicuous central vacuole surrounded by a layer of protoplasm. Any vegetative cell can divide into biflagellate swarmers. Although zygotes have been seen, fusion of gametes has not, but it is presumed that meiosis occurs in the cells immediately before the formation of swarmers.

*Valonia* is a common tool for investigators interested in the relationship between the vacuole and protoplasm because it is relatively



**Fig. 5.52** Segregative cell division in *Siphonocladus tropicus*: (a) germling; (b) cytoplasm in spherical masses; (c) expansion of cytoplasmic masses; (d) lateral branches forming; (e) mature thallus. *Dictyosphaeria cavernosa*: (f) young aseptate vesicle; (g) secondary vesicle attached to primary vesicle. (h) *Ventricaria ventricosa* (*Valonia ventricosa*). (p) Primary vesicle cell; (r) rhizoidal cell. ((f),(g) after Egerod, 1952; (h) after Taylor, 1960.)

easy to remove the vacuolar contents from the cell. The osmotic values of the vacuolar sap are 1 to 3 atm higher than seawater, with the concentration of potassium in the vacuolar sap being 66 times higher than in seawater (Mimietz et al., 2003).

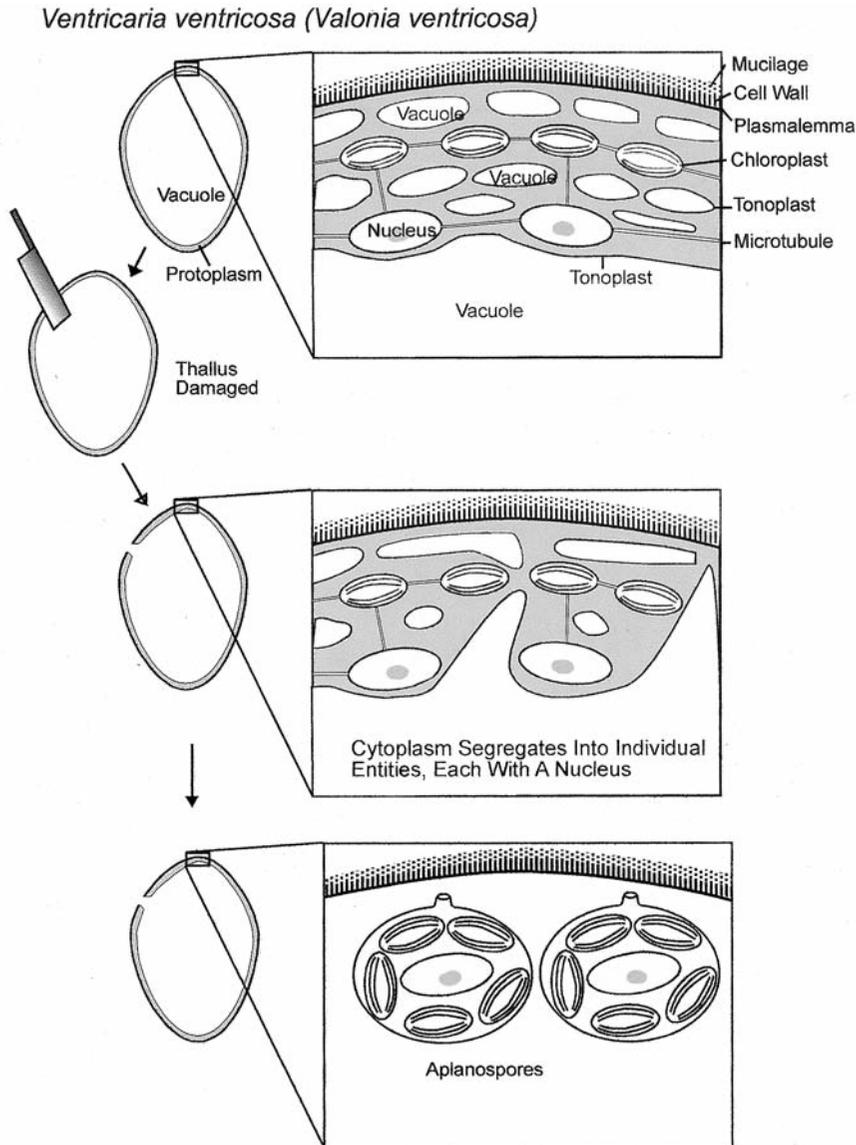
Damage to the thallus of algae in this order results in the formation of aplanospores through a modified process of segregative cell division. *Ventricaria ventricosa* (*Valonia ventricosa*) lives in coral rubble in tropical reef environments such as the Great Barrier Reef. The alga has a large central vacuole with peripheral protoplasm under the cell wall (Fig. 5.53) (Shepard et al., 2004). The protoplasm has chloroplasts to the outside and nuclei to the inside. These organelles are surrounded by the highly convoluted tonoplast of the central vacuole. The organelles are essentially in islands in the vacuole held together by cytoplasmic strands containing microtubules. The tonoplast is “multifolded” by a factor of nine, giving the protoplasm a spongy appearance. Each nucleus is associated with a certain number of chloroplasts and other organelles,

which together can be considered as a fundamental protoplasmic domain. Damage to the thallus results in cytoplasm aggregating around the nuclei to produce aplanospores, with the tonoplast becoming the plasma membrane of the aplanospores.

## Chlorophyceae

The distinguishing characteristics of the Chlorophyceae are the **theca outside of the cells** and a **collapsing telophase spindle** that brings the daughter cells close together, followed by **cell division by a phycoplast**. The flagellar root system is cruciate.

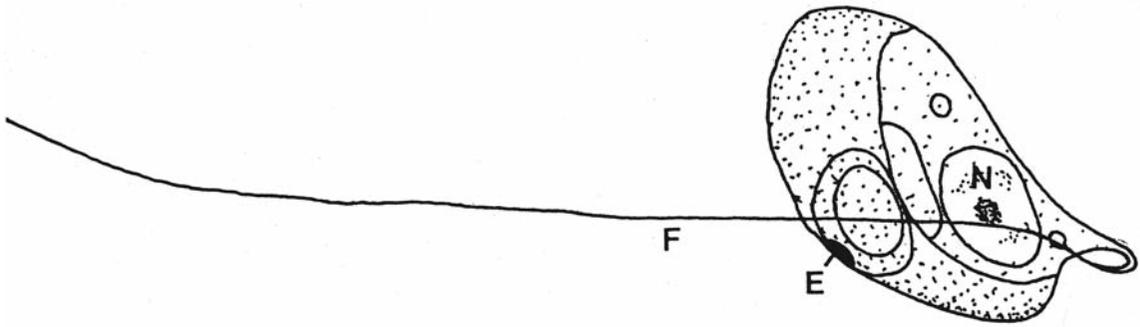
Some of the flagellates in the class do not have a theca, but these are assumed to have lost the theca in evolution because the cells have the other characteristics of the class. The Chlorophyceae are predominantly freshwater. The few unicellular, planktonic species that occur in coastal seawater are members of genera that have a much greater number of freshwater species,



**Fig. 5.53** The coenocyte *Ventricaria ventricosa* (*Valonia ventricosa*) has a large central vacuole with a thin layer of protoplasm under the cell wall. The nuclei in the protoplasm, and associated chloroplasts, are held apart by microtubules. The tonoplast is highly convoluted, resulting in the vacuole weaving between the organelles and giving a spongy appearance to the protoplasm. Damage to the thallus results in aggregation of chloroplasts around the nuclei. Aplanospores are formed with the tonoplast of the original cell becoming the plasmalemma of the aplanospores. (Adapted from Shepherd et al., 2004.)

such as *Chlamydomonas*. The Chlorophyceae whose sexual reproduction is known produce a dormant zygote, with meiosis usually occurring when the zygote germinates.

The other characteristics of the Chlorophyceae were described earlier in this chapter and include motile cells with radial or near-radial external symmetry, flagella attached at the anterior end of the cell, the possibility of a rhizoplast, no multi-layered structure, eyespots common, glycolate breakdown by glycolate dehydrogenase, and urea breakdown by urea amidolyase.



**Fig. 5.54** *Mantoniella squamata*. The cell is covered with scales and has a second, very short flagellum (neither of which is shown).

*Mantoniella* (Fig. 5.54) is a scaly green flagellate with two subapically attached flagella (one of the flagella is very short) (Barlow and Cattolico, 1980). There is one microtubular root composed of four microtubules and a second composed of two microtubules. Duplication of a microtubular root system probably resulted in the cruciate root system of the Chlorophyceae.

## Classification

The Chlorophyceae are divided into the following important orders:

- Order 1 **Volvocales**: vegetative cells flagellated and motile.
- Order 2 **Tetrasporales**: non-filamentous colonies with immobile vegetative cells capable of cell division; pseudocilia may be present.
- Order 3 **Prasiolales**: marine, freshwater or terrestrial algae composed of unicells, short filaments or small sheet-like thalli.
- Order 4 **Chlorellales**: unicells or non-filamentous colonial algae; vegetative cells non-motile.
- Order 5 **Trebouxiales**: green algae involved in a lichen symbiosis.
- Order 6 **Sphaeropleales**: unbranched filaments with new walls formed inside the old filament walls, resulting in H-shaped wall pieces.

- Order 7 **Chlorosarcinales**: daughter cells retained within parent cell wall; no plasmodesmata present.
- Order 8 **Chaetophorales**: branched or unbranched filaments; plasmodesmata present.
- Order 9 **Oedogoniales**: uninucleate filamentous freshwater algae with a unique type of cell division; motile spores and gametes with a whorl of flagella at one pole.

## Volvocales

In the Volvocales the vegetative cells are flagellated and motile. The algae can be unicellular or multicellular. If they are multicellular, then the number of cells in the vegetative colony is a multiple of two. Almost all of these organisms are freshwater, being abundant in waters high in nitrogenous compounds.

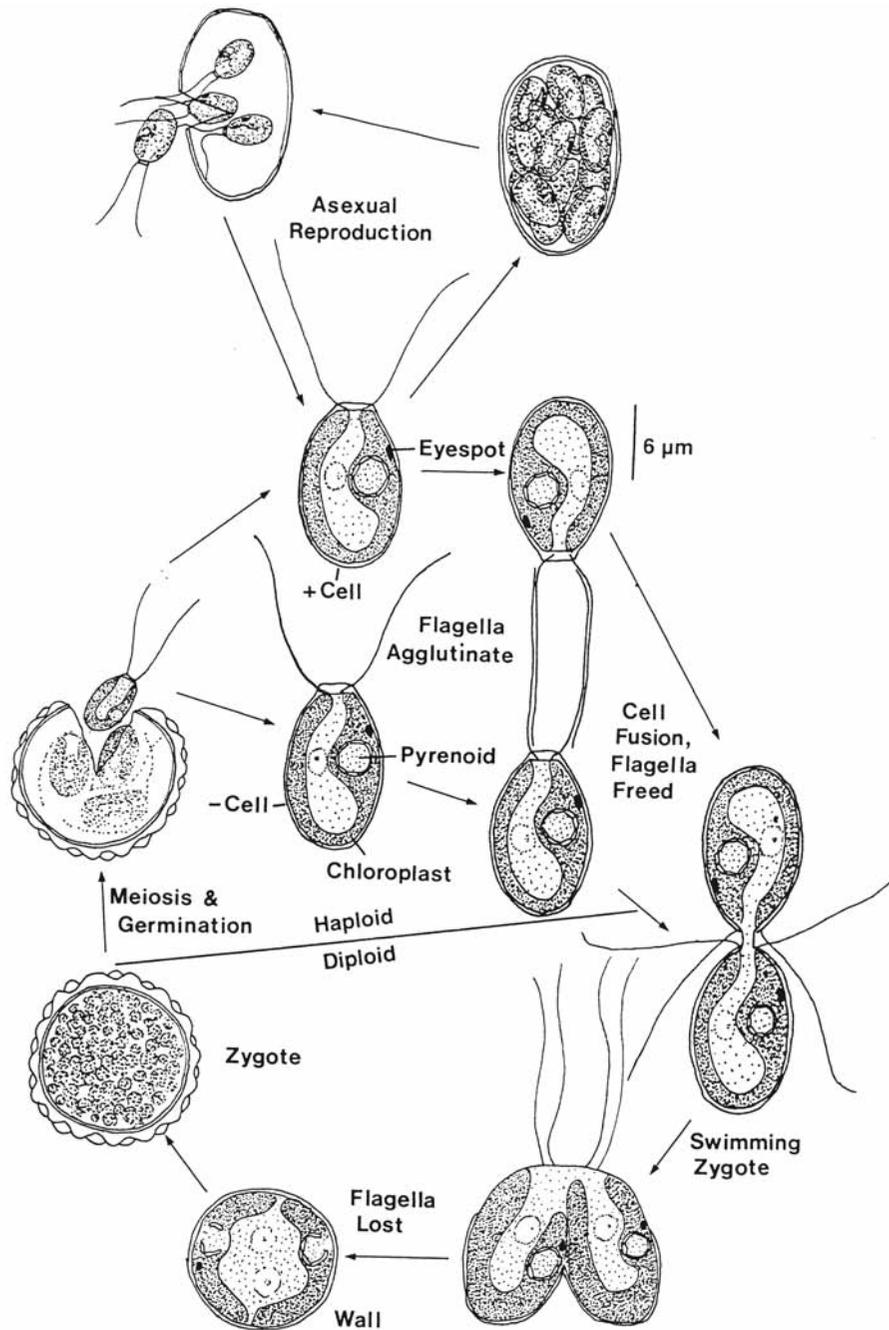
There are two important families in the order:

- Family 1 **Chlamydomonadaceae**: unicellular algae.
- Family 2 **Volvocaceae**: colonial algae formed into **coenobia**, colonies with a definite number of cells arranged in a specific manner.

### Chlamydomonadaceae

In this family are all the unicellular Volvocales. All of the genera are uninucleate, and usually the chloroplast is cup-shaped.

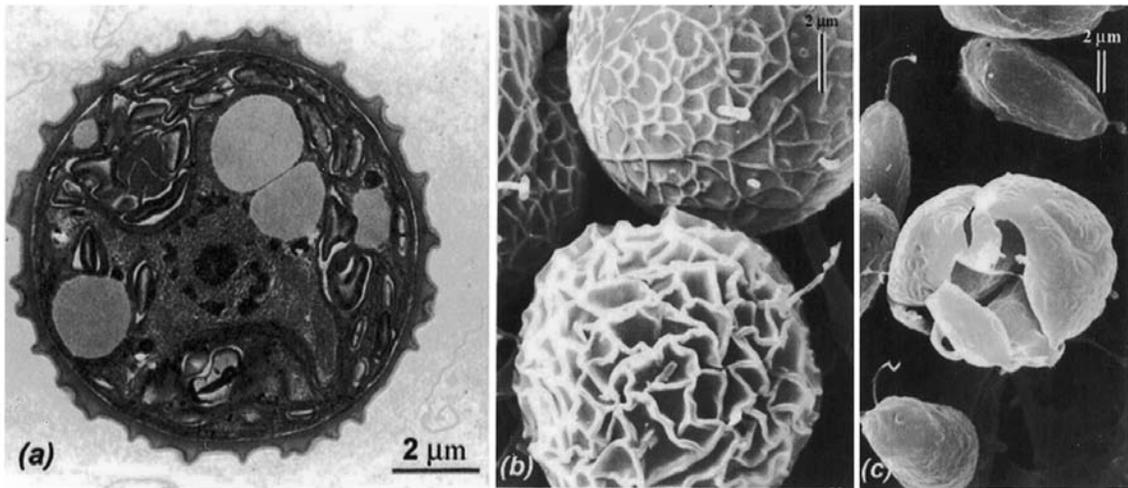
*Chlamydomonas* is a unicellular, biflagellate organism that can be easily grown and manipulated in culture (Harris, 1989, 2001). The uninucleate cells have a cup-shaped, basal chloroplast with a central pyrenoid (Fig. 5.55). There are two



**Fig. 5.55** The life cycle of *Chlamydomonas moewusii*.  
(Adapted from Brown et al., 1968.)

contractile vacuoles at the base of the flagella, and there may, or may not, be an anterior eyespot in the chloroplast. A cell wall usually surrounds the protoplast. When growing on soil, or on agar in the

laboratory, *Chlamydomonas* is non-motile and grows in gelatinous colonies. If these colonies are flooded, motile unicells are formed. The cells are widely distributed in freshwaters and in damp soils where they are commonly found in areas with high concentrations of nitrogen, such as farmyard soils.



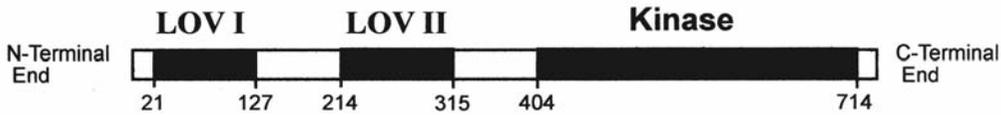
**Fig. 5.56** Zygospores of *Chlamydomonas monoica*. (a) Transmission electron micrograph of a section of zygospore showing a cytoplasm full of lipid droplets and starch grains. (b) Scanning electron micrograph showing a resting zygospore at the bottom of the micrograph. The zygospore swells and the wall becomes less ornamented as the zygospores begin to germinate, as illustrated by the upper zygospore in the micrograph. (c) An empty zygospore wall is in the center of the scanning electron micrograph. Notice that after germination, the remaining zygospore wall is smooth. ((a) from Van Winkle-Swift and Rickoll, 1997; (b),(c) from Malmberg and Van Winkle-Swift, 2001.)

Asexual reproduction begins by the *Chlamydomonas* cell coming to rest and usually retracting or discarding the flagella. The protoplast then divides into 2, 4, 8, or 16 daughter protoplasts within the parent wall (Fig. 5.55). These protoplasts develop walls and flagella, and are released on gelatinization of the parent wall, if growing in liquid medium.

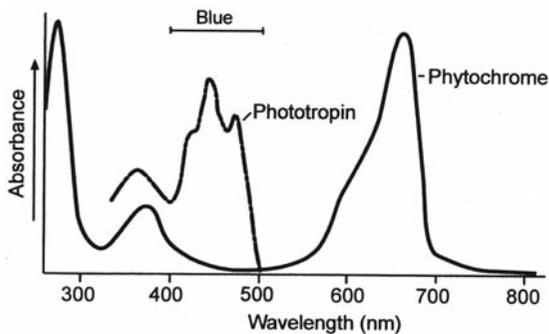
Most species of *Chlamydomonas* exhibit isogamous sexual reproduction (Musgrave, 1993). In *C. moewusii* (*C. eugametos*), there is no structural difference between the gametes and the vegetative cells, and gametogenesis involves the production of agglutinins that cover the flagella (Demets et al., 1990). When gametes of a plus strain are mixed with those of a minus strain, the flagella of the opposite strains adhere because of the agglutinins on the flagella. Initially the gametes clump in groups of up to 50 cells with their flagella toward the center and with different numbers of plus and

minus gametes in each clump. Eventually pairs of opposite gametes fuse at their anterior ends, the flagella become free, and the pair swims away from the clump. Before fusion of gametes, a hole is dissolved in the wall at the interior end of the gametes, and a fertilization tubule is pushed out through the hole. These tubules fuse at their tips, beginning fusion in the gametes. This connecting tubule shortens, causing the apical portion of the gametes to fuse, followed by jackknifing of the two gametes and lateral fusion. The quadriflagellate zygote swims for a while before settling down and forming the primary zygote wall. The nuclei fuse within 24 hours, and a secondary zygote wall with horns and spines is laid down. As the zygote matures, it may or may not enlarge, depending on the species, but it always accumulates large quantities of oils and starch, which may turn it reddish. Light and carbon dioxide are required for development of the zygote (Lewin, 1957). The wall of the zygospore (zygote) (Fig. 5.56) is multi-layered and contains sporopollenin (Malmberg and VanWinkle-Swift, 2001). Zygospore germination occurs in the dark, the inner layer of the zygospore is dissolved away and the sculptured exine splits open to liberate the motile zoospores. The zoospores are formed by meiosis. Usually 4 or 8 zoospores are released, but there can be as many as 16 or 32.

The sequence of events in *Chlamydomonas reinhardtii* is slightly different (Martin and Goodenough, 1975; Huang and Beck, 2003). The



**Fig. 5.57** The structure of phototropin. The protein contains two LOV (Light, Oxygen, Voltage) domains that bind flavin mononucleotide (FMN). The kinase is involved in the autophosphorylation of phototropin in the presence of blue light.

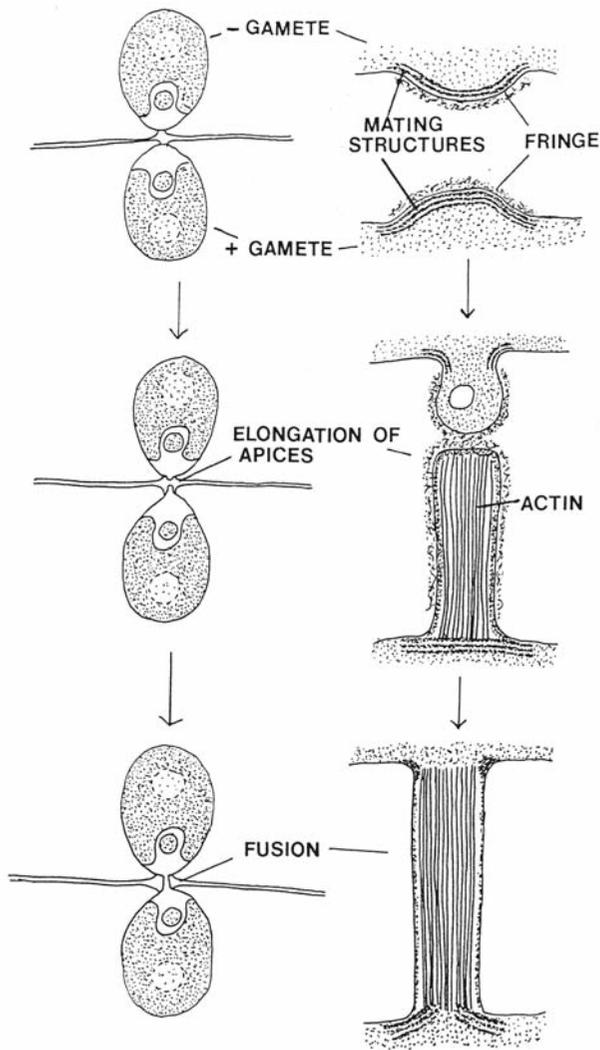


**Fig. 5.58** Stylized action spectra typically observed for phototropin-mediated and phytochrome-mediated responses. An action spectrum represents the effectiveness of different wavelengths of light at eliciting the desired response.

formation of mating type “plus” ( $mt^+$ ) and “minus” ( $mt^-$ ) pregametes is induced by nitrogen starvation in the dark. The pregametes are mating incompetent and require exposure to blue light in a relatively slow process that results in the formation of gametes. Incubation of the gametes in the dark results in rapid loss of mating competence, probably because of deactivation of flagella proteins involved in sexual agglutination. Exposure to blue light results in rapid reactivation of mating competence. Phosphorylation of the phototropin (Figs. 5.57, 5.58) appears to be the blue-light receptor for activation of pregametes to gametes.

The mating reaction is initiated when mating-type “plus” ( $mt^+$ ) and minus ( $mt^-$ ) gametes are mixed together (Snell, 1985). The resulting interactions can be divided into seven stages, all of which are completed within 30 seconds (Fig. 5.59).

- 1 The cells adhere to one another via *mt*-specific flagellar surface agglutinins, which are glycoproteins of extremely high molecular mass. The **agglutinins** are rich in hydroxyproline and appear as extended rods bearing globular domains. The agglutinins of the minus gametes have a protein that recognizes a particular sugar sequence in the glycoprotein on the flagellum of the plus gamete (possibly a mannose-containing polysaccharide) (Wiese and Hayward, 1972). The agglutinins cover the flagellar surface and are continuously lost and replaced from a large cytoplasmic pool. Agglutination results in activation of a flagellar adenyl cyclase (Fig. 2.9) in each gamete and a ten fold elevation in intracellular cyclic adenosine monophosphate (cAMP) levels (Pasquale and Goodenough, 1987). There is an eightfold increase in the adhesion of the flagellar surfaces by the translocation of inactive agglutinin molecules from the plasma membrane of the cell body onto the contiguous flagellar membrane, where the agglutinins become active (Pan and Snell, 2000).
- 2 In a process called “flagellar tip activation”, the tips of the flagella acquire amorphous material between the flagellar membrane and the underlying doublet microtubules of the flagellum. The A microtubules in each of the nine outer doublet microtubules extend, causing extension of the flagellar tips.
- 3 One or more “signals” are transmitted to the cell bodies of the paired cells.
- 4 As a first response to signaling, cells release an **autolysin** that dissolves the crystalline glycoprotein wall (Imam et al., 1985) surrounding the gametes. The autolysin is an enzyme composed of a single polypeptide with a molecular mass of 62 kilodaltons. Autolysin occurs in an inactive soluble form in vegetative cells. Autolysin is converted to a soluble active form when the cell is transformed into a gamete (Matsuda et al., 1987).

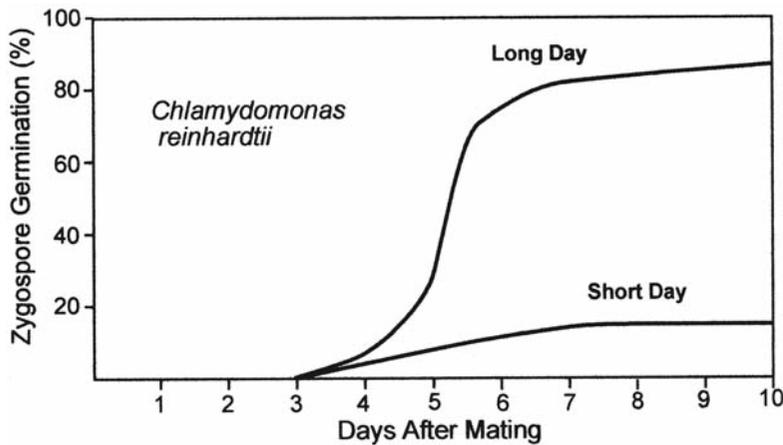


**Fig. 5.59** Drawings of fusing cells of *Chlamydomonas reinhardtii* on the left. On the right are enlargements of the apical areas of the fusing cells. (Adapted from Goodenough et al., 1982.)

5 As a second response to signaling, the cells activate their **mating structures**. The plus gamete has a mating structure composed of three electron-dense layers (Fig. 5.59) that lie under the plasma membrane in an apical bud, approximately 1  $\mu\text{m}$  in diameter (Goodenough et al., 1982). The minus gamete has a mating structure composed of two electron-dense plates in a similar apical bud. Covering the plasma membrane of the apical buds of the plus and minus gametes is a **fringe** of fuzzy material that probably contains the recognition apparatus of the plus and minus gametes.

6 The apical bud of the minus gamete elongates slightly into a dome-shaped structure. In the plus gametes, actin microfilaments extend from the mating structure into the bud to form a long, narrow fertilization tubule. Cell fusion occurs when the fringe at the tip of the fertilization tubule of the plus gamete strikes the fringe at the tip of the apical bud of the minus gamete. A narrow cytoplasmic bridge is formed, which opens up to allow full cytoplasmic confluence.

7 The adhering flagella of the resulting quadriflagellate cell lose their agglutinative properties, presumably in response to a



**Fig. 5.60** Germination of zygospores of *Chlamydomonas reinhardtii* is stimulated by long days but suppressed in short days. (Modified from Suzuki and Johnson, 2001.)

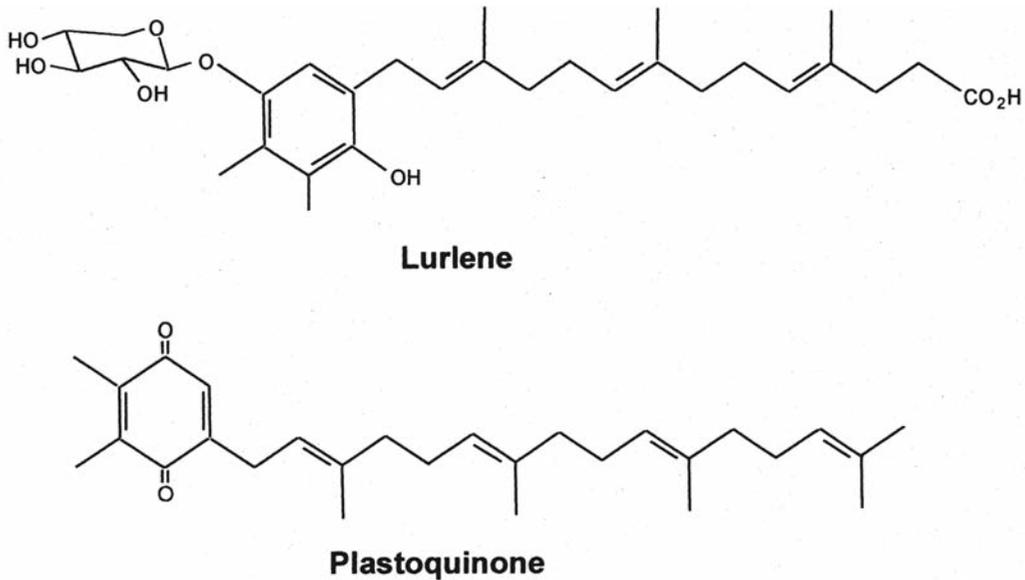
“signal to disadhere,” which is transmitted at the time of cell fusion. The remaining steps leading to the formation of a zygote are similar to those of *Chlamydomonas moewusii*.

Zygospores resist stressful environmental conditions, such as freezing, darkness, desiccation, and starvation. Zygospores undergo meiosis to produce four haploid vegetative cells. Germination of zygospores of *Chlamydomonas reinhardtii* is enhanced under long-day conditions and suppressed under short-day conditions (Fig. 5.60) (Suzuki and Johnson, 2001; Huang and Beck, 2003). The suppression of germination during short days is an adaptive response for overwintering in *Chlamydomonas*. This is an example of **photoperiodic time measurement** in the algae. Photoperiodic time measurement is defined as the ability of plants or animals to sense the season of the year by measuring the duration of the day and/or night in the natural environment and respond appropriately so as to adapt to seasonal changes in the environment (Huang and Beck, 2003).

There have been no reports of a chemical attractant (**pheromone**) being secreted by gametes of *Chlamydomonas moewusii* (*C. eugametos*) or *C. reinhardtii*. However, the larger female gamete in *C. allenworthii* secretes the pheromone **lurlene** (Fig. 5.61) which attracts the smaller male gamete (Starr et al., 1995). Sexual reproduction in *C. allenworthii* is triggered by nitrogen starvation, accompanied by fading of cells from green to brownish as the photosynthetic system is

degraded. Plastoquinone (the chloroplast electron shuttle between photosystem II and I) in the thylakoid membrane is degraded to the chemically similar lurlene at this time (the name lurlene is taken from **Lurley**, the attractive flaxen-haired Rhenish maiden who lured fishermen to their death) (Jaenicke and Starr, 1996; Mori and Takanashi, 1996). Lurlene at a concentration of 1 pM is sufficient to attract the male gamete to the female. Lurlene ceases to be secreted after the male gamete fuses with the female.

Most species of *Chlamydomonas* are found in small ponds and puddles. An unusual habitat is in the snows of some mountains in western North America (Jones et al., 2001; Hoham et al., 2002). Here *C. nivalis* is responsible for what is known as “red snow.” Great numbers of this alga produce the watermelon color associated with these alpine snowfields. The algae grow most abundantly near the surface of old, melting snow in spring and summer, although the resting spores can be found to a depth of more than 60 cm. The algal cells remain until heavy snow covers the surface of the old snow. Upon the arrival of spring, the algal cells migrate upward to the surface of the snow to form another bloom. The algae need melt-water before they will grow, and this need explains their lack of growth in the winter. These snow algae support a unique type of fauna, including various species of protozoans, ciliates, rotifers, nematodes, spiders, and springtails. The last organism in this food chain is the segmented snow worm (*Mesenchytraeus*), which like the algae



**Fig. 5.61** The structure of lurlene and plastoquinone.

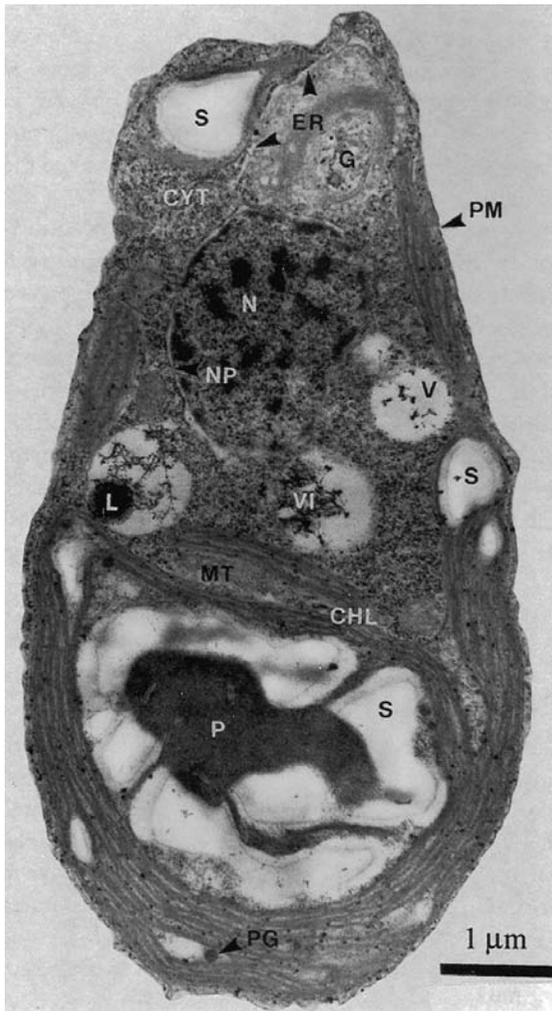
is not prevalent during the winter. During an 1891 expedition to the Malaspina Glacier in Alaska, Dr Israel Russel wrote: "In the early morning before the sunlight touched the snow, its surface was literally covered with small slim black worms, about an inch long and having a remarkable snakelike appearance. These creatures were wriggling over the snow in thousands."

*Dunaliella* (Figs. 5.62, 5.63), a green alga that looks like *Chlamydomonas*, has species that have adapted to life in acid waters and species that have adapted to waters high in salt. *Dunaliella acidophila* is an acid-resistant species that exhibits optimal growth at pH 1.0 (Geib et al., 1996). *Dunaliella salina* (Fig. 5.63) has adapted to waters high in salt, where it is common in places such as the Great Salt Lake of Utah. *D. salina* has two varieties. The first variety has small green halotolerant cells that can grow at NaCl concentrations of 0.5M and above. The second variety has large red halophilic cells that can only grow in saline concentrations above 2M.

Species of *Dunaliella* (Gonzalez et al., 2001) are possibly the most salt-tolerant eukaryotic photosynthetic organisms. *Dunaliella* has two mechanisms that allow it to live in waters of varying salinities (Fisher et al., 1994):

- 1 *Ion pumps in the plasma membrane* – Plasma membrane proteins are produced when the alga is moved from a low-salinity environment to one of high salinity. These proteins are ion pumps that expel sodium from the protoplasm and control intracellular ion levels.
- 2 *Production of glycerol* – Lacking a cell wall, *Dunaliella* cells respond to increases or decreases in the external salinity by immediate shrinking or swelling, respectively. Subsequent synthesis or elimination of glycerol results in an intracellular concentration that balances the external salinity and permits the cells to regain their original volume. At the ultrastructural level (Fig. 5.62), an increase in the size of the Golgi body accompanies an increase in cellular glycerol when the cells are placed in a solution of high salinity (Bérubé et al., 1999).

The unicellular *Hematococcus* (Fig. 5.63) accumulates the yellow carotenoid astaxanthin (Fig. 5.1) in the cytoplasm at concentrations up to 5% of the dry weight of the cells. The alga can be added to fish and poultry feed to impart a yellow color to the skin. High concentrations of astaxanthin can be induced by growing the cells under high O<sub>2</sub> concentrations, nitrogen



**Fig. 5.62** Transmission electron micrograph of a longitudinal section of a cell of *Dunallia bioculata*. (CHL) Chloroplast; (CYT) cytoplasm; (ER) endoplasmic reticulum; (G) Golgi body; (L) lipid globule; (MT) mitochondrion; (N) nucleus; (NP) nuclear pore; (P) pyrenoid; (PG) plastoglobuli; (PM) plasma membrane; (S) starch; (V) vacuole; (VI) vacuolar inclusion. (From Bérubé et al., 1999.)

limitation or high irradiance (Lee and Ding, 1995; Tan et al., 1995; Ben-Amotz, 1996; Grünewald et al., 1997).

The zoospores of *Phacotus lenticularis* (Fig. 5.64) are surrounded by a lorica containing crystals of calcite (Fig. 4.7). *Phacotus* can occur in high quantities in some lakes (up to 5 million cells per liter) where they have a strong influence on the lake ecosystems (Schlegel et al., 2000).

### Volvocaceae

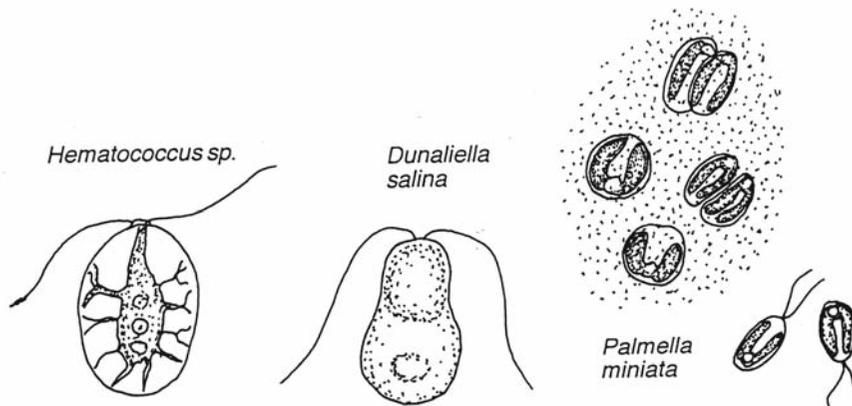
This family includes those colonial Volvocales with a formation of a flat plate (**plakea**) during early development of the colony (Fig. 5.65). The family evolved about 50 million years ago (Nishii, et al., 2003). All of the members of the family undergo a process of inversion after completing embryonic cleavage. The number of cells in a colony is a multiple of two, and each of the cells is surrounded by a gelatinous sheath.

Evolution in the Volvocaceae has resulted in colonies of increasing complexity. The basic cell unit in these organisms (Fig. 5.2) is that of *Chlamydomonas* (Coleman, 1999), the cells joining together to form a colony. The individual cells in the volvocacean colony are held together by an extracellular matrix of hydroxyproline-rich glycoproteins. The extracellular matrix is divided into four zones (Fig. 5.66) (Hallman, 2003): (1) flagellar zone, (2) boundary zone, (3) cellular zone, and (4) deep zone.

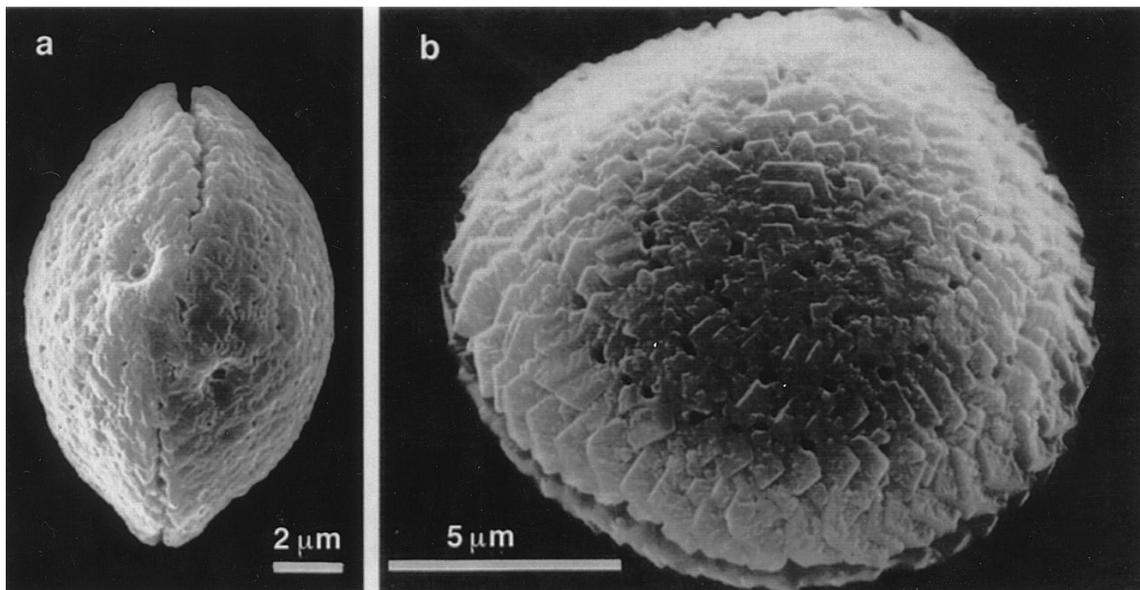
Coenobia of most genera exhibit a polarity when swimming, the anterior pole directed forward. The two flagella of each somatic cell beat toward the posterior, and slightly to the right, which causes the colony to revolve to the left as it swims. Hence, the name *Volvox*, which means “fierce roller.” Photoreception and flagellar activity permit *Volvox* to swim in the water at rates exceeding 5 meters per hour to collect sunlight during the day near the surface, and essential minerals during the night at the bottom of the lake (Kirk, 1999).

In *Volvox*, the anterior cells change the frequency of the beat of the flagella as the light intensity changes, orienting the colony toward the light source (Fig. 5.67) (Hoops et al., 1999). The beat frequency of the flagella of the posterior cells does not change.

There may be a morphological difference in the size of the cells or eyespots in the posterior versus the anterior ends. The saucer-shaped cells of *Gonium* (Fig. 5.65(c)), with 4 to 32 cells, swim with the convex surface forward and have eyespots of a uniform size. In the rest of the Volvocaceae, size differentiation occurs among the eyespots, with larger eyespots in cells in the anterior end. This is so in the spherical coenobium of *Pandorina* with 4 to 32 cells (Fig. 5.65(d)),



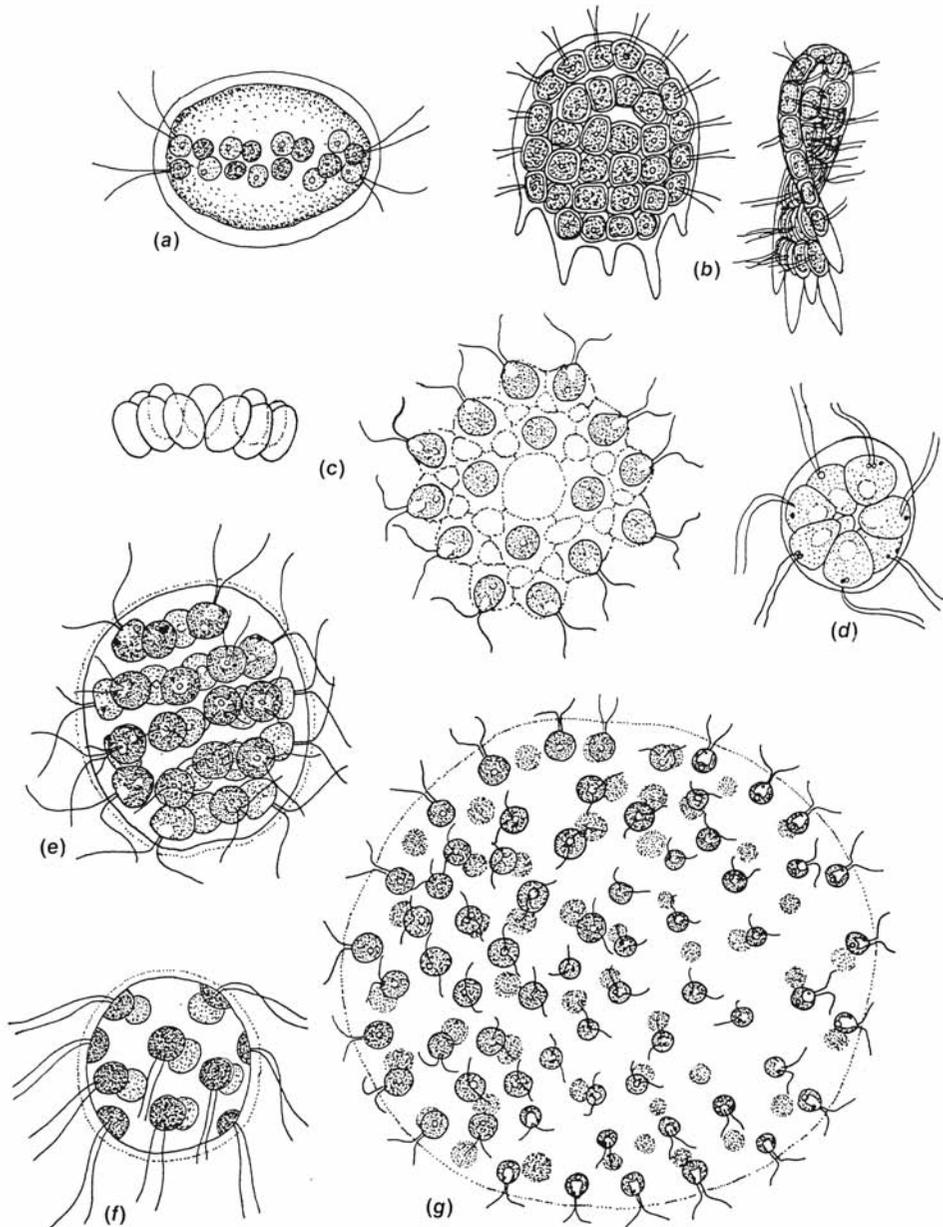
**Fig. 5.63** Drawings of motile cells of *Hematococcus* sp. and *Dunaliella salina*, and palmelloid and motile cells of *Palmella miniata*.



**Fig. 5.64** Scanning electron micrographs of zoospores of *Phacotus lenticularis* covered with a lorica composed of crystals of calcite. (From Hepperle and Krienitz, 1996.)

in *Volvulina* (Fig. 5.65(f)) with 18 cells in four ranks of four, in *Stephanoon* (Fig. 5.65(a)) with 8 to 16 cells in equatorial ranks, in *Eudorina* with a spherical coenobium of 16 to 128 cells, in *Volvox* (Figs. 5.68, 5.69) with spherical colonies of 500 to 10 000 cells, and in *Platydorina* (Fig. 5.65(b)) with flattened colonies.

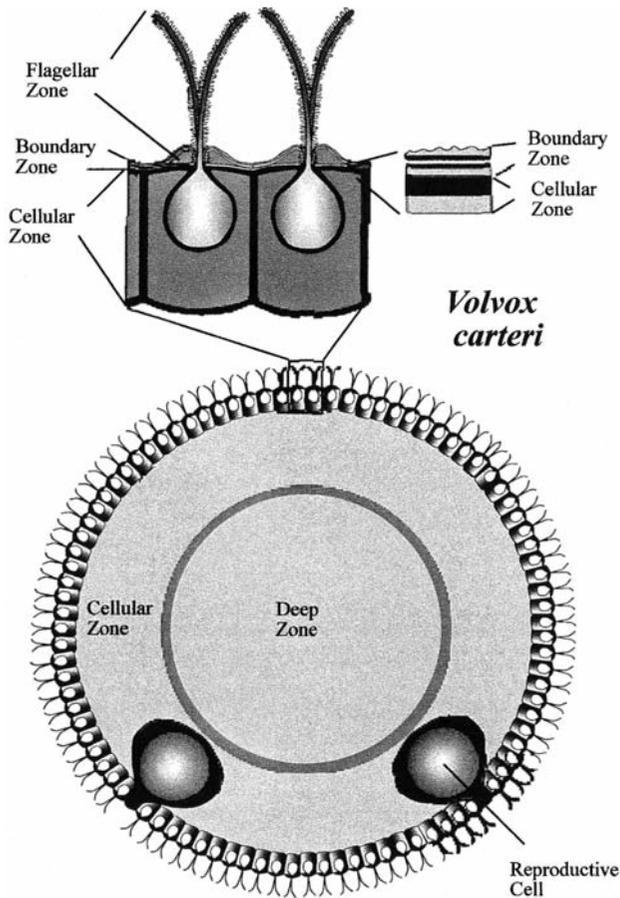
The most highly evolved volvocacean genus is *Volvox*, both in its morphological complexity and in its complex oogamous sexual reproduction. Colonies of *Volvox carteri* are oval to spherical with 2000 to 6000 cells arranged in a single layer (Figs. 5.68, 5.69). Each colony contains a large number of somatic cells and a smaller number of reproductive cells. The protoplast of each somatic cell is typically chlamydomonad and has a single cup-shaped nucleus with a basal



**Fig. 5.65** Colonial Volvocales. (a) *Stephanoon askenasyi*. (b) Front and side views of *Platydorina caudata*. (c) Side and front views of *Gonium* sp. (d) *Pandorina morum*. (e) *Eudorina unicocca*. (f) *Volvulina steinii*. (g) *Pleodorina* sp. ((a),(d) after Huber-Pestalozzi, 1961.)

pyrenoid, an anterior eyespot, a central nucleus, two contractile vacuoles, and two flagella (Fig. 5.2). Daughter colonies are enclosed in vesicles that expand into the interior of the colony.

Vegetative, female, and male colonies of *V. carteri* have similar somatic structure, but each contains a different type of reproductive cell. Vegetative colonies contain cells (gonidia) that give rise to daughter colonies by repeated division; female colonies have eggs, which form zygotes after fertilization; and male colonies contain male initial cells, which divide to form sperm bundles. Female and male colonies develop from gonidia as well as the vegetative colonies.



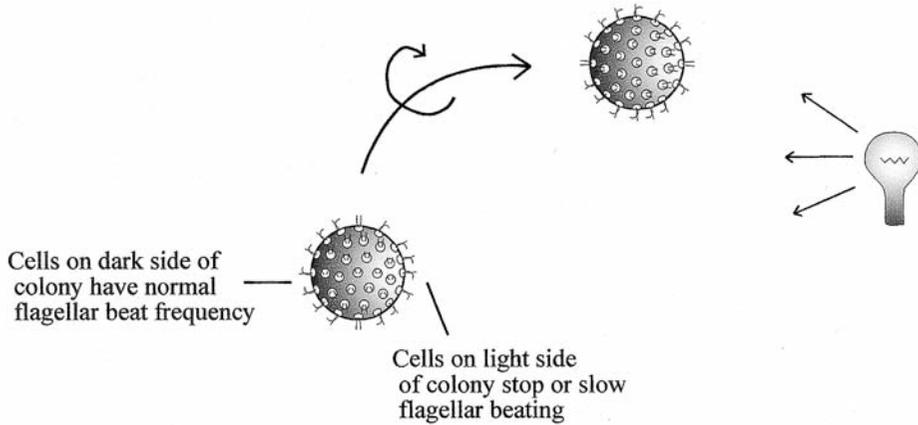
**Fig. 5.66** The extracellular matrix comprises more than 95% of a colony of *Volvox carteri* and is divided into four zones: (1) flagellar, (2) boundary, (3) cellular, and (4) deep. (Modified from Hallman, 2003.)

Asexual reproduction occurs in *Volvox* as follows. Each young asexual adult colony of *Volvox carteri* consists of about 2000 small biflagellate somatic cells embedded in the surface and 16 large gonidia (asexual reproductive cells) (Fig. 5.69), which are positioned slightly below the surface of a transparent sphere of extracellular matrix. The biflagellate somatic cells are specialized for motility and phototaxis, are incapable of dividing, and are programmed to die at the end of two asexual reproductive cycles. In contrast, the gonidia are non-motile, specialized for growth and reproduction, and potentially immortal. Asexual reproduction in *Volvox carteri* resembles that of classical metazoan models, such as snails, sea urchins, and nematodes. As such, it has been used as a model for embryogenesis research.

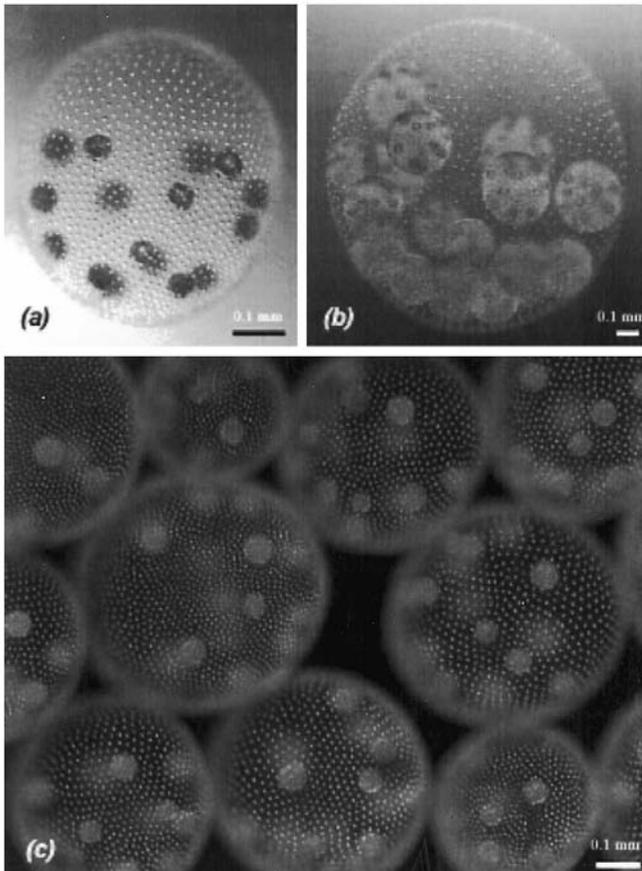
Asexual reproduction is completed in 48 hours (Fig. 5.70) and begins with a series of 11–12 rapid cleavage divisions of the gonidium to pro-

duce an embryo, with each cell division preceded by a round of DNA replication. The first five divisions are symmetrical, resulting in an embryo with 32 cells of equal size and shape. At the sixth division, 16 cells in the anterior hemisphere divide asymmetrically, producing large-small sister pairs. Each large cell is a gonidial initial destined to produce a gonidium, whereas the smaller sister cells and each of the symmetrically dividing cells of the posterior hemisphere are somatic initials that will differentiate to mature somatic (vegetative) cells. The gonidial initials divide asymmetrically two or three more times, generating an additional somatic cell at each division. Then the gonidial initials withdraw from the division cycle, whereas the somatic initials continue cleaving until they have completed 11 or 12 division cycles altogether. As a consequence of asymmetric divisions, and a different number of divisions completed, gonidial

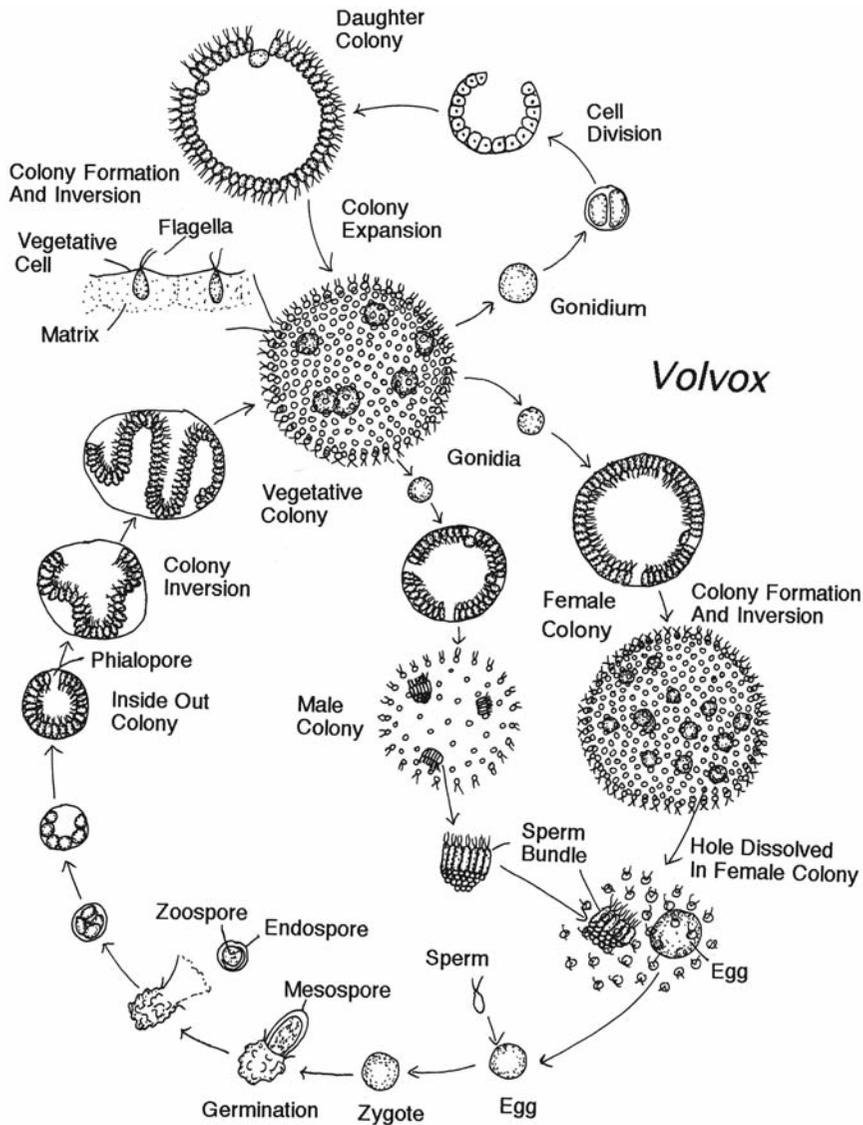
**Phototactic Steering By Change In Flagellar Beat Frequency In *Volvox carteri***



**Fig. 5.67** Colonies of *Volvox carteri* swim toward a light source by changing the frequency of the beat of the flagella. (Adapted from Hoops et al., 1999.)



**Fig. 5.68** *Volvox carteri*. Asexual individuals. (a) Sixteen large asexual reproductive cells are in a colony of ~2000 small biflagellate somatic cells. More than 95% of the volume is composed of extracellular matrix. (b) Juvenile colonies within the parent spheroid. Reproductive cells of the next generation are already visible within the juvenile colonies. (c) Dark-field image of spheroids with reproductive cells. (From Hallman, 2003.)

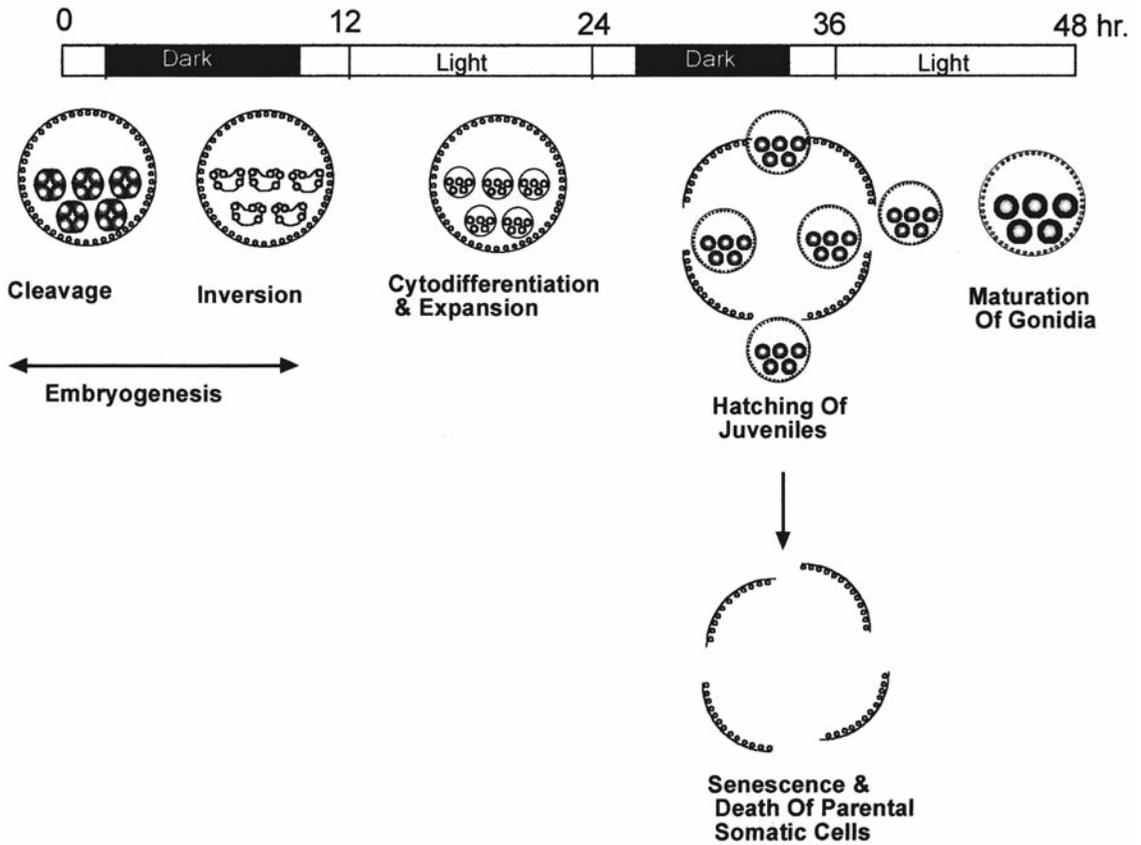


**Fig. 5.69** The life history of *Volvox carteri*. (Adapted from Smith, 1944; Kochert, 1968.)

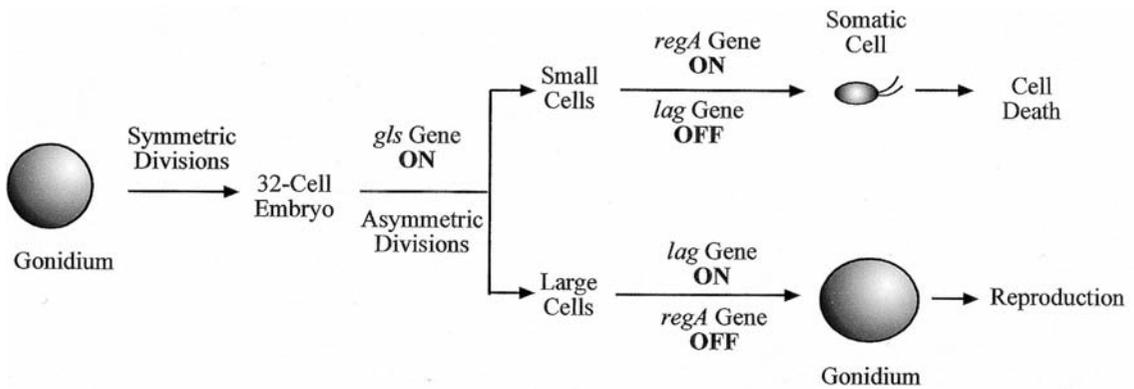
initials are around 30 times the volume of somatic initials at this point. It is the difference in the size of the cells at the end of cleavage, and not a difference in the quality of the cytoplasm, that determines whether the cells will follow the gonidial or somatic pathway of development (Kirk, 1998; Kirk and Nishii, 2001).

There are three categories of genes that control asexual reproduction in *Volvox carteri* (Fig. 5.71) (Kirk, 2001; Schmitt, 2003).

1 *gls* (gonidialess) gene products act in the sixth division in cleaving the embryo to bring about asymmetric division into somatic cells and gonidia. The cleavage plane is determined by the location of the basal body of the flagella. During cell division the flagella are retracted and the basal bodies move under the plasmalemma to the cleavage plane. It is thought the products of the *gls* gene function to position the basal bodies asymmetrically in the cell ensuring that the next cleavage division produces a large and small cell.



**Fig. 5.70** Linear representation of the 48-hour asexual cycle of *Volvox carteri*, as synchronized by a 16:8 hour light:dark regime. (Modified from Stark and Schmitt, 2002.)

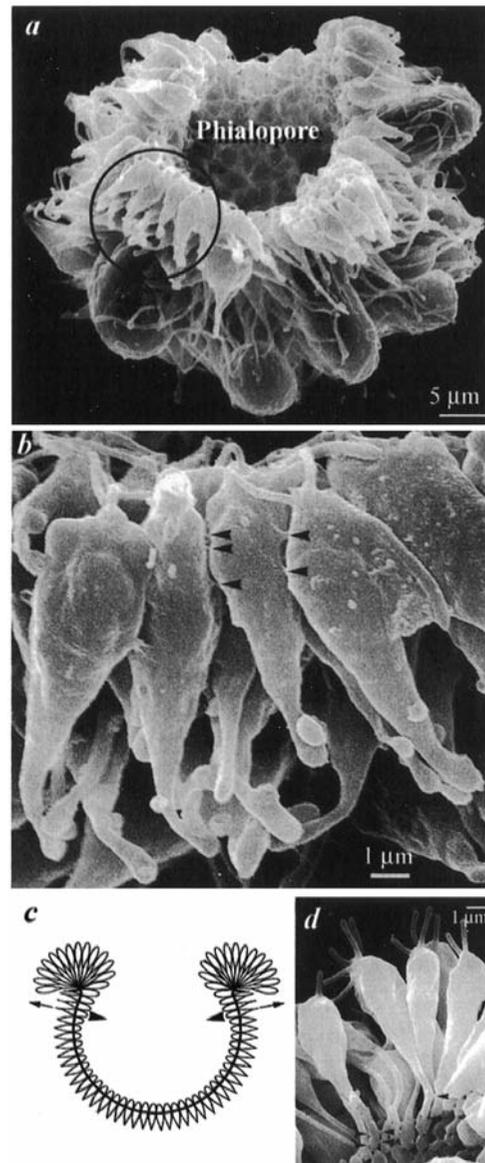


**Fig. 5.71** Model of the genetic program controlling the differentiation of somatic and gonidial cell development in *Volvox carteri*.

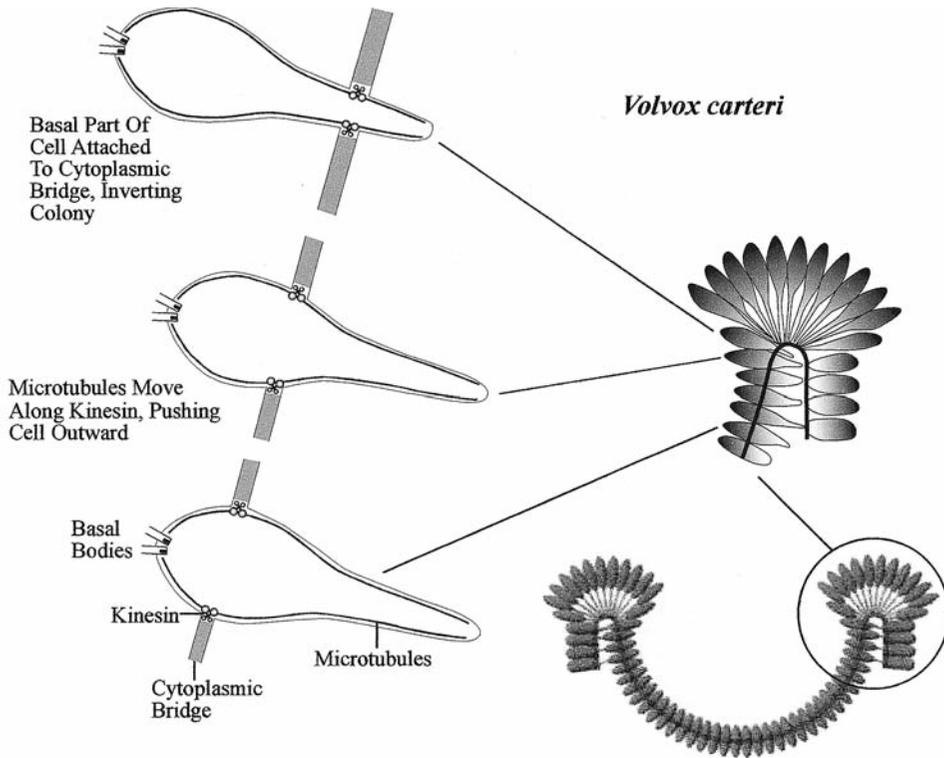
- 2 *regA* (**reg**enerator) gene products prevent the development of the small somatic cells into gonidia. The *regA* gene product, RegA, appears early in somatic cell differentiation to direct suppression of reproductive activity (Kirk, 2001; Stark and Schmitt, 2002). RegA is not detectable in embryos or gonidia. The gene *regA* encodes a protein in the nucleus of *V. carteri* cells. The protein arrests the transcription of genes required for chloroplast biogenesis, thereby making it impossible for the photoautotrophic cells to grow enough to reproduce. The minute amounts (0.05%) of the initial gonidial chloroplast that a somatic cell inherits is sufficient to keep it alive and functional until it senesces and dies the fourth day after the first cleavage division.
- 3 *lag* (**late gonidia**) gene products act in the large pregonidial cells to repress somatic development.

At the end of cleavage, a *V. carteri* embryo contains all of the cells that will be present in an adult, but the embryo is inside out with respect to the adult configuration; the flagellar ends of the somatic cells are all pointing toward the interior and the presumptive gonidia are on the outside. This awkward condition is soon corrected by a morphogenetic rearrangement in which the embryo turns itself right-side out to assume the adult configuration. This process is called “**inversion**”, which is unfortunate because “**eversion**” is a more accurate term. The resemblance of inversion to gastrulation in amphibians has long been noted.

Inversion begins soon after cleavage, when a wave of contraction sweeps over the embryo and a swastika-shaped opening called the **phialopore** appears at the anterior pole of the embryo. Inversion is initiated by cells throughout the colony becoming flask-shaped with thin stalks at their outer ends (Figs. 5.72, 5.73). The cells are connected by a network of cytoplasmic bridges. Incomplete cytokinesis results in each cell linked to its neighbor by a band of about 25 bridges that girdle the equator. Bending of the colony through the phialopore is initiated by the cells moving inward relative to the cytoplasmic bridges. The bridges cannot move because they are part of



**Fig. 5.72** *Volvox carteri*. (a) Scanning electron micrograph of a colony beginning to go through the process of inversion. The flask-shaped cells are joined at their base by cytoplasmic bridges. (b) Higher magnification of the area circled in (a). Arrowheads point to the cytoplasmic bridges. (c) Diagram showing how the protoplasm moves towards the base of the cells (arrowheads), elongating the base and moving the cytoplasmic bridges toward the outside of the colony (arrows). The mechanical force generated causes the colony to invert. (d) Scanning electron micrograph of flask-shaped cells in a colony in the latter stages of inversion. The cytoplasmic bridges are now at the apices of the cells (arrowheads). (From Nishii et al., 2003.)



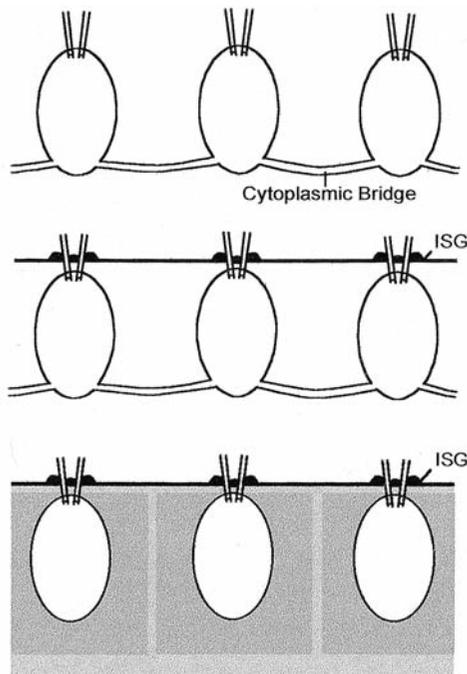
**Fig. 5.73** Inversion of a gonidium (embryo) begins when microtubules under the plasmalemma of *Volvox carteri* are drawn apically by kinesin. This brings the whole cell forward, placing forces on the colony, resulting in inversion through the phialopore.

a coherent interconnected network that runs through the entire colony. The inward movement causes the cells to go from being linked to their neighbors at their equator to being linked at their narrow, outermost tips, which in turn forces the cell sheet to bend sharply backward on itself.

Inward movement of the cells relative to the cytoplasmic bridges is caused by the kinesin *invA* interacting with microtubules (Nishii et al., 2003). A **kinesin** is a *mechanochemical protein capable of utilizing chemical energy from ATP hydrolysis to generate mechanical force*. In the presence of ATP, kinesin can bind to and move on microtubules. The *Volvox* cell has a system of peripheral microtubules under the plasmalemma that course in the cytoplasm from the area of the basal bodies to the other side of the cell. The minus (–) end of the microtubules occurs next to the basal bodies while the plus (+) end of the microtubules is at the other end of the

cell (Fig. 5.73). Activated kinesin molecules attempt to move toward the plus end of the microtubules. The kinesin, however, is anchored in the cytoplasmic bridge area of the cell so they cannot move. Instead, the kinesin molecules exert a force that causes the microtubules and consequently the entire cell to move upward, resulting in bending of the colony through the phialopore. The bending progresses across the surface of the colony until the colony has turned itself inside out.

At this stage, the cells are held apart by cytoplasmic bridges at their bases. These connections break down in early post-embryogenesis concurrent with the transient production of a hydroxyproline-rich glycoprotein called **ISG (inversion-specific glycoprotein)** (Schmitt, 2003). Inversion-specific glycoprotein establishes an extracellular matrix that traps the somatic cells by their flagella (Fig. 5.74), thereby making it impossible for them to change their positioning as their cytoplasmic bridges break down. The inversion-specific glycoprotein also acts as a scaffold for the proper assembly of the rest of the



**Fig. 5.74** The maintenance of cellular position in *Volvox carteri*.

extracellular matrix (Kirk, 1999; Hallman and Kirk, 2000).

While still contained within the original gonidial wall the daughter colony expands, the gelatinous matrix of the daughter colony forms, and the cells separate from one another. Eyespots develop, and the flagella protrude to their full length. Mature daughter colonies rotate slowly in their vesicles before release through pores in the parent colony matrix over each mature daughter colony (Kochert, 1968). It is possible to synchronize asexual division in *Volvox carteri* with alternating periods of light and dark (Fig. 5.70).

In the formation of female colonies, a gonidium cleaves to form a colony similar to a vegetative colony up to the 16- to 32-cell stage (Fig. 5.69). The next division results in cells larger than the somatic cells, the egg initials, which subsequently develop into eggs. Eggs are flagellated at maturity, but the flagella are not of sufficient length to protrude from the matrix of the colony. The maturation and release of the daughter colony are the same as for the vegetative colony.

Usually in *V. carteri*, male colonies are produced from gonidia that are initially the same size as the vegetative and female colonies, but later do not expand to the same size as these other colonies (Fig. 5.69). These are called dwarf male colonies. Dwarf male colonies are formed from gonidia the same way as the vegetative colonies up to the 32- to 64-cell stage. The next division results in larger cells, the male initials, scattered over the surface of the daughter colony. The dwarf male matures similarly to the vegetative cell except that the male colony does not expand as much. Before release of the dwarf male colony from the parent colony, the male initial divides to form a bowl-shaped mass of 64 to 128 cells that undergoes partial inversion to form a sperm bundle which is convex on one side. Each sperm has two flagella and an eyespot. Release of sperm bundles from the dwarf male colonies occurs through individual escape pores after the dwarf male colonies have been released from the parent colonies.

Strains of *V. carteri* are normally heterothallic (male and female colonies are formed in separate parent colonies). In sexual reproduction, the sperm bundles are released from the male colony and swim in the medium. If the sperm bundles come in contact with vegetative colonies, they swim over the surface of the colony with the flagella in contact with the surface. They soon swim off, however. When the sperm bundle comes in contact with a female colony, the flagella of the sperm bundle and the flagella of the female somatic cells bind, causing the sperm bundle to stick to the female colony (Coggin et al., 1979). A fertilization pore is dissolved in the sheath of the female colony, probably by secretion of a proteolytic enzyme (Hutt and Kochert, 1971). A few somatic cells are usually dissolved out of the female colony and swim off. The sperm bundle breaks down into individual sperm, which move in an amoeboid fashion between the somatic cells of the colony or swim with a corkscrew motion through the watery material in the interior of the colony. The sperm fuse with the eggs, forming zygotes that enlarge, develop an orange coloration, and secrete a thick crenulate wall. The parent female colony persists for some time, but eventually it dissociates and releases the zygotes.

After a resting period, the zygote germinates by splitting the outer zygote wall, with the middle layer of the wall (mesospore) protruding through the fissure in the outer layer. The protoplast has two flagella that beat weakly in the watery interior of the mesospore. After the zoospore is released from the outer zygote wall, the mesospore wall breaks down, leaving the protoplast inside the endospore wall. This protoplast then behaves similarly to a gonidial protoplast and divides to form a young colony of approximately 1000 somatic cells with four gonidia in a single tier.

Sexual type is inherited in a 1:1 ratio, and there is no parthenogenetic development of the eggs.

In *Volvox carteri*, the males make and accumulate a sexual inducer that is a 30-kilodalton glycoprotein (Starr and Jaenicke, 1974). The sexual inducer is released when the sperm are released from the sperm packets. The inducer is effective at  $6 \times 10^{-17}$  M. One sexual male releases enough inducer to convert all the related males and females in a volume of 1000 liters from asexual to sexual reproduction. Under the moderate growth conditions present in a large stable body of water, it is normally only the males that produce the sexual inducer. However, it is possible to force asexual males and females to make the inducer by subjugating the cells to a high temperature for a period of time. One hour at 42.5 °C is a sufficient heat shock to induce the formation of sexuality in *V. carteri* asexual males and females. Without the sexual inducer, the asexual males and females will produce asexual gonidia. In the presence of sexual inducer, the males and females produce sperm packets and eggs, respectively. The heat shock response is an adaptation to life in shallow temporary bodies of water where *Volvox* is often found. In the spring, in such bodies of water there is abundant water and the temperature is relatively low. *Volvox* grows asexually under these conditions. As summer progresses, the temperature in these bodies of water rises, and the organisms begin to dry out. The increase in temperature shocks the *Volvox* into producing the sexual inducer and initiating sexual reproduction. This results in the formation of drought-resistant zygospores, which sur-

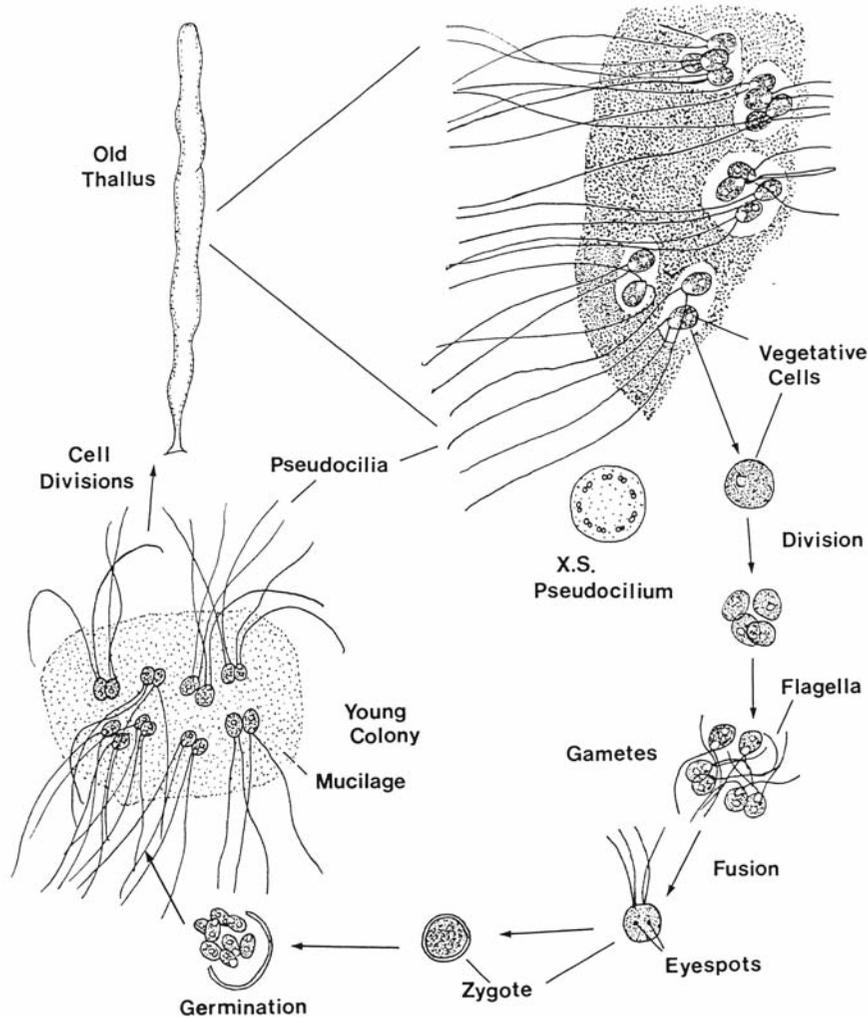


**Fig. 5.75** **David Kirk** Born 1934 in Clinton, Massachusetts. He obtained his B.Sc. from Northeastern University in 1956 and his Ph.D. from the University of Wisconsin in 1962. From 1962 to 1969 he was at the University of Chicago, eventually becoming Associate Professor. In 1969 he joined the faculty in the Department of Biology at Washington University in St. Louis where he is a tenured professor. Dr. Kirk has been a leading researcher in the cell biology of *Volvox*.

vive the dry conditions and serve as an overwintering spore. The above-described research by Kirk and Kirk (1986) (Fig. 5.75) has provided an explanation for Powers's (1908) observation that he had great difficulty finding sexual *Volvox* in large bodies of water. Powers further noted that "in the full blaze of Nebraska sunlight, *Volvox* is able to appear, multiply and riot in sexual reproduction in pools of rainwater of scarcely a fortnight duration." It took another 80 years for Kirk and Kirk to discover the heat-shock phenomenon and to explain Powers's observations.

### Tetrasporales

These algae have immobile vegetative cells that are capable of cell division, unlike those in the Chlorellales or Volvocales. The colonies are non-filamentous, and flagellated cells are formed by many genera. Asexual reproduction occurs via the formation of zoospores, aplanospores, or akinetes. Sexual reproduction is isogamous, by



**Fig. 5.76** The life cycle of *Tetraspora gelatinosa*. (Adapted from Klyver, 1929.)

fusion of biflagellate gametes. Almost all the organisms are freshwater.

Conventional wisdom has stated that the Tetrasporales evolved from the Volvocales by loss of motility in the vegetative condition. However, data from small subunit ribosomal DNA indicate the line leading to the Tetrasporales is more primitive than the line leading to the Volvocales (Booton et al., 1998).

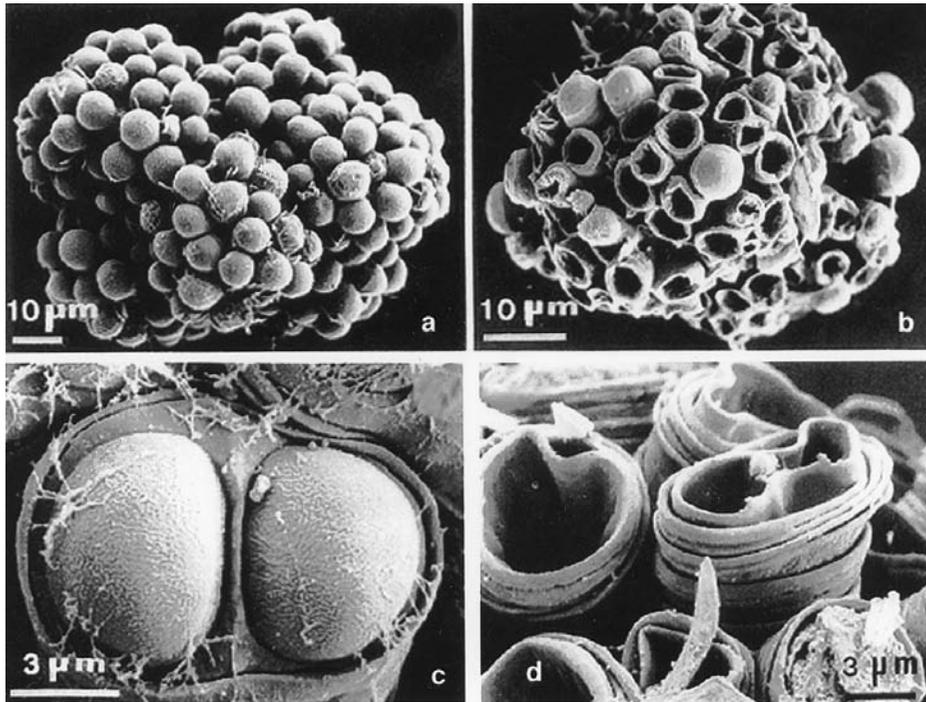
Two families will be considered here:

- Family 1 Tetrasporaceae: cells with pseudocilia.
- Family 2 Palmellaceae: cells without pseudocilia.

### Tetrasporaceae

The elongated gelatinous thalli of the Tetrasporaceae have vegetative cells in groups of two to four, with each cell having two pseudocilia. **Pseudocilia** are longer than flagella but are evidently related to them because the pseudocilia have a normal basal body but an abnormal 9 + 0 configuration of microtubules near the base of the pseudocilium (Lembi and Herndon, 1966; Wujek, 1968). The number of microtubules lessens and becomes more irregular as the end of the pseudocilium is approached.

Colonies of *Tetraspora gelatinosa* (Fig. 5.76) are green, amorphous masses with an outer layer of vegetative cells (Klyver, 1929). They are found in quiet freshwater and can be attached or



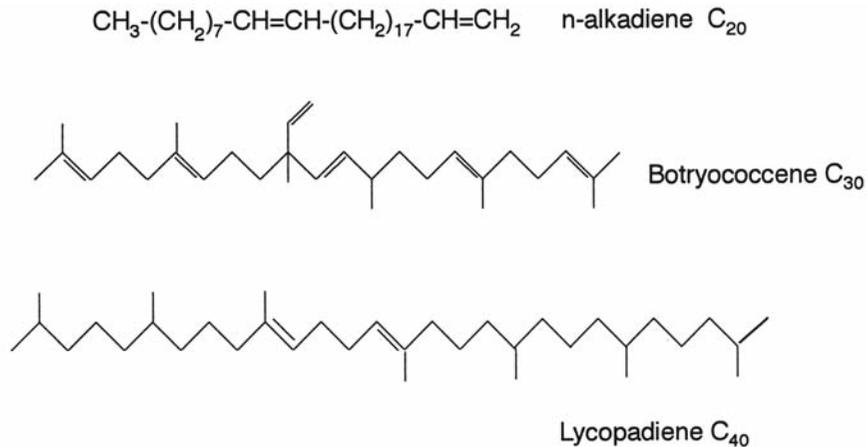
**Fig. 5.77** Scanning electron micrographs of *Botryococcus braunii*. (a) Colony from the wild. (b) Colony showing the cup-shaped mucilaginous bases. (c) Two cells in mucilaginous bases. (d) Mucilaginous base with no cells. (From Plain et al., 1993.)

free-floating. Each cell has a large cup-shaped chloroplast with a central pyrenoid and two pseudocilia. Growth of the thallus results from vegetative division of the cells. In the formation of isogametes, a vegetative cell divides two to three times, resulting in four or eight pyriform gametes, each with an eyespot and a cup-shaped chloroplast. The biflagellate gametes break free from the colonial mucilage and fuse with each other at their anterior ends. The quadriflagellate zygote swims for a while before settling, retracting its flagella, and forming a cell wall. The zygote germinates, forming four or eight aplanospores without pseudocilia. These aplanospores enlarge, and when they have reached the size of vegetative cells, they divide to form daughter cells that have pseudocilia. The aplanospores and their daughter cells are held together by mucilage, and the aggregation makes up the typical thallus of *Tetraspora*.

### Palmellaceae

Members of the Palmellaceae have their cells united in small gelatinous colonies that are generally amorphous but may be of definite shape. *Palmella* (Fig. 5.63) is a freshwater alga composed of cells united by a gelatinous matrix forming colonies of indefinite shape. Asexual division involves the formation of zoospores, and sexual reproduction occurs by formation of isogametes.

*Botryococcus braunii* (Fig. 5.77) is a free-floating colony of indefinite shape within a hyaline or orange envelope. The colonies are composed of radially arranged cells embedded in a tough mucous envelope. It forms water blooms that have been implicated in the death of fish (Chiang et al., 2004). The cells accumulate a large amount of oil in the autumn, which often obscures the cell contents. This alga has been postulated as the cause of the boghead coals (e.g., torbonite) and the oil shales of the Tertiary Period (Blackburn and Temperley, 1936; Cane, 1977; Wolf et al., 1985). If these deposits are examined under a microscope, it is possible to see plant remains that are similar to extant colonies of *B. braunii*. A hydrocarbon derivative exclusively attributable to *Botryococcus* comprises 1.4% of a Sumatran petroleum



**Fig. 5.78** The structure of hydrocarbons isolated from *Botryococcus braunii*. (Modified from Metzger et al., 1990.)

(Moldowan and Seifert, 1980). The cultivation of the alga has been proposed as a renewable source of liquid hydrocarbon fuel (Wake and Hillen, 1980; Yamaguchi, 1997). The resting stage of living *B. braunii* contains up to 70% of its dry weight as alkadienes, botryococcenes or lycopadiene (Fig. 5.78) (Metzger et al., 1990). The hydrocarbons are produced primarily during the exponential and linear growth phases. The dense matrix surrounding the cells is impregnated with the hydrocarbons.

### Prasiolales

The Prasiolales and the following two orders, the Chlorellales and the Trebouxiiales, are closely related and sometimes placed in a separate class, the Trebouxiophyceae (Sherwood et al., 2000; Lewis and McCourt, 2004).

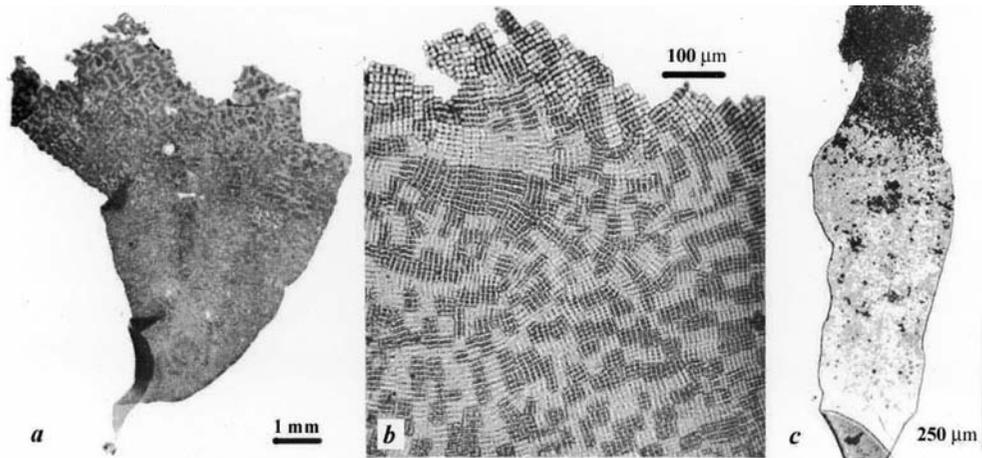
The members of the order are characterized by a stellate chloroplast with a central pyrenoid. These algae occur in a large variety of habitats, including freshwater, marine, and terrestrial habitats (such as concrete walls, rocks, and tree bark (Rindi and Guiry, 2003)). The principal genus, *Prasiola*, has a unique type of life history, with meiotic divisions in mature sexual thalli resulting in a haploid apex and diploid base.

*Prasiola stipitata* consists of small, thin, broadly ovate blades appearing as dirty green patches at or above high-tide level, commonly in the spray zone or areas fouled by bird excrement. The cells have

a central stellate chloroplast. The diploid thalli can form either diploid spores or haploid gametes (Fig. 5.79), the spore-forming plants growing higher on the shore than the sexual gamete-forming plants (Friedmann, 1959; Friedmann and Manton, 1959).

In the formation of the diploid aplanospore, the vegetative cells in the upper part of the thallus divide, making the upper part of the thallus multilayered. Each cell in this area forms a non-flagellated spore that settles, germinates, and develops into a new diploid plant like the parent (Fig. 5.80).

In the production of gametes, the cells in the upper part of a diploid thallus undergo meiosis, with the subsequent division of the haploid cells resulting in a multilayered upper part of the thallus. The haploid tissue is divided into a patchwork of rectangular darker and lighter areas containing the male and female cells, respectively. The difference in shading is due to the difference in size of the chloroplasts, the larger female cells having the larger chloroplasts. The gametes are liberated after the thalli are wet by the incoming tide. More male gametes are released because of their smaller size and greater production per unit area. The anteriorly biflagellate male gametes swim around the non-motile egg, and one of the male flagella touches the egg. The flagellum fuses with the egg, a step followed by fusion of the bodies of the gametes. The pear-shaped zygote swims vigorously by means of the posteriorly directed remaining male flagellum. At 5 °C, the



**Fig. 5.79** *Prasiola stipitata*. (a) Sporophyte producing male and female gametes. (b) Apical part of sporophyte thallus with male (dark areas) and female (light areas) gametes. (c) Sporophyte producing spores from the apical part of the thallus. (From Friedmann, 1959.)

zygote swims for several hours, retracts the flagellum, sinks, attaches firmly to the substratum, and develops a cell wall. The zygotes germinate into diploid thalli, completing the life cycle (Friedmann, 1959).

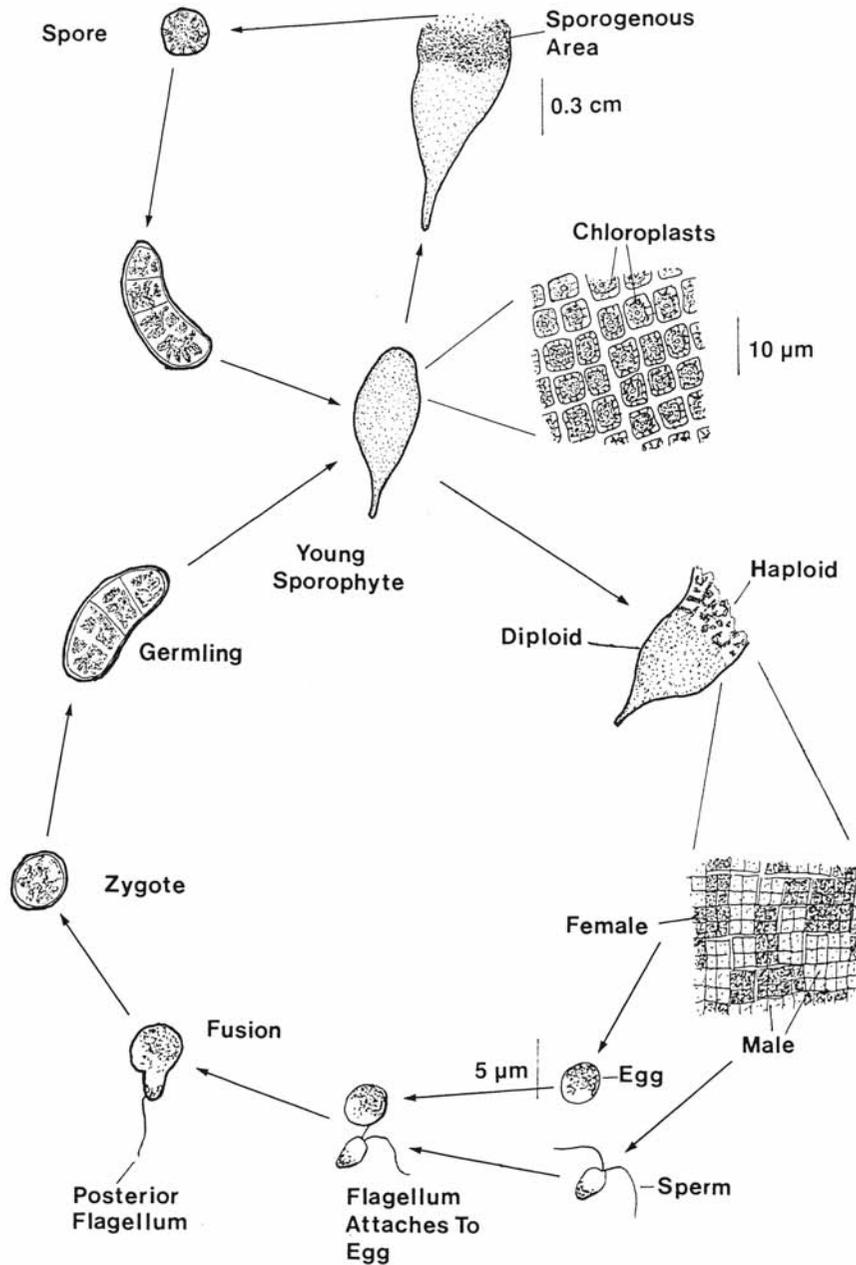
### Chlorellales

In this order are the algae that have a non-motile vegetative thallus where the thallus comprises only a single cell or a coenobium composed of a definite number of cells arranged in a specific manner. Asexual reproduction occurs by zoospores or aplanospores that are commonly autospores. These autospores are probably no more than daughter cells of the parent thallus. Sexual reproduction can be isogamous, anisogamous, or oogamous. The order is exclusively freshwater.

*Chlorococcum* (Fig. 5.81) is an alga frequently isolated from beneath the surface of the soil. It sometimes occurs in abundance on damp soil or brickwork. The cells can survive for a long time in the soil. In one study they grew after being in desiccated soil for 59 years (Trainor, 1970). The cells of the same species vary considerably in size, young cells having a thin cell wall and older cells a thick one. The chloroplast in young cells is a massive parietal cup with a single pyrenoid. In older

cells the chloroplast becomes diffuse. Asexual reproduction is by zoospores, there never being any vegetative division. The biflagellate zoospores have a cup-shaped chloroplast and an eyespot. Sexual reproduction occurs by the formation of isogametes. Under certain conditions, aplanospores can be produced, which eventually liberate two to four biflagellate gametes. Nearly dormant cells of *Chlorococcum echinozygotum* that have been deprived of nitrogen can be induced to undergo gametogenesis by being placed in fresh medium in darkness without nitrogen (O'Kelley, 1984). If nitrogen is supplied 6 hours after the cells are placed in darkness, zoosporogenesis occurs instead of gametogenesis.

*Hydrodictyon reticulatum* (Fig. 5.82), or the water net, is a free-floating, relatively rare freshwater alga that forms colonies that are net-like with polygonal or hexagonal meshes. The net is formed by multinucleate elongated cells joined end to end, probably by lectins such as concanavalin A (Hatano and Ueda, 2000). Each cell has a large central vacuole and a reticulate chloroplast with pyrenoids. In asexual reproduction a daughter net is produced inside, and subsequently released from the parent cell (Pocock, 1960; Marchant and Pickett-Heaps, 1971, 1972a–d). Asexual reproduction begins with the disappearance of pyrenoids, coinciding with the accumulation of starch grains in the fragmenting chloroplasts. Regularly spaced nuclei are surrounded by fragments of chloroplasts. A vesicle forms around and outside of the tonoplast, restricting most of the proto-

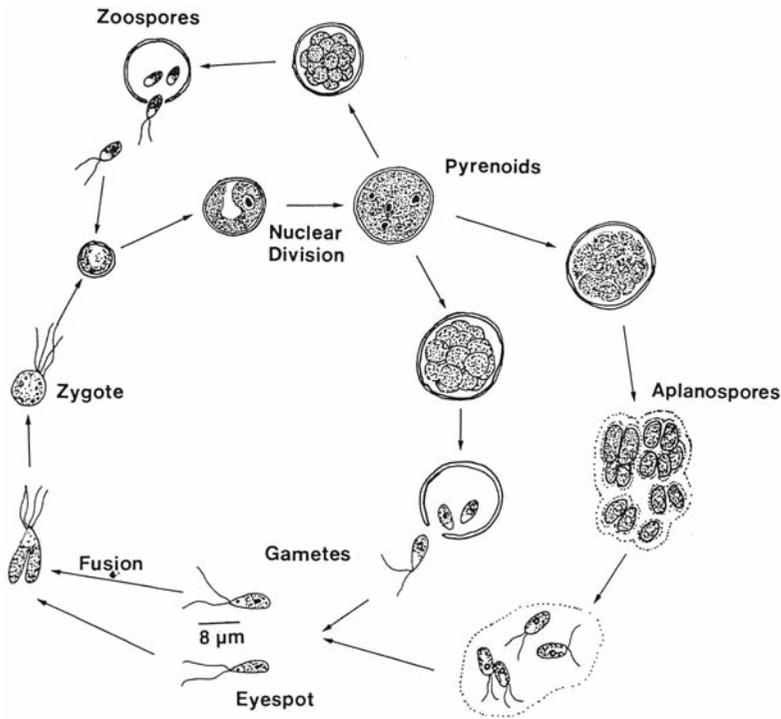


**Fig. 5.80** The life cycle of *Prasiola stipitata*. (Adapted from Friedmann, 1959.)

plasm to a small area around the periphery of the cell. The protoplasm divides to produce uninucleate, biflagellate zoospores that actively swim about inside the parent cell. The zoospores stop swimming, become joined in certain areas, and retract their flagella. A daughter net has now been

formed within the parent cell, which is released to enlarge to a mature colony.

*Chlorella* cells are spherical with a cup-shaped chloroplast (Fig. 5.83). The only method of reproduction is by daughter cells that resemble the parent cell. *Chlorella* often forms intracellular symbioses with aquatic invertebrates and protozoa (such as *Paramecium*). In these symbioses, *Chlorella* is held in host vacuoles where the



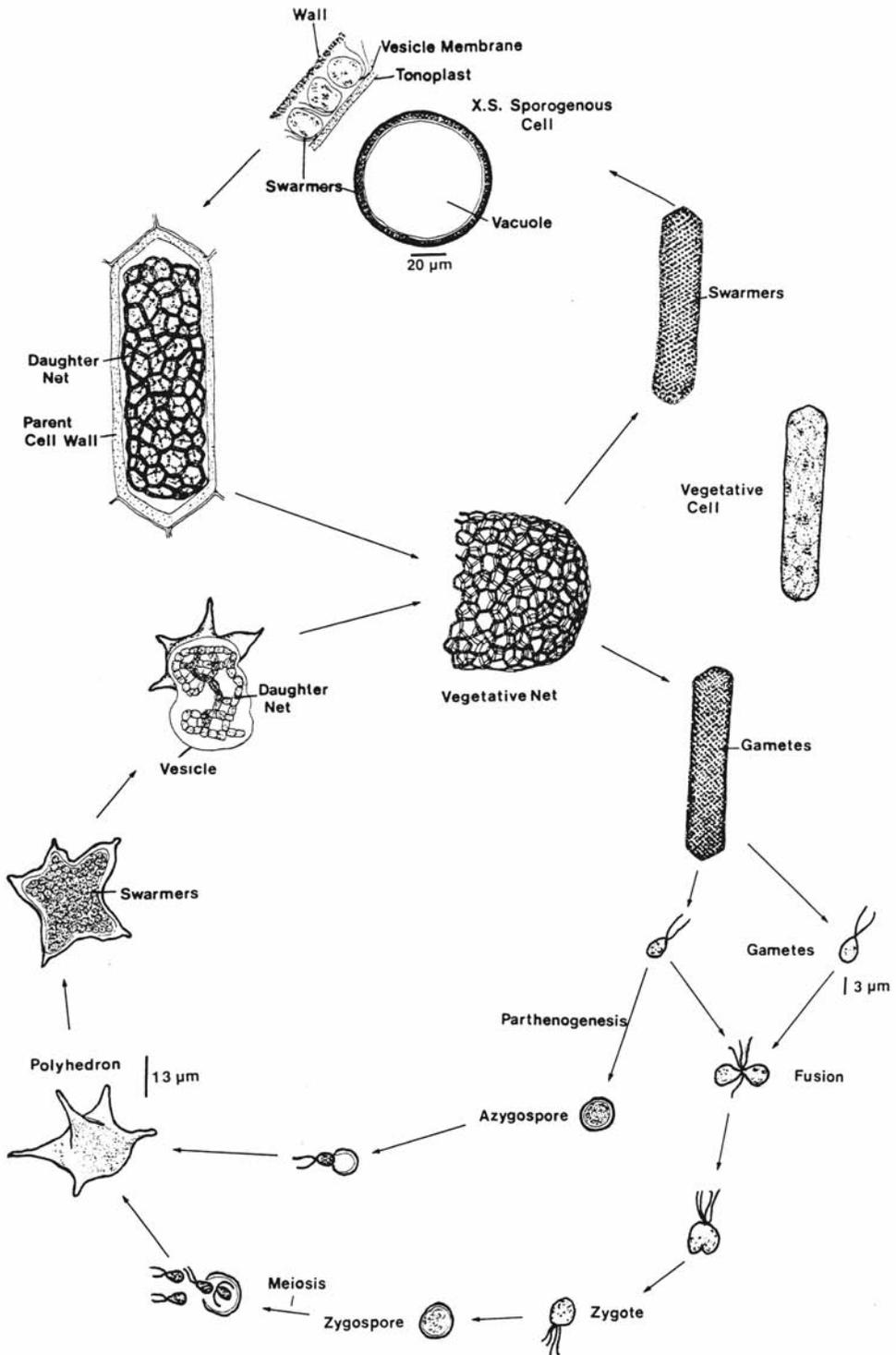
**Fig. 5.81** The life cycle of *Chlorococcum humicola*. (Adapted from Bristol, 1919.)

*Chlorella* synthesizes and releases maltose into the host vacuole. *Chlorella* is held at a low pH in the host vacuoles. Studies on isolated *Chlorella* cells have shown that maximum synthesis and release of maltose occurs at pH 4–5 (Dorling et al., 1997).

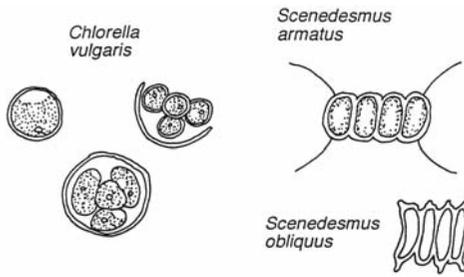
*Prototheca* lacks chlorophyll and resembles a colorless *Chlorella* although the *Prototheca* cells do have starch-containing amyloplasts (Webster et al., 1968). *Prototheca* causes **protothecosis** in animals and humans. This disease is more common than is supposed, but because the small round colorless cells resemble yeasts, the disease, at least in animals, is often incorrectly diagnosed. *Prototheca* cells are fairly common in the soil, where they presumably live by digesting organic matter, and it is from here that most infections occur. In animals most of the reported cases have been severe systemic infections, such as massive invasion of the bloodstream, that have resulted in the death of the animal within a short time (van Kruiningen et al., 1969). Such massive infections have been reported in humans but usually only as secondary invaders after the primary invader has seriously lowered the body's defenses. More common in humans is the subcutaneous type of infection

that starts initially as a small lesion and spreads slowly through the lymph glands, covering large areas of the body and preventing the sufferer from performing normal work duties (Fig. 5.84) (Mars et al., 1971).

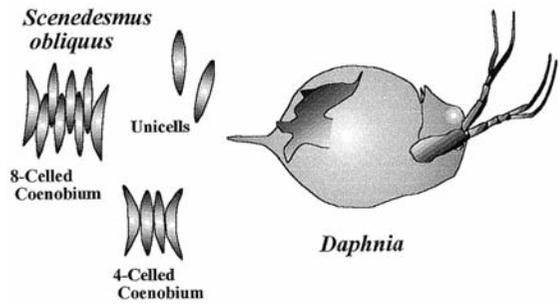
*Scenedesmus* is a common alga (Fig. 5.83), often occurring as almost a pure culture in plankton. Cells in the colony occur in multiples of two, with four or eight cells being most common. The species differ mostly in the number and type of spines on the cells and the texture of the wall. The uninucleate cells have a single laminate chloroplast. The morphology of the colony can be varied considerably by varying the medium in which the cells are growing (Egan and Trainor, 1989). In a medium with low phosphorus or low salt concentration, the *Scenedesmus* is induced to grow as unicells, resembling the genera *Chodatella* and *Franceia* (Trainor, 1992) and the same species can also be induced to grow with or without spines. Composed of aggregated proteinaceous tubules, the spines probably aid in the flotation of the colonies (Stahelin and Pickett-Heaps, 1975). *Scenedesmus* occasionally forms zoospores when deprived of nitrogen (Trainor, 1963).



**Fig. 5.82** The life cycle of *Hydrodictyon reticulatum*.  
 (Adapted from Pocock, 1960; Marchant and Pickett-Heaps, 1971, 1972a-d.)



**Fig. 5.83** Drawing of *Chlorella vulgaris* (with autospores), *Scenedesmus armatus*, and *S. obliquus*.



**Fig. 5.85** *Scenedesmus obliquus* without grazers occurs primarily as unicells. When grazers such as *Daphnia* are present, *S. obliquus* grows primarily as coenobial colonies.

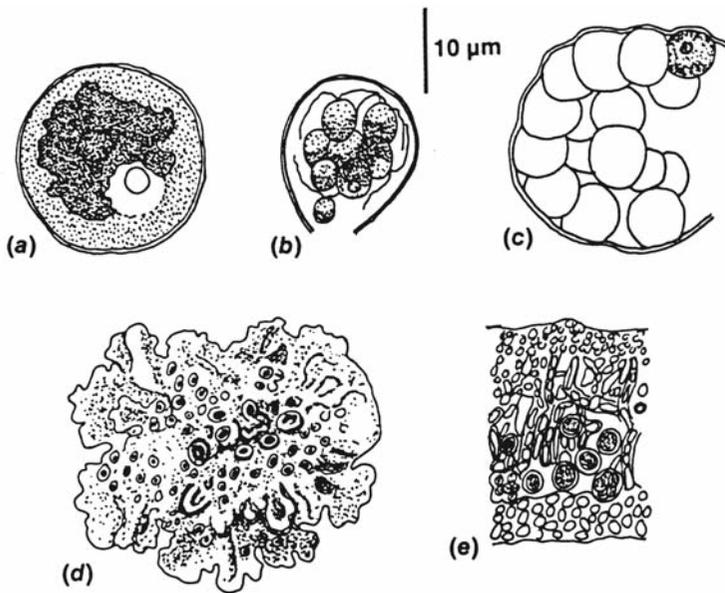


**Fig. 5.84** Lesions of protothecosis on the foot and lower leg of a man from Sierra Leone. (From Davies and Wilkinson, 1967.)

Many species of *Scenedesmus* grow as unicells in an environment without grazers. In the presence of grazers, such as *Daphnia* (Fig. 5.85), *Scenedesmus* forms multicellular colonies that are not grazed as heavily. **Infochemicals** produced by the grazers induce the formation of coenobia. *Infochemicals* are chemicals that, in the natural context, convey information in an interaction between two organisms, evoking a behavioral or physiological response in the receiving organism, which is adaptively favorable to one or both organisms in the interaction. In the case of

*Scenedesmus*, the infochemicals originate from the digestive tract of the grazer. Starved grazers do not produce infochemicals. In the multicellular state, *Scenedesmus* is not grazed as heavily by *Daphnia*. However, the advantages to *Scenedesmus* in the unicellular state include smaller sinking velocity and advantageous surface to volume ratio in terms of nutrient uptake and light harvesting (Lurling, 1998, 1999).

Unicellular green algae, such as *Chlorella*, have been extensively investigated as possible new sources of food for an increasing world population (Yamaguchi, 1997). *Chlorella* produces little cellulose or other carbohydrate wall material; thus more of the cell is digestible in *Chlorella* than in an alga with a large amount of cellulose. Exponentially growing *Chlorella* cells contain about 50% protein, 5% chlorophyll, and a large number of vitamins. Much greater quantities of biomass can be obtained per unit area with algae than with higher plants. When the growth of algae is linked to the purification of sewage in oxidation ponds, yields of 112000 kg hectare<sup>-1</sup> year<sup>-1</sup> of dried algae can be obtained. At the same time, the algae are taking up elements such as nitrogen and phosphorus, and reducing the biological oxygen demand (an indication of the organic material in the water) by 85% (McGarry and Tongkasame, 1971). The algae obtained make a very good stock feed, and attempts have been made to utilize it as human food, although the algae in the human digestive tract lead to undesirable intestinal floras that often result in gas in the intestine. A second line of investigation in this



**Fig. 5.86** *Trebouxia* sp.: (a) vegetative cell; (b) zoospores being released; (c) aplanospores being released. The *Trebouxia*-containing lichen *Xanthoria parietina*; (d) whole thallus; (e) section of thallus showing spherical *Trebouxia* cells. ((a)–(c) after Ahmadjian, 1960; (d),(e) after Fünfstück, 1907.)

area has been to provide food and oxygen for space and submarine travel. In such a setup, carbon dioxide from human respiration is taken up for use in photosynthesis, with the oxygen produced being respired by humans. A man uses about 600 liters of oxygen daily, and a gas exchange unit that would produce this amount of oxygen would also produce almost enough biomass to provide sufficient food for one man as well (Fogg, 1971).

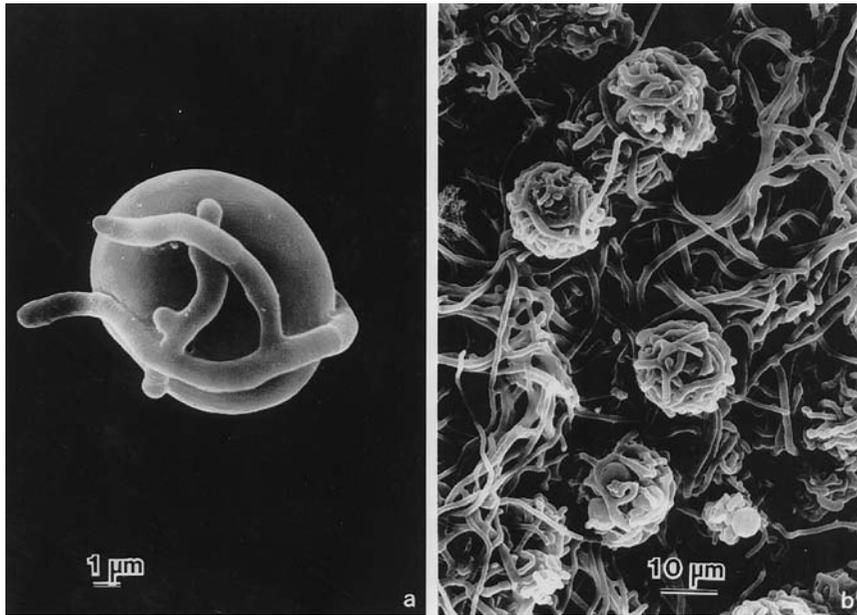
### Trebouxiiales

This order represents the “lichen algae group” (Lewis and McCourt, 2004). Note that not all lichenized algae are in this group: other **phycobionts** (algal partner in a lichen symbiosis, the fungus is the **mycobiont**) include cyanobacteria, *Trentepohlia* (Ulvophyceae), and *Heterococcus* (Xanthophyceae).

*Trebouxia* (Figs. 5.86, 5.87) is the most common green alga as a phycobiont in a lichen symbiosis. The genus also is found as a free-living alga, and, when free-living, it is usually twice the size of the alga in the lichen symbiosis (see Ahmadjian, 1993, for a review). *Trebouxia* has a massive chloroplast with a single pyrenoid. When it is growing in the lichen association, reproduction is normally by autospores, although under wet conditions zoospores may be formed. When the alga is grown in liquid culture, zoospores are formed. Sexual reproduction is isogamous or anisogamous by the

fusion of biflagellate gametes. Lichenized *Trebouxia* produces primarily sugar alcohols (80% ribitol) from photosynthetic processes, whereas free-living *Trebouxia* forms much smaller amounts of sugar alcohols (15% ribitol) and greater quantities of other carbohydrates (Green, 1970). Richardson (1973) has summarized the differences between algae growing as phycobionts (algae in the lichen association) and the free-living ones: the free-living algae (1) synthesize less sugar or sugar alcohol, (2) form more polysaccharides, (3) develop cell sheaths not seen in phycobionts, and (4) release less photosynthate into the surrounding medium. *Trebouxia* is able to grow saprophytically in the dark in the absence of light (Ahmadjian, 1960).

Lichen mycobionts are able to discriminate between suitable and unsuitable algal partners. The mycobiont *Xanthoria parietina* secretes a protein that will bind only to the cell walls of species of *Trebouxia* and *Pseudotrebouxia*, algal genera that normally make up this lichen symbiosis (Bubrick and Galun, 1980). The cell walls of these algae are characterized by high levels of acidic polysaccharide and a protein coat on the cell wall surface. Members of other algal families do not bind the lichen protein. In the lichen *Cladonia cristatella*, compatible phycobionts have fungal haustoria in most of the phycobiont cells. Fungal haustoria are usually used by the fungus to transfer nutrients



**Fig. 5.87** The beginning of a lichen. (a) Scanning electron micrograph of the envelopment of a cell of the phycobiont *Trebouxia erici* by the hyphae of the mycobiont *Cladonia cristatella*. (b) The mycobiont has completely enveloped the cells of the phycobiont, and the hyphae have formed the thallus of the lichen. (From Ahmadjian and Jacobs, 1981.)

from a host to the fungus when the host is a parasitized organism. However, in lichens, transfer of metabolites to the fungus is minimal (Collins and Farrar, 1978; Hessler and Peveling, 1978). This is probably a case of controlled parasitism (Ahmadjian and Jacobs, 1981).

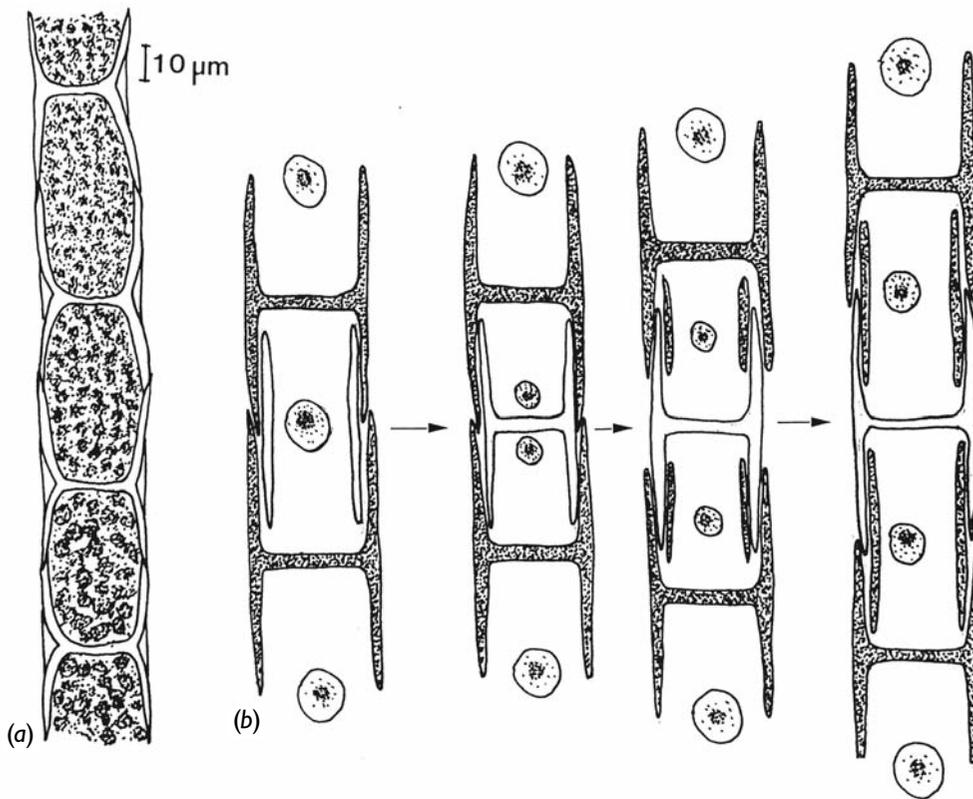
### Sphaeropleales

These filamentous Chlorophyceae have a mature lateral wall that consists of segments, rather than having a continuous structure. In *Microspora*, there are H-shaped wall segments that arise from two separate phases of wall secretion (Fig. 5.88) (Pickett-Heaps, 1973). During interphase, cell expansion is accommodated by the interlocking H-segments moving apart. At the same time, a new cylindrical wall is secreted inside these segments. Then during cytokinesis, the newly formed cross wall turns this cylinder into the typical wall segment. A similar wall occurs in the Oedogoniales, but the algae in the Sphaeropleales lack the unusual structure of the Oedogoniales.

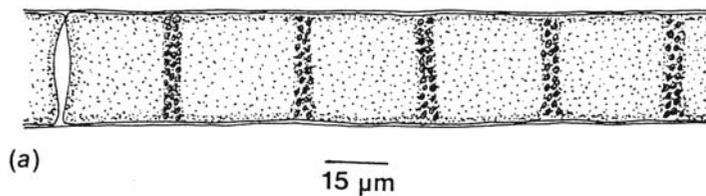
The wall structure in the Sphaeropleales is similar to that in the Xanthophyceae, and it is probable that the filaments in both algal groups evolved from a unicellular alga, although independently of each other. The Sphaeropleales probably evolved from an alga in the Chlorellales (Cáceres et al., 1997).

Algae in the Sphaeropleales (Figs. 5.88, 5.89) typically occur in shallow freshwater habitats that are inundated only periodically. The ephemeral vegetative stage (often lasting a few weeks or less) occurs during short intervals of flooding, whereas the thick-walled, resistant zygotes persist in the soil through long dry periods, thereby enabling long-term survival of these algae. The zygotes are ornamented and sometimes have *cirri* (Fig. 5.90), curled appendages composed of organic material, on their surface (Hoffman and Buchheim, 1989).

*Sphaeroplea* has multinucleate cells arranged end to end in unbranched filaments (Buchheim et al., 2001) (Fig. 5.89). The alga is freshwater, occurring on periodically wet ground, completing its life cycle within 4 to 5 weeks. Within a cell the cytoplasm is restricted to a number of transverse bands, each band separated by a vacuole. Each cytoplasmic band has several nuclei and a band-shaped chloroplast with several pyrenoids, or numerous discoid chloroplasts. There is also a

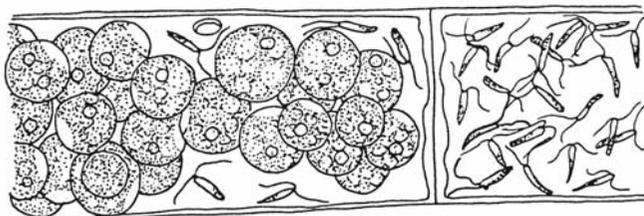


**Fig. 5.88** *Microspora crassior*. (a) Filament. (b) Diagrammatic representation of wall deposition. Initially the interphase cell is enclosed by two H-shaped segments and a central cylinder. At cytokinesis, a cross wall is deposited in the middle of the cylinder. During cell expansion, the two H-shaped segments move apart while a new cylinder is secreted inside the H-shaped segments. ((b) after Pickett-Heaps, 1973.)



(a)

15 µm

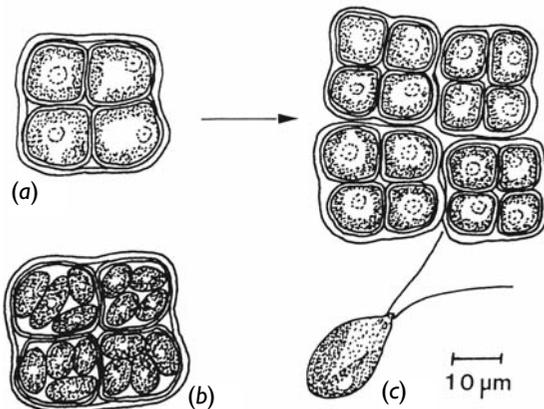


(b)

**Fig. 5.89** *Sphaeroplea annulina*. (a) Part of a vegetative cell. (b) Portion of an oogonium in which the eggs are being fertilized, and a portion of an adjoining antheridium. (After Smith, 1955.)



**Fig. 5.90** Scanning electron micrograph of a zygote of *Sphaeoplea fragilis* showing the sculptured exterior and a long curved cirrus. (From Hoffman and Buchheim, 1989.)



**Fig. 5.91** *Chlorosarcina* sp. (a) Packet of cells dividing by desmoschisis to produce daughter colonies, each enclosed within the cell wall of the parent cell. (b) Colony-forming zoospores. (c) Zoospore.

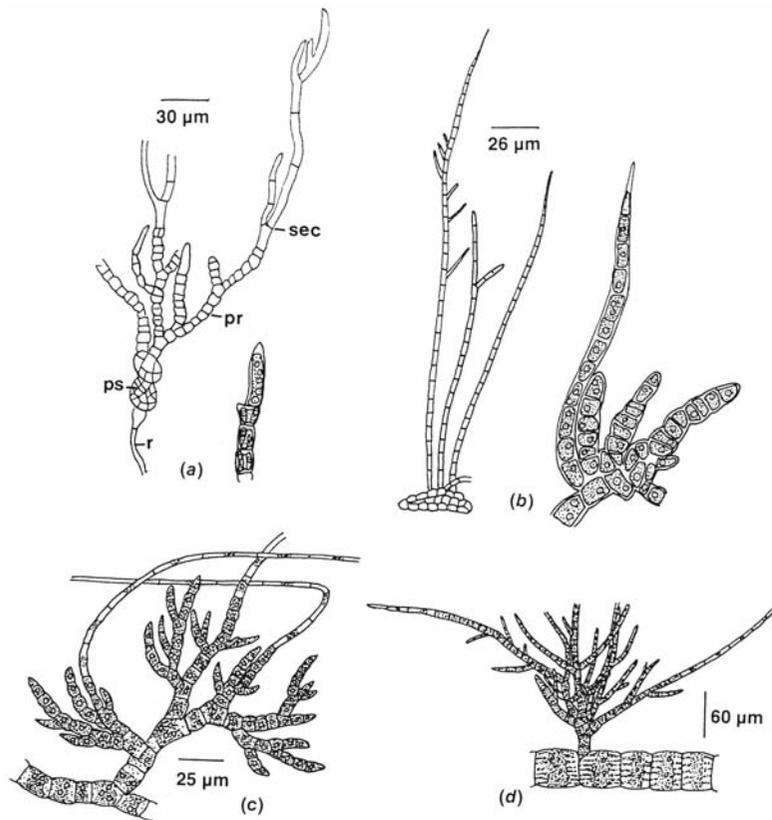
thin layer of cytoplasm without chloroplasts between the vacuoles and the side walls. Asexual reproduction occurs by fragmentation of the filaments. Sexual reproduction is usually oogamous, with eggs and spermatozooids formed in alternate cells of the same filament or in different filaments. The spindle-shaped biflagellate spermatozooids escape through pores in the side walls of the antheridial cell and swim to the oogonial cells. The eggs, when first formed, are multinucleate, with all the nuclei but one disintegrating. The oogonial cells have pores in their walls through

which the spermatozooids enter, swim about the eggs, and eventually fuse with them. Zygotes form a thick ornamented cell with a reddish protoplast, and are released by the decay of the oogonial cell. Zygotes germinate by forming four biflagellate ovoid zoospores, which settle and germinate into a new filament.

### Chlorosarcinales

The algae in this order have a type of cell division (desmoschisis) that results in the formation of a number of cells, each with its own cell wall, within the cell wall of the parent cell. The genera in the Chlorosarcinales lack the plasmodesmata and complexity found in the Chaetophorales and Oedogoniales.

*Chlorosarcina* occurs free-living in the soil, or it can occur as an endophyte within the epidermis of aquatic vascular plants. Each cell contains a parietal chloroplast and has a cell wall that separates the cell from the other cells inside the old parental cell wall (Fig. 5.91). During **desmoschisis**, the protoplasm of a cell divides at successively perpendicular planes to build up cuboidal packets of cells. Cell walls are formed around the newly divided protoplast, next to the parent cell wall which remains intact. Eventually the colonies fragment to distribute the alga. Asexual reproduction can occur by the formation of four to eight biflagellate zoospores per cell,



**Fig. 5.92** (a) *Fritschiella tuberosa* showing prostrate system (ps), protonema (pr), projecting secondary branches (sec), and a rhizoid (r). Also shown is the tip of a branch. (b) *Stigeoclonium farctum*, old and young plants with erect and prostrate portions. (c) *Chaetophora incrassata*. (d) *Draparnaldia glomerata*. ((a) after Iyengar, 1932; (b) after Butcher, 1932; (c),(d) after Smith, 1920.)

which are released by softening in one area of the cell wall.

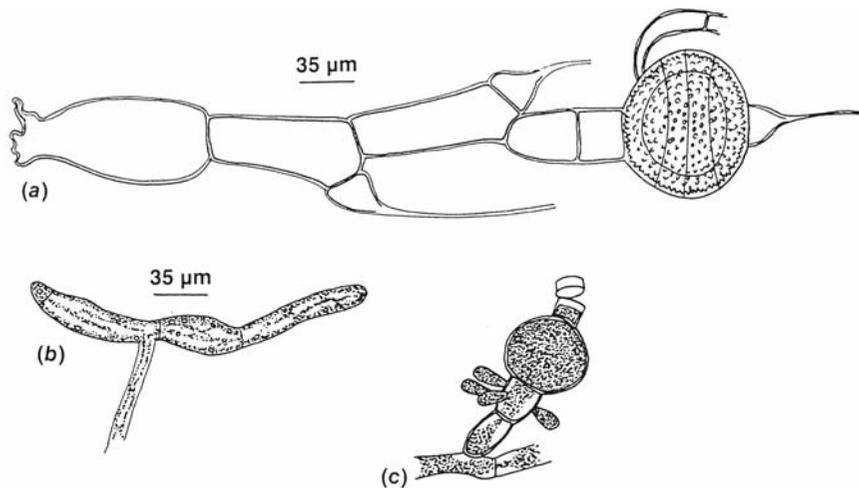
### Chaetophorales

The Chaetophorales, along with the Oedogoniales, mark the most complex organization attained within the Chlorophyceae. The Chaetophorales have plasmodesmata, a characteristic of true multicellularity. In many ways, the level of organization in the Chaetophorales parallels that attained by the Coleochaetales in the Charophyceae.

The Chaetophorales have branched filaments with uninucleate cells that contain a single parietal chloroplast. Many of the plants exhibit **heterotrichy**; that is, there are *two different types of filaments, one constituting the prostrate system and the other the erect system*. In the past, these organisms were postulated to be the precursors of higher plants because they show the differentiation of filaments into different systems. An evolutionary progression from *Stigeoclonium* (Fig. 5.92(b)) to

*Chaetophora* (Fig. 5.92(c)) to *Draparnaldia* (Fig. 5.92(d)) to *Fritschiella* (Fig. 5.92(a)) illustrates increasing differentiation toward what one would expect a primitive land plant to look like. As stated earlier, however, the above algae divide by a phycoplast and are therefore not in a direct line to higher plants.

*Stigeoclonium* (Fig. 5.92(b)) is a common freshwater alga found attached in flowing water. It has a very wide tolerance to organic pollution and can be indicative of heavily polluted water (McLean, 1974; McLean and Benson-Evans, 1974) although it will also grow in unpolluted water. The thallus is differentiated into prostrate and erect portions, with the terminal parts of the branches usually drawn out into colorless hairs. Vegetative reproduction can take place by fragmentation, although the erect portion does not grow well after detachment from the prostrate portion. Quadriflagellate zoospores are frequently formed when a thallus is brought into the laboratory, with most of the cells in the smaller



**Fig. 5.93** (a) *Bulbochaete gigantea*, filament with zygote. (b),(c) *Oedocladium hazenii*, germling with rhizoid (b) and portion of a filament with empty androsporangia, immature dwarf males, and an unfertilized egg (c). (After Smith, 1950.)

branches sporulating a day after being brought in. A cell forms a single zoospore. After settling on its anterior end, the zoospore develops into a new filament. In sexual reproduction, gametes are produced in cells of erect filaments after a cruciate, presumably meiotic, process of division (Simons and van Beem, 1987). The gametes can be biflagellate or quadriflagellate. Fusion of gametes produces a zygote which germinates into a juvenile plant in which the original zygote remains visible as an embryonic cell.

### Oedogoniales

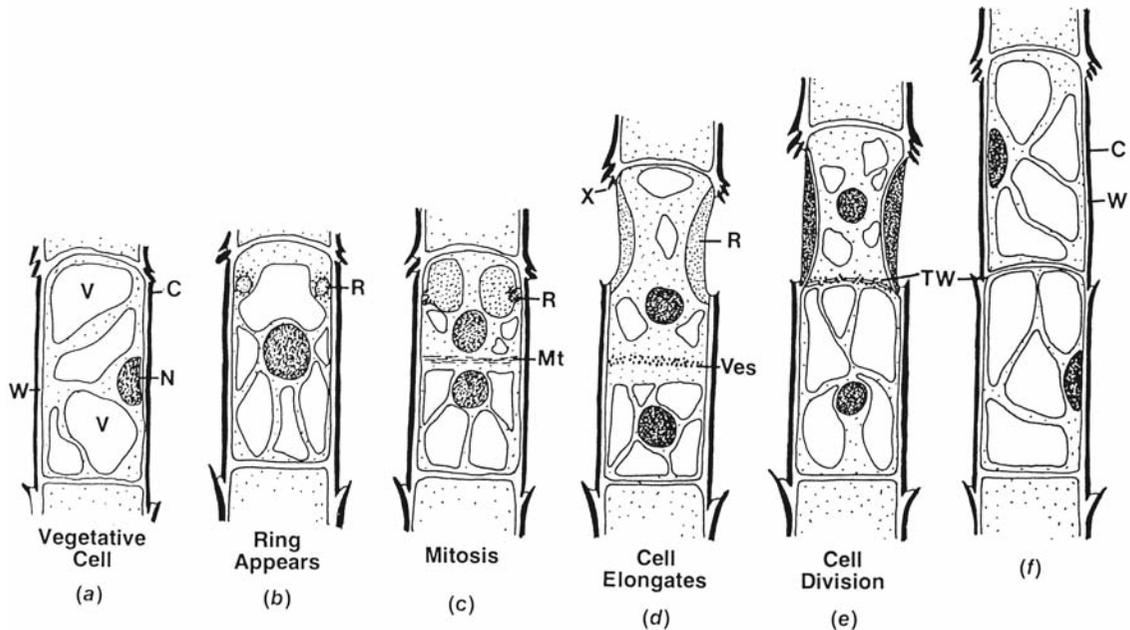
The filamentous, freshwater, uninucleate algae in this order are characterized by a unique type of cell division and the production of motile reproductive cells with a whorl of flagella at one pole (stephanokonts). Sexual reproduction is oogamous, and asexual reproduction can be by zoospores or akinetes.

The Oedogoniales and its single family, the Oedogoniaceae, have only three genera - *Oedogonium* (Fig. 5.94), *Oedocladium* (Fig. 5.93(b), (c)), and *Bulbochaete* (Fig. 5.93(a)). *Oedogonium* is unbranched, whereas *Oedocladium* and *Bulbochaete* are branched. *Bulbochaete* has long colorless hairs (Fig. 5.93(a)); *Oedocladium* lacks hairs (Fig. 5.93(b), (c)). These algae are usually present in permanent bodies of

water such as ponds or lakes. If they are growing in moving water, they are seldom in the fruiting condition. Normally fruiting takes place in the summertime. The plants may be epiphytic on aquatic plants and other algae, or they may be free-floating.

Chloroplasts in this order are reticulate, extending from one end of the cell to the other. The many pyrenoids are at the intersections of the reticulum. Plasmodesmata are present between cells, and the ones in *Bulbochaete* are similar to those of higher plants (Fraser and Gunning, 1969).

Cell division involves the breaking of the parent wall and the formation of apical caps. In *Oedogonium*, cell division (Fig. 5.94) is initiated by the formation of a ring under the wall in the upper part of the cell (Hill and Machlis, 1968). The ring enlarges by the coalescence of material produced in the cytoplasm. While the ring is being produced, the nucleus migrates to the center of the cell and divides mitotically. During late telophase, the new cross wall begins to form by means of a phycoplast. The daughter cells elongate, causing a split in the parent wall near the apical ring. This rent in the cell wall is covered by the material in the apical ring, which expands as the cells elongate. Each daughter cell eventually elongates to about the same length as the mother cell, elongation being completed within 15 minutes. The material of the ring becomes the cuticle, and a new cell wall is laid down under it. During the elongation of the daughter cells, the new transverse wall has moved up to the base of the



**Fig. 5.94** Cell division in *Oedogonium*. (C) cuticle; (Mt) microtubules; (N) nucleus; (R) ring; (TW) transverse wall; (V) vacuole; (Ves) vesicles; (W) wall; (X) cap. (After Hill and Machlis, 1968.)

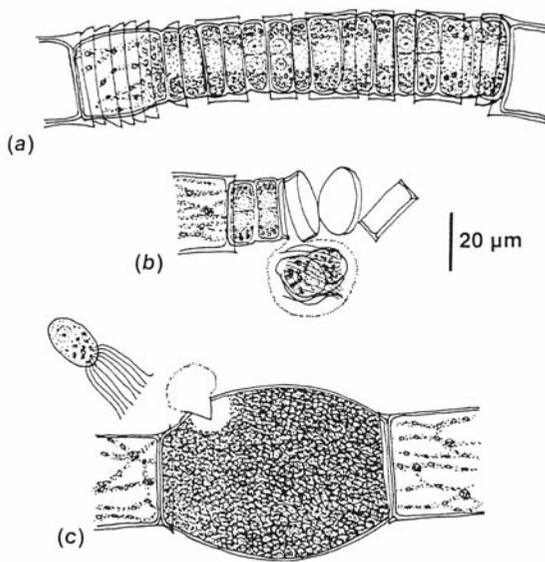
newly formed secondary wall and fused with it. Because cell division is intercalary in *Oedogonium*, division of every cell in the filament and repeated division of the daughter cells result in alternate cells with and without caps of old cell walls. This theoretical condition usually does not occur in nature. Repeated division of the distal daughter cell commonly results in filaments in which a cell with an apical cap is successively followed by several cells without caps.

Asexual reproduction (Fig. 5.95) is by means of zoospores in all three genera. Zoospores are formed singly within a cell and usually only in those cells with apical caps. The earliest sign of zoosporogenesis is the appearance of a small electron-dense mass in an invagination of the nuclear envelope (Pickett-Heaps, 1971). From this the centrioles appear and multiply rapidly, forming two adjacent rows near the nucleus. The nucleus, with its two rows of centrioles, moves to the lateral wall. The two rows of centrioles move apart in the center to form a circle of centrioles under the plasmalemma. The centrioles extrude the flagella and

the flagella roots. The Golgi secrete a fibrillar hyaline layer around the zoospore, which makes up the vesicle in which the zoospore is initially encased. The Golgi also secrete mucilage to the base of the zoospore, which probably aids in extrusion of the zoospore. The lateral wall of the parent cell splits at the apical cap, and the zoospore in a vesicle emerges through the aperture. The vesicle has two layers with a ring (Retallack and Butler, 1970); it opens in the area of the ring, releasing the zoospore. The zoospore has about 30 flagella linked together by a striated root at the apical end (Hoffman and Manton, 1962); it swims for about an hour, settles, retracts its flagella, and develops a holdfast that attaches to the substrata. This then develops into a new filament. Aplanospores can also be formed, and resemble oogonia.

Sexual reproduction is oogamous and, depending on the behavior of the male filaments, either macrandrous if the male filament forms the sperm directly, or nannandrous if the sperm are produced in a special dwarf male filament. In many species of *Oedogonium*, it is relatively easy to induce sexual reproduction by placing old filaments in fresh media and saturating the atmosphere with carbon dioxide. Nitrogen limitation has also been reported to induce the formation of oogonia (Singh and Chaudhary, 1990).





**Fig. 5.96** Gametogenesis in the macrandrous *Oedogonium crassum*. (a) Antheridia. (b) Liberation of sperm. (c) Oogonium with a single egg attracting a spermatozoid. (After Smith, 1955.)

matures, it swells and becomes globose, forming a small pore or crack in the oogonial wall. The protoplast develops into an egg. Just prior to fertilization the central nucleus moves to just under the fertilization pore, and gelatinous material is extruded through the pore.

Macrandrous species form terminal or intercalary antheridia by an unequal division of an antheridial mother cell (Fig. 5.96). In this division, the upper cell is much shorter than the lower. The lower cell then divides repeatedly to produce a series of 2 to 40 antheridia. Each antheridium forms two sperm, which are liberated in a vesicle by transverse splitting of the wall. The sperm then escape from the vesicle. The sperm are similar in structure to the zoospores but smaller and more elongated with a crown of about 30 flagella (Hoffman and Manton, 1963), and are yellowish-green because of their reduced plastids. In a macrandrous species such as *Oedogonium cardiacum* (Hoffman, 1960), the sperm are attracted to the oogonium by a chemotactic substance secreted by the oogonium.

In nannandrous species (Leonardi et al., 1998), male filaments form **androsporangia** in a

manner similar to the formation of antheridia by macrandrous species, except only one **androspore** is produced per sporangium. The androspore can develop in one of three ways depending on the environment (Hill et al., 1989).

- 1 In an environment with low nitrate or ammonium concentration, the androspore produces a cyst that will eventually produce another androspore. The cycle will continue until the nitrate or ammonium concentration in the environment increases.
- 2 If there is a large amount of nitrate or ammonium in the water, the androspore will produce a vegetative male thallus that is indistinguishable from the parent. This is similar to the situation in nature when a rainstorm washes nutrients into a nutrient-depleted environment.
- 3 If the environment contains the female pheromone “circein,” the androspore is attracted to the female oogonium that is secreting the pheromone. The androspore attaches to the oogonial mother cell and causes the oogonial mother cell to divide into the oogonium and a lower **suffultory cell** to which the androspore is now attached. The production of the substance that attracted the androspore now ceases. If the attachment of the androspores to the oogonial mother cell is prevented by coating the oogonial mother cell with agar, then the oogonial mother cell continues to attract androspores, but the division of the oogonial mother cell does not occur. Female differentiation beyond the oogonial mother cell stage must be triggered by direct contact of the attached androspore. The main advantage of a nannandrous system is that differentiation of the male is a prerequisite for differentiation of the female, thus ensuring the presence of a male for the receptive female.

The attached androspore on the suffultory cell next elongates and develops into a two-celled dwarf male filament. The growth of the dwarf male is toward the oogonium under the influence of a chemotactic substance secreted by the oogonium. The apical cell of the male filament is an

antheridium, which forms two sperm. The oogonium secretes a thick gelatinous sheath that includes the tip of the dwarf male. The sperm are released and trapped in this gel. After about 1 hour of random movement in the gel, the sperm aggregate at a certain spot on the upper third of the oogonium. The sperm extend their anterior end so that this end is flexible (Hoffman, 1973). A protoplasmic papilla suddenly appears through the wall of the oogonium, and all the spermatozooids in the vicinity attach to this papilla by their anterior ends. Within a second the papilla is withdrawn through the pore in the wall, taking with it a single sperm. The remaining sperm hover about the pore for about 5 minutes before they disperse in the gel. Four hormone-controlled steps are thus involved in fertilization: (1) the chemotaxis of the androspores; (2) the directed growth of the dwarf males; (3) the triggering of the division of the oogonial mother cell; and (4) the chemotaxis of the sperm to the prospective opening of the oogonium (Rawitscher-Kunkel and Machlis, 1962).

Nannandrous species are thought to have evolved from macrandrous ones by parthenogenetic germination of sperm to form dwarf male filaments.

The zygote is separated from the oogonial wall by a space, and after fertilization a wall is soon formed around the zygote. As the zygote matures, there is an accumulation of a reddish oil in the protoplasm. Eventually the zygote is liberated by decay of the oogonial wall. It undergoes a rest period during which its nucleus divides by meiosis to form four haploid nuclei. Shortly before germination, the protoplast becomes green and forms four daughter protoplasts, each of which becomes a zoospore. The zoospores are released and develop into filaments in the same manner as in asexual reproduction. The life cycle is thus mostly haploid, the zygote being the only diploid structure.

## REFERENCES

- Adolph, S., Jung, V., Rattke, J., and Pohnert, G. (2005). Wound closure in the invasive green alga *Caulerpa taxifolia* by enzymatic activation of a protein crosslinker. *Angew. Chem. Int. Ed.* 44:2–4.
- Ahmadjian, V. (1960). Some new and interesting species of *Trebouxia*, a genus of lichenized algae. *Am. J. Bot.* 47:677–83.
- Ahmadjian, V. (1993). *The Lichen Symbiosis*. New York: John Wiley.
- Ahmadjian, V., and Jacobs, J. B. (1981). Relationship between fungus and alga in the lichen *Cladonia cristatella* Tuck. *Nature* 289:169–72.
- Barlow, S. B., and Cattolico, R. A. (1980). Fine structure of the scale-covered green flagellate *Mantoniella squamata* (Manton et Parke) Desikachary. *Br. Phycol. J.* 15:321–33.
- Bean, B. (1977). Geotactic behaviour of *Chlamydomonas*. *J. Protozool.* 24:394–401.
- Becker, B., Perasso, L., Kammer, A., Salzburg, M., and Melkonian, M. (1996). Scale-associated glycoproteins of *Scherffelia dubia* (Chlorophyta) form high-molecular-weight complexes between the scale layers and the flagellar membrane. *Planta* 199:503–10.
- Belcher, J. H., Pennick, N. C., and Clarke, K. J. (1974). On the identity of *Asteromonas propulsum* Butcher. *Br. Phycol. J.* 9:101–6.
- Ben-Amotz, A. (1996). Effect of low temperature on the stereoisomer composition of  $\beta$ -carotene in the halotolerant alga *Dunaliella bardawil*. *J. Phycol.* 32:272–5.
- Bendix, S. W. (1960). Phototaxis. *Bot. Rev.* 26:145–208.
- Benson, E. E., Rutter, J. C., and Cobb, A. H. (1983). Seasonal variation in frond morphology and chloroplast physiology of the intertidal alga *Codium fragile* (Suringar) Hariot. *New Phytol.* 95:569–80.
- Berger, S., and Kaefer, M. J. (1992). *Dasycladales: An Illustrated Monograph of a Fascinating Algal Order*. Stuttgart: Thieme.
- Berger, S., Fetweiss, U., Gleissberg, S., et al. (2003). 18S rDNA phylogeny and evolution of cap development in Polyphysaceae (formerly Acetabulariaceae; Dasycladales, Chlorophyta). *Phycologia* 42:506–61.
- Berger-Perrot, Y., Thomas, J.-Cl., and L'Hardy-Halos, M.-Th. (1993). Gametangia, gametes, fertilization and zygote development in *Ulothrix flacca* var. *roscoffensis* (Chlorophyta). *Phycologia* 32:356–66.
- Berry, H. A., and Lembi, C. A. (2000). Effects of temperature and irradiance on the seasonal variation of a *Spirogyra* (Chlorophyta) population in a Midwestern lake (U.S.A.). *J. Phycol.* 36:842–51.
- Bérubé, K. A., Dodge, J. D., and Ford, T. W. (1999). Effects of chronic salt stress on the ultrastructure of *Dunaliella bioculata* (Chlorophyta, Volvocales): mechanisms of response and recovery. *Eur. J. Phycol.* 34:117–23.

- Betsche, T., Schaller, D., and Melkonian, M. (1992). Identification and characterization of glycolate oxidase and related enzymes from the endocyanotic alga *Cyanophora paradoxa* and from pea leaves. *Plant Physiol.* 98:887–93.
- Blackburn, K., and Temperley, B. N. (1936). *Botryococcus* and the algal coals. *Proc. R. Soc. Edinburgh* 58:841–6.
- Blomster, J., Back, S., Fewer, D. P., et al. (2002). Novel morphology in *Enteromorpha* (Ulvophyceae) forming green tides. *Amer. J. Bot.* 89:1756–63.
- Booton, G. C., Floyd, G. L., and Fuerst, P. A. (1998). Polyphyly of tetrasporalean green algae inferred from nuclear small-subunit ribosomal DNA. *J. Phycol.* 39:306–11.
- Borowitzka, M. A. (1982). Morphological and cytological aspects of algal calcification. *Int. Rev. Cytol.* 74:127–62.
- Borowitzka, M.A., and Larkum, A. W. D. (1974). Chloroplast development in the caulerpalean alga *Halimeda*. *Protoplasma* 81:131–44.
- Boscov, J. S., and Feinleib, M. E. (1979). Phototactic response of *Chlamydomonas* to flashes of light. II. Response of individual cells. *Photochem. Photobiol.* 30:499–505.
- Bouck, G. B., and Morgan, E. (1957). The occurrence of *Codium* in Long Island waters. *Bull. Torrey Bot. Club* 84:384–7.
- Bråten, T. (1971). The ultrastructure of fertilization and zygote formation in the green alga. *Ulva mutabilis* Føyn. *J. Cell Sci.* 9:621–35.
- Bråten, T. (1973). Autoradiographic evidence for the rapid disintegration of one chloroplast in the zygote of the green alga *Ulva mutabilis*. *J. Cell Sci.* 12:385–9.
- Braun, A. (1851). *Betrachtungen über die Erscheinung der Verjungung in der Natur.*, Leipzig.
- Braun, M., and Richter, P. (1999). Relocalization of the calcium gradient and a dihydropyridine receptor is involved in upward bending by bulging *Chara* protonema, but not in downward bending by bowing of *Chara* rhizoids. *Planta* 209:414–23.
- Bristol, B. M. (1919). On a Malay form of *Chlorococcum humicola* (Naeg.) Rabenh. *J. Linn. Soc. Bot.* 44:473–82.
- Brook, A. J. (1989). Barium sulphate crystals and desmids. *Brit Phycol. J.* 24:299–300.
- Brown, R. M., Johnson, C., and Bold, H. C. (1968). Electron and phase-contrast microscopy of sexual reproduction in *Chlamydomonas moewusii*. *J. Phycol.* 4:100–20.
- Bubrick, P., and Galun, M. (1980). Proteins from the lichen *Xanthoria parietina* which bind to phycobiont cell walls. Correlation between binding patterns and cell wall cytochemistry. *Protoplasma* 104:167–73.
- Buchheim, M. A., Michalopoulos, E. A., and Buchheim, J. A. (2001). Phylogeny of the Chlorophyceae with special reference to the Sphaeropleales: a study of 18S and 26S rDNA data. *J. Phycol.* 37:819–35.
- Butcher, R. N. (1932). Notes on new and little-known algae from the beds of rivers. *New Phytol.* 31:289–309.
- Cáceres, E. J., Hoffman, L. R., and Leonardi, P. I. (1997). Fine structure of the male and female gametes of *Atractomorpha porcata* (Sphaeropleaceae, Chlorophyta) with emphasis on the development and absolute configuration of the flagellar apparatus. *J. Phycol.* 33:948–59.
- Cane, R. F. (1977). Coorgongite, balkashite and related substances – An annotated bibliography. *Trans. R. Soc. S. Aust.* 101:153–64.
- Carothers, Z. B., and Kreitner, G. L. (1967). Studies of spermatogenesis in the Hepaticae. I. Ultrastructure of the Vierergruppe in *Marchantia*. *J. Cell Biol.* 33:43–51.
- Casanova, M. T. (1997). Oospore variation in three species of *Chara* (Charales, Chlorophyta). *Phycologia* 36:274–80.
- Cavalier-Smith, T. (1981). Eukaryote kingdoms: seven or nine? *BioSystems* 14:461–81.
- Chapman, V. J. (1970). *Seaweeds and Their Uses*, 2nd edn. London: Methuen.
- Chen, Y.-C. (1998). Development of protoplasts from holdfasts and vegetative thalli of *Monostroma latissimum* (Chlorophyta, Monostromataceae) for algal seed stock. *J. Phycol.* 34:1075–81.
- Chiang, I.-Z., Huang, W.-Y., and Wu, J.-T. (2004). Allelochemicals of *Botryococcus braunii* (Chlorophyceae). *J. Phycol.* 40:474–80.
- Chihara, M. (1963). The life history of *Prasinocladus ascus* as found in Japan, with special reference to the systematic position of the genus. *Phycologia* 3:19–28.
- Chihara, M. (1969). *Ulva arasakii*, a new species of green algae: its life history and taxonomy. *Bull. Nat. Sci. Mus. (Japan)* 12:849–62.
- Christie, A. O., and Evans, L. V. (1962). Periodicity in the liberation of gametes and zoospores of *Enteromorpha intestinalis* Link. *Nature* 193:193–4.
- Cimino, M. T., and Delwiche, C. F. (2002). Molecular and morphological data identify a cryptic species complex in endophytic members of the genus *Coleochaete* Breb. (Charophyta: Coleochaetaceae). *J. Phycol.* 38:1213–21.
- Coggin, S. J., Hutt, W., and Kochert, G. (1979). Sperm bundle–female somatic cell interaction in the fertilization process of *Volvox carteri* f. *weismannii* (Chlorophyta). *J. Phycol.* 15:247–51.

- Coleman, A. W. (1999). Phylogenetic analysis of "Volvocaceae" for comparative genetic studies. *Proc. Natl. Acad. Sci., U.S.A.* 96:13892-7.
- Collings, D. A., Wasteneys, G. B., and Williamson, R. E. (1996). Actin microtubule interactions in the alga *Nitella*: analysis of the mechanism by which microtubule depolymerization potentiates cytochalasin's effects in streaming. *Protoplasma* 191:178-90.
- Collins, C. R., and Farrar, J. F. (1978). Structural resistances to mass transfer in the lichen *Xanthoria parietina*. *New Phytol.* 81:71-83.
- Cooper, J. J., and Mandoli, D. F. (1999). Physiological factors that aid differentiation of zygotes and early juveniles of *Acetabularia acetabulum* (Chlorophyta). *J. Phycol.* 35:143-51.
- Courties, C., Perasso, R., Chretiennot-Dinet, M.-J., Govy, M., Guillou, L., and Troussellier, M. (1998). Phylogenetic analysis and genome size of *Ostreococcus tauri* (Chlorophyta, Prasinophyceae). *J. Phycol.* 34:844-9.
- Daugbjerg, N., Moestrup, Ø., and Arctander, P. (1995). Phylogeny of genera of Prasinophyceae and Pedinophyceae (Chlorophyta) deduced from molecular analysis of the *rbs* gene. *Phycological Research* 43:203-13.
- Davies, R. R., and Wilkinson, J. L. (1967). Human protothecosis: Supplementary studies. *Ann. Trop. Med. Parasitol.* 61:112-15.
- Deason, T. R. (1983). Cell wall structure and composition as taxonomic characters in the coccoid Chlorophyceae. *J. Phycol.* 19:248-51.
- deJesus, M. D., Tabatabai, F., and Chapman, D. J. (1989). Taxonomic distribution of copper-zinc superoxide dismutase in green algae and its phylogenetic importance. *J. Phycol.* 25:767-72.
- Demets, R., Tomson, A. M., Stegwee, D., and van de Ende, H. (1990). Cell-cell coordination in conjugating *Chlamydomonas* gametes. *Protoplasma* 158:188-99.
- Dodds, W. K., and Gudder, D. A. (1992). The ecology of *Cladophora*. *J. Phycol.* 28:415-27.
- Domozych, C. R., Plante, K., Blair, P., Paliules, L., and Domozych, D. S. (1993). Mucilage processing and secretion in the green alga *Closterium*. I. Cytology and biochemistry. *J. Phycol.* 29:650-9.
- Domozych, D. S., Stewart, K. D., and Mattox, K. R. (1981). Development of the cell wall in *Tetraselmis*: Role of the Golgi apparatus and extracellular wall assembly. *J. Cell Sci.* 52:351-71.
- Dorling, M., McAuley, P. J., and Hodge, H. (1997). Effect of pH on growth and carbon metabolism of maltose-releasing *Chlorella* (Chlorophyta). *Eur. J. Phycol.* 32:19-24.
- Douglas, A. E. (1985). Growth and reproduction of *Convoluta roscoffensis* containing different naturally occurring algal symbionts. *J. Mar. Biol. Assoc. UK* 65:871-9.
- Duncan, T. M., Renzaglia, K. S., and Garbary, D. J. (1997). Ultrastructure and phylogeny of the spermatozoid of *Chara vulgaris* (Charophyceae). *Pl. Syst. Evol.* 204:125-40.
- Ebnet, E., Fischer, M., Deininger, W., and Hegemann, P. (1999). Volvoxrhodopsin, a light-regulated sensory photoreceptor of the spheroidal alga *Volvox carteri*. *Plant Cell* 11:1473-84.
- Egan, P. F., and Trainor, F. R. (1989). The role of unicells in the polymorphic *Scenedesmus armatus* (Chlorophyceae). *J. Phycol.* 25:65-70.
- Egerod, L. E. (1952). An analysis of the siphonaceous Chlorophycophyta. *Univ. Calif. Publ. Bot.* 25:325-454.
- Ernst, A. (1902). *Dichotomosiphon tuberosus* (A. Br.) Ernst, eine neue oogame Süßwasser-Siphonee. *Beih. Bot. Zentralbl.* 13:115-48.
- Fawley, M. W., Yun, Y., and Qin, M. (2000). Phylogenetic analyses of 18S rDNA sequences reveal a new coccoid lineage of the Prasinophyceae (Chlorophyta). *J. Phycol.* 36:387-93.
- Feldmann, J. (1950). Sur l'existence d'une alternance de générations entre *l'Halicystis parvula* Schmitz et *le Derbesia tenuissima* (De Not.) Crn. *C. R. Séances Acad. Sci. Paris* 230:322-33.
- Fisher, M., Pick, U., and Zamir, A. (1994). A salt-induced 60-kilodalton plasma membrane protein plays a potential role in extreme halotolerance of the alga *Dunaliella*. *Plant Physiol.* 106:1359-65.
- Floyd, G. L., Stewart, K. D., and Mattox, K. R. (1972). Cellular organization, mitosis and cytokinesis in the ulotrichalean alga, *Klebsormidium*. *J. Phycol.* 8:176-84.
- Fogg, G. E. (1971). Recycling through algae. *Proc. R. Soc. Lond. [B]* 179:201-7.
- Fraser, T. W., and Gunning, B. E. S. (1969). The ultrastructure of plasmodesmata in the filamentous green alga *Bulbochaete hiloensis* (Nordst.) Tiffany. *Planta* 88:244-54.
- Friedmann, I. (1959). Structure, life history and sex determination of *Prasiola stipitata* Suhr. *Ann. Bot. N. S.* 23:571-94.
- Friedmann, I., and Manton, I. (1959). Gametes, fertilization and zygote development in *P. stipitata*. *Nova Hedwigia* 1:333-44.
- Fukumoto, R., Fuji, T., and Sekimoto, H. (2003). Cloning and characterization of a cDNA encoding a sexual cell division-inducing pheromone from a unicellular green alga *Closterium ehrenbergii* (Chlorophyta). *J. Phycol.* 39:931-6.

- Fünfstück, M. (1907). Lichenes. In *Die Natürlichen Pflanzenfamilien*, ed. A. Engler, and K. Prantl. Leipzig: Engelmann.
- Geib, K., Gollmack, D., and Gimmler, H. (1996). Is there a requirement for an external carbonic anhydrase in the extremely acid-resistant green alga *Dunaliella acidophila*? *Eur. J. Phycol.* 31:273–84.
- Goldstein, M., and Morral, S. (1970). Gametogenesis and fertilization in *Caulerpa*. *Ann. NY Acad. Sci.* 175:660–72.
- Gontcharov, A. A., Marin, B., and Melkonian, M. (2002). Molecular phylogeny of conjugating green algae (Zygnemophyceae, Streptophyta) inferred from SSU rDNA sequence comparisons. *J. Mol. Evol.* 56:89–104.
- Gonzalez, M. A., Coleman, A. W., Gomez, P. J., and Montoya, R. (2001). Phylogenetic relationship among various strains of *Dunaliella* (Chlorophyceae) based on nuclear ITS rDNA sequences. *J. Phycol.* 37:604–11.
- Goodenough, U. W., and Heuser, J. E. (1985). The *Chlamydomonas* cell wall and its constituent glycoproteins analyzed by the quick-freeze, deep-etch technique. *J. Cell Biol.* 101:1550–68.
- Goodenough, U. W., Detmers, P. A., and Hwang, C. (1982). Activation for cell fusion in *Chlamydomonas*: Analysis of wild-type gametes and nonfusing mutants. *J. Cell Biol.* 92:378–86.
- Goreau, T. F. (1963). Calcium carbonate deposition by coralline algae and corals in relation to their roles as reef-builders. *Ann. NY Acad. Sci.* 109:127–67.
- Graham, J. M., Arancibia-Avile, P., and Graham, L. E. (1996). Physiological ecology of a species of the filamentous green alga *Mougeotia* under acid conditions: light and temperature effects on photosynthesis and respiration. *Limnol. Oceanogr.* 41:253–62.
- Graham, J. M., Lembi, C. A., Adrian, H. L., and Spencer, D. F. (1995). Physiological responses to temperature and irradiance in *Spirogyra* (Zygnematales, Charophyceae). *J. Phycol.* 31:334–40.
- Graham, L. E. (1984). *Coleochaete* and the origin of land plants. *Am. J. Bot.* 71:603–8.
- Graham, L. E., and McBride, G. E. (1979). The occurrence and phylogenetic significance of a multilayered structure in *Coleochaete* spermatozoids. *Am. J. Bot.* 66:887–94.
- Graham, L. E., and Kranzfelder, J. A. (1986). Irradiance, daylength and temperature effects on zoosporogenesis in *Coleochaete scutata* (Charophyceae). *J. Phycol.* 22:35–9.
- Green, T. G. A. (1970). The biology of lichen symbionts, DPhil. thesis, Oxford.
- Griffin, N. J., and Aken, M. E. (1993). Rhythmic settling behavior in *Pyramimonas parkae* (Prasinophyceae). *J. Phycol.* 29:9–15.
- Gruber, P. J., Frederick, S. E., and Tolbert, N. E. (1974). Enzymes related to lactate metabolism in green algae and lower land plants. *Plant Physiol.* 53:167–70.
- Grünewald, K., Hagen, C., and Braune, W. (1997). Secondary carotenoid accumulation in flagellates of the green alga *Haematococcus lacustris*. *Eur. J. Phycol.* 32:387–92.
- Gulliksen, O. M., Hushovd, O. T., Texmon, I., and Nordby, Ø. (1982). Changes in respiration, photosynthesis and protein composition during induced synchronous formation of gametes and zoospores in *Ulva mutabilis* Føyn. *Planta* 156:33–40.
- Hagen, C., Grünewald, K., Schmidt, S., and Müller, J. (2000). Accumulation of secondary carotenoid in flagellates of *Haematococcus pluvialis* (Chlorophyta) is accompanied by an increase in per unit chlorophyll productivity of photosynthesis. *Eur. J. Phycol.* 35:75–82.
- Hallman, A. (2003). Extracellular matrix and sex-inducing pheromone in *Volvox*. *Int. Rev. Cytol.* 227:131–82.
- Hallman, A., and Kirk, D. L. (2000). The developmentally regulated ECM glycoprotein ISG plays an essential role in organizing the ECM and orienting cells of *Volvox*. *J. Cell Sci.* 113:4605–17.
- Hanisak, M. D. (1979). Nitrogen limitation of *Codium fragile* ssp. *tomentosoides* as determined by tissue analysis. *Mar. Biol.* 50:333–7.
- Harris, E. H. (1989). *The Chlamydomonas Sourcebook: A Comprehensive Guide To Biology And Laboratory Use*. San Diego: Academic Press.
- Harris, E. H. (2001). *Chlamydomonas* as a model organism. *Annu. Rev. Plant Physiol. Mol. Biol.* 52:363–506.
- Hatano, K., and Ueda, J. (2000). Effects of concanavalin A on net formation of zoospores in *Hydrodictyon reticulatum* (Chlorococcales, Chlorophyceae). *Phycol. Res.* 48:155–60.
- Hayden, H. S., Blomster, J., Maggs, C. A., Silva, P. C., Stanhope, M. J., and Waaland, J. R. (2003). Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *Eur. J. Phycol.* 38:277–94.
- Head, W. D., and Carpenter, E. J. (1975). Nitrogen fixation associated with the marine macroalga *Codium fragile*. *Limnol. Oceanogr.* 20:815–23.
- Hegemann, P., Fuhrmann, M., and Kateriya, A. (2001). Algal sensory photoreceptors. *J. Phycol.* 37:668–76.
- Hepperle, D., and Krienitz, L. (1996). The extracellular calcification of zoospores of *Phacotus lenticularis*

- (Chlorophyta, Chlamydomonadales). *Eur. J. Phycol.* 31:11–21.
- Hessler, R., and Peveling, E. (1978). Die Lokalisation von  $^{14}\text{C}$ -Assimilaten von Flechtenthalli von *Cladonia incassata* Floerke und *Hypogymnia physodes* (L.) Ach. *Z. Pflanzenphysiol.* 86:287–302.
- Hill, G. J. C., and Machlis, L. (1968). An ultrastructural study of vegetative cell division in *Oedogonium borisianum*. *J. Phycol.* 4:261–71.
- Hill, G. J. C., Cunningham, M. R., Byrne, M. M., Ferry, T. P., and Halvorsen, J. S. (1989). Chemical control of androspore morphogenesis in *Oedogonium donneli* (Chlorophyta, Oedogoniales). *J. Phycol.* 25:368–76.
- Hiraoka, M., Obata, S., and Ohno, M. (1998). Pigment content of the reproductive cells of *Ulva pertusa* (Ulvales, Ulvophyceae): evidence of anisogamy. *Phycologia* 37:222–6.
- Hodick, D. (1994). Negative gravitropism in *Chara* protonemata: a model integrating the opposite gravitropic responses of protonemata and rhizoids. *Planta* 195:43–9.
- Hoffman, L. (1960). Chemotaxis of *Oedogonium* sperms. *Southwest. Nat.* 5:111–16.
- Hoffman, L. (1973). Fertilization in *Oedogonium*. I. Plasmogamy. *J. Phycol.* 9:62–84.
- Hoffman, L., and Manton, I. (1962). Observations on the fine structure of the zoospore of *Oedogonium cardiacum* with special reference to the flagellar apparatus. *J. Exp. Bot.* 13:443–9.
- Hoffman, L., and Manton, I. (1963). Observations on the fine structure of *Oedogonium* II. The spermatozoid of *O. cardiacum*. *Am. J. Bot.* 50:455–63.
- Hoffman, L. R., and Buchheim, M. A. (1989). Zygote appendages (cirri), a new structural feature in the Sphaeropleaceae (Chlorophyceae). *J. Phycol.* 25:149–59.
- Hoham, R. W. (1973). Pleiomorphism in the snow alga *Raphidonema nivale* Lagerh. (Chlorophyta), and a revision of the genus *Raphidonema* Lagerh. *Syesis* 6:255–63.
- Hoham, R. W., Bonome, T. A., Martin, C. W., and Leebens-Mack, J. H. (2002). A combined 18S and *rbcL* phylogenetic analysis of *Chloromonas* and *Chlamydomonas* (Chlorophyceae, Volvocales) emphasizing snow and other cold-temperature habitats. *J. Phycol.* 38:1051–64.
- Hoops, H. J., Brighton, M. C., Stickles, S. M., and Clement, P. R. (1999). A test of two possible mechanisms for phototactic steering in *Volvox carteri* (Chlorophyceae). *J. Phycol.* 35:539–47.
- Hori, T., Norris, R. E., and Chihara, M. (1982a). Studies on the ultrastructure and taxonomy of the genus *Tetraselmis* (Prasinophyceae). I. Subgenus *Tetraselmis*. *Bot. Mag. (Tokyo)* 95:49–61.
- Hori, T., Norris, R. E., and Chihara, M. (1982b). Studies on the ultrastructure and taxonomy of the genus *Tetraselmis* (Prasinophyceae). II. Subgenus *Prasinocladia*. *Bot. Mag. (Tokyo)* 96:385–92.
- Hoskin, C. M. (1963). Recent carbonate sedimentation on Alacran Reef, Yucatan, Mexico. *Nat. Acad. Sci. Nat. Res. Council Publ.* 1089.
- Huang, K., and Beck, C. F. (2003). Phototropin is the blue-light receptor that controls multiple steps in the sexual life cycle of the green alga *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci., USA* 100:6269–74.
- Huber-Pestalozzi, G. (1961). Volvocales. In *Die Binnengewässer*, Vol. 16, Part 5. Stuttgart, Germany: E. Schweizerbart'sche Verlagsbuchhandlung.
- Huizing, H. J., Rietema, H., and Sietsma, J. H. (1979). Cell wall constituents of several siphonaceous green algae in relation to morphology and taxonomy. *Br. Phycol. J.* 14:25–32.
- Hutt, W., and Kochert, G. (1971). Effects of some protein and nucleic acid synthesis inhibitors on fertilization in *Volvox carteri*. *J. Phycol.* 7:316–20.
- Imam, S. H., Buchanan, M. J., Shin, H. C., and Snell, W. J. (1985). The *Chlamydomonas* cell wall: Characterization of the wall framework. *J. Cell Biol.* 101:1599–607.
- Inouye, I., Hori, T., and Chihara, M. (1990). Absolute configuration analysis of the flagellar apparatus of *Pterosperma cristatum* (Prasinophyceae) and consideration of its phylogenetic position. *J. Phycol.* 26:329–44.
- Iyengar, M. O. P. (1932). *Fritschella*, a new terrestrial member of the Chaetophoraceae. *New Phytol.* 31:329–35.
- Jaenicke, L., and Starr, R. C. (1996). The lurlenes, a new class of plastoquinone-related pheromones from *Chlamydomonas allensworthii* (Chlorophyceae). *Eur. J. Biochem.* 241:581–5.
- Johnson, H. J. (1961). *Limestone-Building Algae and Algal Limestones*. Boulder, Colo: Johnson Publ.
- Jones, H. G., Pomeroy, J. W., Walker, D. A., and Hoham, R. W. (2001). *Snow Ecology*. Cambridge: Cambridge University Press.
- Kamitsubo, E. (1980). Cytoplasmic streaming in characean cells: role of subcortical fibrils. *Can. J. Bot.* 58:760–5.
- Kamiya, R., and Witman, G. B. (1984). Submicromolecular levels of calcium control the

- balance of beating between the two flagella in demembrated models of *Chlamydomonas*. *J. Cell Biol.* 98:97–107.
- Karol, K. G., McCourt, R. M., Cimino, M. T., and Delwiche, C. F. (2001). The closest living relatives of land plants. *Science* 294:2351–3.
- Kim, G. H., Yoon, M., and Klotchkova, T. A. (2005). A moving mat: phototaxis in the filamentous green alga *Spirogyra* (Chlorophyta, Zygnemataceae). *J. Phycol.* 41:232–7.
- Kim, K. Y., Choi, T. S. W., Kim, J. H., Han, T., Shin, H. W., and Garbary, D. J. (2004). Physiological ecology and seasonality of *Ulva pertusa* on a temperate rocky shore. *Phycologia* 43:483–92.
- Kim, Y-S., Oyaizu, H., Matsumoto, S., Watanabe, M-M., and Nozaki, H. (1994). Chloroplast small-subunit ribosomal RNA gene sequence from *Chlamydomonas parkae* (Chlorophyta): Molecular phylogeny of a green alga with a peculiar pigment composition. *Eur. J. Phycol.* 29:213–17.
- Kingsley, R. J., Van Gilder, R., LeGeros, R. Z., and Watabe, N. (2003). Multimineral calcareous deposits in the marine alga *Acetabularia acetabulum* (Chlorophyta, Dasycladaceae). *J. Phycol.* 39:937–47.
- Kirk, D. L., and Kirk, M. M. (1986). Heat shock elicits production of sexual inducer in *Volvox*. *Science* 231:51–4.
- Kirk, D. L. (1998). *Volvox: Molecular-Genetic Origins of Multicellularity and Cellular Differentiation*. Cambridge: Cambridge University Press.
- Kirk, D. L. (1999). Evolution of multicellularity in the volvocine algae. *Curr. Opin. Plant Biol.* 2:496–501.
- Kirk, D.L. (2001). Germ-soma differentiation in *Volvox*. *Dev. Biol.* 238:213–23.
- Kirk, D. L., and Nishii, I. (2001). *Volvox carteri* as a model for studying the genetic and cytological control of morphogenesis. *Dev. Growth Differ.* 43:621–31.
- Kleinig, H. (1969). Carotenoids of siphonous green algae: A chemotaxonomical study. *J. Phycol.* 5:281–4.
- Klotchkova, T. A., Chah, O., West, J. A., and Kim, G. H. (2003). Cytochemical and ultrastructural studies on protoplast formation from disintegrated cells of the marine alga *Chaetomorpha aerea* (Chlorophyta). *Eur. J. Phycol.* 38:205–16.
- Klyver, F. D. (1929). Notes on the life history of *Tetraspora gelatinosa* (Vauch.) Desv. *Arch. Protistenk.* 66:290–6.
- Kochert, G. (1968). Differentiation of reproductive cells in *Volvox carteri*. *J. Protozool.* 15:438–52.
- Koop, H-U. (1975a). Über den Ort der Meiose bei *Acetabularia mediterranea*. *Protoplasma* 85:109–14.
- Koop, H-U. (1975b). Germination of cysts of *Acetabularia mediterranea*. *Protoplasma* 84:137–46.
- Kranz, H. D., Mikš, D., Sieglar, M-L., Capesius, I., Sensen, C. W., and Huss, V. A. R. (1995). The origin of land plants: Phylogenetic relationships among charophytes, bryophytes, and vascular plants inferred from complete small-subunit ribosomal RNA gene sequences. *J. Mol. Evol.* 41:74–84.
- Kriemer, G., and Melkonian, M. (1990). Reflection confocal laser scanning microscopy of eyespots in flagellated green algae. *Eur. J. Cell Biol.* 53:101–11.
- Kuroiwa, T. (1985). Mechanisms of maternal inheritance of chloroplast DNA: An active digestion hypothesis. *Microbiol. Sci.* 2:267–70.
- Kwiatkowska, M. (2003). Plasmodesmal changes are related to different developmental stages of antheridia of *Chara* species. *Protoplasma* 222:1–11.
- Lee, S-H., Motomura, T., and Ichimura, T. (2000). Nuclear phase alternation in the life cycle of *Derbesia* (Chlorophyta). *Phycologia* 39:441–7.
- Lee, Y-K., and Ding, S-Y. (1995). Effects of dissolved oxygen partial pressure on the accumulation of astaxanthin in chemostat cultures of *Haematococcus lacustris* (Chlorophyta). *J. Phycol.* 31:922–4.
- Lembi, C. A., and Herndon, W. R. (1966). Fine structure of the pseudocilia of *Tetraspora*. *Can. J. Bot.* 44:710–12.
- Lemieux, C., Otis, C., and Turmel, M. (2000). Ancestral chloroplast genome in *Mesostigma viride* reveals an early branch of green plant evolution. *Nature* 403:649–52.
- Leonardi, P. I., Caceres, E. J., and Velez, C. G. (1998). Fine structure of dwarf males in *Oedogonium pluviale* (Chlorophyceae). *J. Phycol.* 34:250–6.
- Lewin, R. A. (1957). The zygote of *Chlamydomonas moewusii*. *Can. J. Bot.* 35:795–807.
- Lewin, R. A. (1958). The cell walls of *Platymonas*. *J. Gen. Microbiol.* 19:87–90.
- Lewis, L. A., and McCourt, R. M. (2004). Green algae and the origin of land plants. *Amer. J. Bot.* 91:1535–56.
- Lucas, W. C. (1979). Alkaline band formation in *Chara corallina*. *Plant Physiol.* 63:248–54.
- Lurling, M. (1998). Effect of grazing-associated infochemicals as growth and morphological development in *Scenedesmus acutus* (Chlorophyceae). *J. Phycol.* 34:578–86.
- Lurling, M. (1999). Grazer-induced coenobial formation in clonal cultures of *Scenedesmus*

- obliquus* (Chlorococcales, Chlorophyceae). *J. Phycol.* 35:19–23.
- Lynn, R., and Brock, T. D. (1969). Notes on the ecology of a species of *Zygonium* (Kütz) in Yellowstone National Park. *J. Phycol.* 5:181–5.
- McCourt, R. M. (2000). Phylogeny of the conjugating green algae (Zygnemophyceae) based on *rbcl* sequences. *J. Phycol.* 36:747–58.
- McCourt, R. M., Karol, K. G., Kaplan, S., and Hoshaw, R. W. (1995). Using *rbcl* sequences to test hypotheses of chloroplast and thallus evolution in conjugating green algae (Zygnematales, Charophyceae). *J. Phycol.* 31:989–95.
- McGarry, M. G., and Tongkasame, C. (1971). Water reclamation and algae harvesting. *Water Pollut. Control Fed. J.* 43:824–35.
- McLean, R. O. (1974). The tolerance of *Stigeoclonium tenue* Kütz. to heavy metals in South Wales. *Br. Phycol. J.* 9:91–5.
- McLean, R. O., and Benson-Evans, K. (1974). The distribution of *Stigeoclonium tenue* Kütz. in South Wales in relation to its use as an indicator of organic pollution. *Br. Phycol. J.* 9:83–9.
- Maeda, M., Kuroda, K., Iriki, Y., Chihara, M., Nisizawa, K., and Miwa, T. (1966). Chemical nature of major cell wall constituents of *Vaucheria* and *Dichotomosiphon* with special reference to their phylogenetic positions. *Bot. Mag. Tokyo* 79:634–43.
- Malmberg, A. E., and VanWinkle-Swift, K. P. (2001). Zygospor germination in *Chlamydomonas monoica* (Chlorophyta): timing and pattern of secondary zygospor wall degradation in relation to cytoplasmic events. *J. Phycol.* 37:86–94.
- Mandoli, D. F. (1995). Whatever happened to *Acetabularia*? Bringing a once classic model system into the age of molecular genetics. *Int. Rev. Cytol.* 182:1–68.
- Manton, I., and Ettl, H. (1965). Observations on the fine structure of *Mesostigma viride* Lauterborn. *J. Linn. Soc. (Bot.)* 59:175–84.
- Manton, I., and Parke, M. (1960). Further observations on green flagellates with special reference to possible relatives of *Chromulina pusilla* Butcher. *J. Mar. Biol. Assoc.* 39:275–98.
- Marchant, H. J. (1977). Ultrastructure, development and cytoplasmic rotation of seta-bearing cells of *Coleochaete scutata*. *J. Phycol.* 13:28–36.
- Marchant, H. J., and Pickett-Heaps, J. D. (1971). Ultrastructure and differentiation of *Hydrodictyon reticulatum*. II. Formation of zoids within the coenobium. *Aust. J. Biol. Sci.* 24:471–86.
- Marchant, H. J., and Pickett-Heaps, J. D. (1972a). Ultrastructure and differentiation of *Hydrodictyon reticulatum*. III. Formation of the vegetative daughter net. *Aust. J. Biol. Sci.* 25:265–78.
- Marchant, H. J., and Pickett-Heaps, J. D. (1972b). Ultrastructure and differentiation of *Hydrodictyon reticulatum*. IV. Conjugation of gametes and the development of zoospores and azygospores. *Aust. J. Biol. Sci.* 25:279–91.
- Marchant, H. J., and Pickett-Heaps, J. D. (1972c). Ultrastructure and differentiation of *Hydrodictyon reticulatum*. V. Development of polyhedra. *Aust. J. Biol. Sci.* 25:1187–97.
- Marchant, H. J., and Pickett-Heaps, J. D. (1972d). Ultrastructure and differentiation of *Hydrodictyon reticulatum*. VI. Formation of the germ net. *Aust. J. Biol. Sci.* 25:1199–213.
- Marin, B., and Melkonian, M. (1999). Mesostigmatophyceae, a new class of streptophyte green algae revealed by SSU rRNA sequence comparisons. *Protist* 150:399–417.
- Mars, P. W., Rabson, A. R., Rippey, J. J., and Ajello, L. (1971). Cutaneous protothecosis. *Br. J. Dermatol.* 85, Suppl. 7:76–84.
- Martin, N. C., and Goodenough, U. W. (1975). Gametic differentiation in *Chlamydomonas reinhardtii*. I. Production of gametes and their fine structure. *J. Cell Biol.* 67:587–605.
- Matsuda, Y., Saito, T., Yamaguchi, T., Koseki, M., and Hayashi, K. (1987). Topography of cell wall lytic enzyme in *Chlamydomonas reinhardtii*: Form and location of the stored enzyme in vegetative cell and gamete. *J. Cell Biol.* 104:321–9.
- Matsuo, Y., Imagawa, H., Nishizawa, M., and Shizuri, Y. (2005). Isolation of an algal inducer from a marine bacterium. *Science* 307:1598.
- Mattox, K. R., and Stewart, K. D. (1973). Observations on the zoospores of *Pseudodendroclonium basilense* and *Trichosarcina polymorpha* (Chlorophyceae). *Can. J. Bot.* 51:1425–30.
- Mattox, K. R., and Stewart, K. D. (1977). Cell division in the scaly green flagellate *Heteromastix angulata* and its bearing on the origin of the Chlorophyceae. *Am. J. Bot.* 64:931–45.
- Mattox, K. R., and Stewart, K. D. (1984). Classification of the green algae; A concept based on comparative cytology. In *Systematics of the Green Algae* ed. D. E. G. Irvine, and D. M. John, pp. 29–72. London and Orlando: Academic Press.
- Melkonian, M. (1980a). Flagellar roots, mating structure and gametic fusion in the green alga *Ulva lactuca* (Ulvales). *J. Cell Sci.* 46:149–69.

- Melkonian, M. (1980b). Flagellar apparatus, mating structure and gametic fusion in *Ulva lactuca* (Ulvales, Chlorophyceae). *Br. Phycol. J.* 15:197.
- Melkonian, M. (1989). Flagellar apparatus ultrastructure in *Mesostigma viride* (Prasinophyceae). *Plant Syst. Evol.* 164:93–122.
- Melkonian, M., and Robenek, H. (1980a). Eyespot membranes in newly released zoospores of the green alga *Chlorosarcinopsis gelatinosa* (Chlorosarcinales) and their fate during zoospores settlement. *Protoplasma* 104:129–40.
- Melkonian, M., and Robenek, H. (1980b). Eyespot membranes of *Chlamydomonas reinhardtii*: A freeze-fracture study. *J. Ultrastruct. Res.* 72:90–102.
- Melkonian, M., Schulze, D., McFadden, G. I., and Robenek, H. (1988). A polyclonal antibody (anti-centrin) distinguishes between the two types of fibrous flagellar roots in green algae. *Protoplasma* 144:56–61.
- Menzel, D. (1980). Plug formation and peroxidase accumulation in two orders of siphonous green algae (Caulerpales and Dasycladales) in relation to fertilization and injury. *Phycologia* 19:37–48.
- Menzel, D. (1986). Visualization of cytoskeletal changes through the life cycle in *Acetabularia*. *Protoplasma* 134:30–42.
- Metzger, P., Allard, B., Casadevall, E., Berkaloff, C., and Couté, A. (1990). Structure and chemistry of a new chemical race of *Botryococcus braunii* (Chlorophyceae) that produces lycopadiene, a tetraterpenoid hydrocarbon. *J. Phycol.* 26:258–66.
- Miller, D. H. (1978). Cell wall chemistry and ultrastructure of *Chlorococcum oleofaciens* (Chlorophyceae). *J. Phycol.* 14:189–94.
- Mimietz, S., Heidecker, M., Krohne, G., Wegner, L.-H., and Zimmerman, U. (2003). Impact of hypoosmotic challenges on spongy architecture of the cytoplasm of the giant marine alga *Valonia utricularis*. *Protoplasma* 222:117–28.
- Mineyuki, Y., Kataoka, H., Masuda, Y., and Nagai, R. (1995). Dynamic changes in the actin cytoskeleton during the high-fluence rate response of the *Mougeotia* chloroplast. *Protoplasma* 185:222–29.
- Miyamura, S. (2004). Visualization of the rapid fertilization process of the marine green alga, *Ulva arasaki* Chihara (Ulvophyceae, Chlorophyta) using high-speed video microscopy. *Cytologia* 69:197–201.
- Miyamura, S., Hori, T., and Nagumo, T. (2003). Eyespot behavior during the fertilization of gametes in *Ulva arasaki* Chihara (Ulvophyceae, Chlorophyta). *Phycol. Res.* 51:143–6.
- Moestrup, Ø. (1978). On the phylogenetic validity of the flagellar apparatus in green algae and other chlorophyll *a* and *b* containing plants. *BioSystems* 10:117–44.
- Moestrup, Ø., and Hoffman, L. R. (1973). Ultrastructure of the green alga *Dichotomosiphon tuberosus* with special reference to the occurrence of striated tubules in the chloroplast. *J. Phycol.* 9:430–7.
- Moestrup, Ø., and Hoffman, L. R. (1975). A study of the spermatozooids of *Dichotomosiphon tuberosus* (Chlorophyceae). *J. Phycol.* 11:225–35.
- Moldowan, J. M., and Seifert, W. K. (1980). First discovery of botryococcane in petroleum. *J.S.C. Chem. Comm.*, 912–14.
- Mori, K., and Takanashi, S. (1996). Synthesis of lurlene, the sex pheromone of the green flagellate *Chlamydomonas allwensworthii*. *Tetrahedron Lett.* 37:1821–4.
- Musgrave, A. (1993). Mating in *Chlamydomonas*. *Prog. Phycol. Res.* 9:193–237.
- Nelson, T. A., Lee, D. J., and Smith, B. C. (2003). Are “green tides” harmful algal blooms? Toxic properties of water soluble extracts from two bloom-forming macroalgae, *Ulva fenestrata* and *Ulvaria obscura* (Ulvophyceae). *J. Phycol.* 39:874–9.
- Neumann, K. (1969). Protonema mit Riesenkern bei der siphonalen Grünalge *Bryopsis hypnoides* und weitere cytologische Befunde. *Helgol. Wiss. Meeresunters.* 19:45–57.
- Nishii, I., Ogihara, S., and Kirk, D. L. (2003). A kinesin, *invA*, plays an essential role in *Volvox* morphogenesis. *Cell* 113:743–53.
- Nitecki, M. H. (1971). *Ischadites abbottae*, a new North American Silurian species (Dasycladales). *Phycologia* 10:263–75.
- Nossag, J., and Kasprk, W. (1993). The movement of *Micrasterias thomasiana* (Desmidiaceae, Zygnematophyceae) in directed blue light. *Phycologia* 32:332–7.
- O’Kelley, J. C. (1984). Nitrogen and gamete production in *Chlorococcum echinozygotum*. *J. Phycol.* 20:220–5.
- Okuda, K., and Brown, R. M. (1992). A new putative cellulose-synthesizing complex of *Coleochaete scutata*. *Protoplasma* 168:51–63.
- Okuda, K., Tsekos, I., and Brown, R. M. (1994). Cellulose microfibril assembly in *Erythrocladia subintegra* Rosenv.: An ideal system for understanding the relationship between synthesizing complexes (TCs) and microfibril crystallization. *Protoplasma* 180:49–58.
- Oltmanns, F. (1898). Die Entwicklung der Sexualorgane bei *Coleochaete pulvinata*. *Flora* 85:1–14.

- Orset, S., and Young, A. J. (1999). Low-temperature-induced synthesis of  $\alpha$ -carotene in the microalga *Dunaliella salina* (Chlorophyta). *J. Phycol.* 35:320–7.
- Oschman, L. J. (1966). Development of the symbiosis of *Convoluta roscoffensis* Graff and *Platymonas* sp. *J. Phycol.* 2:105–11.
- Page, J. Z., and Kingsbury, J. M. (1968). Culture studies on the marine green alga *Halicystis parvula* – *Derbesia tenuissima*. II. Synchrony and periodicity in gamete formation and release. *Am. J. Bot.* 55:1–11.
- Pan, J., and Snell, W. J. (2000). Signal transduction during fertilization in the unicellular green alga, *Chlamydomonas*. *Curr. Opin. Microbiol.* 3:596–602.
- Parke, M., and Manton, I. (1967). The specific identity of the algal symbiont in *Convoluta roscoffensis*. *J. Mar. Biol. Assoc. UK* 47:445–64.
- Parker, B. C. (1970). Significance of cell wall chemistry to phylogeny in the algae. *Ann. NY Acad. Sci.* 175:417–28.
- Pasquale, S. M., and Goodenough, U. W. (1987). Cyclic AMP functions as a primary sexual signal in gametes of *Chlamydomonas reinhardtii*. *J. Cell Biol.* 105:2279–92.
- Philips, J. A., and Price, I. R. (2002). How different is Mediterranean *Caulerpa taxifolia* (Caulerpales: Chlorophyta) to other populations of the species? *Mar. Ecol. Progr. Ser.* 238:61–71.
- Piazzi, L., Ceccherelli, G., and Cinelli, F. (2001). Threat to macroalgal diversity: effect of the introduced green alga *Caulerpa racemosa* in the Mediterranean. *Mar. Ecol. Progr. Ser.* 210:149–59.
- Pickett-Heaps, J. D. (1970). Some ultrastructural features of *Volvox* with particular reference to the phenomenon of inversion. *Planta* 90:174–90.
- Pickett-Heaps, J. D. (1971). Reproduction by zoospores in *Oedogonium*. I. Zoosporogenesis. *Protoplasma* 72:275–314.
- Pickett-Heaps, J. D. (1973). Cell division and wall structure in *Microspora*. *New Phytol.* 72:347–55.
- Pickett-Heaps, J. D. (1974). Cell division in *Stichococcus*. *Br. Phycol. J.* 9:63–73.
- Pickett-Heaps, J. D. (1976). Cell division in *Raphidonema longiseta*. *Arch. Protistenkd.* 118:209–14.
- Plain, N., Largeau, C., Derenne, S., and Couté, A. (1993). Variabilité morphologique de *Botryococcus braunii* (Chlorococcales, Chlorophyta) corrélations avec les conditions de croissance et la teneur en lipides. *Phycologia* 32:259–65.
- Pocock, M. A. (1960). *Hydrodictyon*: A comparative biological study. *J. S. Afr. Bot.* 26:167–319.
- Powers, J. H. (1908). Further studies in *Volvox*, with description of three new species. *Trans. Am. Microsc. Soc.* 28:141–75.
- Proctor, V. W. (1962). Viability of *Chara* oospores taken from migrating water birds. *Ecology* 43:528–9.
- Proskauer, J. (1950). On *Prasinocladus*. *Am. J. Bot.* 37:59–66.
- Provasoli, L., and Pinter, I. J. (1980). Bacteria induced polymorphism in an axenic laboratory strain of *Ulva lactuca* (Chlorophyceae). *J. Phycol.* 16:196–201.
- Rawitscher-Kunkel, E., and Machlis, L. (1962). The hormonal integration of sexual reproduction in *Oedogonium*. *Am. J. Bot.* 49:177–83.
- Retallack, B., and Butler, R. D. (1970). The development and structure of the zoospore vesicle in *Bulbochaete hiloensis*. *Arch. Mikrobiol.* 72:223–37.
- Richardson, D. H. S. (1973). Photosynthesis and carbohydrate movement. In *The Lichens*, ed. V. Ahmadjian, and M. E. Hale, pp. 249–88. New York and London: Academic Press.
- Rindi, F., and Guiry, M. D. (2003). Composition and distribution of subaerial algal assemblages in Galway City, Western Ireland. *Cryptogamie Algologie* 24:245–67.
- Roberts, K. (1974). Crystalline glycoprotein cell walls of algae: Their structure, composition and assembly. *Philos. Trans. R. Soc. Lond. [B]*, 268:129–46.
- Roberts, K. R., Sluiman, H. J., Stewart, K. D., and Mattox, K. R. (1981). Comparative cytology and taxonomy of the Ulvophyceae. III. The flagellar apparatuses of the anisogametes of *Derbesia tenuissima* (Chlorophyta). *J. Phycol.* 17:330–40.
- Ross, C., Vreeland, V., Waite, J. H., and Jacobs, R. S. (2005). Rapid assembly of a wound plug: stage one of a two-stage wound repair mechanism in the giant unicellular chlorophyte *Dasycladus vermicularis* (Chlorophyceae). *J. Phycol.* 41:46–54.
- Russell, F. S., and Yonge, C. M. (1963). *The Seas*. 2nd edn. London and New York: Frederick Warne and Co.
- Sabnis, D. D., and Jacobs, W. P. (1967). Cytoplasmic streaming and microtubules in the coenocytic marine alga, *Caulerpa prolifera*. *J. Cell Sci.* 2:465–72.
- Salisbury, J. L., and Floyd, G. L. (1978). Calcium-induced contraction of the rhizoplast of a quadriflagellate green alga. *Science* 202:975–6.
- Sawada, T. (1972). Periodic fruiting of *Ulva pertusa* at three localities in Japan. *Proc. 7th Int. Seaweed Symp.*, pp. 229–30. Tokyo: University of Tokyo Press.
- Sawitzky, H., Gleissberg, S., and Berger, S. (1998). Phylogenetic implications of patterns of cap development in selected species of *Acetabularia/Polyphysa* (Dasycladaceae, Chlorophyta). *Phycologia* 37:478–85.

- Scagel, R. F., Bandoni, R. J., Rouse, G. E., Scofield, W. B., Stein, J. R., and Taylor, T. M. C. (1965). *An Evolutionary Survey of the Plant Kingdom*. Belmont, CA: Wadsworth.
- Schlegel, I., Krienitz, L., and Hepperle, D. (2000). Variability of calcification of *Phacotus lenticularis* (Chlorophyta, Chlamydomonadales) in nature and culture. *Phycologia* 39:318–22.
- Schmitt, R. (2003). Differentiation of germinal and somatic cells in *Volvox carteri*. *Curr. Opin. Microbiol.* 6:608–13.
- Schnetter, R., and Eckhardt, R. (2000). Does karyogamy follow plasmogamy in the life cycle of *Derbesia tenuisima* (Chlorophyta). *Phycologia* 39:355–7.
- Shepherd, V. A., Beilby, M. J., and Bisson, M. A. (2004). When is a cell not a cell? A theory relating coenocytic structure to the unusual electrophysiology of *Ventricaria ventricosa* (*Valonia ventricosa*). *Protoplasma* 223:79–91.
- Sherwood, A. R., Garbary, D. J., and Sheath, R. G. (2000). Assessing the phylogenetic position of the Prasiolales (Chlorophyta) using *rbcL* and 18S rRNA gene sequence data. *Phycologia* 39:139–46.
- Shimmen, T., and MacRobbie, E. A. C. (1987). Demonstration of two proton translocating systems in tonoplast of permeabilized *Nitella* cell. *Protoplasma* 136:205–7.
- Simons, J., and van Beem, A. P. (1987). Observations on asexual and sexual reproduction in *Stigeoclonium helveticum* Vischer (Chlorophyta) with implications for the life history. *Phycologia* 26:356–62.
- Simons, J., van Beem, A. P., and de Vries, P. J. R. (1982). Structure and chemical composition of the spore wall in *Spirogyra* (Zygnemataceae, Chlorophyceae). *Acta Bot. Neerl.* 31:359–70.
- Singh, H. V., and Chaudhary, B. R. (1990). Nutrient effects on the formation of oogonia in *Oedogonium hatei* (Chlorophyta). *Phycologia* 29:332–7.
- Sluiman, H. J. (1983). The flagellar apparatus of the zoospore of the filamentous green alga *Coleochaete pulvinata*: Absolute configuration and phylogenetic significance. *Protoplasma* 115:160–75.
- Sluiman, H. J. (1985). A cladistic evaluation of the lower and higher green plants (Viridiplantae). *Plant Syst. Evol.* 149:217–32.
- Sineshchekov, O. A., Jung, K.-H., and Spudich, J. L. (2002). Two rhodopsins mediate phototaxis to low- and high-intensity light in *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci., USA* 99:8689–94.
- Smith, G. M. (1920). *Phytoplankton of the Inland Lakes of Wisconsin*, Part I. State of Wisconsin Publ.
- Smith, G. M. (1944). A comparative study of the species of *Volvox*. *Trans. Am. Microsc. Soc.* 63:265–310.
- Smith, G. M. (1947). On the reproduction of some Pacific Coast species of *Ulva*. *Am. J. Bot.* 34:80–7.
- Smith, G. M. (1950). *The Freshwater Algae of the United States*. New York: McGraw-Hill.
- Smith, G. M. (1955). *Cryptogamic Botany*, Vol. 1, 2nd edn. New York: McGraw-Hill.
- Smith, G. M. (1969). *Marine Algae of the Monterey Peninsula*. Stanford, Calif: Stanford University Press.
- Snell, W. J. (1985). Cell-cell interactions in *Chlamydomonas*. *Annu. Rev. Plant Physiol.* 36:287–315.
- Sormus, L., and Bicudo, C. E. M. (1974). Polymorphism in the desmid *Micrasterias pinnatifida* and its taxonomical implications. *J. Phycol.* 10:274–9.
- Staehelin, L. A., and Pickett-Heaps, J. D. (1975). The ultrastructure of *Scenedesmus* (Chlorophyceae) I. Species with the “reticulate” or “warty” type of ornamental layer. *J. Phycol.* 11:163–85.
- Stanley, S. M. (1979). *Macroevolution*. San Francisco: W. H. Freeman.
- Stark, K., and Schmitt, R. (2002). Genetic control of germ-soma differentiation in *Volvox carteri*. *Protist* 153:99–107.
- Stark, L. M., Almodovar, L., and Krauss, R. W. (1969). Factors affecting the rate of calcification in *Halimeda opuntia* (L.) Lamouroux and *Halimeda discoidea* Decaisne. *J. Phycol.* 5:305–12.
- Starr, R. C. (1954). Heterothallism in *Cosmarium botrytis* var. *subtuumidum*. *Am. J. Bot.* 41:601–7.
- Starr, R. C. (1972). A working model for the control of differentiation during development of the embryo of *Volvox carteri* f. *nagariensis*. *Soc. Bot. Fr. Memoires*, pp. 175–82.
- Starr, R. C., and Jaenicke, L. (1974). Purification and characterization of the hormone initiating sexual morphogenesis in *Volvox carteri* f. *nagariensis* Iyengar. *Proc. Natl. Acad. Sci. USA* 71:1050–4.
- Starr, R. C., Marner, F. J., and Jaenicke, L. (1995). Chemoattraction of male gametes by a pheromone produced by female gametes of *Chlamydomonas*. *Proc. Natl. Acad. Sci., USA*. 92:641–5.
- Steinkötter, J., Bhattacharya, D., Semmelroth, I., Bibeau, C., and Melkonian, M. (1994). Prasinophytes form independent lineages within the Chlorophyta: Evidence from ribosomal RNA sequence comparisons. *J. Phycol.* 30:340–5.
- Stratmann, J., Paputsoglu, G., and Oertel, W. (1996). Differentiation of *Ulva mutabilis* (Chlorophyta) gametangia and gamete release are controlled by extracellular inhibitors. *J. Phycol.* 32:1009–21.
- Suzuki, L., and Johnson, C. H. (2001). Algae know the time of day: circadian and photoperiodic programs. *J. Phycol.* 37:933–42.

- Suzuki, K., Iwamoto, K., Yokoyama, S., and Ikawa, T. (1991). Glycolate-oxidizing enzymes in algae. *J. Phycol.* 27:492–8.
- Syrett, P. J., and Al-Houty, F. A. A. (1984). The phylogenetic significance of the occurrence of urease/urea amidolyase and glycolate oxidase/glycolate dehydrogenase in green algae. *Br. Phycol. J.* 19:11–21.
- Takatori, S., and Imahori, K. (1971). Light reactions in the control of oospore germination of *Chara delicatula*. *Phycologia* 10:221–8.
- Tan, S., Cunningham, F. X., Youmana, M., Grabowski, B., Sun, Z., and Gantt, E. (1995). Cytochrome loss in astaxanthin-accumulating red cells of *Hematococcus pluvialis* (Chlorophyceae): Comparison of photosynthetic activity, photosynthetic enzymes, and thylakoid membrane polypeptides in red and green cells. *J. Phycol.* 31:897–905.
- Taylor, W. R. (1960). *Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas*. Ann Arbor: University of Michigan Press.
- Tolbert, N. E. (1976). Glycolate oxidase and glycolate dehydrogenase in marine algae and plants. *Aust. J. Plant Physiol.* 3:129–32.
- Trainor, F. R. (1963). Zoospores on *Scenedesmus obliquus*. *Science* 142:1673–4.
- Trainor, F. R. (1970). Survival of algae in a desiccated soil. *Phycologia* 9:111–13.
- Trainor, F. R. (1992). Cyclomorphosis in *Scenedesmus armatus* (Chlorophyta): an ordered sequence of ecomorph development. *J. Phycol.* 28:552–8.
- Trench, R. K., Greene, R. W., and Bystrom, B. G. (1969). Chloroplasts as functional organelles in animal tissues. *J. Cell Biol.* 42:404–17.
- Trench, R. K., Boyle, J. E., and Smith, D. C. (1973a). The association between chloroplasts of *Codium fragile* and the mollusc *Elysia viridis*. I. Characteristics of isolated *Codium* chloroplasts. *Proc. R. Soc. Lond. [B]* 184:51–61.
- Trench, R. K., Boyle, J. E., and Smith, D. C. (1973b). The association between chloroplasts of *Codium fragile* and the mollusc *Elysia viridis*. II. Chloroplast ultrastructure and photosynthetic carbon fixation in *E. viridis*. *Proc. R. Soc. Lond. [B]* 184:63–81.
- Tsekos, I. (1999). The sites of cellulose synthesis in algae: diversity and evolution of cellulose-synthesizing enzyme complexes. *J. Phycol.* 35:635–55.
- Van Kruiningen, H. J., Garner, F. M., and Schiefer, B. (1969). Protothecosis in a dog. *Pathol. Vet.* 6:348–54.
- vanWinkle-Swift, K. P., and Rickoll, W. L. (1997). The zygospore wall of *Chlamydomonas monoica* (Chlorophyceae): morphogenesis and evidence for the presence of sporopollenin. *J. Phycol.* 33:655–65.
- Verlaque, M., Durand, C., Huisman, J. M., Boudouresque, C.-F., and LeParco, Y. (2003). On the identity and origin of the Mediterranean invasive *Caulerpa racemosa* (Caulerpaceae, Chlorophyta). *Eur. J. Phycol.* 38:325–39.
- Wake, L. V., and Hillen, L. W. (1980). Study of a “bloom” of the oil-rich alga *Botryococcus braunii* in the Darwin River Reservation. *Biotech. Bioeng.* 22:1637–56.
- Wang, B., Zarka, A., Trebst, A., and Boussiba, S. (2003). Astaxanthin accumulation in *Haematococcus pluvialis* (Chlorophyceae) as an active photoprotective process under high irradiance. *J. Phycol.* 39:1116–24.
- Watanabe, S., Kuroda, N., and Maiwa, F. (2001). Phylogenetic status of *Helicodictyon planctonicum* and *Desmochloris halophila* gen. et comb. nov. and the definition of the class Ulvophyceae (Chlorophyta). *Phycologia* 40:421–34.
- Webster, D. A., Hackett, D. P., and Park, R. B. (1968). The respiratory chain of colorless algae. III. Electron microscopy. *J. Ultrastruct. Res.* 21:514–23.
- Wheeler, A. E., and Page, J. Z. (1974). The ultrastructure of *Derbesia tenuissima* (de Notaris) Crouan. I. Organization of the gametophyte protoplast, gametangium, and gametangial pore. *J. Phycol.* 10:336–52.
- Wiese, L., and Hayward, P. C. (1972). On sexual agglutination and mating-type substances in isogamous dioecious chlamydomonads. III. The sensitivity of sex cell contact to various enzymes. *Am. J. Bot.* 59:530–6.
- Wilbur, K. M., Colinvaux, L. H., and Watabe, N. (1969). Electron microscope study of calcification in the alga *Halimeda* (order Siphonales). *Phycologia* 8:27–35.
- Wilcox, L. W., Fuerst, P. A., and Floyd, G. L. (1993). Phylogenetic relationships of four charophycean green algae inferred from complete nuclear-encoded small subunit rRNA gene sequences. *Amer. J. Bot.* 80:1028–33.
- Wolf, F. R., Nonomura, A. M., and Bassham, J. A. (1985). Growth and branched hydrocarbon production in a strain of *Botryococcus braunii* (Chlorophyta). *J. Phycol.* 21:388–96.
- Woodcock, C. L. F. (1971). The anchoring of nuclei by cytoplasmic microtubules in *Acetabularia*. *J. Cell Sci.* 8:611–21.
- Woodcock, C. L. F., and Miller, G. J. (1973a). Ultrastructural features of the life cycle of *Acetabularia mediterranea*. I. Gametogenesis. *Protoplasma* 77:313–29.

- Woodcock, C. L. F., and Miller, G. J. (1973b). Ultrastructural features of the life cycle of *Acetabularia mediterranea*. II. Events associated with the division of the primary nucleus and the formation of cysts. *Protoplasma* 77:331–41.
- Wray, J. L. (1977). *Calcareous Algae*. Amsterdam: Elsevier.
- Wujek, D. E. (1968). Some observations on the fine structure of three genera in the Tetrasporaceae. *Ohio J. Sci.* 68:187–91.
- Wustman, B. A., Melkonian, M., and Becker, B. (2004). A study of cell wall and flagella formation during cell division in the scaly green alga, *Scherffelia dubia* (Chlorophyta). *J. Phycol.* 46:895–910.
- Yamaguchi, K. (1997). Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. *J. Appl. Phycol.* 8:487–502.
- Yoshii, Y., Takaichi, S., Maoka, T., and Inouye, I. (2003). Photosynthetic pigment composition in the primitive green alga *Mesostigma viride* (Prasinophyceae): phylogenetic and evolutionary implications. *J. Phycol.* 39:570–6.
- Zechman, F. W. (2003). Phylogeny of the Dasycladales (Chlorophyta, Ulvophyceae) based on analyses of Rubisco large subunit (*rbcL*) gene sequences. *J. Phycol.* 39:819–27.
- Zeigler, J. R., and Kingsbury, J. M. (1964). Cultural studies on the marine green alga *Halicystis parvula*–*Derbesia tenuissima*. I. Normal and abnormal sexual and asexual reproduction. *Phycologia* 4:105–16.
- Zuljevic, A., and Antollic, B. (2000). Synchronous release of male gametes of *Caulerpa taxifolia* (Caulerpales, Chlorophyta) in the Mediterranean Sea. *Phycologia* 39:257–9.



# Part IV

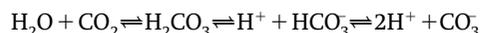
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## Evolution of one membrane of chloroplast endoplasmic reticulum

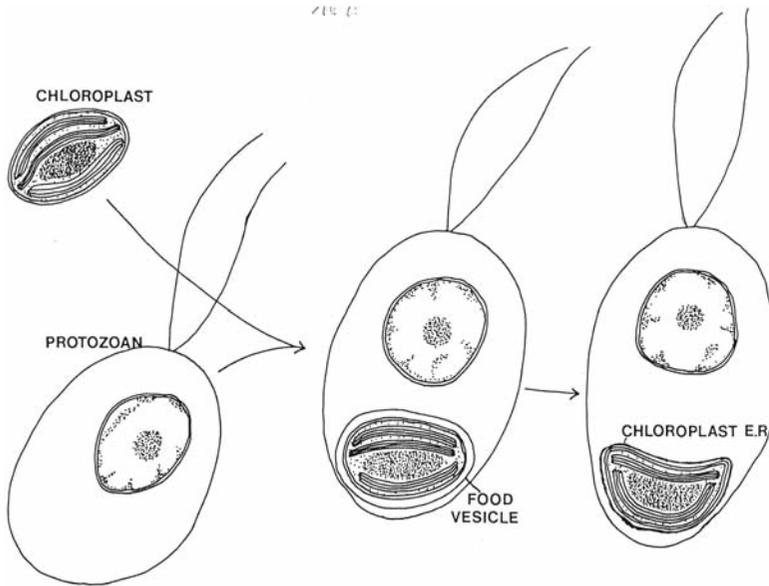
The Euglenophyta (euglenoids), Apicomplexa, and Dinophyta (dinoflagellates) are a natural grouping in that they are the only algal groups to have one membrane of chloroplast endoplasmic reticulum. Chloroplast endoplasmic reticulum evolved when a chloroplast from a eukaryotic alga was taken into a food vesicle by a phagocytotic euglenoid, apicomplexan or dinoflagellate (Fig. IV.1) (Lee, 1977; Gibbs, 1978). Normally the cell would have digested the chloroplast as a source of food. However, in this case the chloroplast remained in the cytoplasm of the host as an endosymbiont. The host benefited from the association by receiving photosynthate from the endosymbiotic chloroplast. The endosymbiotic chloroplast benefited from the high concentration of carbon dioxide in the acidic environment of the host vesicle. Eventually the food vesicle membrane of the host became the single membrane of chloroplast endoplasmic reticulum surrounding the chloroplast. It is probable that the plastid of the euglenoids evolved by the capture of a green-algal chloroplast. The plastids of the dinoflagellates and apicomplexans probably are derived from an endosymbiotic red-algal chloroplast.

It appears that algae with chloroplast endoplasmic reticulum were selected for in evolution because of their ability to outcompete other algae in environments which are low in dissolved  $\text{CO}_2$ . Before explaining the mechanism by which these algae are able to outcompete, it is necessary to understand the equilibria governing the distribution of carbon species in water.

Carbon occurs in water as dissolved inorganic carbon (DIC) which is composed of  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$  and  $\text{CO}_2$ .



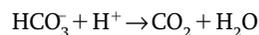
Seawater in equilibrium with the atmosphere at pH 8.2 and 25 °C contains about 2200  $\mu\text{M}$  of dissolved inorganic carbon. At this pH, 10  $\mu\text{M}$



**Fig. IV.1** Drawing illustrating the probable sequence of evolutionary events that led to a chloroplast being surrounded by a single membrane of chloroplast endoplasmic reticulum. Initially a chloroplast was taken up by a phagocytotic protozoan into a food vesicle, with the food vesicle membrane eventually evolving into the single membrane of chloroplast endoplasmic reticulum surrounding the chloroplast

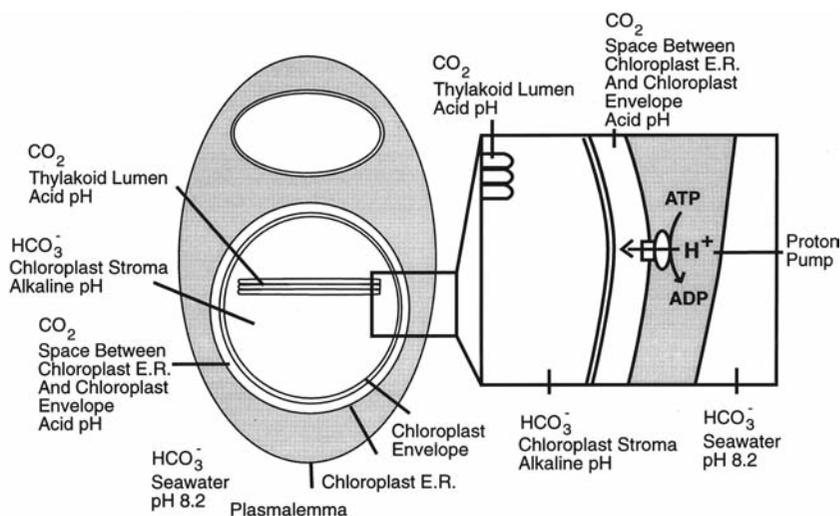
occurs as  $\text{CO}_2$ , 200  $\mu\text{m}$  occurs as  $\text{CO}_3^{-2}$  with the remainder occurring as  $\text{HCO}_3^-$ . As the pH is lowered, more of the dissolved inorganic carbon occurs as  $\text{CO}_2$ . At pH 1 virtually all the dissolved inorganic carbon occurs as  $\text{CO}_2$ , with  $\text{HCO}_3^-$  completely absent.

Carbon dioxide is the form of carbon that is fixed by the carboxylating enzyme in photosynthesis, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Falkowski and Raven, 1998). Many microalgae have developed a  $\text{CO}_2$  concentrating mechanism (CCM) that concentrates  $\text{CO}_2$  inside cells to a level several times higher than the  $\text{CO}_2$  in the external medium (Fridlyand, 1997; Sukenik et al., 1997). The  $\text{CO}_2$  concentrating mechanisms pumps bicarbonate ( $\text{HCO}_3^-$ ) outside the cell through the plasma membrane and chloroplast membrane into the chloroplast. Once inside the chloroplast, the dissolved inorganic carbon stays mostly as  $\text{HCO}_3^-$  because the stroma is alkaline. A significant amount of the  $\text{HCO}_3^-$  passes into the lumen of the thylakoids, where in illuminated chloroplasts, the pH is about 5 (Fridlyand, 1997). The enzyme carbonic anhydrase is attached to the thylakoid membranes (Raven, 1997). In the lumen of the thylakoids, carbonic anhydrase converts the  $\text{HCO}_3^-$  to  $\text{CO}_2$  at a rate hundreds of times faster than the non-enzymatic conversion.



The result is a  $\text{CO}_2$  concentration that is ten times higher than  $\text{HCO}_3^-$  in the lumen of the thylakoids (Raven, 1997).

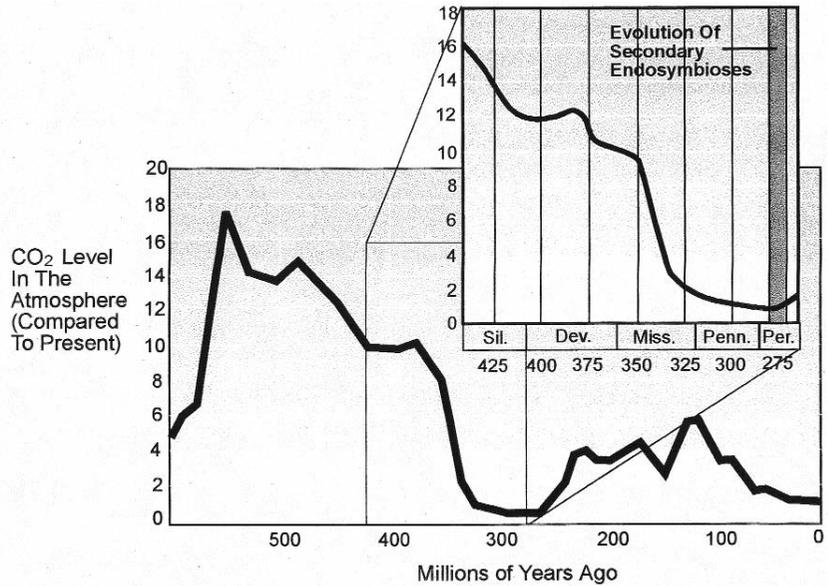
Efflux of the  $\text{CO}_2$  from the thylakoid lumen to the chloroplast stroma suppresses Rubisco oxygenase activity and stimulates carboxylase activity in the stroma (or pyrenoid if one is present). Through carboxylation by Rubisco, the  $\text{CO}_2$  enters into the carbon reduction cycle. Thus the



**Fig. IV.2** Diagrammatic representation of the compartments present in an alga with one membrane of chloroplast endoplasmic reticulum. The pH and predominant form of dissolved inorganic carbon are indicated for each compartment.

evolutionary goal of the carbon concentrating mechanism is to increase the concentration of  $CO_2$  at the location of Rubisco.

Once generated by carbonic anhydrase, the  $CO_2$  rapidly diffuses away from the thylakoid lumen and out of the chloroplast, reducing the effectiveness of the carbon concentrating mechanism. Algae with chloroplast E.R. have limited the egress of  $CO_2$  by the following mechanism (Fig. IV.2). The volume between the chloroplast envelope and chloroplast endoplasmic reticulum most likely has an acid pH since the membrane of chloroplast E.R. next to the chloroplast envelope was the vacuolar membrane in the original endosymbiosis leading to the establishment of chloroplast E.R. (Lee, 1977; Gibbs, 1978). An acidic pH in the volume between the chloroplast envelope and the adjacent membrane of chloroplast E.R. is therefore probable since the pH of digestive vacuoles of phagocytic protozoa is usually acidic when tested for acid phosphatase (optimal pH 5). An acid pH in the volume between the chloroplast envelope and chloroplast E.R. would contain a relatively high concentration of  $CO_2$ , and a relatively low concentration of  $HCO_3^-$ , because of the effect of pH on the balance between the two entities. The chloroplast membranes are relatively permeable to  $CO_2$  so the  $CO_2$  in this space would freely move into the chloroplast stroma. A relatively high concentration of  $CO_2$  would, therefore, be maintained in the chloroplast with  $CO_2$  entering both from the acidic thylakoid lumen and the acidic space between the chloroplast E.R. and chloroplast envelope. This would provide algae with chloroplast E.R. with an abundant supply of  $CO_2$  for photosynthesis, particularly in the



**Fig. IV.3** The amount of CO<sub>2</sub> in the atmosphere over the past 600 Ma. The time of evolution of secondary endosymbioses around 275 Ma is shown in the insert.

marine environment where at pH 8.2, the dissolved CO<sub>2</sub> concentration is particularly low. In such an environment, algae with chloroplast endoplasmic reticulum had a competitive advantage and were selected for in evolution.

On the basis of a molecular clock, Medlin et al. (1997) postulated that the original secondary endosymbiosis occurred between 260 and 285 million years ago (Ma). Atmospheric CO<sub>2</sub> levels were at record lows at this time (Fig. IV.3), levels not reached again until Recent times. Such an environment favored algae that were better able to photosynthesize in a low CO<sub>2</sub> environment, e.g., those algae with chloroplast endoplasmic reticulum. Thus, the radiation of algae with chloroplast endoplasmic reticulum occurred at a time when the more efficient CO<sub>2</sub> mechanism of these algae enabled them to outcompete other algae without chloroplast endoplasmic reticulum.

## REFERENCES

- Falkowski, P. G., and Raven, J. (1997). *Aquatic Photosynthesis*. Oxford: Blackwell Science.
- Fridlyand, L. E. (1997). Models of CO<sub>2</sub> concentrating mechanisms in microalgae taking into account cell and chloroplast structure. *BioSystems* 44:41–57.
- Gibbs, S. P. (1978). The chloroplasts of *Euglena* may have evolved from symbiotic green algae. *Canadian J. Bot.* 56:2883–9.
- Lee, R. E. (1977). Evolution of algal flagellates with chloroplast endoplasmic reticulum from the ciliates. *South African J. of Sci.* 73:179–82.

- Medlin, L. K., Kooistra, W. H. C. F., Gersonde, R., Sims, P. A., and Wellbrock, U. (1997). Is the origin of the diatoms related to the end-Permian mass extinction? *Nova Hedwigia* 65:1–11.
- Raven, J. (1997). CO<sub>2</sub> concentrating mechanisms: a direct role for thylakoid lumen acidification? *Plant Cell Env.* 20:147–54.
- Sukenik, A., Tchernov, D., Kaplan, A., Huertas, E. Lubian, L. M., and Livne, A. (1997). Uptake, efflux, and photosynthetic utilization of inorganic carbon by the marine eustigmatophyte *Nannochloropsis* sp. *J. Phycol.* 33:969–74.



## Euglenophyta

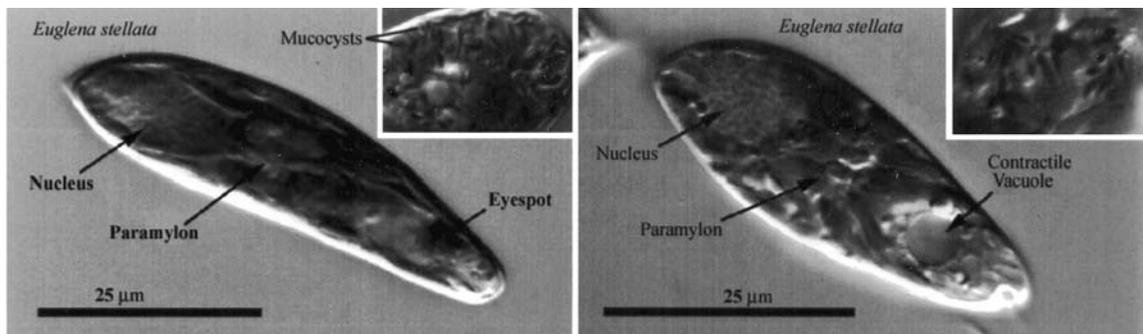
### EUGLENOPHYCEAE

Euglenoid flagellates occur in most freshwater habitats: puddles, ditches, ponds, streams, lakes, and rivers, particularly waters contaminated by animal pollution or decaying organic matter (Buetow, 1968). Usually larger bodies of purer water, such as rivers, lakes, and reservoirs, have sparser populations of less common euglenoids as planktonic organisms. Marine euglenoids are more common than supposed, with *Eutreptia*, *Eutreptiella* (Figs. 6.11, 6.14(c)), and *Klebsiella* occurring exclusively in marine or brackish water, and many other genera having one or a few marine species. These occur in the open sea, in tidal zones among seaweeds, and as sand inhabitants on beaches. Brackish species of *Euglena* (Figs. 6.1, 6.2, 6.3, 6.7, 6.14(c)) often color estuarine mud flats green when light intensity is low, the green color

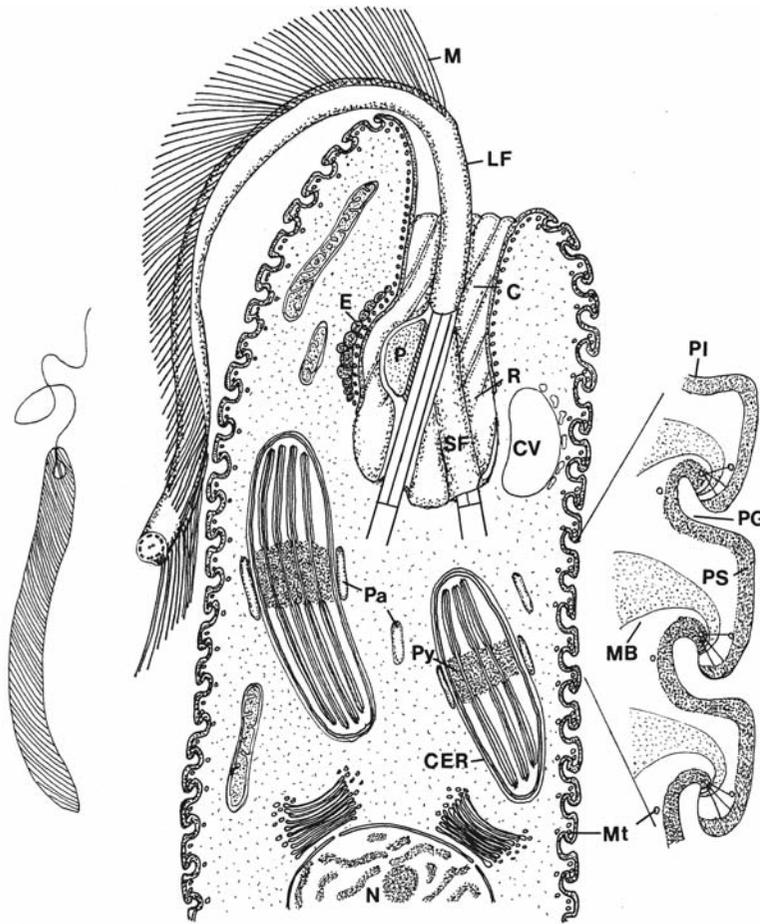
disappearing in full sunlight as the euglenoids creep away from the surface. There are also several parasitic euglenoid flagellates, mostly species of *Khawkinea*, *Euglenamorpha*, and *Hegneria*.

Euglenoids are characterized by chlorophylls *a* and *b*, one membrane of chloroplast endoplasmic reticulum, a mesokaryotic nucleus, flagella with fibrillar hairs in one row, no sexual reproduction, and paramylon or chrysolaminarin as the storage product in the cytoplasm.

Euglenoid cells have two basal bodies and one or two emergent flagella (Fig. 6.2). The flagella are similar to those of trypanosomes in having a **paraxonemal rod (paraxial rod)** that runs the length of the flagellum inside the flagellar membrane (Ngô and Bouck, 1998; Bastin and Gul, 1999; Talke and Preisfeld, 2002). The paraflagellar rod is composed of two major proteins forming an elongated alpha-helical stalk that parallels the axoneme. The one emergent flagellum in *Euglena*



**Fig. 6.1** Light micrographs of *Euglena stellata*. (From Shin and Triemer, 2004.)



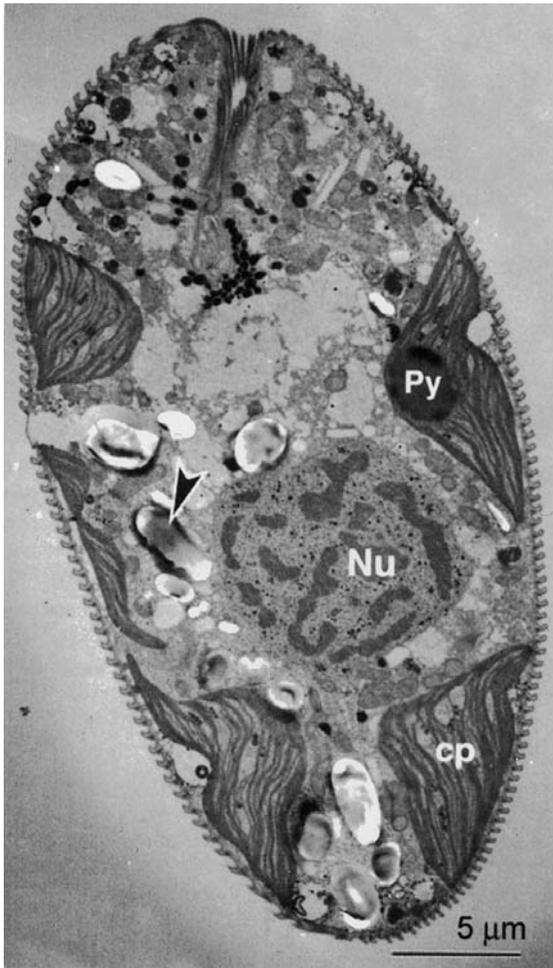
**Fig. 6.2** A semidiagrammatic drawing of the fine structure of the anterior part of a *Euglena* cell. (C) Canal; (CER) chloroplast endoplasmic reticulum; (CV) contractile vacuole; (E) eyespot; (LF) long flagellum; (M) mastigonemes; (MB) muciferous body; (Mt) microtubules; (N) nucleus; (P) paraflagellar swelling; (Pa) paramylon; (PG) pellicle groove; (PI) plasmalemma; (PS) pellicle strip; (Py) pyrenoid; (R) reservoir; (SF) short flagellum. (Adapted from Mignot, 1966; Jahn and Bovee, 1968.)

has helically arranged fibrillar hairs (no microtubules) attached along the length of the flagellar membrane. The fibrillar hairs are of two lengths: there is a single helical row of long ( $3 \mu\text{m}$ ) hairs and two helical rows of short ( $1.5 \mu\text{m}$ ) hairs in *Euglena* (Bouck et al., 1978). Other genera have flagella similar to *Euglena* (Hilenski and Walne, 1985). There are two basic types of flagellar movement in the class. The first group (including the Eutreptiales and Euglenales) has the flagellum continually motile from base to apex, resulting in cell gyration with the anterior end of the cell tracing a wide circle. The swimming rate of *Euglena gracilis* (Figs. 6.7, 6.14(a)) depends on the temperature: at  $10^\circ\text{C}$  it is  $15 \mu\text{m s}^{-1}$  while at  $30^\circ\text{C}$  it is  $84 \mu\text{m s}^{-1}$  (Lee, 1954). The second group (including *Peranema* (Fig. 6.13), *Entosiphon*, and *Sphenomonas*) has the flagellum held out straight in front of the cell with just the tip motile, resulting in smooth

swimming or gliding locomotion in contact with the substratum or air-water interface at rates up to  $30 \mu\text{m s}^{-1}$  (Saito et al., 2003).

Euglenoid cells are surrounded by a pellicle that has four main components: the plasma membrane, repeating proteinaceous units called strips, subtending microtubules, and tubular cisternae of endoplasmic reticulum (Figs. 6.2, 6.3, 6.4, 6.5) (Leander and Farmer, 2000, 2001a). The strips are arranged in parallel, are characteristic of the species, and are composed primarily of the proteinaceous articulins. Below each strip is a set of parallel microtubules, where each microtubule occupies a discrete position relative to the strip. The cisterna of endoplasmic reticulum is also intimately associated with each strip and appears to function as a reservoir for calcium.

Each strip of the pellicle has a thick side and a thinner flange side (Figs. 6.5, 6.6). In the



**Fig. 6.3** *Euglena rustica*. Transmission electron micrograph of a mid-sagittal section of a cell. (Arrow) Paramylon granule; (cp) chloroplast; (Py) pyrenoid; (Nu) nucleus. (From Brown et al., 2002.)

construction of the pellicle, the thick end of one strip fits under the thin flange end of the second strip. This structure gives the pellicle an alternating pattern of ridges and grooves. In *Euglena gracilis* (Figs. 6.5, 6.7, 6.14(a)) there are about 40 of the S-shaped strips overlapping at their lateral margins, under the plasma membrane. The region of strip overlap is occupied by a set of microtubule-associated bridges and microtubule-independent bridges.

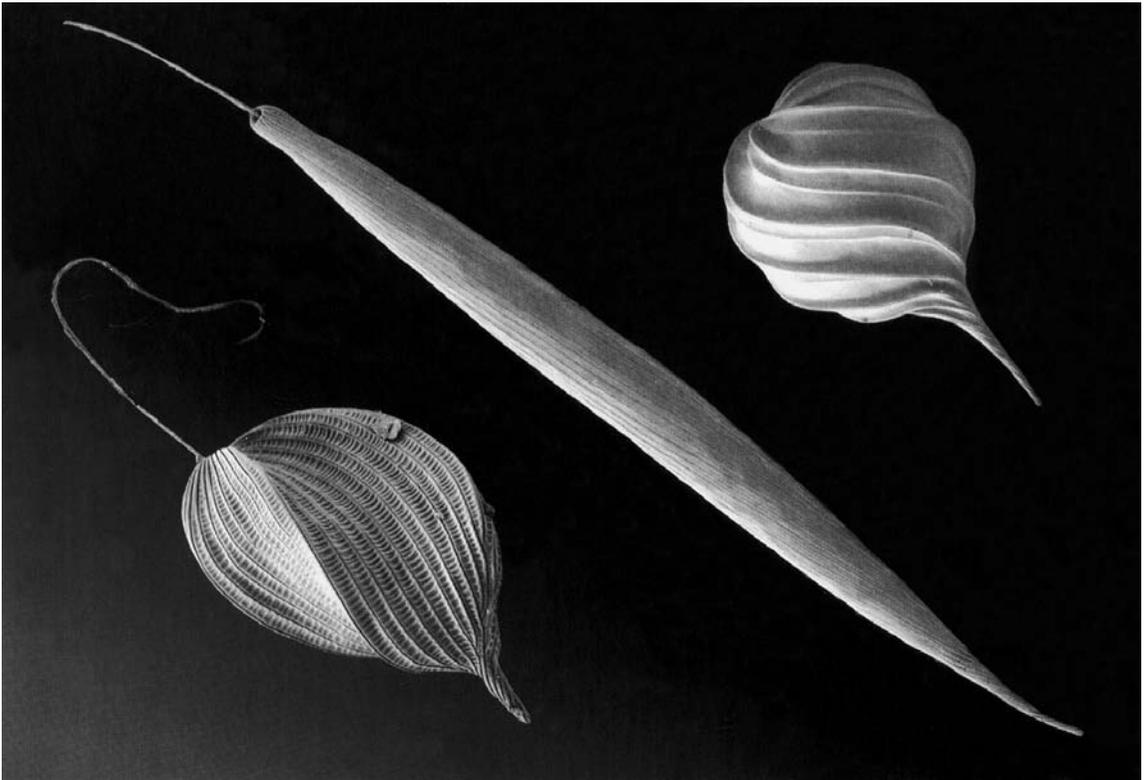
Muciferous bodies occur under the pellicle strips and contain a water-soluble mucopolysaccharide. The muciferous bodies open to the

outside through pores between the strips of the pellicle. The muciferous bodies function in the formation of the stalk in *Colacium*, lorica formation in *Trachelomonas*, cyst formation and lubrication during euglenoid movement (Leander and Farmer, 2000).

Some euglenoids have a flexible pellicle that allows the cells to undergo a flowing movement known as **euglenoid movement** (Fig. 6.7). This type of movement occurs only when the cells are not swimming and results from sideways movement of the pellicle strips. The movement may be induced by the binding of  $\text{Ca}^{2+}$  from the endoplasmic reticulum directly under the strips of the pellicle (Murata et al., 2000).

*Euglena gracilis* changes its shape two times per day when grown under the synchronizing effect of a daily light–dark cycle. At the beginning of the light period, when photosynthetic capacity is low (as measured by the ability of the cells to evolve oxygen), the population of cells is largely **spherical**. The **mean cell length** of the population **increases to a maximum** in the middle of the light period when photosynthetic capacity is greatest, and then decreases for the remainder of the 24-hour period. The population becomes **spherical** by the end of the 24-hour period when the cycle reinitiates. These changes are also observed under dim light conditions and are therefore controlled by a biological clock and represent a circadian rhythm in cell shape (Lonergan, 1983).

The locomotory flagellum or flagella emerge from an anterior invagination of the cell, which consists of a narrow tubular portion, the **canal**, and a spherical or pyriform chamber, the **reservoir** (Figs. 6.2, 6.8). The canal is a rigid structure, whereas the reservoir easily changes shape and is regularly distorted by the discharge of the contractile vacuole. The rigidity of the canal is maintained by microtubules that form a flat helix around the canal, in much the same position as hoops on a barrel. The pellicle lines the canal but not the reservoir, the reservoir being the only part of the cell covered solely by the plasmalemma. A cylindrical pocket arises as an infolding of the plasma membrane of the reservoir in *Euglena*, *Colacium*, and *Peranema* and is probably common in the euglenoids (Willey and Wibell, 1985a; Surek



**Fig. 6.4** Scanning electron micrographs of euglenoids showing the helical pellicle of *Phacus triqueteter* (lower left), *Euglena acus* (middle), and *Lepocincilis ovata* (upper right). (From Leander and Farmer, 2001b.)

and Melkonian, 1986). The reservoir pocket is similar to the cytosome found in protozoa assigned to the Kinetoplastida (Willey and Wibel, 1985b). In the Kinetoplastida (bodonids and trypanosomatids), the cytosome is associated with the uptake of food organisms by phagotrophy, and the reservoir pocket has a similar function in the phagotrophic euglenoids (e.g., *Peranema*; Fig. 6.13) where the pocket is called a **cytosome**.

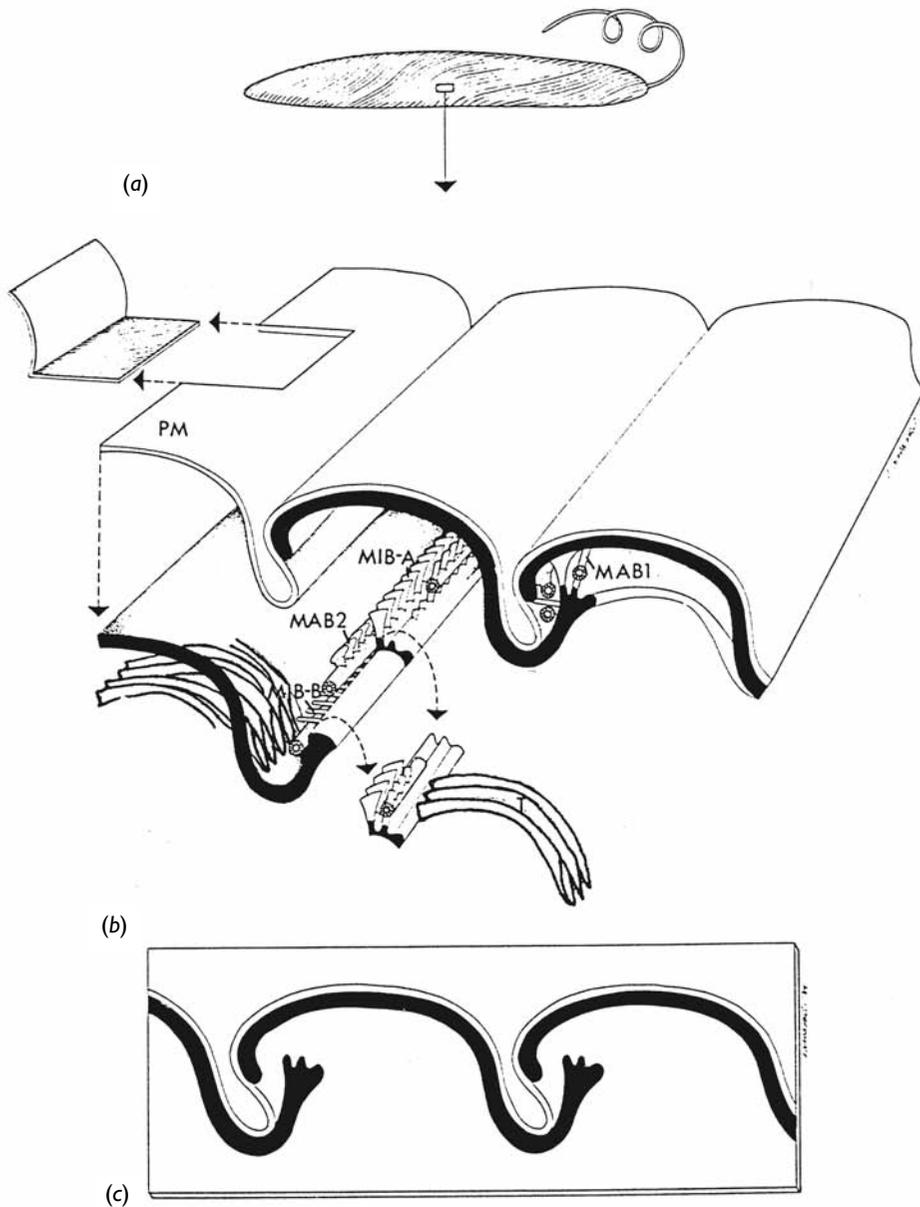
The contractile vacuole (Figs. 6.1, 6.2, 6.13, 6.14), in the anterior part of the cell next to the reservoir, has an osmoregulatory function, expelling excessive water taken into the cell. The contractile vacuole fills and empties at regular intervals of 15 to 60 seconds. It empties into the reservoir, from which the water is carried out through the canal (Leedale, 1967).

Mitochondria are of typical algal type. Colorless euglenoids always have more mitochondria

than do equivalent-sized green ones. When green cells are decolorized by heat or streptomycin, they have a sevenfold increase in mitochondrial volume, reflecting a change from autotrophic to heterotrophic nutrition. The formation of two mitochondrial enzymes, fumarase and succinate dehydrogenase, necessary for dark respiration of substrates, is repressed by light (Davis and Merrett, 1974).

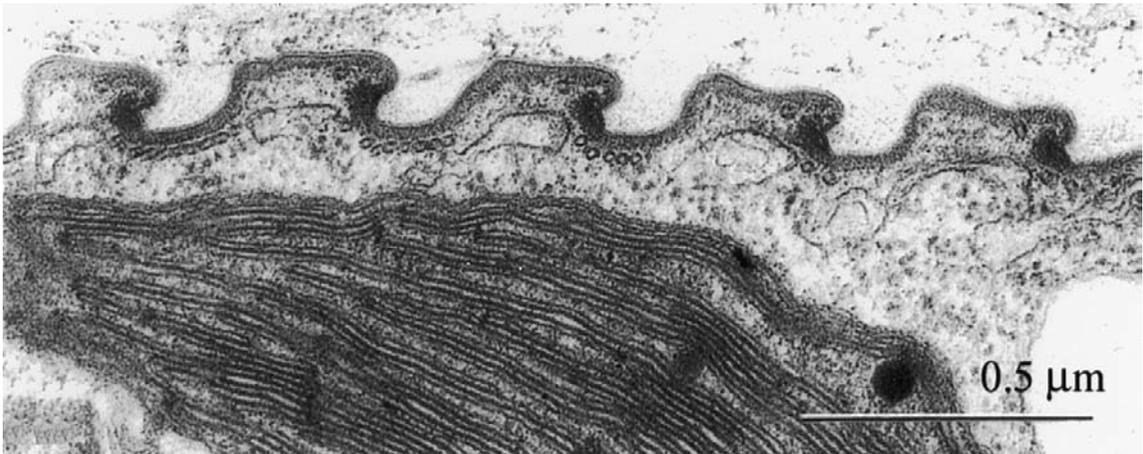
## Nucleus and nuclear division

The euglenoid nucleus is of the mesokaryotic type, having chromosomes that are permanently condensed during the mitotic cycle, a nucleolus (endosome) that does not disperse during nuclear division, no microtubules from chromosomes to pole spindles, and a nuclear envelope that is intact during nuclear division (Fig. 6.9). The chromosome number is usually high, and polyploidy probably occurs in some genera (Gravilá, 1996).



**Fig. 6.5** *Euglena gracilis*. Diagrams of a whole swimming cell (a) and transverse sections of the cell surface (b) and (c), illustrating details of the articulating S-shaped strips of the membrane skeleton and the infrastructure associated with strip overlap. The position of the skeleton and bridges seems well suited to mediate the sliding of adjacent strips that is presumed to occur during shape changes. (MAB1, MAB2) Microtubule-associated bridges between the pellicle strips; (MIB-A, MIB-B) microtubule-independent bridges between the pellicle strips; (PM) plasma membrane; (T) transverse fiber. (From Dubreuil and Bouck, 1985.)

Mitosis in euglenoids (Leedale, 1970; Chaly et al., 1977) begins during early prophase with the nucleus migrating from the center of the cell to an anterior position. Microtubules appear in the nucleus, but they do not attach to the chromosomes. At metaphase, bundles of microtubules are among the chromosomes, and the nucleolus has started to elongate along the division axis. In anaphase, the intact nuclear envelope elongates along the division axis, the nucleolus divides, and



**Fig. 6.6** Transmission electron micrograph of a section of the pellicle area of *Euglena terricola*. (From Leander and Farmer, 2001a.)

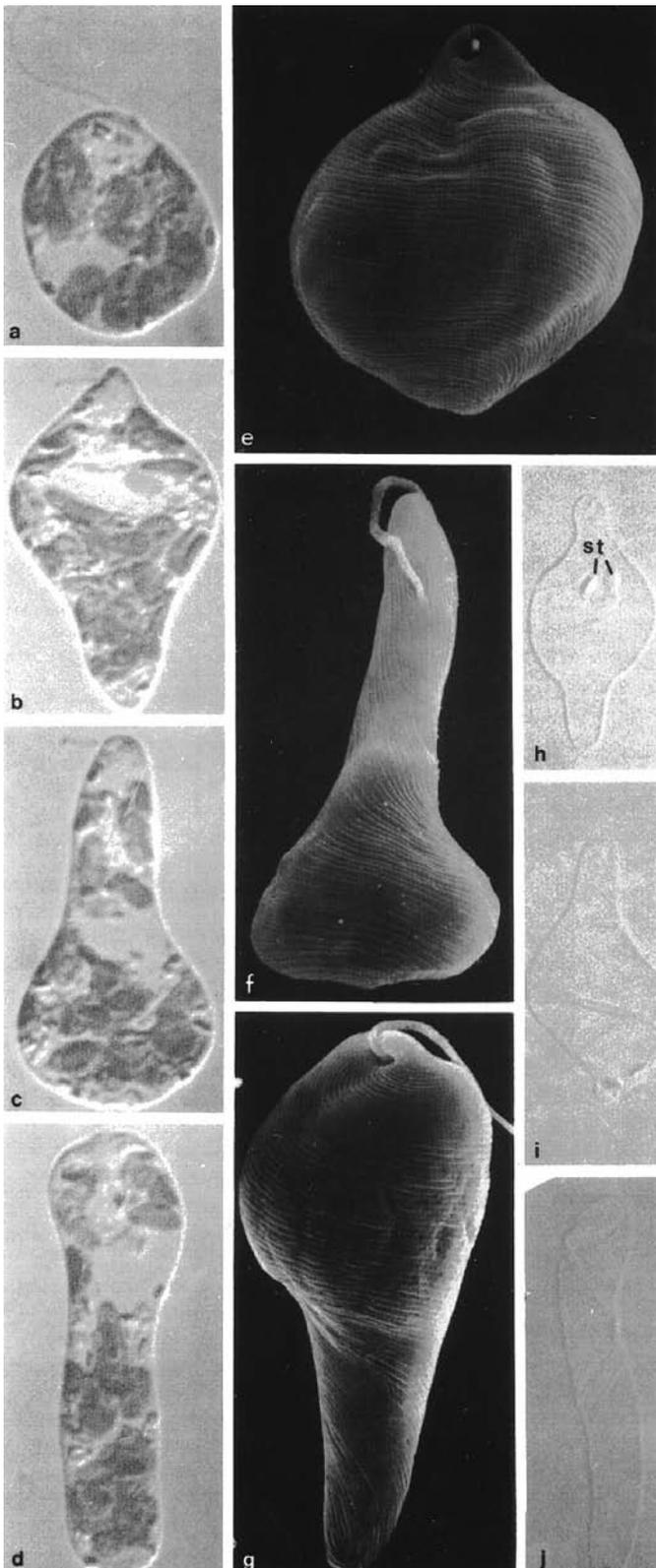
the daughter chromosomes disperse into the two daughter nuclei.

## Eyespot, paraflagellar swelling, and phototaxis

The **eyespot (stigma)** is a collection of orange-red lipid droplets, independent of the chloroplast (Figs. 6.1, 6.2, 6.14). The eyespot is in the anterior part of the cell, curving to ensheath the neck of the reservoir on the dorsal side. In most euglenoids the eyespot consists of a compact group of 20 to 50 droplets, although in *Eutreptia* and *Khawkinea* it may consist of just one or two large droplets. The eyespot has been reported to contain  $\alpha$ -carotene and seven xanthophylls (Sperling Pagni et al., 1981), mainly  $\beta$ -carotene (Batra and Tollin, 1964), or a  $\beta$ -carotene derivative, echinone (Krinsky and Goldsmith, 1960). The independence of the eyespot is emphasized by the existence of colorless species with an eyespot but no plastids. One flagellum of all green euglenoids bears a lateral swelling near the transition zone from canal to reservoir; in *Euglena* (Figs. 6.2, 6.14(a)), the swelling is on the longer flagellum. The swelling is composed of a crystalline body next to the axoneme and inside the flagellar membrane.

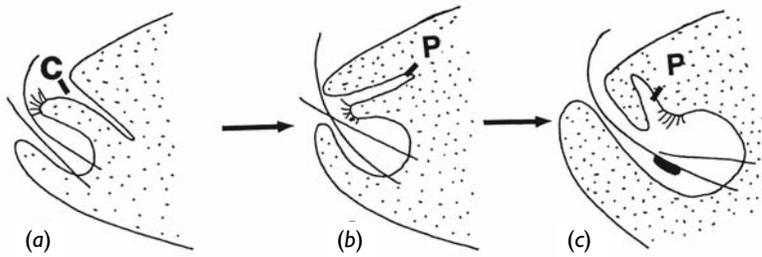
All euglenoid species with an eyespot and flagellar swelling exhibit phototaxis, usually swimming away from bright light (negative phototaxis) and away from darkness toward subdued light (positive phototaxis) to accumulate in a region of low light intensity. Upon sudden changes in its environment, the cell responds with a transient sideways turn by swinging out its one emergent flagellum. At low light intensities (less than  $1.4 \text{ W m}^{-2}$ ), the alga swims toward the light source, whereas at higher light intensities, it moves away from the light source. According to the shading hypothesis (Häder, 1987), positive phototaxis is brought about by repetitive step-down photophobic responses. During forward locomotion, the cells rotate helically with a frequency of 1 to 2 hertz (cycles per second). In lateral light, each time the stigma (eyespot) intercepts the light beam impinging on the paraflagellar body, the flagellum swings out temporarily and turns the front end of the cell toward the light source by a fraction until the cell is aligned in the light direction.

*Euglena* bleached of its chlorophyll but retaining its eyespot and photoreceptor (paraflagellar swelling) is still positively phototactic, eliminating chlorophyll and chloroplasts in the phototaxis directly. A *Euglena* bleached of all pigments but retaining its photoreceptor is negatively phototactic; this rules out the possibility that the carotenoids of the eyespot are directly stimulatory in phototaxis. A *Euglena* lacking a photoreceptor and all pigments, like *Astasia* (Fig. 6.14(b)), is no

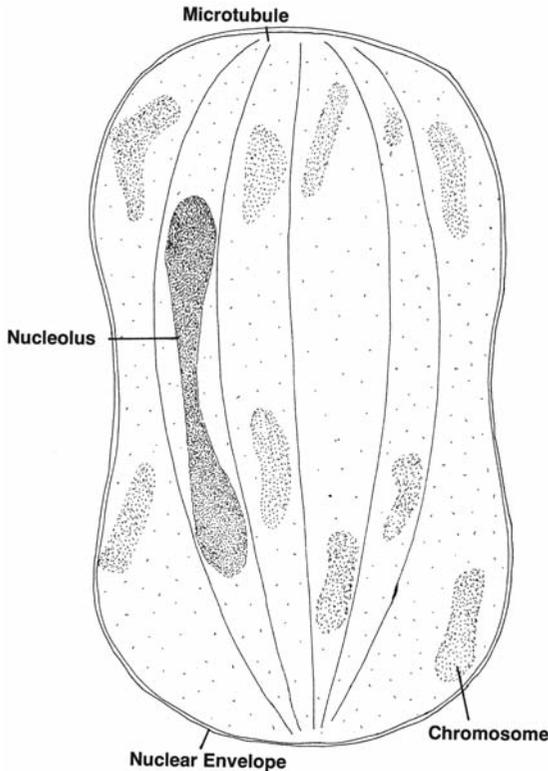


**Fig. 6.7** *Euglena gracilis*. (a)–(d).

Sequence of shape changes photographed at 5-second intervals of a cell undergoing euglenoid movements. The nearly spherical cell in (a) initiates a forward wave of dilation in (b), which reaches the anterior of the cell, and then recovers by an inward flow of cytoplasm to initiate a new wave at (c). The new wave progresses forward, and the cell recovers in (d). Scanning electron microscope micrographs in (e)–(g) illustrate the positions of surface ridges and grooves during selected stages of deformation. Nearly horizontal strips in (e) reorient to longitudinal in cells, initiating (f) or completing (g) the cycle. In (h)–(j), cell ghosts are shown with the changes in cell shape. (From Dubreuil and Bouck, 1985.)



**Fig. 6.8** Semidiagrammatic drawings of the anterior portion of *Bodo* (Kinetoplastida) (a), *Peranema* (Euglenophyceae) (b), and *Colacium* (Euglenophyceae) (c), illustrating probable evolution of the euglenoids. (C) Cytosome; (P) pocket. (After Willey and Wibel, 1985b.)



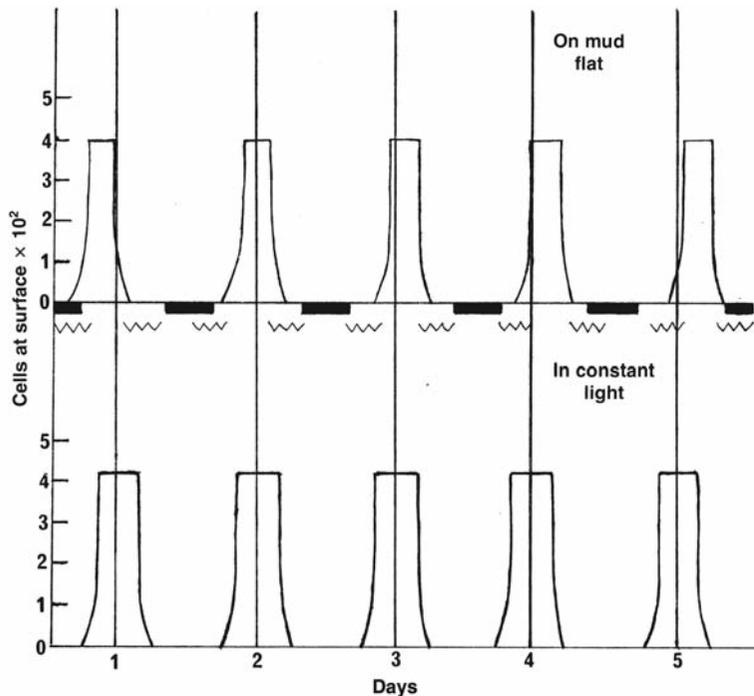
**Fig. 6.9** A semidiagrammatic drawing of an anaphase nucleus of *Euglena* with nuclear envelope intact, the nucleolus pinching in two, and the chromosomes not attached to spindle microtubules.

longer phototactic (Jahn and Bovee, 1968). The flagellar swelling is therefore the photoreceptor or light-sensitive organelle. It has a sensitive maximum at 410 nm, explaining the phototactic peak at that wavelength. Positive phototaxis occurs only if the eyespot, with its absorptive range of 400 to 630 nm, periodically shades the photoreceptor.

There is a circadian rhythm in phototaxis in *Euglena*, with phototaxis operative during the light period and not operative during the dark

period. Even if light is introduced during the normal dark period, the *Euglena* cell does not respond phototactically. The phototactic response in the direction of the light source plainly involves the photoreceptor, but why this is incapable of reacting during dark periods when the light is reintroduced is not known. Continuous darkness does not eliminate the rhythm unless continued for so long a period that complete bleaching results, and the cells become photonegative. It is possible that the non-operative nature of photaxis during this time is related to mitotic division occurring during this period. Leedale (1959) found that green euglenoids have almost perfectly circadianly synchronized mitotic cycles, mitosis occurring at the beginning of the dark period, requiring one hour of dark to trigger the division. During mitotic division, *Euglena* normally rounds up and loses its flagellum; but even if the flagellum is not shed, the cells are still unable to swim ably during this period. Because phototactic insensitivity coincides with the generation time of *Euglena* during dark hours, it perhaps is of no surprise that motility loss, generation time, and absence of phototaxis should coincide as they do (Jahn and Bovee, 1968).

A phototactic circadian rhythm also exists in the movement of mud flat *Euglena*. The mouth of the River Avon in Bristol, England, is an estuary with very large tidal differences in water level. The mud flats which are exposed at low tide become green in spots owing to an accumulation of *Euglena obtusa* on the surface of the mud (Bracher, 1937; Palmer and Round, 1965). Before the incoming tide floods the mud flats, the *Euglena* cells move back down into the mud, a pattern of behavior that avoids the washing away of the *Euglena* by the tide. *Euglena proxima* exhibits a similar movement and it has been shown that the



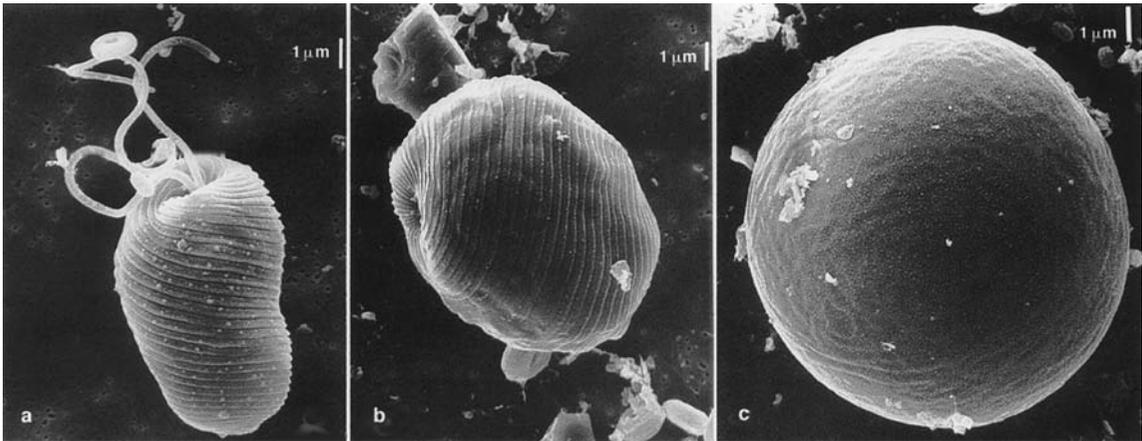
**Fig. 6.10** The vertical migration rhythm in *Euglena obtusa* on a mud flat (top curve), and in constant light in the laboratory (bottom curve). The dark bars on the upper abscissa represent night, whereas the wavy lines indicate when the tide covered the mud flats. (After Palmer and Round, 1965.)

cells absorb a significant amount of nutrients in the mud during this part of the migration cycle (Kingston, 2002). *Euglena* cells accumulate at the surface of the mud only when the low tides occur during daylight, no cells being found on the surface during low tides (Fig. 6.10). The above observation indicate that phototaxis is the main mechanism involved, with the *Euglena* cells swimming toward the light during the day to reach the surface of the mud. If the *Euglena* cells are brought into the laboratory and placed in constant light (980 lux), the cells display a circadian rhythm in their vertical migration. The phase of this circadian rhythm is determined by the last dark period in nature, be it night or a dark period resulting from the covering of the mud flat by murky water at high tide. Thus the rhythm is actually circadian, entrained by the tidally caused light–dark cycle, rather than truly tidal.

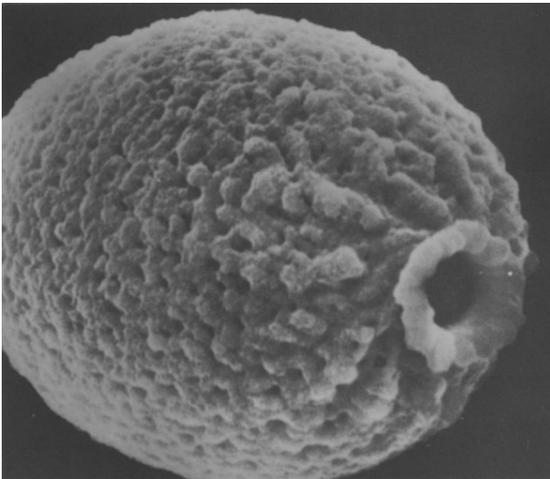
### Muciferous bodies and extracellular structures

**Muciferous bodies (mucocysts)**, containing water-soluble polysaccharides, occur in helical

rows under the pellicle in all species of euglenoid flagellates (Fig. 6.2). The muciferous bodies open to the outside of the cell through pores that course between the strips of the pellicle. Euglenoid cells are permanently coated with a thin slime layer from the muciferous bodies (Rosowski, 1977). It sometimes accumulates at the posterior end of the cell as a trailer of slime, and some species have the habit of sticking to a substratum by their posterior ends. Species with large muciferous bodies eject the contents on irritation and produce a copious slime layer around the cell (Hilenski and Walne, 1983). In *Euglena gracilis* (Figs. 6.7, 6.14(a)) the slime is composed of glycoproteins and polysaccharides (Cogburn and Schiff, 1984). The envelopes and stalks of *Colacium* (Figs. 6.15, 6.16) are formed of carbohydrate extruded by mucocysts in the anterior portion of the cell (Willey, 1984). The cylindrical stalks are composed of an inner and outer core of mildly acidic carbohydrate. The stalk is continuous with the canal and anterior part of the cell (Willey et al., 1977). The envelopes of such species as *Trachelomonas* (Figs. 6.12, 6.14(d)) are built up by inorganic deposition on a foundation of mucilaginous threads.



**Fig. 6.11** Scanning electron micrographs of the encystment of *Eutreptiella gymnastica*. The vegetative cell (a) loses its flagella, forms a large number of paramylon grains, and begins to round up (b). The cell swells and produces a mucilaginous covering (c). (From Olli, 1996.)



**Fig. 6.12** Scanning electron micrograph of the mineralized envelope of *Trachelomonas lefevrei*. (From Dunlap et al., 1983.)

Cysts are formed by euglenoids as a means of surviving unfavorable periods. The cell rounds off and secretes a thick sheath of mucilage (Fig. 6.11) that survives for months until the cell emerges by cracking the cyst. In conditions of partial desiccation or excessive light, the slime sheath sometimes acts as a temporary cyst, cells emerging from the sheath as soon as conditions improve. In certain genera (*Euglena* and *Eutreptia*), cell divi-

sion within the slime layer leads to the formation of a palmelloid colony, which may form extensive sheets of cells covering many square feet of mud surface (Leedale, 1967).

*Trachelomonas* is a large genus of free-swimming green euglenoids, characterized by encasement of the cell in a patterned mineralized envelope with a rimmed apical pore through which the flagellum emerges (Figs. 6.12, 6.14(d)). Most of the species are defined by the form and ornamentation of the envelopes, characteristics that can be changed by varying conditions of growth, especially iron and manganese supply. This has resulted in some described species being growth forms of other species (Pringsheim, 1953). The process of envelope formation involves mineralization of an initial fibrillar envelope which is probably derived as a secretion of the mucilaginous bodies (Pringsheim, 1953; Leedale, 1975). It begins with longitudinal division of the parent protoplast, the two daughter cells rotating within the envelope, and one or both squeezing out through the pore. Each naked daughter cell then secretes a new envelope externally, at first delicate and colorless but already the size and shape of the old one. Under good growth conditions, the envelope slowly becomes thicker and ornamented, first yellow, then brown. Under conditions of manganese deficiency, the envelope usually remains thin and unornamented. Envelope substructure is species specific, with a given species producing one of two general types of substructure: (1) weft or fibrillar deposits composed primarily of manganese, or (2) fine

granules composed primarily of iron (Dodge, 1975; Leedale, 1975; Walne, 1980; West and Walne, 1980; West et al., 1980).

Pellicular ornamentation occurs in a number of euglenoids, particularly in species of *Phacus* (Figs. 6.4, 6.14(e)) and in the *Euglena spirogyra* complex. The process is related to envelope formation in *Trachelomonas* (Figs. 6.12, 6.14(d)) and stalk formation in *Colacium* (Figs. 6.15, 6.16).

## Chloroplasts and storage products

Euglenoid chloroplasts arose from a secondary endosymbiosis. The chloroplasts originated from the chloroplasts of a scaly flagellate in the green algal class Prasinophyceae (Marin, 2004). The euglenoid chloroplasts are surrounded by two membranes of the chloroplast envelope plus one membrane of chloroplast endoplasmic reticulum; the latter membrane is not continuous with the nuclear membrane (Figs. 6.2, 6.3, 6.14). The chloroplasts are usually discoid or plate-like with a central pyrenoid. The thylakoids are grouped into bands of three, with two thylakoid bands traversing the pyrenoid.

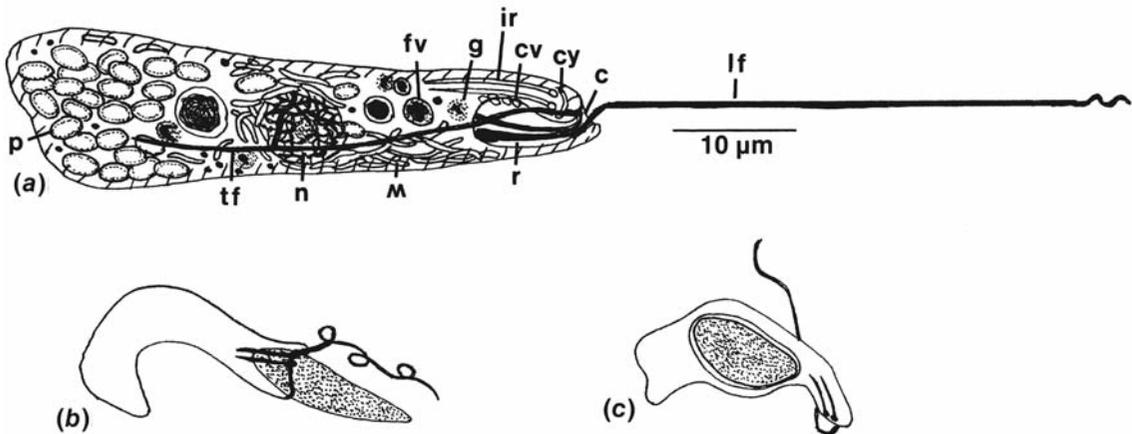
A shield of paramylon grains surrounds the pyrenoid, but outside the chloroplast, in phototrophically grown cells (Figs. 6.2, 6.3, 6.14). Paramylon granules are distributed throughout the cytoplasm in heterotrophically grown cells in the dark (Bäumer et al., 2001). Gottlieb isolated the granules in 1850, and showed that they were composed of a carbohydrate that although isomeric with starch (amylopectin), was not stained with iodine. For this reason, they were termed **paramylon granules**. They have since been shown to be composed of a  $\beta$ -1,3 linked glucan (Barsanti et al., 2001). The paramylon granule is a membrane-bounded crystal composed of two types of segments – rectangular solids and wedges (Kiss et al., 1987). The liquid storage product, chrysolaminarin, can be an alternative storage product in some Euglenophyceae such as *Eutreptiella gymnastica* (Fig. 6.11) and *Sphenomonas laevis* where it can occur with solid paramylon grains in the same cell (Leedale, 1967; Throndsen, 1969). Whereas the paramylon usually occurs as a shield of grains, the chrysolaminarin occurs in vacuoles

primarily in the anterior part of the cell (Throndsen, 1973).

## Nutrition

The Euglenophyceae have a number of modes of nutrition, depending on the species involved. No euglenoid has yet been demonstrated to be fully **photoautotrophic** – capable of living on a medium devoid of all organic compounds (including vitamins), with carbon dioxide as a carbon source, nitrates or ammonium salts as a nitrogen source, and light as an energy source. All green euglenoid flagellates so far studied are **photoauxotrophic** – capable of growing in a medium devoid of organic nutrients, with carbon dioxide, ammonium salts, and light, but needing at least one vitamin. *Euglena gracilis* has an absolute requirement for vitamin B<sub>12</sub> (Hutner and Provasoli, 1955), it having been calculated that between 4900 and 22000 molecules of vitamin B<sub>12</sub> are necessary for cell division (Carell, 1969). Vitamin B<sub>12</sub>-starved cells increase in cell volume, sometimes to 10 times the size of control organisms, the cells in the final stage of vitamin B<sub>12</sub> starvation often being polylobed, polynucleate, and containing more than the normal number of chloroplasts per cell (Bertaux and Valencia, 1971, 1973; Carell, 1969). During vitamin B<sub>12</sub> starvation, total cellular RNA and protein increase 400% to 500% compared with controls (Carell et al., 1970). The chloroplast number per cell increases during this period, although the ratio of chloroplast protein to total cellular protein remains constant, evidence for the independence of chloroplast division from nuclear division (Bré and Lefort-Tran, 1974). Although the protein increase is 400% to 500% during vitamin B<sub>12</sub> starvation, the total DNA increases only about 180%, suggesting that a particular step in DNA replication may be preferentially affected by the vitamin (Bré et al., 1975).

As *Euglena* cells age, they become immobile and spherical, with a tendency to form enlarged “giant” cells and to accumulate orange to black pigment bodies. Aging also results in the formation of larger numbers of lysosomes and microbodies with an increase in the degradation of organelles (Gomez et al., 1974). The older cells



**Fig. 6.13** *Peranema trichophorum*. (a) General cell structure. (b),(c) Two stages in the ingestion of a cell of *Euglena* (stippled cell). (c) Canal; (cv) contractile vacuoles; (cy) rim of cytosome; (fv) food vesicle; (g) Golgi; (ir) ingestion rods; (lf) leading flagellum; (m) mitochondrion; (n) nucleus; (p) paramylon; (tf) trailing flagellum. (After Leedale, 1967.)

undergo a shift from carbohydrate to fat oxidation, as evidenced by an increase in malate synthetase, the enzyme involved in the glycolate bypass, important in the oxidation of fats.

The euglenoids belong to the osmotrophic acetate flagellates, having the ability to grow photosynthetically in the light or heterotrophically in the dark. In either state, the fixed carbon is used as a source of energy or as building blocks for cell constituents. The substrates that can be used for heterotrophic growth vary from one species to another, with the permeability of the substrate into the cell probably being the most important factor. As a rule, the most readily utilized substrates are acetic and butyric acids and the corresponding alcohols (e.g., ethanol). The two most commonly used substrates are acetate and ethanol.

## Classification

Studies on rRNA of euglenoids support a monophyletic origin of the Euglenophyceae with the kinetoplastids as a sister clade (Preisfeld et al., 2000; Nudelman et al., 2003). The phagotrophic euglenoids (e.g., *Peranema*; Fig. 6.13) evolved before

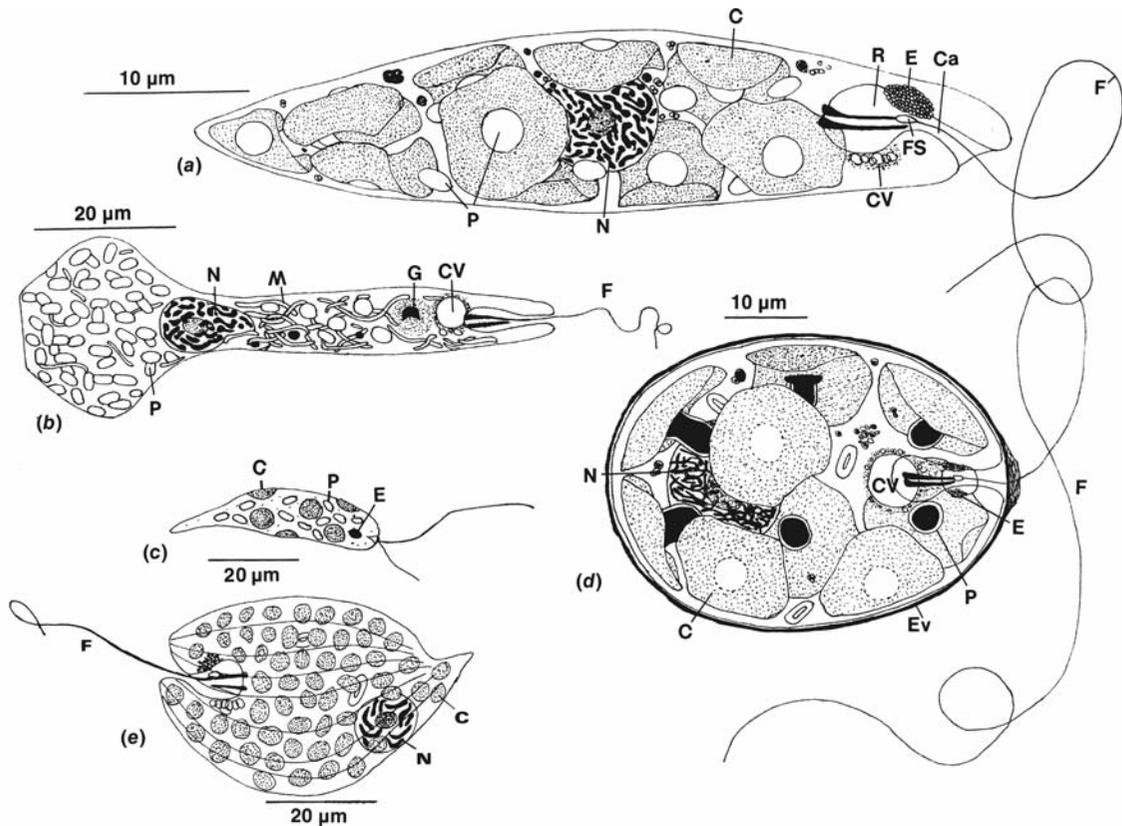
the phototrophic and osmotrophic species. The phototrophic species evolved from a single secondary endosymbiotic event involving a chloroplast from a green alga in the Prasinophyceae (Marin, 2004). Osmotrophic genera such as *Astasia* (Fig. 6.14(b)) contain chloroplast genetic material indicating they evolved from photosynthetic euglenoids (Sekiguchi et al., 2002).

Three orders of euglenoids are presented here. Investigations utilizing nucleic acid sequencing have shown the organisms in the Euglenales to have evolved most recently (Marin, 2004).

- Order 1 Heteronematales: two emergent flagella, the longer flagellum directed anteriorly and the shorter one directed posteriorly during swimming; special ingestion organelle present.
- Order 2 Eutreptiales: two emergent flagella, one directed anteriorly and the other laterally or posteriorly during swimming; no special ingestion organelle.
- Order 3 Euglenales: two flagella, only one of which emerges from the canal; no special ingestion organelle.

## Heteronematales

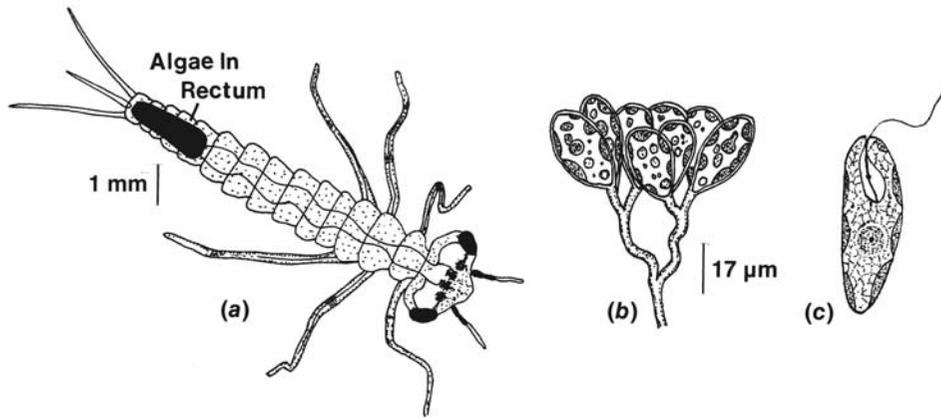
Here the colorless cells have a special ingestion organelle (Triemer, 1997), and are phagocytic, taking up food particles whole and digesting them in food vesicles. *Peranema trichophorum* is a euglenoid that ingests other cells and detritus (Leedale, 1967) (Fig. 6.13). The ingestion apparatus consists



**Fig. 6.14** (a) *Euglena gracilis*. (b) *Astasia klebsii*. (c) *Eutreptiella marina*. (d) *Trachelomonas grandis*. (e) *Phacus triqueter*. (C) Chloroplast; (Ca) canal; (CV) contractile vacuole; (E) eyespot; (Ev) envelope; (F) emergent flagellum; (FS) flagellar swelling; (M) mitochondrion; (N) nucleus; (P) paramylon grains or paramylon sheath around chloroplast; (R) reservoir. (After Leedale, 1967.)

of two parallel tapering rods, the hooked anterior ends of which are attached to the stiffened rim of the **cytosome**. The latter is a permanent “mouth” situated in a subapical position independent of the canal opening. There is no permanent “gullet,” and food vacuoles are formed at the cytosome only when feeding takes place. *Peranema* normally ingests food particles and living organisms by engulfing them whole into food vacuoles. The ingestion rods are protruded and attached to the surface of the prey, which is then pulled through the cytosome in connection with a wave of euglenoid movement from the *Peranema* cell. With a large prey, such as *Euglena*, the rods are detached, moved, and attached again farther along the prey,

so that more of it can be pulled into the predator. By repeated pullings, the whole *Euglena* is engulfed, the process taking up to 15 minutes. A second form of attack, reserved for larger algal cells, consists of cutting and sucking rather than engulfing. Several *Peranema* cells converge on their prey, with their ingestion rods protruded and used to rasp a way through the prey’s wall or periplast. *Euglena spirogyra* pellicle is cut through in about 10 minutes, with the cell contents sucked out into a temporary food canal below the cytosome. If the prey is large enough, the predators finally enter the cell and engulf what remains of the prey. The food vacuoles decrease in size as digestion proceeds, the indigestible remains being finally ejected through a “defecation area” of constant position at the posterior end of the cell. It is possible to show that chemotaxis is important in directing *Peranema* to its prey by bursting open living algal cells in a suspension of *Peranema*, the peranemas streaming in from all directions for the meal.



**Fig. 6.15** (a) Larva of the damselfly *Ischnura verticalis* with a plug of *Colacium libellee* in the rectum. (b), (c) Colony and single swimming cell of *Colacium vesiculosum*. ((a) adapted from Rosowski and Willey, 1975; (b), (c) after Stein and Johnson in Huber-Pestalozzi, 1955.)

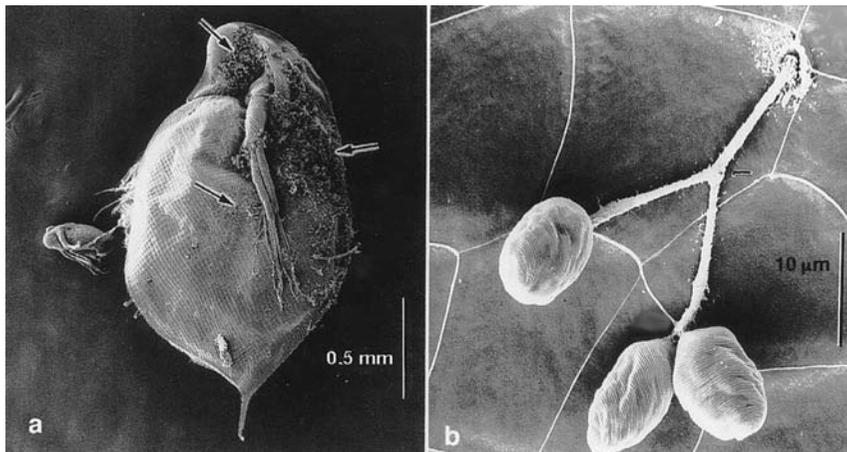
### Eutreptiales

The organisms in the Eutreptiales have two emergent flagella and no special ingestion organelles. *Eutreptia* and *Eutreptiella* (Figs. 6.11, 6.14(c)) are estuarine or marine genera, while *Distigma* is characteristic of acid freshwaters.

### Euglenales

In this primarily freshwater order, the flagellum without the paraflagellar swelling has been reduced so that it does not emerge from the canal. Common genera in the order are the green photosynthetic *Euglena* (Figs. 6.1, 6.2, 6.3, 6.7, 6.14(a)), *Trachelomonas* (Figs. 6.12, 6.14(d)), and *Phacus* (Figs. 6.4, 6.14(d)), as well as the colorless osmotrophic *Astasia* (Fig. 6.14(b)).

*Colacium libellee* is a member of this order that establishes itself in the rectum of damselfly nymphs during the winter in colder lakes (Figs.



**Fig. 6.16** Scanning electron micrographs of the euglenoid *Colacium vesiculosum* on the freshwater arthropod *Daphnia pulex*. (a) Arrows point to large concentrations of *Colacium* on *Daphnia*. (b) A colony of *Colacium* attached by mucilage stalks to *Daphnia*. (From Al-Daheri and Willey, 1996.)

6.15, 6.16). During the warm summer months the damselfly nymphs and *C. libellae* live separately. With the onset of winter, the *Colacium* cells attach to the cuticle of the rectum of the damselfly larvae, forming a conspicuous green plug that colors the terminal four segments of the abdomen dark green. In the rectum, the *Colacium* is in a palmelloid state, lacking a flagellar swelling and eyespot. As the peripheral waters of the lake freeze, the damselfly nymphs move to deeper water. Here the damselfly nymphs form a protected, motile, translucent microhabitat for the *Colacium*; the damselfly nymphs probably also provide a source of nutrients for the *Colacium* cells, which helps the alga to survive the unfavorable winter conditions. In spring, the damselfly nymphs swim to warmer water, at which time the *Colacium* cells swim out of the rectum to establish a free-living existence in the summer. If algal-free damselfly nymphs are placed in water with *C. libellae*, the alga will establish itself within 36 hours in the damselfly nymph rectum. Other species are not able to infect the nymphs (Willey et al., 1970, 1973; Willey, 1972; Rosowski and Willey, 1975).

## REFERENCES

- Al-Dhaheri, R. S., and Willey, P. L. (1996). Colonization and reproduction of the epibiotic flagellate *Colacium vesiculosum* (Euglenophyceae) in *Daphnia pulex*. *J. Phycol.* 32:770–4.
- Barsanti, L., Vismara, R., Passarelli, V., and Gualtieri, P. (2001). Paramylon ( $\beta$ -1,3- glucan) content in wild type and WZSL mutant of *Euglena gracilis*. Effects of growth conditions. *J. Appl. Phycol.* 13:59–65.
- Bastin, P., and Gull, K. (1999). Assembly and function of complex flagellar structures illustrated by the paraflagellar rod of trypanosomes. *Protist* 150:113–23.
- Batra, P. P., and Tollin, G. (1964). Phototaxis in *Euglena*. I. Isolation of the eyespot granules and identification of the eye-spot pigments. *Biochem. Biophys. Acta* 79:371–8.
- Bäumer, D., Preisfeld, A., and Ruppel, H.G. (2001). Isolation and characterization of paramylon synthase from *Euglena gracilis* (Euglenophyceae). *J. Phycol.* 37:38–46.
- Bertaux, O., and Valencia, R. (1971). Effects de la carence  $B_{12}$  sur les cellules synchrones de *Euglena gracilis*: Blocage de la division cellulaire, polyploidie et gigantisme. *J. Physiol. (London)* 63:167A.
- Bertaux, O., and Valencia, R. (1973). Blocage de la division cellulaire et malformations induites par carence  $B_{12}$  chez les cellules synchrones de *Euglena gracilis* Z. C. R. Séances Acad. Sci. Paris 276:753–6.
- Bouck, G. B., Rogalski, A., and Valaitis, A. (1978). Surface organization and composition of *Euglena*. II. Flagellar mastigonemes. *J. Cell Biol.* 77:805–26.
- Bracher, R. (1937). The light relations of *Euglena limosa* Gard. Part I. The influence of intensity and quality of light on phototaxy. *J. Linn. Soc. Bot.* 51:23–42.
- Bré, M. H., and Lefort-Tran, M. (1974). Influence de l'avitaminose  $B_{12}$  sur les chloroplastes de l'*Euglena gracilis* Z. en milieu lactate. C. R. Séances Acad. Sci. Paris 278:1349–52.
- Bré, M. H., Diamond, J., and Jacques, R. (1975). Factors mediating the vitamin  $B_{12}$  requirement of *Euglena*. *J. Protozool.* 22:432–4.
- Brown, P. J. P., Leander, B. S., and Farmer, M. A. (2002). Redescription of *Euglena rustica* (Euglenophyceae), a rare euglenophyte from the intertidal zone. *Phycologia* 41:445–52.
- Carell, E. F. (1969). Studies on chloroplast development and replication in *Euglena*. I. Vitamin  $B_{12}$  and chloroplast replication. *J. Cell Biol.* 41:431–40.
- Carell, E. F., Johnston, P. L., and Christopher, A. R. (1970). Vitamin  $B_{12}$  and the macromolecular composition of *Euglena*. *J. Cell Biol.* 47:525–30.
- Chaly, N., Lord, A., and Lafontaine, J. G. (1977). A light- and electron-microscope study of nuclear structure throughout the cell cycle in the euglenoid *Astasia longa* (Jahn). *J. Cell Sci.* 27:23–45.
- Cogburn, J. N., and Schiff, J. A. (1984). Purification and properties of the mucus of *Euglena gracilis* (Euglenophyceae). *J. Phycol.* 20:533–44.
- Davis, B., and Merrett, M. J. (1974). The effect of light on the synthesis of mitochondrial enzymes in division-synchronized *Euglena* cultures. *Plant Physiol.* 53:575–80.
- Dodge, J. D. (1975). The fine structure of *Trachelomonas* (Euglenophyceae). *Arch. Protistenk.* 117:65–77.
- Dubreuil, R. R., and Bouck, G. B. (1985). The membrane skeleton of a unicellular organism consists of bridged, articulating strips. *J. Cell Biol.* 101:1884–96.
- Dunlap, J. R., Walne, P. L., and Bentley, J. (1983). Microarchitecture and elemental spatial segregation of envelopes of *Trachelomonas lefevrei* (Euglenophyceae). *Protoplasma* 117:97–106.
- Gomez, M. P., Harris, J. B., and Walne, P. L. (1974). Studies of *Euglena gracilis* in aging cultures. I. Light microscopy and cytochemistry. *Br. Phycol. J.* 9:163–74.

- Gottlieb, J. (1850). Ueber eine neue, mit Starkmehl isomere Substanz. *Ann. Chem. Pharm.* 75:51–61.
- Gravilă, L. (1996). Light and electron microscope studies of euglenoid nuclei. In *Cytology, Genetics and Molecular Biology of Algae*, ed. B. R. Chaudhary, and S. B. Agrawal, pp. 193–213. Amsterdam, The Netherlands: SPB Academic Pub.
- Häder, D-P. (1987). Polarotaxis, gravitaxis and vertical phototaxis in the green flagellate, *Euglena gracilis*. *Arch. Microbiol.* 147:179–83.
- Hilenski, L. L., and Walne, P. L. (1983). Ultrastructure of mucocysts in *Peranema trichophorum* (Euglenophyceae). *J. Protozool.* 30:491–6.
- Hilenski, L. L., and Walne, P. L. (1985). Ultrastructure of the flagella of the colorless phagotroph *Peranema trichophorum* (Euglenophyceae). I. Flagellar mastigonemes. *J. Phycol.* 21:114–25.
- Huber-Pestalozzi, G. (1955). Euglenophycean. *Das Phytoplankton des Süßwassers*, Vol. 16, Part 4. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung.
- Hutner, S. H., and Provasoli, L. (1955). Comparative biochemistry of flagellates. In *Biochemistry and Physiology of Protozoa*, ed. S. H. Hutner, and A. Lwoff, Vol. 2, pp. 1–40. New York and London: Academic Press.
- Jahn, T. L., and Bovee, E. C. (1968). Locomotive and motile response in *Euglena*. In *The Biology of Euglena*, ed. D. E. Buetow, 1:45–108. New York and London: Academic Press.
- Kingston, M. B. (2002). Effect of subsurface nutrient supplies on the vertical migration of *Euglena proxima* (Euglenophyta). *J. Phycol.* 38:872–80.
- Kiss, J. Z., Vasconcelos, A. C., and Triemer, R. E. (1987). Structure of the euglenoid storage carbohydrate, paramylon. *Am. J. Bot.* 74:877–82.
- Krinsky, N. I., and Goldsmith, T. H. (1960). The carotenoids of the flagellated alga, *Euglena gracilis*. *Arch. Biochem. Biophys.* 91:271–9.
- Leander, B. S., and Farmer, M. A. (2000). Comparative morphology of the euglenoid pellicle. I. Patterns of strips and pores. *J. Eukary. Microbiol.* 47:469–79.
- Leander, B. S., and Farmer, M. A. (2001a). Comparative morphology of the euglenoid pellicle. II. Diversity of strip substructure. *J. Eukary. Microbiol.* 48:202–17.
- Leander, B. S., and Farmer, M. A. (2001b). Evolution of *Phacus* (Euglenophyceae) as inferred from pellicle morphology and SSU rDNA. *J. Phycol.* 37:143–59.
- Leedale, G. F. (1959). Periodicity of mitosis and cell division in the Euglenineae. *Biol. Bull.* 116:162–74.
- Leedale, G. F. (1967). *Euglenoid Flagellates*. Englewood Cliffs, NJ: Prentice Hall.
- Leedale, G. F. (1970). Phylogenetic aspects of nuclear cytology in the algae. *Ann. NY Acad. Sci.* 175(2):429–53.
- Leedale, G. F. (1975). Envelope formation and structure in the euglenoid genus *Trachelomonas*. *Br. Phycol. J.* 10:17–41.
- Loneragan, T. A. (1983). Regulation of cell shape in *Euglena gracilis*. I. Involvement of the biological clock, respiration, photosynthesis, and cytoskeleton. *Plant Physiol.* 71:719–30.
- Marin, B. (2004). Origin and fate of chloroplasts in the Euglenoida. *Protist* 155:13–14.
- Mignot, J-P. (1966). Structure et ultrastructure de quelques Euglénomonadines. *Protistologica* 2:51–117.
- Murata, K., Okamoto, M., and Suzaki, T. (2000). Morphological change of cell-membrane-integrated crystal structure induced by cell shape change in *Euglena gracilis*. *Protoplasma* 214:73–9.
- Ngô, H. M., and Bouck, G. B. (1998). Heterogeneity and a coiled coil predication of trypanosomatid-like flagellar rod proteins in *Euglena*. *J. Eukary. Microbiol.* 45:323–33.
- Nudelman, A. A., Rossa, M. S., Conforti, V., and Triemer, R. E. (2003). Phylogeny of Euglenophyceae based on small subunit rDNA sequences: taxonomic implications. *J. Phycol.* 39:226–35.
- Olli, K. (1996). Resting cyst formation of *Eutreptiella gymnastica* (Euglenophyceae) in the northern coastal Baltic Sea. *J. Phycol.* 32:535–42.
- Palmer, J. D., and Round, F. E. (1965). Persistent, vertical-migration rhythms in the benthic microflora. I. The effect of light and temperature on the rhythmic behaviour of *Euglena obtusa*. *J. Mar. Biol. Assoc. UK* 45:567–82.
- Preisfeld, A., Berger, S., Busse, I., Liller, S., and Ruppel, H. G. (2000). Phylogenetic analyses of various euglenoid taxa (Euglenozoa) based on 18S rDNA sequence data. *J. Phycol.* 36:220–6.
- Pringsheim, E. G. (1953). Observations on some species of *Trachelomonas* grown in culture. *New Phytol.* 52:93–113, 238–66.
- Rosowski, J. R., and Willey, R. L. (1975). *Colacium libellae* sp. nov. (Euglenophyceae), a photosynthetic inhabitant of the larval damselfly rectum. *J. Phycol.* 11:310–15.
- Saito, A., Suetomo, Y., Arikawa, M., et al. (2003) Gliding movement in *Peranema trichophorum* is powered by flagellar surface motility. *Cell Motil. Cytoskel.* 55:244–53.
- Sekiguchi, H., Moriya, M., Nakayama, T., and Inouye, I. (2002). Vestigial chloroplasts in heterotrophic stramenopiles *Pteridomonas danica* and *Ciliophrys infusionum* (Dictyochophyceae). *Protist* 153:157–67.

- Shin, W., and Triemer, R. E. (2004). Phylogenetic analysis on the genus *Euglena* (Euglenophyceae) with particular reference to the species *Euglena viridis*. *J. Phycol.* 40:759–71.
- Sperling Pagni, P. G., Walne, P. L., and Pagni, R. M. (1981). On the occurrence of  $\alpha$ -carotene in isolated stigmata of *Euglena gracilis* var. *bacillaris*. *Phycologia* 20:431–4.
- Surek, B., and Melkonian, M. (1986). A cryptic cytosome is present in *Euglena*. *Protoplasma* 133:39–49.
- Talke, S., and Preisfield, A. (2002). Molecular evolution of euglenozoan paraxonemal rod genes *par1* and *par2* coincides with phylogenetic reconstruction based on small subunit rDNA data. *J. Phycol.* 38:995–1003.
- Thronsdon, J. (1969). Flagellates of Norwegian coastal waters. *Nytt Mag. Bot.* 16:161–216.
- Thronsdon, J. (1973). Fine structure of *Eutreptiella gymnastica* (Euglenophyceae). *Norw. J. Bot.* 20:271–80.
- Walne, P. L. (1980). Euglenoid flagellates. In *Phytoflagellates*, ed. E. Cox, pp. 165–212. North Holland: Elsevier.
- West, L. K., and Walne, P. L. (1980). *Trachelomonas hispida* var. *coronata* (Euglenophyceae). II. Envelope substructure. *J. Phycol.* 16:498–506.
- West, L. K., Walne, P. L., and Bentley, J. (1980). *Trachelomonas hispida* var. *coronata* (Euglenophyceae). III. Envelope elemental composition and mineralization. *J. Phycol.* 16:582–91.
- Willey, R. L. (1972). The damselfly (*Odonata*) hindgut as a host for the euglenoid *Colacium*. *Trans. Am. Microsc. Soc.* 91:585–93.
- Willey, R. L. (1984). Fine structure of the mucocysts of *Colacium calvum* (Euglenophyceae). *J. Phycol.* 20:426–30.
- Willey, R. L., and Wibel, R. G. (1985a). The reservoir cytoskeleton and a possible cytosomal homologue in *Colacium* (Euglenophyceae). *J. Phycol.* 21:570–7.
- Willey, R. L., and Wibel, R. G. (1985b). A cytosome/cytopharynx in green euglenoid flagellates (Euglenales) and its phylogenetic implications. *BioSystems* 18:369–76.
- Willey, R. L., Bowen, W. R., and Durban, E. M. (1970). Symbiosis between *Euglena* and damselfly nymphs is seasonal. *Science* 170:80–1.
- Willey, R. L., Durban, E. M., and Bowen, W. R. (1973). Ultrastructural observations of a *Colacium palmella*: The reservoir, eyespot, and flagella. *J. Phycol.* 9:211–15.

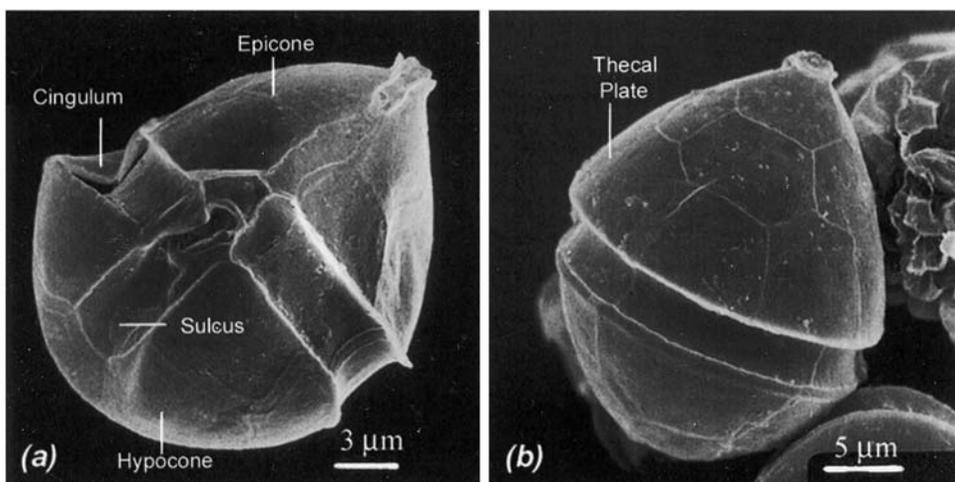
## Dinophyta

### DINOPHYCEAE

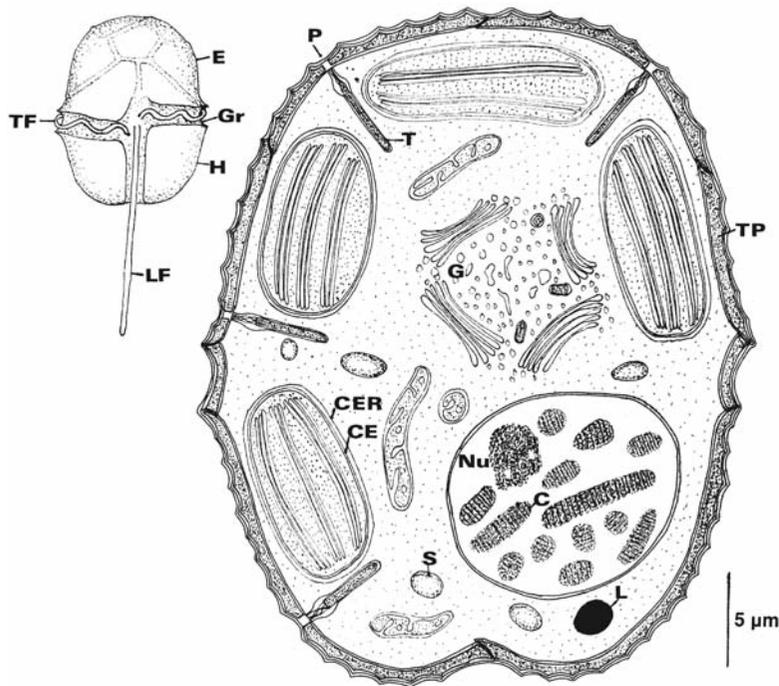
These organisms are important members of the plankton in both fresh and marine waters, although a much greater variety of forms is found in marine members. Generally the Dinophyceae are less important in the colder polar waters than in warmer waters. The highly elaborate Dinophysales (Fig. 7.56(d), (e)) are essentially a tropical group.

A typical motile dinoflagellate (Figs. 7.1, 7.2) consists of an **epicone** and **hypocone** divided by the transverse **girdle** or **cingulum**. The epicone and hypocone are normally divided into a number

of **thecal plates**, the exact number and arrangement of which are characteristic of the particular genus (Figs. 7.1, 7.3, 7.21(b), 7.25(b)). There is a **longitudinal sulcus** running perpendicular to the girdle. The longitudinal and transverse flagella emerge through the thecal plates in the area where the girdle and sulcus meet. The longitudinal flagellum projects out from the cell, whereas the transverse flagellum is wave-like and is closely appressed to the girdle. The cells can be photosynthetic or colorless and heterotrophic. Photosynthetic organisms have chloroplasts surrounded by one membrane of chloroplast E.R., which is not continuous with the outer



**Fig. 7.1** *Scrippsiella trochoidea*. Scanning electron micrographs showing the thecal plates in ventral (a) and dorsal (b) view. (From Janofske, 2000.)



**Fig. 7.2** Light and electron microscopical drawings of *Peridinium* sp., a dinoflagellate showing many of the features of the class. (C) Chromosome; (CE) chloroplast envelope; (CER) chloroplast endoplasmic reticulum; (E) epicone; (G) Golgi apparatus; (Gr) girdle; (H) hypocone; (L) lipid globule; (LF) longitudinal flagellum; (Nu) nucleolus; (P) trichocyst pore; (S) starch; (T) trichocyst; (TF) transverse flagellum; (TP) thecal plate.

membrane of the nuclear envelope. Chlorophylls  $a$  and  $c_2$  are present in the chloroplasts, with **peridinin** and **neoperidinin** being the main carotenoids. About half of the Dinophyceae that have been examined by electron microscopy have pyrenoids in the chloroplasts (Dodge and Crawford, 1970). The storage product is **starch**, similar to the starch of higher plants (Vogel and Meeuse, 1968), which is found in the cytoplasm. An eyespot may be present. The nucleus has permanently condensed chromosomes and is called a **dinokaryotic** or **mesokaryotic** nucleus.

## Cell structure

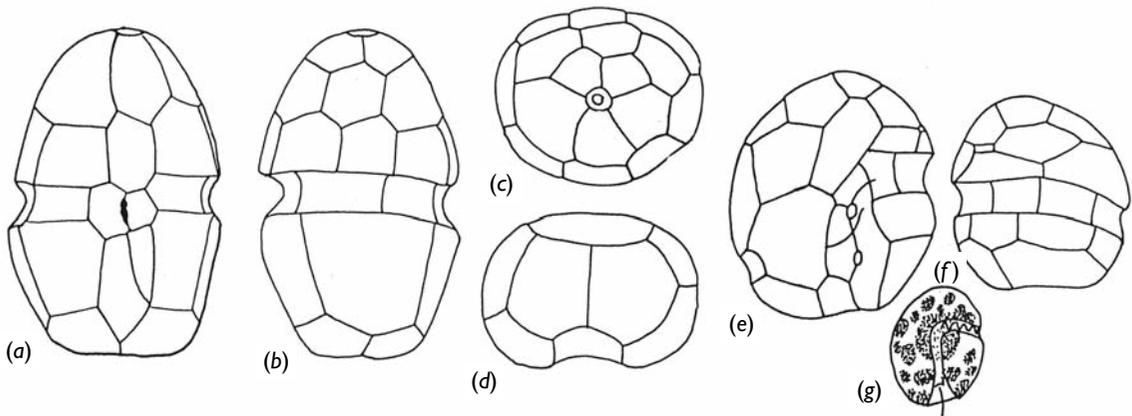
### Theca

The thecal structure of motile Dinophyceae consists of an outer plasmalemma beneath which lies a single layer of flattened vesicles (Figs. 7.2, 7.3(c), 7.5) (Dodge and Crawford, 1970; Sekida et al., 2004). These vesicles, which normally contain cellulosic plates, give the theca its characteristic structure. The actual form and arrangement of the thecal plates varies from none in the phagotrophic *Oxyrrhis marina*, to very thick plates

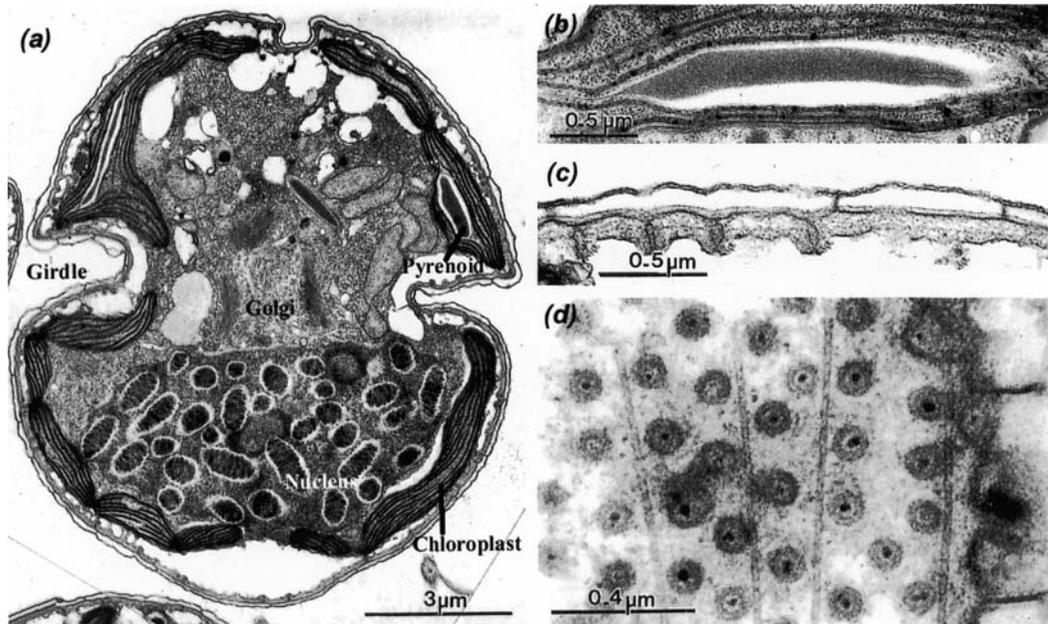
with flanges at the edges in *Ceratium* (Figs. 7.12, 7.57) and *Peridinium* spp. (Figs. 7.2, 7.11).

**Amphiesma** is the term used to specify the outermost layers of the dinoflagellate cell (Schütt, 1895) and includes all of the thecal plates with their lists (ribs), the peripheral vesicles, and the attendant microtubules. All types of amphiesma have the plasma membranes (continuous with the flagellar membrane) on the outside. The most complex type of amphiesma (Fig. 7.5(a)) consists of a single-membrane-bounded vesicle under the plasma membrane. Inside this vesicle are a number of cellulosic thecal plates subtended by a proteinaceous pellicle (Morrill and Loeblich, 1983a). A less complex amphiesma consists of a number of vesicles under the plasma membrane, each vesicle containing a thecal plate (Fig. 7.5(b), (c)). The least complex amphiesma has a number of vesicles without plates under the plasma membrane (Fig. 7.5(d)). Numerous pores generally occur within the amphiesma with a trichocyst beneath each pore (Fig. 7.4).

Outside of the plasma membrane of *Amphidinium carteri* (Fig. 7.16) there is a **glycocalyx** composed of acidic polysaccharides. The glycocalyx probably is formed by the discharge of



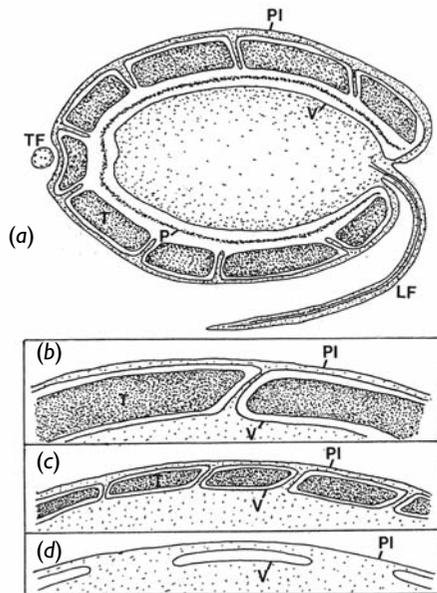
**Fig. 7.3** (a)–(d) *Cachonina niei* showing the arrangement of thecal plates: (a) ventral, (b) dorsal, (c) apical, and (d) posterior views. (e)–(g) *Cryptothecodinium cohnii*: (e) ventral and (f) dorsal views, (g) living cell. ((a)–(d) after Loeblich, 1968; (e)–(g) after Chatton, 1952.)



**Fig. 7.4** Transmission electron micrographs of sections of cells of *Karlodinium veneficum*. (a) Cell. (b) Detail of pyrenoid in chloroplast. (c) Transverse section of amphiesma showing amphiesmal vesicles. (d) Tangential section of cell showing plugs in amphiesma. (From Daugbjerg et al., 2000.)

cytoplasmic mucocysts to the outside of the cell. Such a glycocalyx probably exists in most Dinophyceae (Klut et al., 1985).

In many dinoflagellates, cell division usually involves sharing of the mother cell thecal plates between the daughter cells, with the daughter cells producing the new thecal plates that they lack. However, in certain peridinioid genera the

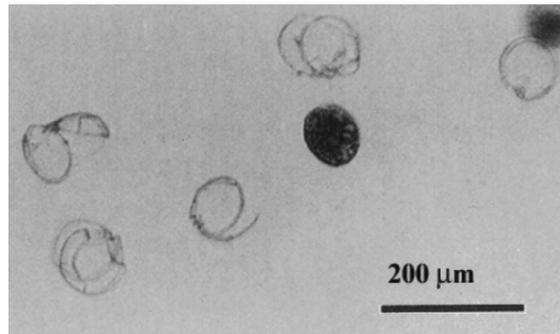


**Fig. 7.5** Representative drawings of different types of amphiesmal arrangements in the Dinophyceae. (a) Cross section of a dinoflagellate with the most complex type of amphiesma. The outer plasma membrane (PI) is continuous with the flagellar membrane. The cellulosic thecal plate (T) and a proteinaceous pellicle (P) are enclosed in a large vesicle (V). (b)–(d) Less complicated types of amphiesma occurring in other Dinophyceae. (LF) Longitudinal flagellum; (TF) transverse flagellum. (After Morrill and Loeblich, 1983a.)

theca is completely shed (ecdysis) at cell division, followed by the formation of a thickened pellicle around the cell to form an ecdysal cyst (Bricheux et al., 1992; Höhfeld and Melkonian, 1992). New thecal vesicles are produced under the pellicle with the cell escaping from the cyst (Figs. 7.6, 7.7). Ecdysis in *Gambierdiscus toxicus* is induced by a glycerol compound (Fig. 7.8) that is released by the green alga *Bryopsis* sp. (Sakamoto et al., 2000). The dinoflagellate occupies the thallosphere of this green alga.

### Scales

Although relatively rare, scales occur outside the plasma membrane in some Dinophyceae (Figs. 7.9, 7.10) (Sekida et al., 2003). In *Heterocapsa*, the scales are formed in Golgi vesicles that migrate to the area of the basal bodies, where the scales are released outside the cell (Morrill and Loeblich, 1983b).

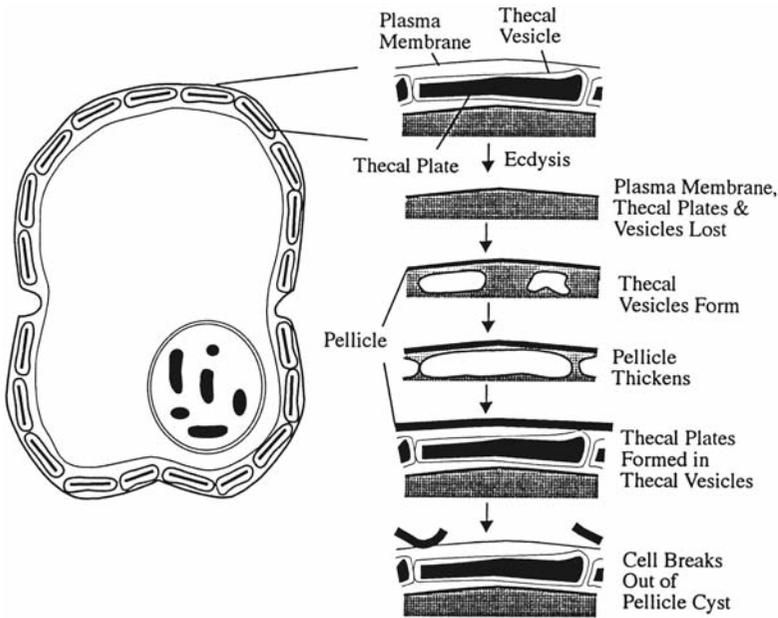


**Fig. 7.6** Ecdysis in *Gambierdiscus toxicus*. (From Sakamoto et al., 2000.)

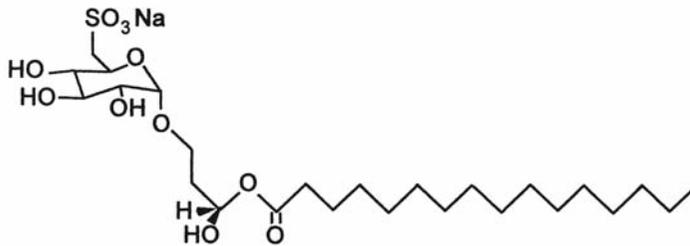
### Flagella

Generally, dinoflagellates have a transverse flagellum that fits into the transverse girdle and a longitudinal flagellum that projects out from the longitudinal sulcus (Figs. 7.2, 7.11, 7.13) (Roberts and Roberts, 1991). The two flagella are inserted into the cell in the area of the intersection of the girdle and sulcus. The longitudinal flagellum usually has a wide basal portion and a thinner apical portion. In the basal portion the flagellar sheath surrounds packing material plus the axoneme, whereas at the apical portion the flagellar sheath surrounds only the axoneme. Fibrillar hairs 0.5  $\mu\text{m}$  long and 10 nm wide may cover the entire flagellum (Leadbeater and Dodge, 1967a). Mechanical stimulus of cells of *Ceratium tripos* (Fig. 7.12) causes the longitudinal flagellum to be retracted and folded so that the flagellum lies along the sulcus (Maruyama, 1982). The longitudinal flagellum contains an R-fiber running along the length of the flagellum. Retraction of the flagellum occurs when the R-fiber contracts to one third of its length. Influx of  $\text{Ca}^{2+}$  into the longitudinal flagellum causes retraction of the R-fiber (Maruyama, 1985).

The transverse flagellum is about two to three times as long as the longitudinal flagellum and has a helical shape (Figs. 7.12, 7.13, 7.14). The transverse flagellum consists of (1) an axoneme whose form approximates a helix, (2) a striated strand that runs parallel to the longitudinal axis of the axoneme but outside the loops of the coil, and (3) a flagellar sheath that encloses both axoneme and striated strand (Berdach, 1977). The striated strand

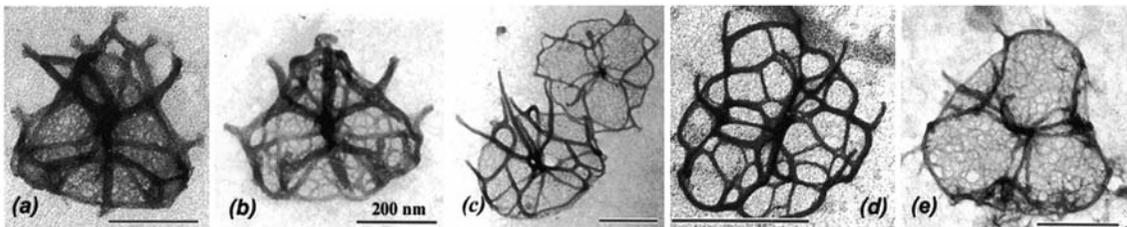


**Fig. 7.7** Ecdysis and the formation of new thecal vesicles in *Glenodinium foliaceum*. Ecdysis results in the loss of the plasma membrane, thecal plates, and vesicles. A pellicle forms from a layer that existed under the thecal vesicles. New thecal vesicles and plates are formed under the thickening pellicle. When the amphiesma is mature, the wall breaks out of the pellicle cyst. (Drawn from transmission electron micrographs in Bricheux et al., 1992.)

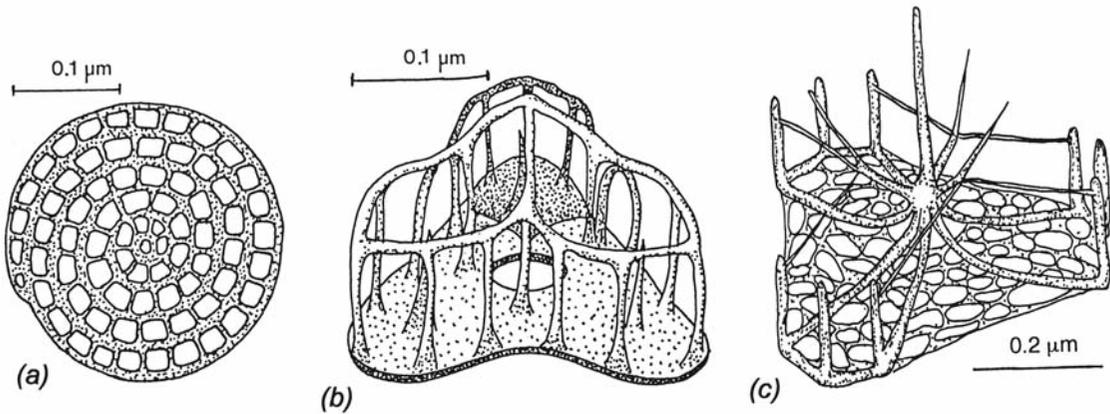


**Fig. 7.8** The compound released by the green alga *Bryopsis* that induces ecdysis in the dinoflagellate *Gambierdiscus toxicus*.

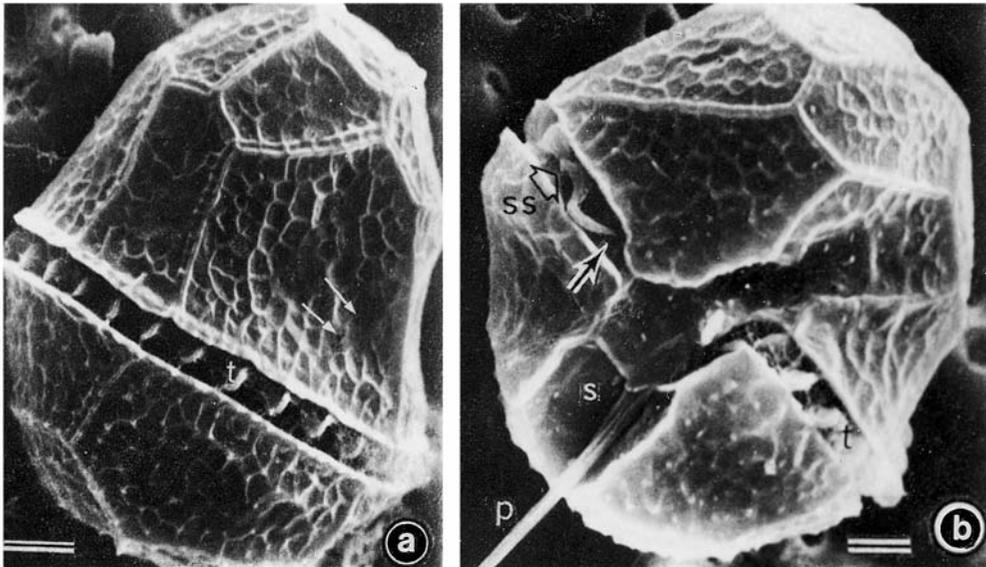
1-O-palmitoyl-3-O-(6'-sulfo-a-D-quinovopyranosyl)-sn-glycerol (PSQG)



**Fig. 7.9** Transmission electron micrographs of scales of different species of *Heterocapsa*. (a) *H. triquetra*. (b) *H. arctica*. (c) *H. circularisquama*. (d) *H. horiguchi*. (e) *H. ildefina*. (From Iwataki et al., 2004.)



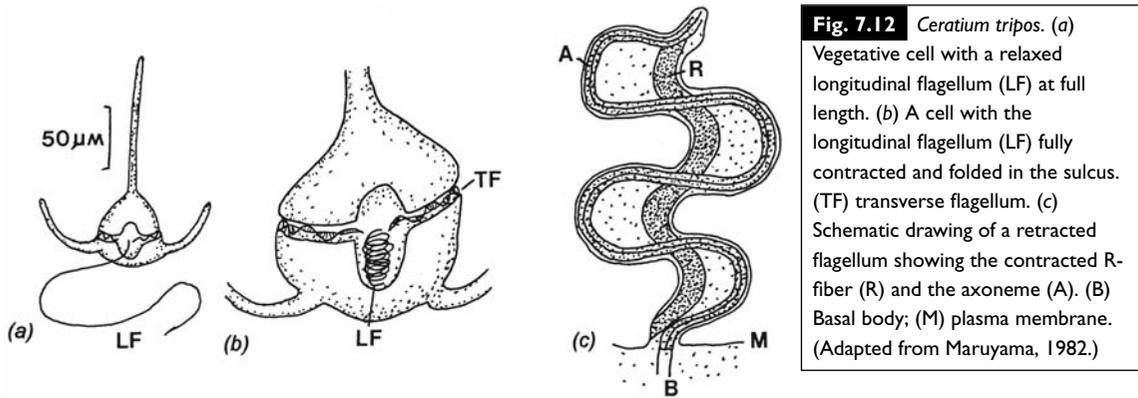
**Fig. 7.10** Dinoflagellate scales. (a) *Oxyrrhis marina*. (b) *Heterocapsa niei*. (c) *Katodinium rotundatum*. ((a) after Clarke and Pennick, 1976; (b) after Morrill and Loeblich, 1983b; (c) after Hansen, 1989.)



**Fig. 7.11** Dorsal and ventral views of cells of *Peridinium cinctum* in the scanning electron microscope. (p) Posterior (longitudinal) flagellum; (s) longitudinal sulcus; (ss) striated strand; (t) transverse flagellum. Bar = 5 µm. (From Berdach, 1977.)

contains **centrin**, a  $\text{Ca}^{2+}$ -modulated contractile protein (Höhfeld et al., 1988). Contraction of the striated strand leads to supercoiling of the axoneme. On one side of the axoneme, a unilateral row of fibrillar hairs, 2 to 4 µm long and 10 nm wide,

is attached to the flagellar sheath. The final micrometer of the flagellum lacks the striated strand and hairs, with the axoneme tightly covered by the flagellar sheath. The axoneme is a left-handed screw; waves propagated from the attached end toward the free end create a downward thrust at the outermost edges of the coil. The flagellum causes a direct forward movement while at the same time causing the cell to rotate. The flagellar beat always proceeds

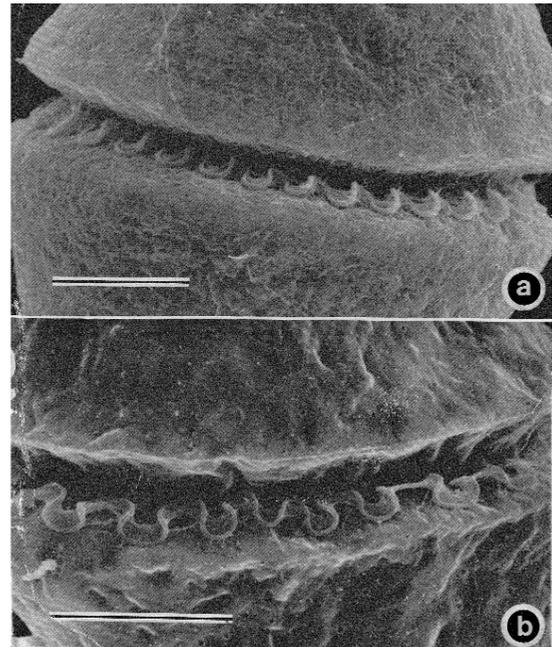


counterclockwise when seen from the cell apex (**leiotropic** direction). The cell always rotates in the direction of the flagellar beat (in the leiotropic direction), with the fluid propelled in the opposite direction (in the **dextrotropic** direction) (Fig. 7.15) (Gaines and Taylor, 1985; Fenchel, 2001). The longitudinal flagellum swings in a narrow orbit acting as a rudder.

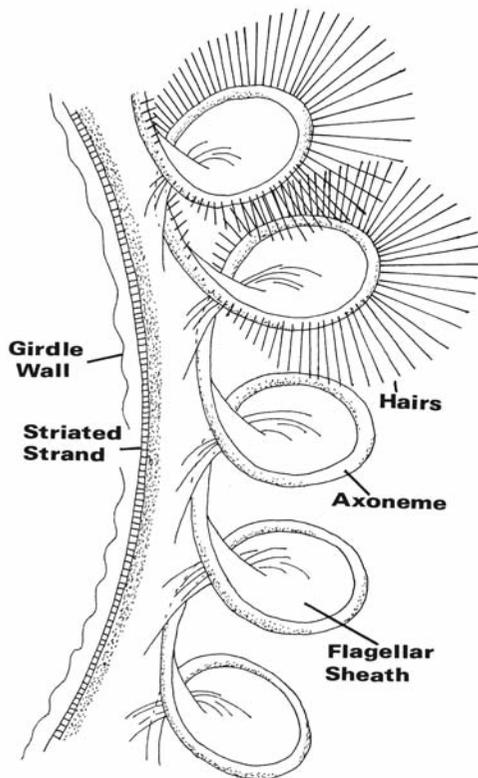
The dinoflagellates are the fastest swimmers among the algae although they are slower than *Mesodinium*, the photosynthetic symbiosis between a ciliate and a cryptophyte (see Chapter 9, Cryptophyta, Symbiotic associations). Dinoflagellates swim from 200 to 500  $\mu\text{m s}^{-1}$  (Raven and Richardson, 1984). The cells of *Lingulodinium polyedrum* (Fig. 7.40) swim at a linear rate of 250  $\mu\text{m s}^{-1}$  at 20°C whereas cells of *Gyrodinium* swim at a mean linear rate of velocity of 319  $\mu\text{m s}^{-1}$  at 20°C. Marine dinoflagellates frequently move into deeper, nutrient-rich waters at night and better illuminated waters near the surface during the day. The **diel** (over a 24-hour period) migrations of dinoflagellates are 5 to 10 m in relatively quiet waters. A dinoflagellate swimming at 500  $\mu\text{m s}^{-1}$  would take about 6 hours of the 24-hour period for 5 m of upward migration around dawn, and a further 6 hours for downward migration around dusk (Raven and Richardson, 1984).

### Pusule

A **pusule** is a sac-like structure that opens by means of a pore into the flagellar canal and probably has an osmoregulatory function similar to that of a contractile vacuole. The pusule of



*Amphidinium carteri* is representative of those dinoflagellates that have a pusule (Fig. 7.16) (Dodge and Crawford, 1968). In this organism there are two pusules, one associated with each flagellar canal. The pusule consists of about 40 vesicles which open by small pores into the flagellar canal. Whereas the flagellar canal is lined by a single membrane continuous with the plasma



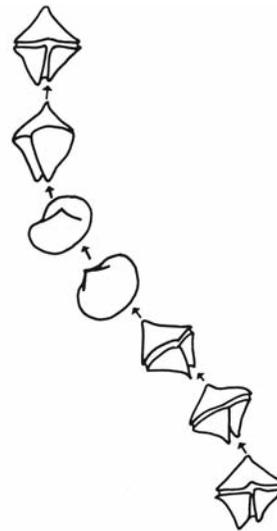
**Fig. 7.14** Diagram of part of the transverse flagellum of *Peridinium cinctum*. Only a portion of the fibrillar hairs have been drawn in. (After Berdach, 1977.)

membrane, the pusule vesicles are lined by a double membrane.

### Chloroplasts and pigments

The plastids of most photosynthetic dinoflagellates originated from a secondary endosymbiosis with a red alga. These plastids are surrounded by three membranes (two membranes of chloroplast envelope and one membrane of chloroplast endoplasmic reticulum) (Fig. 7.2) and contain chlorophyll *a* and *c*, and peridinin (Fig. 7.17) as the major photosynthetic pigments (Ishida and Green, 2002).

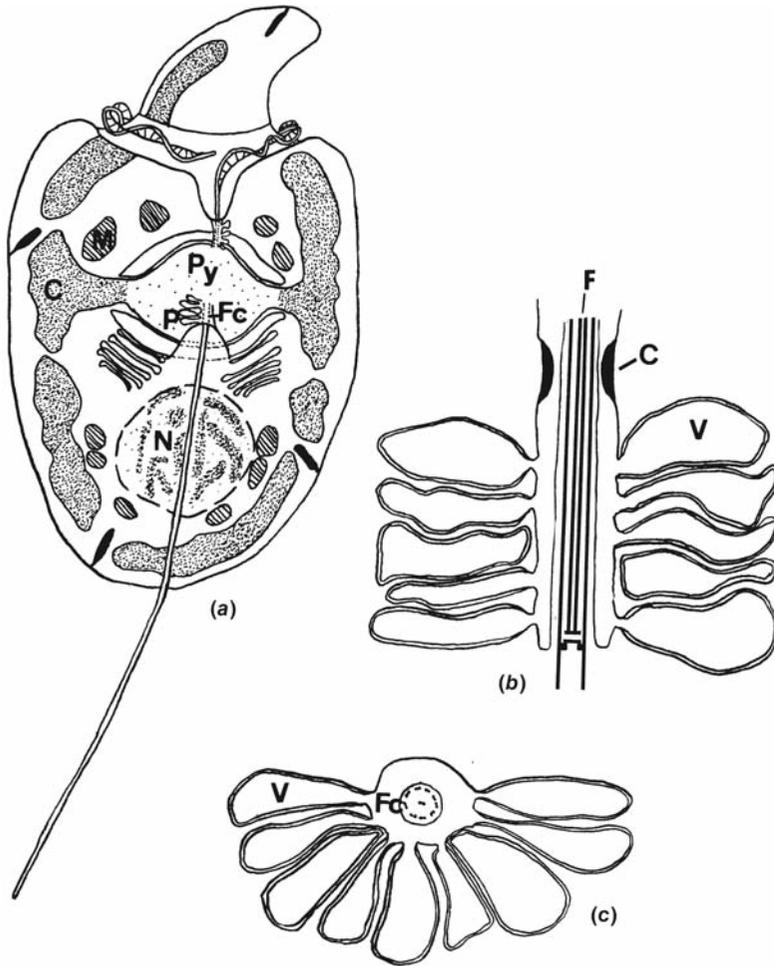
There are a small number of dinoflagellates that have plastids derived from a tertiary endosymbiosis (Fig. 7.18). **Tertiary endosymbiosis** begins with the loss of the plastid (originally derived from a secondary endosymbiosis) from a dinoflagellate followed by endosymbiosis of an



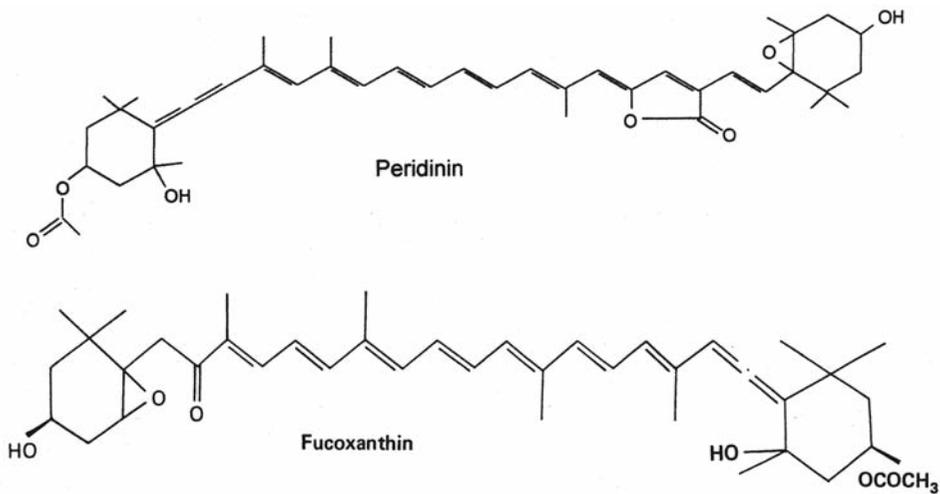
**Fig. 7.15** Tracings from successive video frames of *Protoperidinium conicum* swimming, showing the leiotropic rotation of the cell. Time between intervals is 1/5 second. (From Gaines and Taylor, 1985.)

alga from the Prymnesiophyceae (a haptophyte alga). All of the protoplasm of the endosymbiont was lost, except for the chloroplast surrounded by one membrane of chloroplast endoplasmic reticulum, which became the permanent chloroplast of the dinoflagellate. Instead of peridinin, these algae have 19'-hexanoyloxy-fucoxanthin (Fig. 7.17) and/or 19'-butanoyloxy-fucoxanthin, pigments characteristic of the Prymnesiophyceae. *Karenia brevis* (Fig. 7.19(a)), *Karenia mikimotoi* (Fig. 7.19(b)), and *Karlodinium veneficum* (Fig. 7.34) are dinoflagellates that are derived from tertiary endosymbioses (Ishida and Green, 2002; Takishita et al., 2005; Yoon et al., 2002).

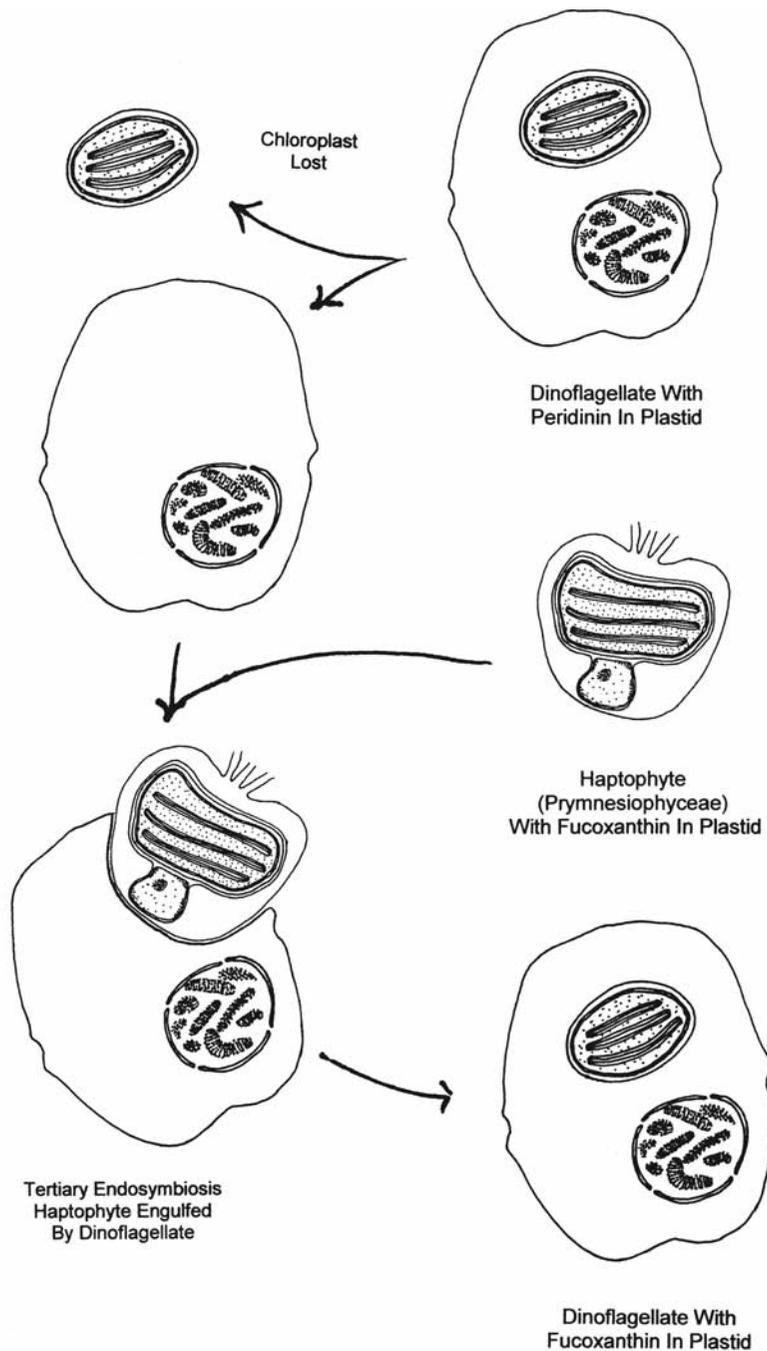
A number of dinoflagellates contain short-term plastids stolen from their food source (**kleptoplastids**) and it is sometimes difficult to distinguish between these plastids and permanent plastids (Keeling, 2004). Dinoflagellates have transferred most of the plastid genome to the nucleus of the cell, making them the only eukaryotes that encode the majority of the plastid genes in the nucleus (Hackett et al., 2004). Having the genes for plastid-targeted proteins may actually be an advantage since it provides the cell with the freedom to replace the original plastid with a new one (Green,



**Fig. 7.16** (a) Drawing of *Amphidinium carteri*. The small epicone is separated from the hypocone by the encircling girdle, which contains the transverse flagellum. The peripheral chloroplast (C) is shown only at the sides of the cell so the positions of the nucleus (N), mitochondria (M), pyrenoid (Py), and the pusule (P) associated with the flagellar canals can be seen. (b) Longitudinal section through a pusule showing the flagellar pore constriction (C), the pusule vesicles (V), and the flagellum (F). (c) Transverse section of a pusule showing the flagellum within the flagellar canal (Fc) and the pusule vesicles (V) opening into the canal. (From Dodge and Crawford, 1968.)



**Fig. 7.17** The chemical structure of peridinin and 19'-hexanoyloxy-fucoxanthin. Peridinin has three rings instead of the two found in other light-harvesting carotenoids.

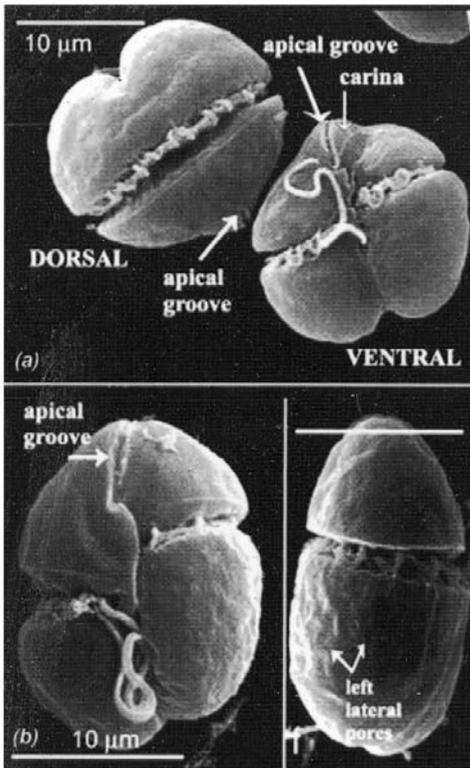


**Fig. 7.18** The sequence of events leading to plastids evolved from a tertiary endosymbiosis.

2004). The plastid genome of peridinin-containing dinoflagellates is reduced and broken up into minicircles that encode only 16 proteins.

Most of the chlorophyll *a* and peridinin occur

together in a water-soluble protein complex called peridinin-chlorophyll *a*-protein (PCP). Within the chromophore the peridinin and chlorophyll *a* are in a 4:1 ratio (Prézelin and Haxo, 1976). PCP is similar to the phycobiliproteins in cyanobacteria



**Fig. 7.19** Scanning electron micrographs of (a) *Karenia (Gymnodinium) brevis* and (b) *Karenia (Gymnodinium) mikimoto*. (From Haywood et al., 2004.)

and red algae in that it is on the surface of thylakoids, is water-soluble, and acts as a light-harvesting pigment. If *Glenodinium* (Fig. 7.22) is cultured under decreasing light intensity (to  $\frac{1}{12}$  of

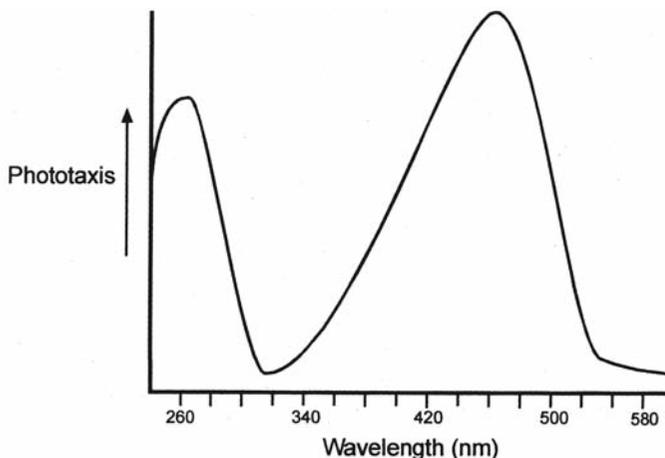
the original light intensity) the amount of PCP increases about seven fold, whereas the amount of chlorophyll *a* and peridinin not associated with PCP increases only 1.5 times. Little change occurs in chlorophyll *c* (Prézelin, 1976). This is a type of chromatic adaptation; as the cells receive less light (i.e., grow in deeper water), they produce more PCP, with the peridinin capturing light and passing it to chlorophyll *a*.

### Phototaxis and eyespots

The action spectra for phototaxis is the same in all dinoflagellates that have been studied, with maximum phototaxis obtained at a wavelength of 450 nm (Fig. 7.20) (Horiguchi et al., 1999). An eyespot is not necessary for a phototactic response, indicating that the phototactic machinery was carried in the host organisms in the endosymbiosis leading to photosynthetic dinoflagellates (e.g., the phototactic receptor is not in the eyespot but is probably associated with the plasma membrane).

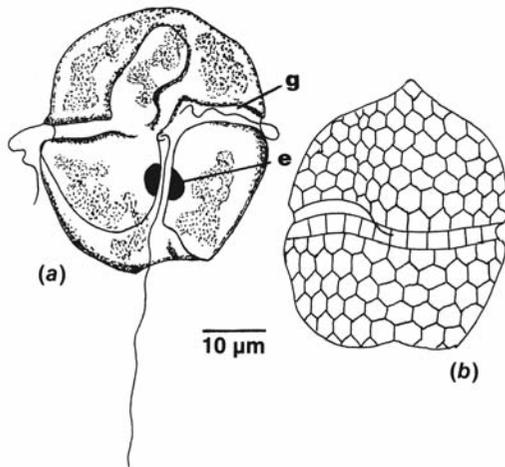
Less than 5% of the Dinophyceae contain eyespots, and those that do are mostly freshwater species; yet the eyespots are among the most complex in the algae.

The simplest type of eyespot consists of a collection of lipid globules in the cytoplasm not surrounded by a membrane (e.g., *Woloszynskia coronata*) (Dodge, 1971) (Fig. 7.21). A second type of eyespot consists of a row of lipid globules in a plastid-like structure at the cell periphery (e.g., *Peridinium westii*, *W. tenuissima*) (Messer and Ben-Shaul, 1969; Crawford et al., 1970).



**Fig. 7.20** The effect of different wavelengths of light on phototaxis in dinoflagellates. Maximum photosynthesis occurs at a wavelength around 450 nm. (Adapted from Horiguchi et al., 1999.)

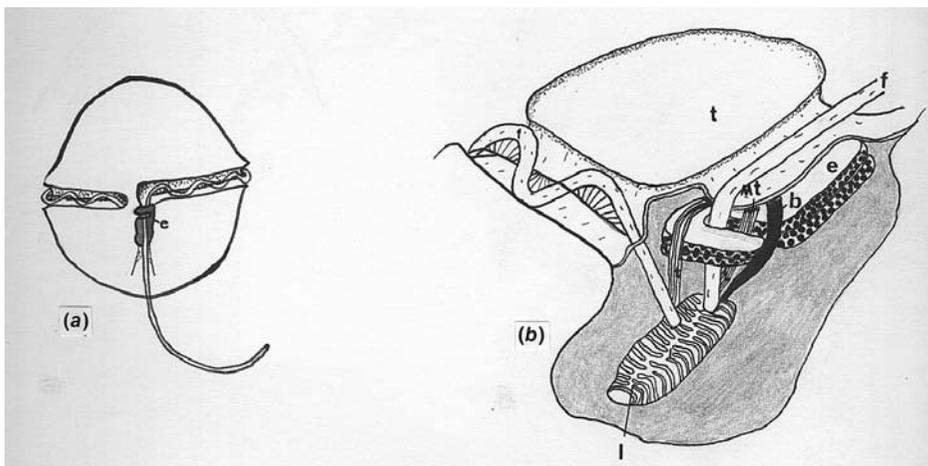
The eyespot of *Glenodinium foliaceum* (Dodge and Crawford, 1969) is immediately under the anterior portion of the sulcus and is about 6  $\mu\text{m}$  long and 3  $\mu\text{m}$  wide (Fig. 7.22). It is more or less rectangular in outline with a hook-shaped projection at the anterior end. This flattened sac-like structure contains



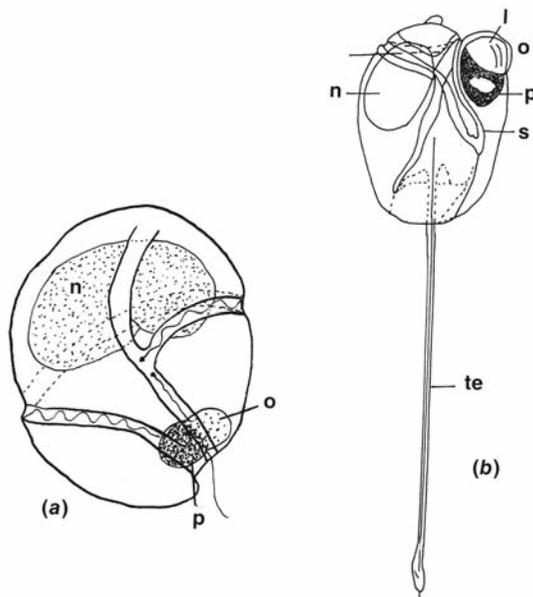
**Fig. 7.21** *Woloszynskia tenuissima*. (a) Ventral view showing the two flagella, the girdle (g), and the eyespot (e). (b) Side view showing the thecal plates, which were not drawn in (a). (After Crawford et al., 1970.)

two rows of large lipid globules separated by a granular space. Surrounding the eyespot is a triple-membrane envelope identical in appearance to that surrounding the chloroplasts. Adjacent to the eyespot is a non-membrane-bounded lamellar body consisting of a stack of flattened vesicles arranged more or less parallel to one another. The lamellar body is about 2  $\mu\text{m}$  long and 0.75  $\mu\text{m}$  wide, and contains up to 50 vesicles. The vesicles are connected at their edges, and also at the ends of the stack, with rough endoplasmic reticulum.

The most complex type of eyespot is found in the Warnowiaceae of the Peridinales. The eyespot in *Nematodinium armatum* (Moronin and Francis, 1967) and that in *Erythroopsis cornuta* (Gruet, 1965) have been studied at the fine-structural level and found to be essentially similar in construction (Figs. 7.23, 7.24). In *N. armatum*, the eyespot is located toward the rear of the cell alongside the girdle, and consists of a lens mounted in front of a pigment cup, oriented so that the axis through the center of the lens and pigment cup is nearly perpendicular to the longitudinal axis of the cell body. The lens lies just below the plasmalemma. The pigment cup is made up of three main parts. Most of its wall consists of

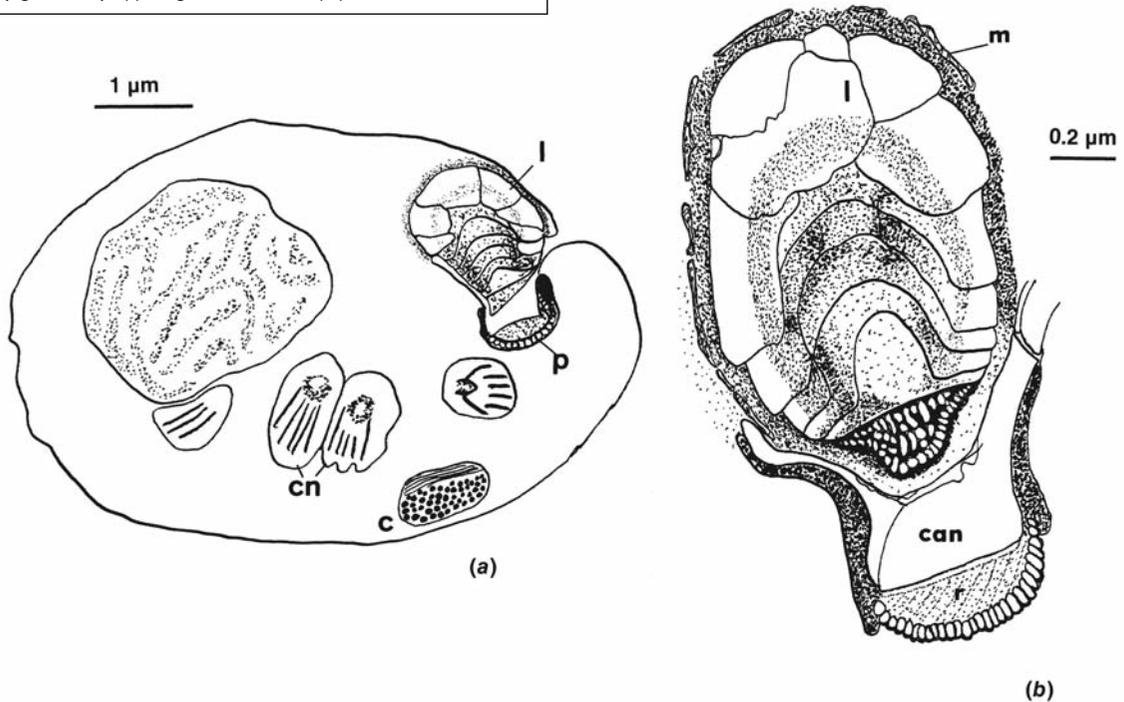


**Fig. 7.22** (a) A ventral view of a cell of *Glenodinium foliaceum* showing the location of the eyespot (e). (b) Three-dimensional diagram of the eyespot area. The two flagella arise just above the lamellar body (l). The eyespot (e) lies beneath the sulcus. (mt) Microtubular roots; (b) banded root; (t) thecal plate. (After Dodge and Crawford, 1969.)



**Fig. 7.23** (a) *Nematodinium armatum*. (b) *Erythroopsis cornuta*. (l) Lens; (n) nucleus; (o) ocellus (eyespot); (p) pigment cup; (s) longitudinal sulcus; (te) tentacle.

a single layer of large oblong pigment granules  $0.3\ \mu\text{m}$  in diameter. Toward the lip of the cup, the pigment granules are smaller and loosely arranged in several layers. Within the base of the cup is a dense layer of fibrils  $33\ \text{nm}$  in diameter, parallel to the axis of the eyespot; this layer is covered by a mat of transverse fibrils and has been named the **retinoid** because of its supposed light-receptive function. Above the retinoid is a canal that opens into a furrow of the transverse flagellum; the outermost membrane on the cell surface is continuous with the membrane of the canal. The lens unit is a complex structure completely surrounded by mitochondria. Inside the mitochondria is a granular layer separated by a membrane from the lens. The central core of the lens consists of membrane-bounded domed or concentric layers of dense material; the major bulk of the lens surrounds this core and consists of several large, nearly empty lobes. Between the core and the lobes is a network of vesicles of intermediate size.



**Fig. 7.24** (a) A cell of *Nematodinium armatum*, showing the lens (l), pigment cup (p), cnidocysts (cn), and a chloroplast (c). (b) A complete eyespot (ocellus) with retinoid (r), canal leading to outside (can), and mitochondria (m) surrounding the lens (l). (After Moronin and Francis, 1967.)

## Nucleus

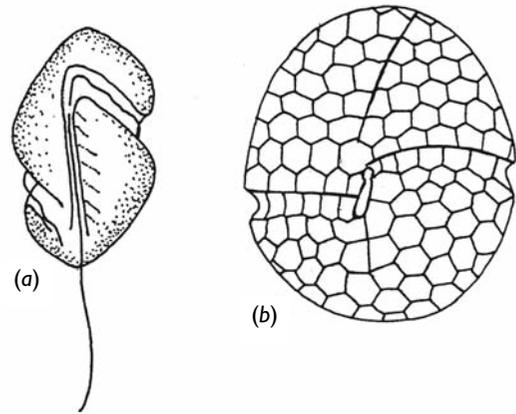
The first dinoflagellates to evolve had nuclei similar to other algae with a closed mitosis and histones associated with the nucleic acids (Taylor, 1999). *Oxyrrhis* (Fig. 7.56(a)) is representative of such a dinoflagellate having microtubules formed inside the nuclear membrane during mitosis (Triemer, 1982; Gao and Li, 1986). Histones were gradually lost as dinoflagellate evolution proceeded and a type of mitosis evolved with the mitotic spindle outside the nuclear membrane.

The nuclei of the more advanced dinoflagellates are striking cytologically in that they have their chromatin condensed into 2.5 nm fibrils in the interphase nuclei (Figs. 7.2, 7.4). The organization of the chromatin is different from either prokaryotic or eukaryotic cells and has been referred to as **mesokaryotic** or **dinokaryotic** (Rizzo, 1991).

Unicellular eukaryotic organisms usually have between 0.046 and 3 picograms (pg) of DNA per nucleus. Dinoflagellates, however, have much more DNA in their nuclei, with values ranging from 3.8 pg per nucleus in *Cryptothecodinium cohnii* (Fig. 7.3(e)–(g)) to 200 pg per nucleus in *Lingulodinium polyedrum* (Fig. 7.40(b)) corresponding to about 200 000 Mb (in comparison, hexploid *Triticum* wheat is 16 000 Mb and the haploid human genome is 3 180 Mb) (Sigee, 1984). This implies that a large amount of the DNA is genetically inactive (structural DNA) in dinoflagellates.

Nuclear division in the more advanced dinoflagellates has the nuclear envelope remaining intact during division, the nucleolus persisting and dividing by pinching in two, and the chromosomes attached to the nuclear envelope (Barlow and Triemer, 1988).

Mitosis in *Syndinium* (Fig. 7.25(a)) can be used as an example of the more advanced type in the dinoflagellates. The onset of division is usually marked by the duplication of the flagellar bases from two to four. Throughout this stage the nucleus enlarges, and many Y- and V-shaped chromosomes are found. The nucleus becomes invaginated, resulting in the formation of 1 to 15 channels traversing the dividing nucleus (Fig. 7.26). The channels contain tunnels of cytoplasm passing through the nucleus outside of the



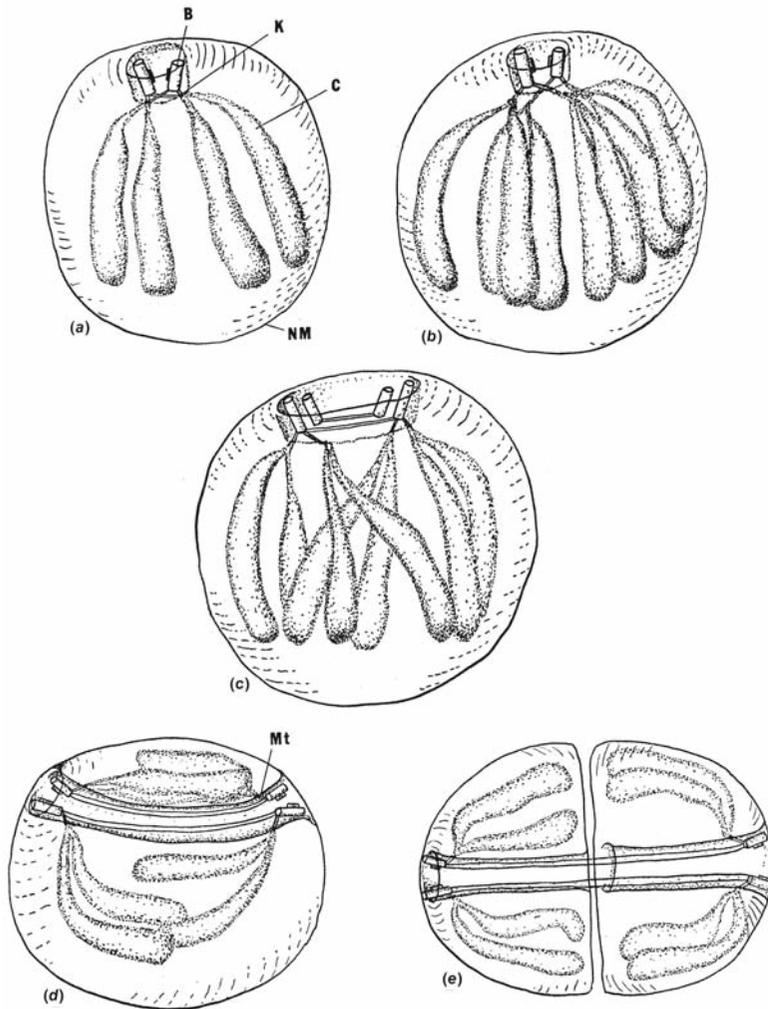
**Fig. 7.25** (a) *Syndinium turbo*. (b) *Gymnodinium neglectum*.  
((a) after Chatton, 1952; (b) after Schiller, 1933.)

nuclear membrane. There are bundles of microtubules in these channels, which are not connected to the intact nuclear envelope. The chromosomes are probably attached to the nuclear membrane or a specialized kinetochore in the nuclear membrane. At metaphase there is no formation of a metaphase plate as is common with eukaryotes, and the chromosomes are still scattered. The nucleolus persists throughout the whole nuclear cycle and divides by constricting in the middle. In anaphase the cell and nucleus expand laterally, and the chromosomes move to opposite ends of the nucleus. With continued lateral expansion, the central isthmus eventually severs, and the two daughter nuclei become independent (Leadbeater and Dodge, 1967b; Kubai and Ris, 1969; Ris and Kubai, 1974).

## Projectiles

The Dinophyceae have a number of different projectiles, which are fired out of the cell when it is irritated, resulting in a sudden movement of the cell in the opposite direction from the discharge.

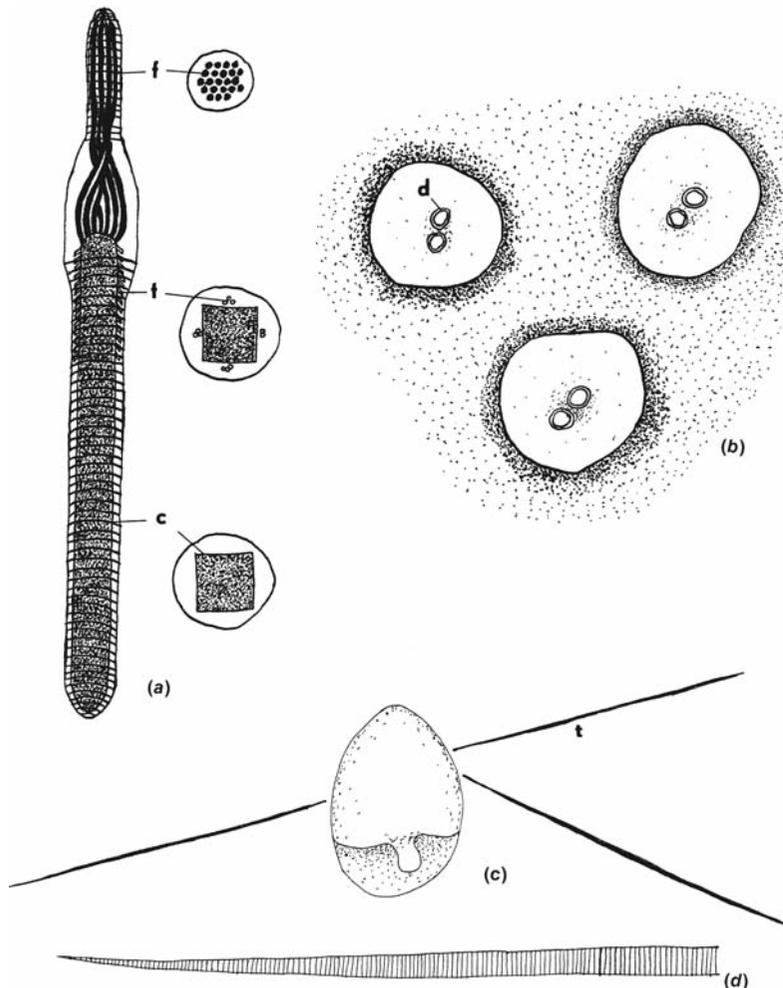
A **trichocyst** has a membrane-bounded, rod-shaped crystalline core (Bouck and Sweeney, 1966) (Fig. 7.27). Along the anterior one-third of the core are short, fine, tubular elements that project slightly downward. At the extreme outer end of the core, a group of 20 to 22 fibers extend from the outer part of the core to the enclosing membrane, and still finer fibrils then connect



**Fig. 7.26** Diagrammatic representation of nuclear division in *Syndinium* sp. (a) Interphase. (b) Early division; kinetochores and chromosomes have duplicated. (c) Early stage of chromosome segregation. Central spindle between separating basal bodies of flagella. (d) Late stage of chromosome separation. Central spindle in cytoplasmic channel through nucleus. (e) Division of nucleus. (B) Basal body of flagellum; (K) kinetochore; (C) chromosome; (Mt) microtubules; (NM) nuclear membrane. (After Ris and Kubai, 1974.)

the larger fibrils to the apical portion of the trichocyst membrane. Just within the enclosing membrane are fine, thread-like, opaque hoops. The outer part of the trichocyst membrane is attached to the plasma membrane between the thecal vesicles or to the thecal vesicles beneath round, thin areas of thecal plates that form trichocyst pores. Trichocysts originate in areas rich in Golgi bodies and are probably derived from them initially as spherical vesicles that eventually become spindle-shaped and develop into the trichocysts. On irritation a “charged” trichocyst is converted to a “discharged” trichocyst in a few milliseconds, possibly by the rapid uptake of water. The discharged trichocysts are straight,

tapering rods many times longer than the charged trichocyst (up to 200  $\mu\text{m}$  long in *Prorocentrum*) (Figs. 7.30(c), 7.55, 7.56(c)). The discharged trichocyst has transverse banding, with a major period of 50 to 80 nm. Although most dinoflagellates have trichocysts, ultrastructural investigations have shown that some do not (i.e., *Gymnodinium neglectum* (Fig. 7.25(b)), *Aureodinium pigmentosum*, *Woloszynskia tylota*, and the symbiotic *Symbiodinium microadriaticum*). The actual benefit of trichocysts to the cell (if any) is still obscure. They could be a mechanism for quick escape as the cells move sharply in the opposite direction of discharge, or they could be able to directly “spear” a naked intruder.



**Fig. 7.27** (a) Diagram of a longitudinal section and cross sections of a charged trichocyst of *Lingulodinium polyedrum*. The single membrane limiting the trichocyst is lined on its inner surface with fine hoops or spirals. Within the membrane is a crystalline core (c) composed of long rods or plates. Along the upper one-third of the core, short tubules (t) protrude downward and outward. At the anterior portion of the core, a series of fibers (f) attach to the core to still finer fibrils, which eventually reach to the anterior portion of the enclosing membrane. (b) Drawing of a segment of a wall plate of *Lingulodinium polyedrum*. Pore-like thin areas in the plate contain two or three slightly ridged discs (d) through which the trichocyst is discharged. (c) Cell of *Oxyrrhis* with discharged trichocysts. (d) Drawing of the tip of a discharged trichocyst showing striations. ((a),(b) after Bouck and Sweeney, 1966.)

### Accumulation body

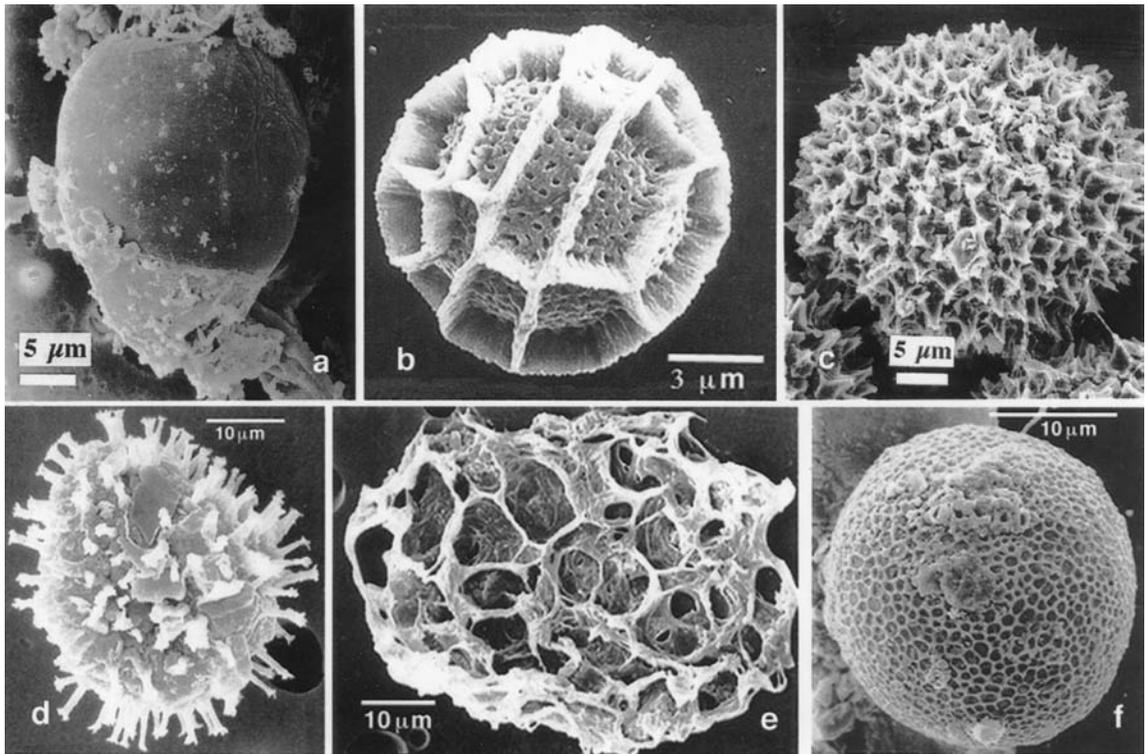
This is a large vesicle containing the remains of digested organelles (Figs. 7.36(c), 7.53(b)). It is probably similar to the Corps de Maupas of the Cryptophyceae and the digestive vesicles of other flagellates (Zhou and Fritz, 1994). An accumulation body is particularly common in symbiotic Dinophyceae.

### Resting spores or cysts or hyrinospores and fossil Dinophyceae

The resting spore or cyst of most dinoflagellates is morphologically distinct from the parent cell. They are 30 to 70  $\mu\text{m}$  in diameter with smooth or spinose bodies (Figs. 7.28, 7.36). The

newly formed cysts of *Scrippsiella trochoidea* have ten times more carbohydrate and 1.5% the respiratory rate of vegetative cells (Brooks and Andersen, 1990).

The cell walls of the cysts are highly resistant to decay and contain **dinosporin**, a chemical similar to sporopollenin in the pollen of higher plants (Kokinos et al., 1998). The cysts are extremely resistant and are preserved in ancient sediments where they are of value in paleoecological studies. Fossilized cysts are called **hystrichosphaerids** (hystrichospores) and are included in fossilized cyst-like structures of unicellular algae called **acritrachs**. These fossilized dinoflagellates first appeared in the Triassic and reached a peak in the Jurassic and Cretaceous, followed by a decrease in the Tertiary (MacRae et al., 1996).



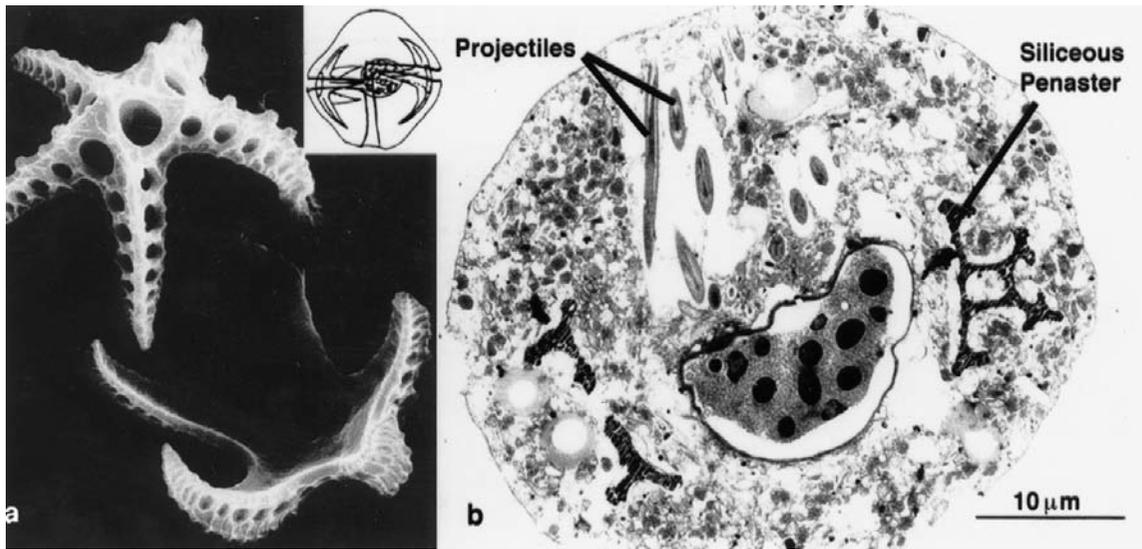
**Fig. 7.28** Dinoflagellate cysts. (a) *Alexandrium catenella*. (b) *Calciodinellum operosum*. (c) *Scrippsiella trophoidea*. (d) *Gonyaulax grindleyi*. (e) *Polykrikos schwartzii*. (f) *Gymnodinium catenatum*. ((d)–(f) from Ellegaard et al., 1994; (b) from Montresor et al., 1997; (a),(c) from Meksumpun et al., 1994.)

Hystrichosphaerids were discovered independently by paleontologists and classified under a separate taxonomic scheme consisting of only fossil species. Many extant resting spores are identical to hystrichosphaerids of the Tertiary and Quarternary, with the result that there are two names for the same structure.

The process of encystment or resting spore formation is regulated by a complex interaction of day length, temperature, and nutrient concentration (Kremp, 2001; Sgrosso et al., 2001). Melatonin levels increase by several orders of magnitude during encystment and may function in preventing oxidation of the lipids in the cyst (Balzer and Hardeland, 1996). In the freshwater dinoflagellate *Woloszynskia tylota*, encystment involves the following changes (Bibby and Dodge, 1972): (1) replacement of the theca by a thin

amorphous outer wall, which gradually thickens by the deposition of material on its inner face; (2) the appearance of a layer of closely packed lipid droplets at the cytoplasmic margin of the mature cyst; (3) the reduction in size or disappearance of cytoplasmic structures such as chloroplasts, Golgi bodies, and pusules; and (4) the enlargement of a central orange-brown body and cytoplasmic vacuoles containing crystals. Cysts are recognizable because they lack chromatophores and have a microgranular brown cytoplasm and a red eyespot (if the organism normally has an eyespot). Calcification of cysts in some genera occurs by the deposition of calcium carbonate crystals in the narrow space between the cell wall and the plasma membrane (Montresor et al., 1997). The cysts of *Ceratium hirundinella* contain an outer silicon layer (Chapman et al., 1982).

A small number of dinoflagellates produce siliceous skeletons, with the protoplasm wrapped around the skeleton. The best known species is the heterotrophic non-armored *Actiniscus pentasterias* (Fig. 7.29) (Hansen, 1993).



**Fig. 7.29** *Actiniscus pentasterias*. (a) Scanning electron micrograph of the siliceous internal skeleton (two pentasters). (b) Transmission electron micrograph showing two pentasters surrounding the nucleus. (From Hansen, 1993; Preisig, 1994.)

## Toxins

Some Dinophyceae have the ability to produce very potent toxins which cause the death of fish and shellfish during **red tides** when there are dinoflagellate blooms that color the water red. The dinoflagellates become lodged in the gills of the shellfish, and when shellfish are eaten by humans or animals, poisoning results.

Historically, red tides and paralytic shellfish poisoning have been mentioned many times (Shilo, 1967). One of the plagues that struck Egypt was described in the Bible: “all the waters that were in the river were turned to blood. And the fish that was in the river died; and the river stank, and the Egyptians could not drink the water of the river . . .” (Exodus 7:17). This description is strongly reminiscent of the poisonous red tides. Darwin, in his description of discolored water in 1832 during his voyage on the *Beagle*, graphically described blooms of algae that were dinoflagellates.

Death and illness caused by consumption of poisonous mussels and clams were reported by Captain Cook and Captain George Vancouver

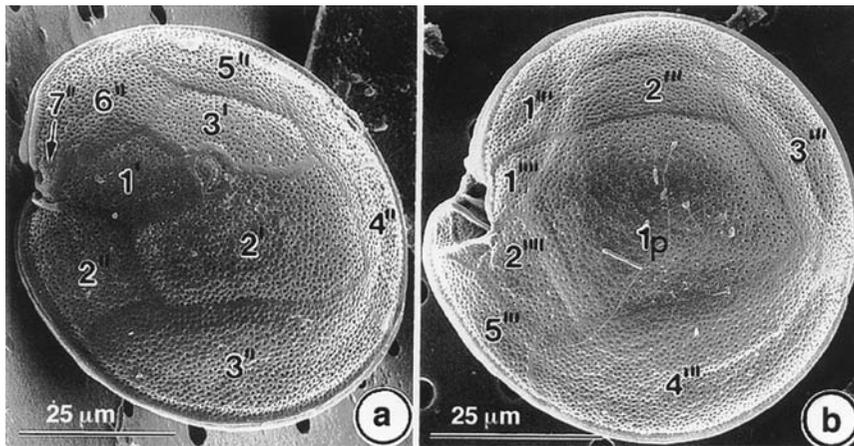
during their expeditions to the coast of the Pacific Northwest. An old custom among Indian tribes along the coast of Alaska was to station sentries to watch for the marine luminescence occurring during hot weather, which they understood to be associated with Kal-Ko-O, their name for mussel poisoning.

All of the dinoflagellates that have been convincingly demonstrated to produce toxins contain chloroplasts, indicating that the ability to produce toxins may have been derived from endosymbiotic cyanobacteria.

**1 Diarrhetic shellfish poisoning.** This occurs primarily in temperate regions and is caused by species of the planktonic dinoflagellates *Exuviaella*, *Dinophysis* (Fig. 7.30(a), (b)), and *Prorocentrum* (Figs. 7.30(c), 7.55, 7.56(c)). Diarrhetic shellfish poisoning is caused by the polyether carboxylic acids okadaic acid, macrolide toxins, and yessotoxin (Figs. 7.31, 23.3, 23.4). The toxins are powerful inhibitors of serine- and threonine-protein phosphatases PP1 and PP2A and induce severe gastroenteritis (Zhou and Fritz, 1994; Morton and Tindall, 1995; Suzuki et al., 1997).

The polyether carboxylic acid okadaic acid is initially formed inside the dinoflagellate cell as dinophysistoxin-4 (Fig. 7.31). This weakly sulfated derivative of okadaic acid is not toxic to the dinoflagellate cell. Dinophysistoxin-4 is





**Fig. 7.32** Scanning electron micrographs of *Gambierdiscus toxicus*, the causative organism of ciguatera fish poisoning. (a) Epithecal view. (b) Hypothecal view. The numbers refer to arrangements of the plates. (From Faust, 1995.)

either excreted by the dinoflagellate cell, or is released on death of the cell. Dinophysistoxin-4 is hydrolyzed in the medium to okadaic acid diol ester, which is lipid soluble and can pass through cell membranes. Thus, okadaic acid diol ester can be taken up by cells which further hydrolyze the compound to okadaic acid. Research has shown that okadaic acid with a free acid moiety is the toxic form of the compound (Hu et al., 1999; Windust et al., 2000).

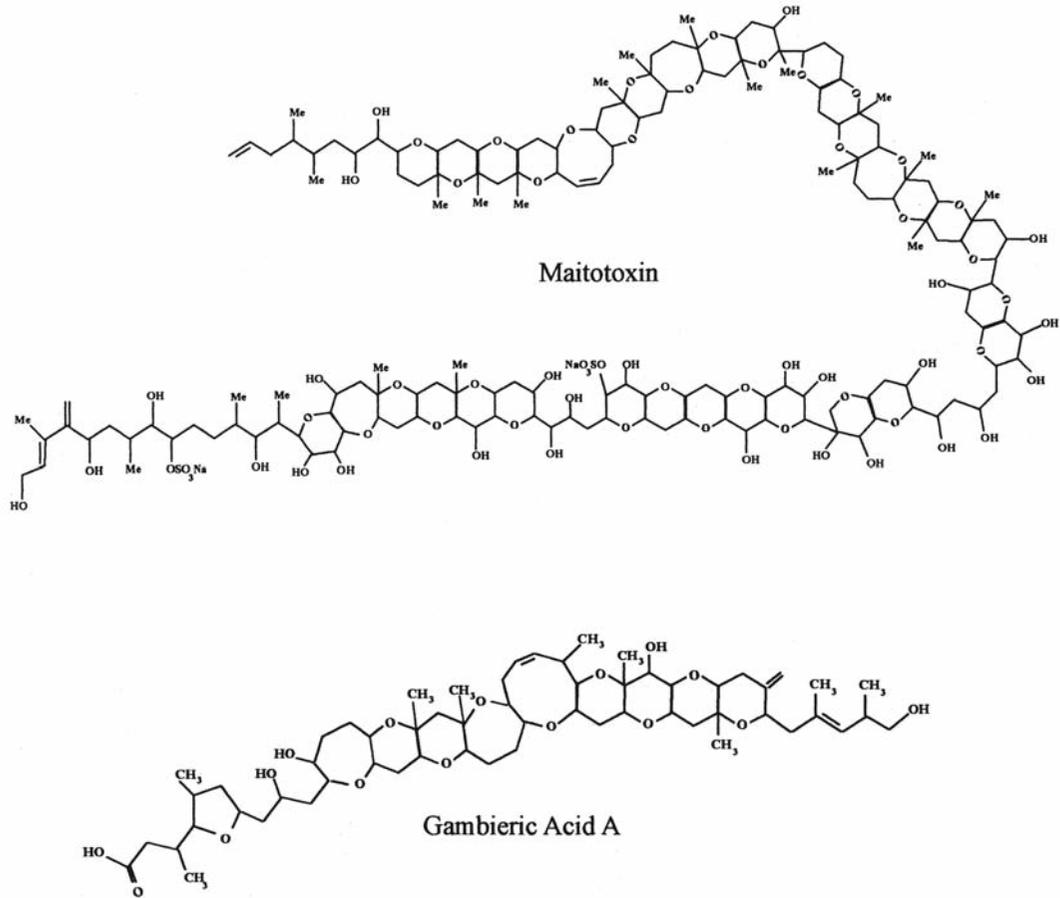
**2 Ciguatera fish poisoning.** This occurs primarily in tropical regions with the common causative agent being *Gambierdiscus* (Figs. 7.6, 7.32) which is common circumtropically between 32° N and 32° S. The dinoflagellate contains gambieric acids, ciguatoxins, and maitotoxins (Fig. 7.33), putative  $\text{Ca}^{2+}$  channel activators that result in breakdown of the cell membrane (Igarashi et al., 1999). *Gambierdiscus* is epiphytic on macroalgae that are eaten by herbivorous fish and shellfish, which, in turn, are eaten by humans. In French Polynesia alone, approximately 1000 cases of ciguatera fish poisoning are reported every year (Chinain et al., 1997). The term ciguatera is derived from the Spanish term “cigua” for the turban shell, which was commonly eaten before the illness developed. The typical course of ciguatera fish

poisoning is diarrhea for two days, followed by general weakness for one to two days.

Occasionally the condition is fatal (Withers, 1982).

**3 Paralytic shellfish poisoning.** This is caused by species of *Alexandrium* (*A. catanella*, *A. acatenella*, *A. excavatum* (Fig. 7.35), *A. tamarensis*), *Pyrodinium bahamense* (Fig. 7.34), and *Gymnodinium catenatum* (Fig. 7.34). These dinoflagellates produce a group of toxins that are derivatives of saxitoxin (Fig. 23.2). Saxitoxins are potent neurotoxins acting upon voltage-gated  $\text{Na}^+$ -channels, preventing influx of  $\text{Na}^+$ , thereby preventing the generation of an action potential (Cembella, 2003).

*Alexandrium excavatum* is a toxic red-tide dinoflagellate (Fig. 7.35). The vegetative cells divide to produce motile gametes that subsequently fuse. In the early stages of gamete fusion, when superficial contact is apparent, fusing pairs swim poorly and settle in the water. The motile quadriflagellate planozygotes swim for a few days before losing their flagella and thecal plates, and encyst to form resting cysts (hypnospores) (Figs. 7.28(a), 7.36) that can survive for 5 to 15 years. The hypnospores contain storage products and have a thick, three-layer wall (Kennaway and Lewis, 2004). Cyst germination is controlled by a biological clock with a 12 month maturation period (Perez, et al., 1998). Each hypnospore undergoes meiosis with two cell divisions to produce haploid vegetative cells, completing the life cycle (Destombe and Cembella, 1990; Nagai et al., 2003).



**Fig. 7.33** The chemical structure of maitotoxin and gambieric acid.

The amount of toxin in cells of *Alexandrium* is relatively low when nutrients are in ready supply (John and Flynn, 2002). The toxin concentration is highest under conditions of phosphorus deficiency, possibly because free amino acids (precursors of toxins) accumulate under conditions of phosphorus deficiency.

Cells of dinoflagellates that produce toxins are avoided by grazing copepods, indicating that the extra energy put into toxin production is more than offset by the decreased grazing of the dinoflagellate cells (Guisande et al., 2002).

A number of factors have been suggested as the cause of red tides:

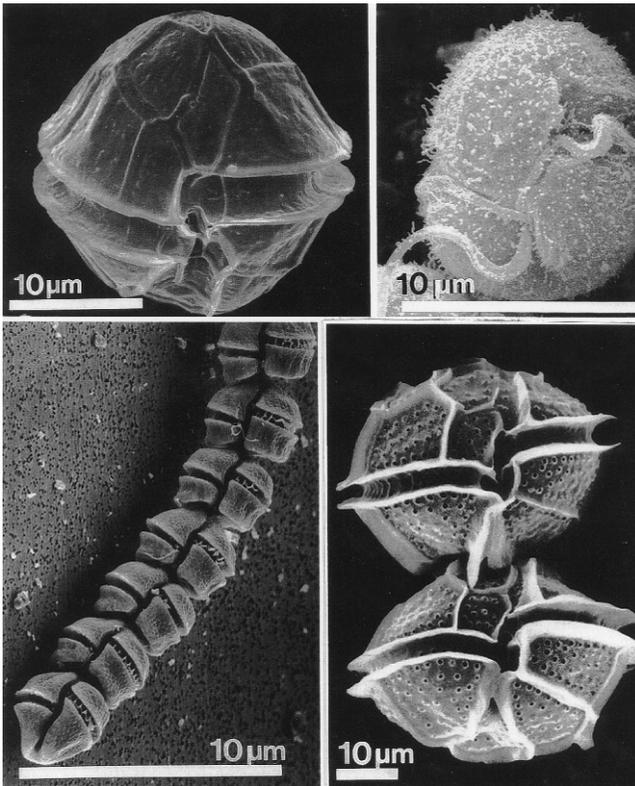
**1 High surface-water temperatures:**

Dinoflagellates favor warm water, and are

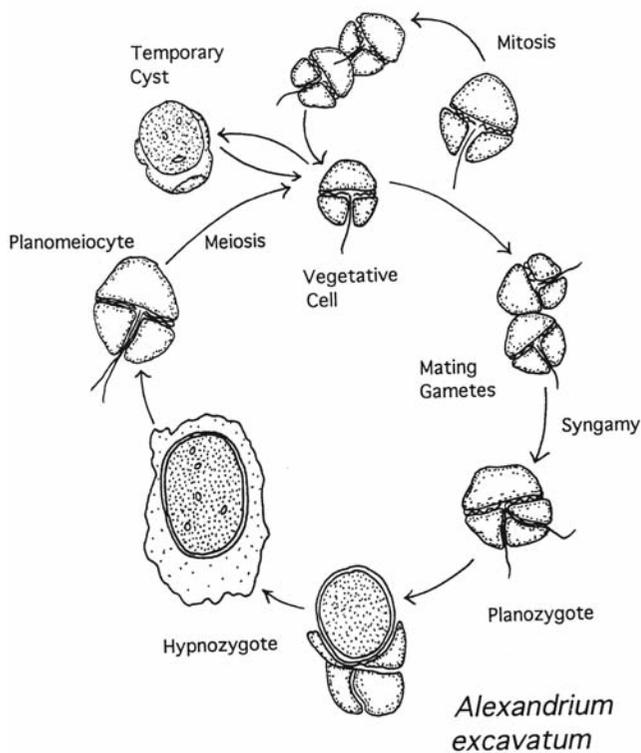
generally more abundant near the surface.

This does not necessarily mean that they occur only in warm seas, because the surface of the sea in normally cool areas may be warmed up during periods of hot, calm weather.

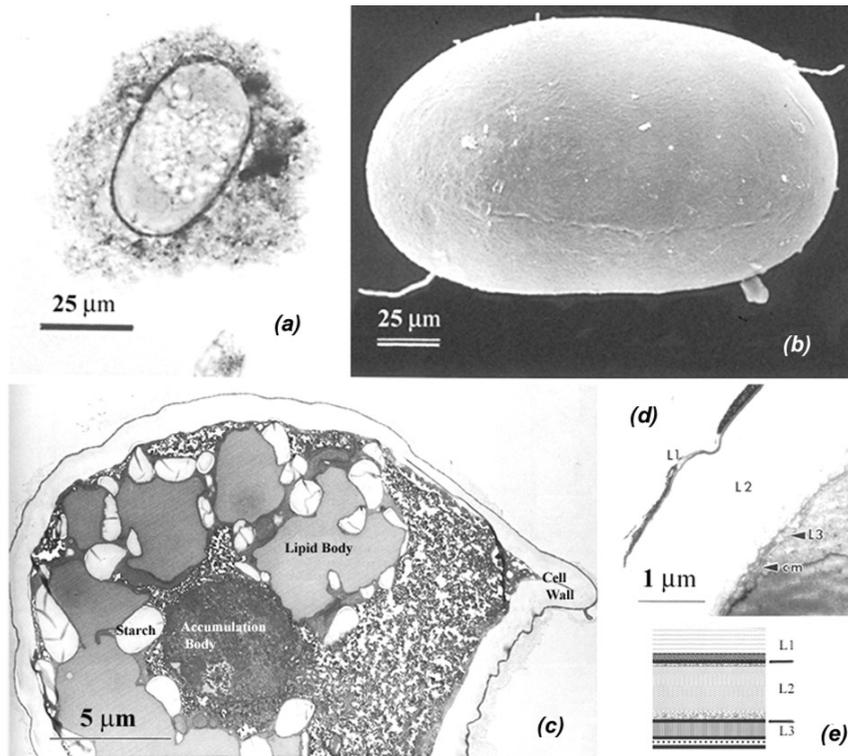
- 2 Wind:** A strong, offshore wind aids upwelling, whereas a gentle onshore wind concentrates the bloom near the coast. On the other hand, heavy weather and strong winds disperse the bloom. Storms also result in the death of dinoflagellates and can prevent the development of red tides (Juhl and Latz, 2002; Sullivan and Swift, 2003).
- 3 Light intensity:** There is usually a period of bright, sunny, calm weather before outbreaks.
- 4 Nutrients:** Red tides usually occur after an upwelling has stopped, but the nutrients brought to the surface do not, themselves, appear to be the direct cause of these blooms (Grindley and Nel, 1970). It is thought that



**Fig. 7.34** Scanning electron micrographs of poisonous dinoflagellates. *Alexandrium minutum* (upper left), *Karlodinium veneficum* (= *Gymnodinium galatheanum*) (upper right), *Gymnodinium catenatus* (bottom left), *Pyrodinium bahamense* var. *compressum* (bottom right). *Karlodinium micrum* has killed cage-reared fish, while the others cause paralytic shellfish poisoning. (From Hallegraeff, 1993.)



**Fig. 7.35** The life cycle of *Alexandrium excavatum*. (Adapted from Destombe and Cembella, 1990.)



**Fig. 7.36** Hypnospores (resting spores) of *Alexandrium*. (a) Light micrograph of a hypnosporium surrounded by mucus. (b) Scanning electron micrograph showing the smooth surface of a hypnosporium. (c) Transmission electron micrograph showing the large amount of storage products in a hypnosporium. (d) Transmission electron micrograph of the layers of the cell wall. (e) Illustration of the layers of the cell wall. (cm) Cell membrane. (From Kennaway and Lewis, 2004.)

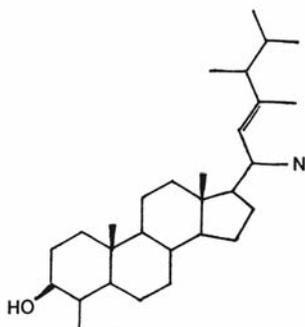
preceding blooms of diatoms may impoverish the water and reduce one or more of the inorganic nutrients to a level favorable for the growth of dinoflagellates (but too low for the diatoms), and also allow the production of organic nutrients such as vitamin B<sub>12</sub>, which are important for their growth.

## Dinoflagellates and oil and coal deposits

Blooms of dinoflagellates have most likely been responsible for some of the oil deposits of the world, including the North Sea oil deposit (Downie, 1956; Gallois, 1976). The best studied oil

deposits are the oil shales of the Kimmeridge Clay deposits in England, which vary from less than 100 m in thickness to over 500 m and are composed of a number of different layers: clays and black and brown shales intermixed with occasional thin limestones. The limestones are composed primarily of coccolithophorids, whereas the shales contain yellow-brown organic matter called **kerogen**. Kerogen contains a large amount of amorphous organic matter, from which it is impossible to determine its biological origin. In addition to the amorphous organic matter, there are found large numbers of dinoflagellates and their hystrichospores, as well as some coccoliths. The best known Kimmeridge Clay deposit is the Kimmeridge Coal, a highly bituminous shale, about 80 cm thick. The Kimmeridge Coal has long been used as a solid fuel, and in the latter half of the last century much interest was taken in it as a source of oil. However, the high sulfur content and the relative thinness of the “coal” bed prevented the economic exploitation of the oil.

The Kimmeridge Clay oil shales were formed from algal blooms in seas that were to some



**Fig. 7.37** The structure of dinosterol, the major sterol in dinoflagellates.

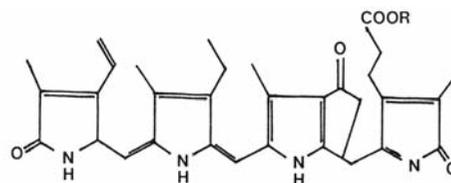
degree land-locked, with salinity a little beneath the average salinity of the open ocean. These seas were rich in land-derived nutrients, allowing the water to support blooms of toxic dinoflagellates. These blooms deoxygenated and poisoned the water, providing the temporary anaerobic bottom conditions required for the preservation of organic matter. It is interesting that one of the bivalves commonly poisoned by toxic dinoflagellate blooms today is *Lucinoma borealis*, and the bivalve that forms the most extensive plasters in the Kimmeridge Clay oil shales is the related *Lucina miniscula*.

Petroleum deposits and ancient sediments contain 4 $\alpha$ -methylsteroidal hydrocarbons, which probably originated from 4 $\alpha$ -methylsterols in dinoflagellates (Robinson et al., 1984). The dinoflagellates differ from other classes of algae with respect to the dominance of 4 $\alpha$ -methylsterols among their sterol components and the uniqueness of certain of these 4 $\alpha$ -methylsterol structures (Leblond and Chapman, 2002; Giner et al., 2003). The principal sterol of several marine dinoflagellates, and organisms with dinoflagellate symbionts, is **dinosterol** (Fig. 7.37).

## Bioluminescence

About, about, in reel and rout  
The death-fires danced at night  
The water, like a witch's oils  
Burnt green, and blue, and white

Samuel Taylor Coleridge  
*The Rime of the Ancient Mariner*

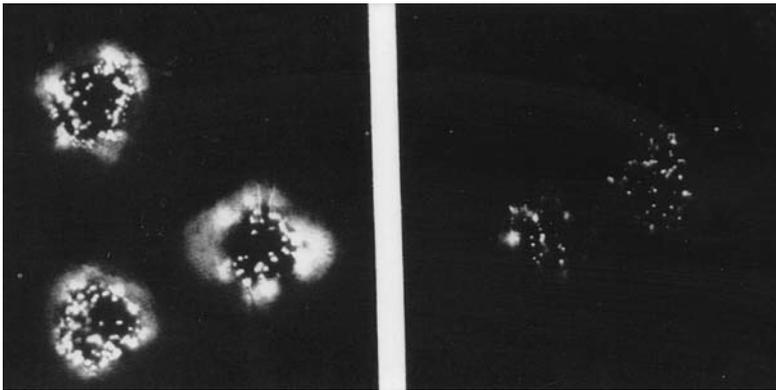


**Fig. 7.38** A possible partial structure of dinoflagellate luciferin. (After Dunlap and Hastings, 1981; Hastings, 1986.)

Mariners from early times have marveled at the displays of bioluminescence that accompany large populations of dinoflagellates. The burning seas were at first thought to be of supernatural origin, omens of the pleasure or displeasure of the gods. As science began to usurp the explanation of natural phenomena from religion, the light emitted from friction between salt molecules or from phosphorus burning in water was invoked; the term **phosphorescence** still survives today from the explanation. By 1800, living cells were suspected, but the last experiments were not settled in favor of a biological origin until 1830 (Sweeney, 1979).

There are two types of light emission in living organisms: (1) **bioluminescence (chemiluminescence)**, in which *energy from an exergonic chemical reaction is transformed into light energy*; and (2) **photoluminescence**, which is dependent on the *prior absorption of light* (Hastings, 1986). Many marine, but no freshwater dinoflagellates are capable of bioluminescence. The Dinophyceae are the main contributors to marine bioluminescence, emitting a bluish-green (maximum wavelength at 474 nm) flash of light of 0.1-second duration when the cells are stimulated. The luminescent wake of a moving ship or the phosphorescence of tropical bays is usually caused primarily by Dinophyceae.

The compound responsible for bioluminescence is **luciferin** (Fig. 7.38), which is oxidized with the aid of the enzyme **luciferase**, resulting in the emissions of light. Luciferin and luciferase are terms for a general class of compounds, and not of a specific chemical structure. Bioluminescence occurs in many organisms in many different phyla, ranging from bacteria to dinoflagellates to jellyfish and brittle stars to worms, fireflies, molluscs, and fish (Hastings, 1986). In bacteria, luciferin is a reduced flavin; in insects it is a (benzo)thiazole nucleus; and in dinoflagellates it is a tetrapyrrole.

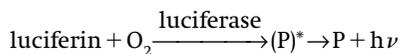


**Fig. 7.39** The white spots in these photographs of *Lingulodinium* cells indicate fluorescence and thus localization of luciferin, the substrate that reacts with the enzyme luciferase and oxygen to produce light. The intensity of the luciferin fluorescence is greater in the night phase (left) of the circadian cycle of bioluminescence than in the day phase (right). The amount of luciferase shows a similar oscillation during the daily cycle. (From Johnson et al., 1985.)

Likewise, luciferase has different structures, although all luciferases share the feature of being **oxygenases** (enzymes that add oxygen to compounds). The necessity for oxygen in bioluminescence was actually discovered by Boyle in 1667, who showed with his air (vacuum) pump that:

a piece of shining (bioluminescent) wood . . . gave a vivid light . . . which was manifestly lessened . . . [at] about the seventh suck, losing its light more and more as the air was still further pumped out. . . . Wherefore we let in outward air and had the pleasure to see the seemingly extinguished light revive so fast and perfectly, that it looked to us almost like a little flash of lightning. (Cited in Harvey, 1952, p. 142).

In the basic reaction of bioluminescence (Hastings, 1983), a luciferin is oxidized by a luciferase, resulting in an electronically excited product (P)\* which emits a photon ( $h\nu$ ) on decomposition:

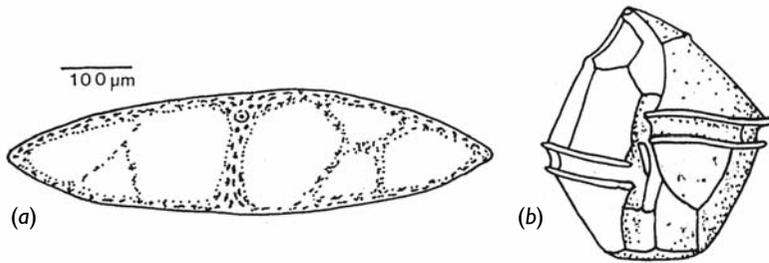


Luciferin in the dinoflagellates has been reported to be a linear tetrapyrrole (Fig. 7.38). Associated with dinoflagellate luciferin is a **luciferin-binding protein (LBP)** which sequesters luciferin at alkaline pH and releases it under acidic conditions (Sulzman et al., 1978). It has been postulated that the flash of bioluminescent light may occur simply by a lowering of the pH from 8.0 to 6.5. Agitation of cells depolarizes the vacuolar membrane, allowing a flux of protons ( $\text{H}^+$ ) and acidification of the peripheral cytoplasm (Johnson et al., 1985). Lowering the pH causes two pH-dependent

reactions to occur: (1) release of luciferin from its binding protein at acidic pH, and (2) activation of luciferase followed by emission of a photon of blue-green light.

Luciferin, luciferase, and luciferase-binding protein occur in particles called **scintillons (flashing units)** that are approximately 0.5 to 1.5  $\mu\text{m}$  in diameter (Sweeney, 1980). Scintillations occur in cytoplasmic invaginations in the vacuolar membrane. Flashes of blue-green light are produced when an action potential proceeds along the vacuolar membrane, causing an outflow of protons from the acidic contents of the vacuole. The resulting drop in the pH in the scintillon causes flashes of light (Nicolas et al., 1991).

In *Lingulodinium polyedrum* (Figs. 7.39, 7.40), most bioluminescence occurs during the night phase of a circadian rhythm (Fig. 7.41) (Fritz et al., 1991). This is due to a ten fold increase in luciferase and luciferin-binding protein during the night phase. Daytime photoinhibition of bioluminescence is considered a mechanism to conserve the energy of cells when ambient light levels are high enough to render the bioluminescent flash ineffective (Buskey and Swift, 1983). The position of bioluminescence in *Pyrocystis fusiformis* (Fig. 7.40(a)) varies over 24 hours (Sweeney, 1980; Seo and Fritz, 2000). During the day, bioluminescence emanates from a spherical mass of tightly packed vesicles near the nucleus, whereas at night bioluminescence occurs in the peripheral cytoplasm. There is a reverse movement of chloroplasts, with the organelles in the cell periphery during the day and the chloroplasts concentrating around the nucleus at night.



**Fig. 7.40** (a) *Pyrocystis fusiformis*.  
(b) *Lingulodinium polyedrum* (5  
*Gonyaulax polyedra*).

Dinoflagellates can emit light in three modes: (1) they can flash when stimulated mechanically, chemically, or electrically; (2) they can flash spontaneously; and (3) late at night they can glow dimly (Sweeney, 1979). The maximum amount of light emitted in a flash differs widely among species, with larger species emitting more light per flash than smaller ones. In a population of dinoflagellates, the cells emit an average of one flash per cell per day (Hastings and Krasnow, 1981). It is not clear whether each cell flashes once and only once during this period, or whether some cells are responsible for a larger share of the flashes whereas others do not emit at all. The nutritional status of a cell influences the brightness of the flash. *Noctiluca* with green algal symbionts (Fig. 7.54) shows an increase in the photons emitted per flash as the intensity of the illumination of the dinoflagellate, and therefore photosynthesis, increases. Different isolates of the same species in genera such as *Dissodinium* and *Pyrocystis* (Figs. 7.40(a), 7.49(c), (d)) can be bioluminescent or not (Swift et al., 1973; Schmidt et al., 1978).

*Pyrocystis* (Figs. 7.40(a), 7.49(c), (d)), one of the most strongly phosphorescent dinoflagellates, is the chief source of diffused phosphorescence in the sea in equatorial regions (Swift et al., 1973). Species of *Pyrocystis* produce at least 1000 times more bioluminescence per cell than members of the genus *Lingulodinium* (Figs. 7.39, 7.40) and about 100 times as much light per cell as *Ceratium fusus*, *Peridinium pentagonium*, and *Pyrodinium bahamense*.

There are two theories concerning the adaptive value of bioluminescence to dinoflagellates, both of which relate to nighttime grazing of the dinoflagellates:

1 “Burglar alarm” hypothesis. This hypothesis argues that dinoflagellates render themselves

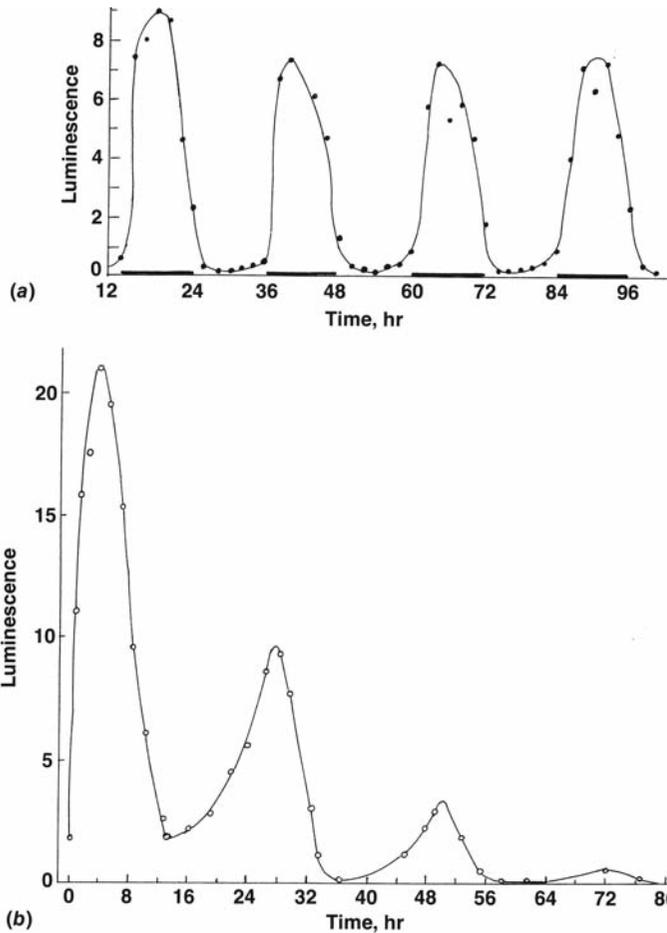
dangerous as prey to invertebrate grazers because they generate a signal identifying the location of invertebrate food to individuals two levels up the food chain from the dinoflagellates (Abrahams and Townsend, 1993). Bioluminescence generated by dinoflagellates serves to attract predators of the grazers of the dinoflagellates.

2 “Startle” hypothesis. In this hypothesis, mechanical stimulation of a bioluminescent dinoflagellate by a grazer produces a flash of light that startles an invertebrate grazer, such as a copepod, and causes the copepod to swim away with its feeding appendage retracted (Buskey and Swift, 1983).

Whichever theory is correct, experiments have shown that copepods consume only half as many dinoflagellates at night, indicating that the bioluminescence is serving as a deterrent to grazing (Buskey et al., 1983).

## Rhythms

Many Dinophyceae exhibit rhythmic processes, with the best known of the rhythms in the algae being those in the dinoflagellate *Lingulodinium polyedrum* (Figs. 7.39, 7.40) (Sweeney, 1969). It produces light via bioluminescence by forming its own dinoflagellate-specific luciferin and luciferase. The cells emit a flash of light when the seawater in which they are swimming is given a sharp shake or stirred vigorously. When the luminescence is measured while the culture is being stirred, the amount of light that the cells emit is not always the same and depends upon their recent history. If they have been growing in natural illumination or on a light–dark cycle, the amount of light emitted will be markedly



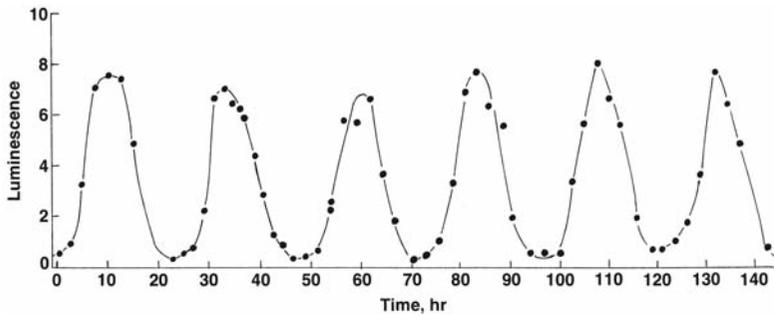
**Fig. 7.41** The rhythm of stimulated luminescence in *Lingulodinium polyedrum* measured in (a) a light-dark cycle of 12:12 and (b) in complete dark. (After Sweeney, 1969.)

dependent on the time of day when measurements are made. If luminescence is elicited during the day, the amount of light will be low, and very hard stirring will be required to bring about any luminescence at all. But if the cells are stimulated at night, a great deal more light will result, and the most delicate shake will produce a flash. When the amount of luminescence is plotted as a function of the time of day, a graph such as that in Fig. 7.41(a), will result. The greatest luminescence will be produced in the middle of the dark period, whereas toward morning, flashes will gradually become smaller and a greater stimulus will be required. The rhythm is circadian (which means literally about (*circa*) a day (*diem*)), as shown by the persistence of changes in brightness of luminescence when the cells are kept in the dark (Fig. 7.41(b)). In continuous darkness, the cycle continues for as long as 4 days, but the

amplitude becomes successively smaller. If the cells are kept in continuous light, the reduction in amplitude is no longer evident. Cycles continue for at least 3 weeks in continuous light of appropriate intensity (Fig. 7.42).

When photosynthesis in *Lingulodinium polyedrum* is measured, either as oxygen production or carbon dioxide fixation, it is also found to be rhythmic (Fig. 7.45). The rhythm is circadian and continues under conditions of continuous light. The maximum rate of photosynthesis occurs, as one would expect, in the middle of the day. The rhythm in photosynthesis is due to changes in photosystem II (Samuelsson et al., 1983) (Fig. 7.43).

A third rhythm with a circadian period in *Lingulodinium polyedrum* is that of cell division (Fig. 7.45); all cell division occurring during 30 minutes when cultures are in a light-dark cycle. When the light-dark cycle is 12:12, then this



**Fig. 7.42** The rhythm of stimulated luminescence in *Lingulodinium polyedrum* measured in continuous light (1000 lux). (After Sweeney, 1969.)

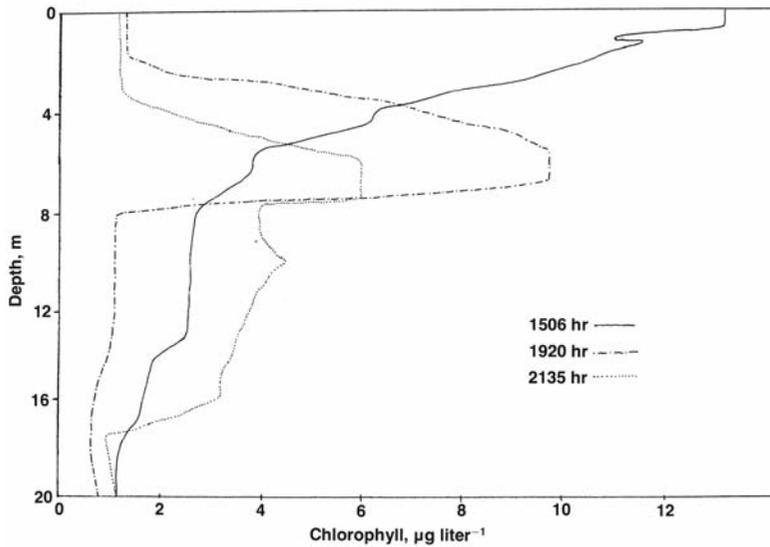


**Fig. 7.43** **Beatrice M. Sweeney** Dr. Sweeney's investigations established the rhythmic phenomena of dinoflagellates. Born August 11, 1914, in Boston, Massachusetts. Died June 30, 1989 in Woods Hole, Massachusetts. She received her A.B. from Smith College (1936) and her Ph.D. from Radcliffe College (1942). She worked as a laboratory and research assistant at the Mayo Clinic (1942–43) and Scripps Institution of Oceanography (1947–55). From 1961 to 1967, she was a biologist and lecturer at Yale University. In 1967 she moved to the Department of Biological Sciences at the University of California, Santa Barbara. She published two editions of her book *Rhythmic Phenomena in Plants*. (Photo from Garbary and Wynne, 1996.)

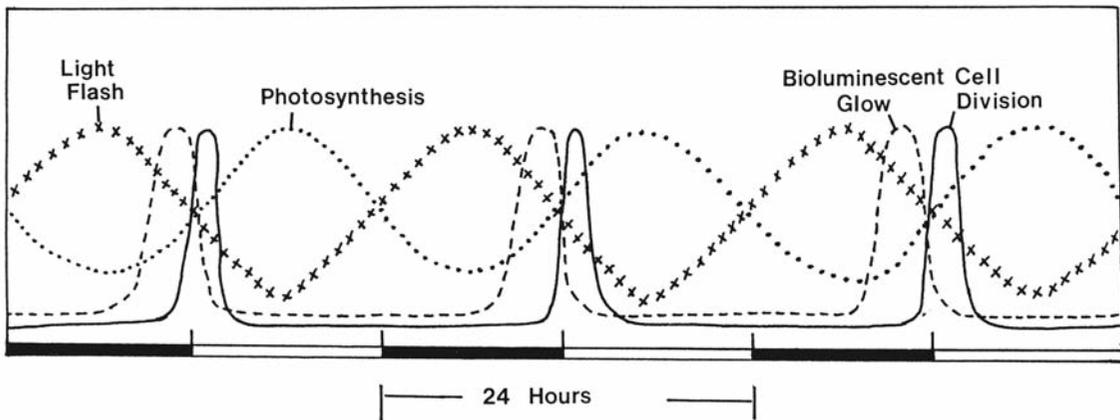
30 minutes spans “dawn.” An investigation of other light–dark cycles, such as 7:7, shows that the dark–light transition is not the determining factor because division takes place about 12 hours after the beginning of the dark period, even though this time is considerably after the beginning of the next light period. In continuous light of low intensity where other rhythms of *Lingulodinium polyedrum* persist, very little cell division takes place, and the average generation time may be as long as 6 days. However, those cells that are ready to divide do so only at the expected time in each 24 hours.

A fourth type of rhythm involves the vertical migration of dinoflagellate cells in the water column (Eppley et al., 1968; Horiguchi and Pienaar, 1988; Lombard and Capon, 1971; Roenneberg and Deng, 1997). Before dawn, the cells rise to the surface, where they form dense clouds (aggregations), and before night fall, they again sink to lower depths (Fig. 7.44). In the marine environment, this vertical migration exposes the cells to several gradients: (1) Nutrients are more concentrated at lower depths (accumulating at the bottom of the ocean or at thermoclines) while surface waters are often practically devoid of nutrients. (2) Temperatures at the surface exceed those in deeper waters. (3) Variations in light intensities. (4) In shallow waters, differences in washout by the tidal waters.

In *Lingulodinium polyedrum*, there exists a control over luminescence, photosynthesis, and cell division, so that each process reaches a maximum and then declines in an orderly fashion during each 24 hours (Fig. 7.45). A single process, a biological clock, may control all of these processes. It appears that the part of the cell that may be the controlling agent is the plasma membrane



**Fig. 7.44** Profiles of chlorophyll *a* during a bloom of dinoflagellates showing the vertical migration of dinoflagellates at different times of the day. (After Eppley et al., 1968.)



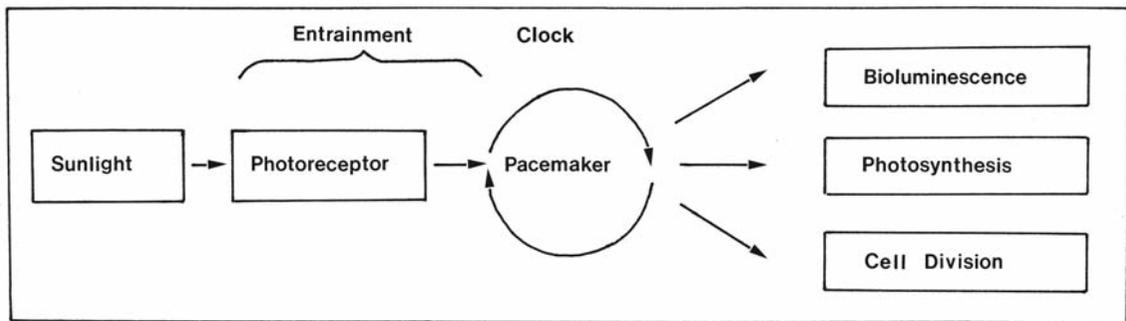
**Fig. 7.45** Circadian rhythmicity has been identified in at least four distinct biological processes of the unicellular *Lingulodinium polyedrum*. Although the four rhythms peak at different times of the 24-hour day, as represented here diagrammatically, they are all synchronized with the circadian clock. Perturbations that reset the clock will phase-shift all four rhythms simultaneously, suggesting that all of these overt rhythms are driven by a single pacemaker. (After Johnson and Hastings, 1986.)

because there is a rhythmic reorganization of the plasma membrane over a 24-hour period in synchronized cells (Adamich et al., 1976).

Although the phases at which the rhythms peak in *Lingulodinium polyedrum* are different, it is generally believed that they are all controlled by a

single pacemaker (Johnson and Hastings, 1986). The rhythmic processes seem to have no feedback mechanism and thus appear to be driven systems. For example, photosynthesis can be completely inhibited with a specific herbicide, but the bioluminescence rhythm continues and its phase is not altered. Such observations on *Lingulodinium polyedrum* suggest models of circadian organization like those in Fig. 7.46. The central pacemaker is phased to the solar light cycle by means of a photoreceptor and an entraining pathway, and it controls the expression of the different rhythms, such as cell division, bioluminescence, and photosynthesis.

Two different systems time the circadian rhythm in dinoflagellates, a red-light sensitive



**Fig. 7.46** Diagram showing how a single endogenous clock within a *Lingulodinium polyedrum* cell can control a multiplicity of observed rhythms. (After Johnson and Hastings, 1986.)

system that delays the timing, and a blue-light sensitive system that advances the timing to dawn (Roenneberg and Deng, 1997). These light systems probably stimulate or depress the production of melatonin in the cells. An increase in the concentration of melatonin appears to mark the end of the light phase. There is a circadian rhythm in the production of melatonin, with a rapid increase in melatonin concentration at the end of the light phase and a subsequent decline during the dark phase, reaching a minimal value in the light phase. The concentration of melatonin in dinoflagellate cells is similar to that in the mammalian pineal gland (Balzer and Hardeland, 1996). Melatonin may represent a common basis for photoperiodism in organisms as distant as dinoflagellates and vertebrates, a fact that suggests an ancient mechanism for mediating information about darkness within the circadian cycle (Pöggeler et al., 1991).

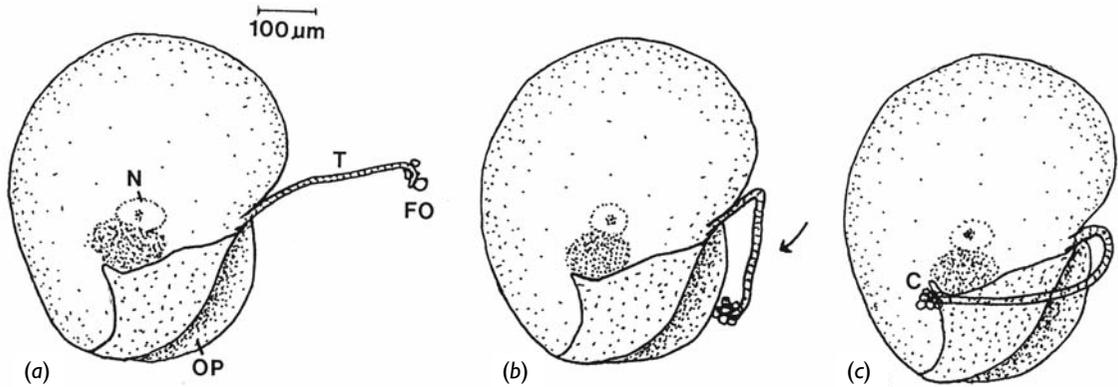
## Heterotrophic dinoflagellates

An estimated half of the more than 2000 living dinoflagellate species lack chloroplasts and are exclusively heterotrophic (Hansen and Calado, 1999; Jacobsen, 1999). In addition, many dinoflagellates that contain chloroplasts are capable of **mixotrophy** where a portion of their nutrients is obtained heterotrophically, particularly when nutrient conditions are low in the environment (Smalley et al., 2003).

The different modes of heterotrophy are: (1) **phagotrophy** through the direct engulfment of prey; (2) **pallium feeding** where the prey is engulfed by a cytoplasmic veil – the pallium – with digestion of the food taking place outside of the dinoflagellate cell; (3) **peduncle feeding (myzocytosis)** involving the uptake of intracellular material of the prey through a cytoplasmic extension – the peduncle – leaving the plasma membrane and extracellular material of the prey behind; and (4) **osmotrophy** or the uptake of dissolved substances (Höhfeld and Melkonian, 1998).

### Direct engulfment of prey

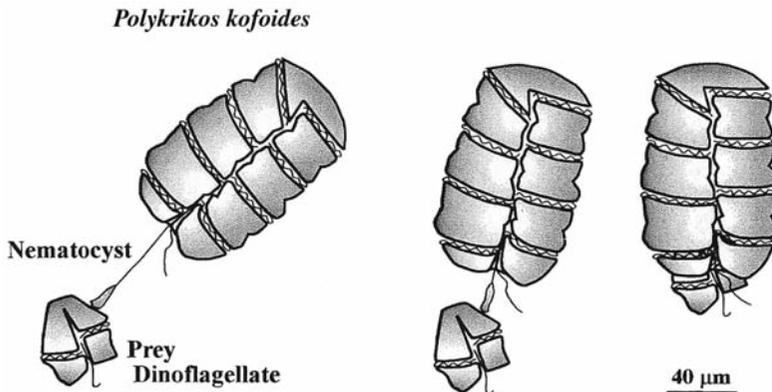
The large cells of *Noctiluca* have a 300- $\mu\text{m}$ -long food-gathering **tentacle** that is covered with a slimy exudate (Fig. 7.47) (Sweeney, 1978; Nawata and Sibaoka, 1983). Two wing-like extensions of the cells form an **oral pouch** at the base of the tentacle. At the bottom of the oral pouch is a **cytosome** that opens like a slit during ingestion of food organisms. Collision of other organisms in the medium with the mucus-covered tentacle tip results in attachment of the other organisms to the tentacle. Continued collisions result in the formation of a clump of cells adhering to the tentacle. The tentacle is primarily in an extended configuration during this time. The tentacle now bends so that the tentacle tip moves to the bottom of the oral pouch. The cytoplasm streams vigorously toward the cytosome and then aggregates around the cytosome (Nawata and Sibaoka, 1987). The cytosome opens, the tentacle tip is inserted into it, the food organisms are swept into the cytosome, and the tentacle tip is retracted. The cytosome opening closes, and the food organisms are engulfed in a food vesicle in



**Fig. 7.47** Drawing of the ingestion of food organisms (other algae, bacteria) by *Noctiluca*. (a) The tentacle (T) is in an extended configuration. Any food organisms (FO) that collide with the mucus-covered tentacle tip, stick to the tentacle. (N) Nucleus; (OP) oral pouch. (b) The tentacle bends back toward the oral pouch. (c) The cytosome (C) at the base of the oral pouch opens, the tentacle tip is inserted into the cytosome, and the food organisms are swept into a food vacuole. (After Nawata and Sibaoka, 1983.)

the cytoplasm. The food organisms are digested in the food vesicle. In the cell under the microscope, long strands of mucus containing food organisms can be seen spirally coiled in the food vacuoles.

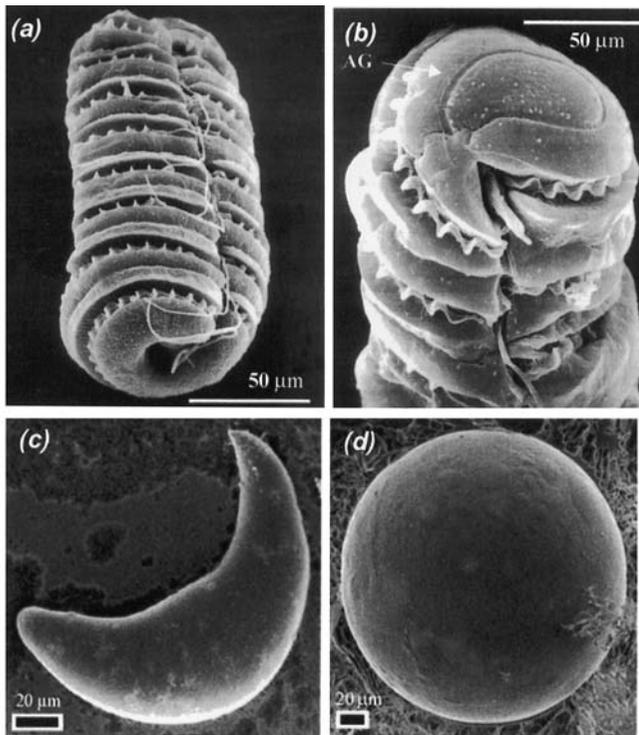
*Polykrikos kofoides* feeds by initially spearing its prey with a nematocyst (Figs. 7.48, 7.49(a), (b)). The prey organism is pulled into the posterior sulcus, with the prey eventually being completely engulfed into a food vacuole.



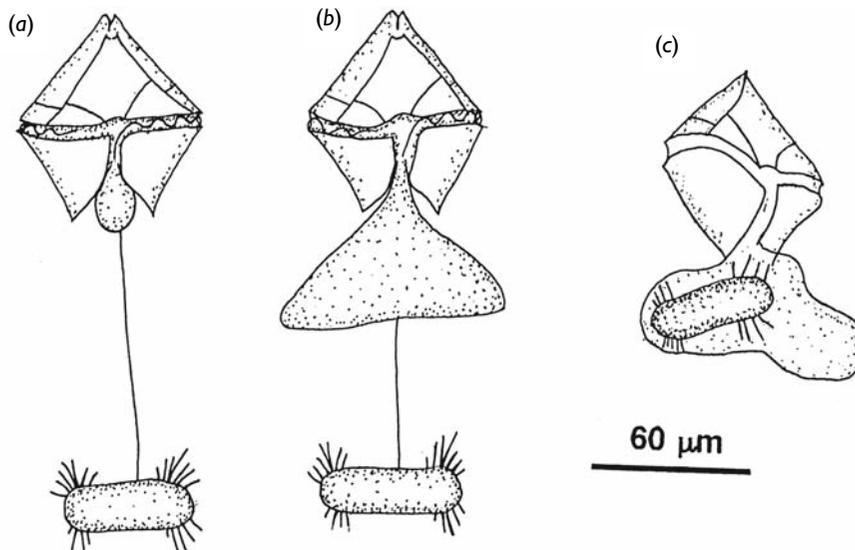
**Fig. 7.48** *Polykrikos kofoides* captures prey by firing a nematocyst into the prey and bringing the tethered prey into the posterior portion of the cell. The prey is engulfed and digested by the dinoflagellate. (Modified from Matsuoka et al., 2000.)

### Pallium feeding

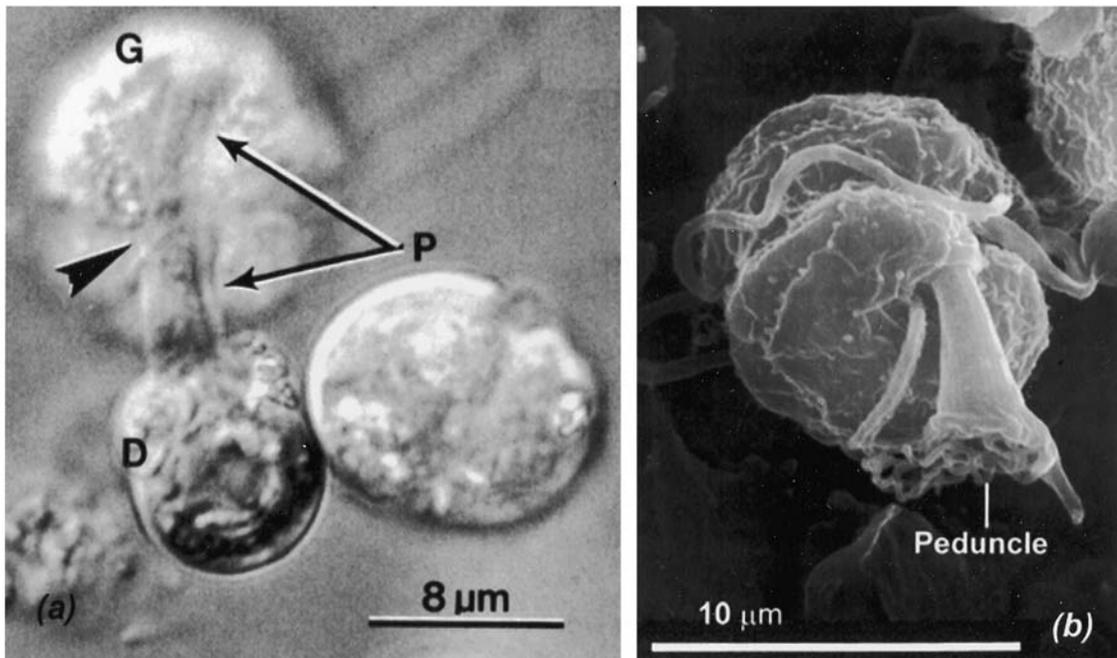
This occurs only in thecate species and utilizes a feeding veil, the **pallium**, which emerges from the flagellar pore and encloses the prey. The prey protoplasm is digested by enzymes released into the pallium, and the digestion products are transported into the feeding cell. *Protoperdinium* (Fig. 7.50) and *Diplopsalis* (Naustvoll, 1998) feed in this manner. These dinoflagellates swim in a straight line until they encounter a prey organism. The dinoflagellate then changes swimming behavior by slowing down and swimming in tight circles around the prey for less than one minute. A thin filament of cytoplasm (about 1  $\mu\text{m}$  in diameter) emerges from the sulcal pore and attaches to the prey (Fig. 7.50). A pseudopod is extended along the filament while the filament is retracted, pulling the prey closer to the dinoflagellate. The pseudopod advances at 2 to 6  $\mu\text{m s}^{-1}$  until it reaches the prey. The pseudopod conforms to projections,



**Fig. 7.49** (a),(b) Scanning electron micrographs of 8-cell pseudocolonies of *Polykrikos schwartzii*. Ventral view (a) and apical view (b) showing the apical groove. (c),(d) Scanning electron micrographs of *Pyrocystis lunula* (c) and *Pyrocystis noctiluca* (d). *Pyrocystis* is unusual among dinoflagellates in existing primarily in the non-motile vegetative state. *Pyrocystis* is a major source of bioluminescence in the ocean. ((a),(b) from Nagai et al., 2002; (c),(d) from Seo and Fritz, 2002.)



**Fig. 7.50** The heterotrophic dinoflagellate *Protoperidinium conicum* feeding on the diatom *Corethron hystria*. Initially the dinoflagellate attaches to the prey by a long thin filament (a). Next a pseudopod extends along the filament (b) and engulfs the prey (c), which is digested. (After Jacobsen and Anderson, 1986.)



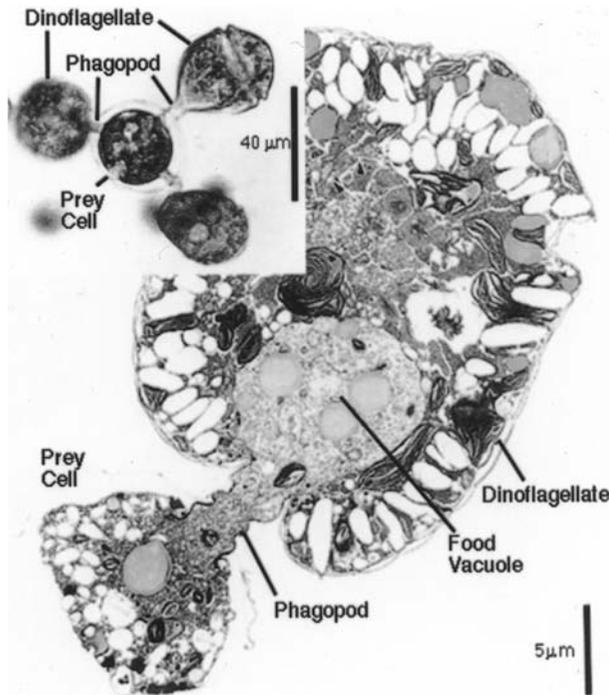
**Fig. 7.51** (a) Light micrograph of the dinoflagellate *Gymnodinium fungiforme* (G) ingesting the protoplasm of *Dunaliella salina* (D). The peduncle (P) of *G. fungiforme* has attached to *D. salina* with the protoplasm of *D. salina* passing through the enlarged and extended peduncle into the dinoflagellate. (b) Scanning electron micrograph of a zoospore of *Pfiesteria piscicida* showing the peduncle. ((a) from Spero, 1982; (b) from Lewitus et al., 1999.)

such as spines, and can engulf an organism many times larger than the dinoflagellate. The prey is digested in the pseudopod, with the pseudopod showing active cytoplasmic streaming at a velocity of about  $5 \mu\text{m s}^{-1}$ . A diatom is digested by the dinoflagellate in about 30 minutes, at which time the pseudopod is rapidly retracted (at a rate of  $10 \mu\text{m s}^{-1}$ ) into the dinoflagellate.

### Peduncle feeding

*Gymnodinium fungiforme* contains an extensible peduncle (Lee, 1977), a projection of cytoplasm full of microtubules, in the epicone just above the intersection of the sulcus and cingulum (Figs. 7.51(a), 7.52) (Spero and Moree, 1981). The peduncle can extend from 8 to  $12 \mu\text{m}$  to attach to, and make a hole into, the prey (Spero, 1982). The cytoplasm of the prey moves through the peduncle to the dinoflagellate cytoplasm. After feeding

is complete, the microtubules of the peduncle, and the peduncle itself, retract back into the dinoflagellate cytoplasm. Feeding is characterized by the aggregation of numbers of the dinoflagellate on the prey organism. A small green alga, such as *Dunaliella*, has five to ten dinoflagellates attached by their peduncles, whereas a ciliate may have 400 to 500 dinoflagellates attached to it. The peduncle of *G. fungiforme* apparently cannot penetrate a cell wall, so the dinoflagellate feeds only on prey that lacks a cell wall, or on injured higher organisms. The marine ciliate *Condylostoma magnum* is not fed on by *G. fungiforme* until it is injured (in the laboratory by piercing it with a micropipette). Within 15 seconds, more than 100 dinoflagellates will congregate around the wound. When a ciliate is taken from a dying culture and placed in a *G. fungiforme* culture, the dinoflagellate cells congregate around the posterior end of the ciliate. Initially small strands of ciliate cytoplasm are pulled off by groups of one to five dinoflagellates. As the ciliate begins to leak cytoplasm through these small wounds, large aggregations of phagotrophic dinoflagellates are formed. Approximately 20 to 30 minutes later, the ciliate is completely digested. The attacking cells are small ( $9 \mu\text{m}$  long and  $6 \mu\text{m}$  wide), clear cells



**Fig. 7.52** *Amphidinium cryophilum* attached to a prey organism by a peduncle. The inset shows a light micrograph of three cells of *A. cryophilum* attached to a prey cell. (From Wilcox and Wedemeyer, 1991.)

that swell to 20 times their original size with food vacuoles after ingesting a victim. These large cells barely move or are motionless. *Gymnodinium funghi-forme* is attracted to a variety of amino acids and other organic compounds (Spero, 1985). Glycine, taurine, and serine attract the dinoflagellate at threshold detection levels of  $10^{-8}$  M, followed by dextrose at  $10^{-7}$  and alanine, proline, and threonine at  $10^{-6}$  M. Glycine, taurine, and alanine are three of the most abundant free amino acids found in invertebrates and protozoa, which are the major food organisms of *G. funghi-forme*.

*Pfiesteria piscicida* is a heterotrophic dinoflagellate that is responsible for many of the fish kills along the Atlantic coast of the Southeastern United States (Burkholder and Glasgow, 1997). *P. piscicida* belongs to the “ambush-predator” group of dinoflagellates which chemically detect their prey and then swarm in a direct attack on the prey. The zoospores have a peduncle that is thin and tapered when the dinoflagellate is not feeding. In the presence of fish, the dinoflagellate zoospores feed on the fish by phagocytizing fish tissue through the peduncle. The peduncle is swollen with haustoria-like penetrating extensions when feeding on the fish (Fig. 7.51(b)).

## Symbiotic dinoflagellates

Symbiotic dinoflagellates (**zooxanthellae**) occur in almost all species of tropical and reef-building corals, jellyfish, and sea anemones (Cnidaria). The dinoflagellates are coccoid spheres in the symbiotic state and have been assigned to the genus *Symbiodinium*. Studies on nucleic acid makeup have revealed the dinoflagellates to be a diverse group, although the dinoflagellate inhabiting one type of Cnidaria remains a constant (Baillie et al., 2000; Rodriguez-Lanetty et al., 2004).

*Symbiodinium* cells change into typical gymnodinoid form when placed in culture. Cultured and symbiotic *Symbiodinium* differ physiologically. The symbiotic cells grow ten times slower than cultured cells. The host exerts strong control over the translocation of metabolites from the dinoflagellate endosymbiont, resulting in 98% of the carbon fixed by the endosymbiont being released to the host. In contrast, cultured cells release only 10% of their photosynthetically fixed carbon (Trench, 1993). The host animal cells secrete the amino acid taurine which causes the

dinoflagellate to release photosynthate outside the cell for absorption by the animal cells (Wang and Douglas, 1997).

Corals with symbiotic dinoflagellates do not incorporate calcium into their skeletons as fast as corals without symbiotic dinoflagellates. During the daytime, corals with and without symbiotic dinoflagellates calcify at the same rate. Calcification occurs three times faster during the day than at night (Gattuso et al., 2001).

In the symbiosis between the marine anemones and marine dinoflagellates, the dry weight ratio of host to alga is about 300:1 (Taylor, 1969). When the anemone is subjected to poor growing conditions, it responds by excreting some of the dinoflagellate cells, which the anemone obviously has difficulty in maintaining. Even under normal growing conditions the algal population divides too rapidly, and the anemone reacts by pruning the population down to a level it can support. In this situation the older dinoflagellate cells in the outer regions of the host (crown and tentacles) respond by thickening the outer secreted layer around the cell wall and subsequently forming cysts. As the thickness of the cyst's outer layer increases, the cells begin to show signs of degeneration. The host then seems to become sensitive to the degenerate condition of the cysts and reacts by removing them from these outer regions and transporting them to the mesenteries as intracellular inclusions of its undifferentiated amoeboid cells. They are stored in the mesenteries in varying states of decomposition until they are finally excreted by the host.

In the flatworm *Amphiscolops langerhansi*, D. L. Taylor (1971) has found that the cells of the dinoflagellate *Amphidinium klebsii* occur exclusively between the cells of the peripheral parenchyma of the host and appear as a conspicuous layer below the composite muscle (Fig. 7.53). Each cell of *Amphidinium* has a specific and uniform orientation within the host. The anterior of the cell is directed toward the central parenchyma, and the posterior with the large nucleus is directed toward the epithelium. By rearing the flatworm from eggs, it is possible to obtain individuals without the dinoflagellate symbiont. When these flatworms are grown in culture with a number of different types of dinoflagellates, only species of

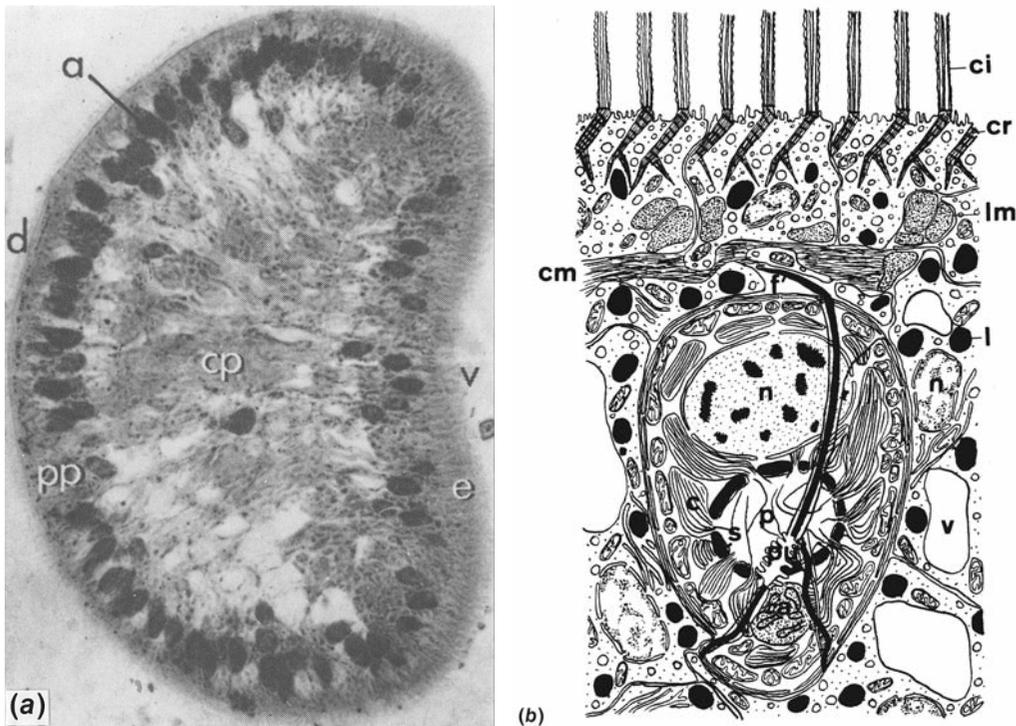
*Amphidinium* are able to infect the host. When contact is made, the animal seizes and ingests the alga. Once inside the flatworm, the alga is not retained in the central digestive paranchyma but passes freely between the animal cells to the peripheral parenchyma, where it comes to lie intercellularly below the composite muscle. The alga remains unchanged within the host and still has its typical two flagella. In nature the association between the flatworm and the dinoflagellate is probably essential for the survival of the animal. It is not known whether the dinoflagellate receives any benefit.

In addition to Dinophyceae living symbiotically inside other organisms, there are other organisms that live inside dinoflagellate cells. *Noctiluca scintillans* is a heterotrophic omnivorous feeder that ingests zooplankton, mesozooplankton, and their eggs (Fig. 7.47) (Hansen et al., 2004). However, in the tropical to subtropical areas of Southeast Asia, a green form of *N. scintillans* is found. The cells have 6 000 to 12 000 green flagellates actively swimming in the fluid within the large vacuoles (Fig. 7.54), especially around the periphery of the vacuole. The green flagellate resembles the green alga *Pedinomonas* in being bright green,  $2 \times 5 \mu\text{m}$  in size, with no eyespot, and having a single posterior flagellum. Whether the dinoflagellate gains any advantage from the association is not known.

## Classification

There is a single class in the Dinophyta, the Dinophyceae. Four orders are considered here. Molecular studies have shown that the Prorocentrales, Peridiniales, and Gymnodiniales represent three clear lines of evolution (Zardoya et al., 1995). The Dinophysiales are probably related to the Prorocentrales since they are both divided vertically into two halves.

- Order 1 Prorocentrales: cell wall divided vertically into two halves; no girdle; two flagella borne at cell apex.
- Order 2 Dinophysiales: cell wall divided vertically into two halves, cells with elaborate extensions of the theca.

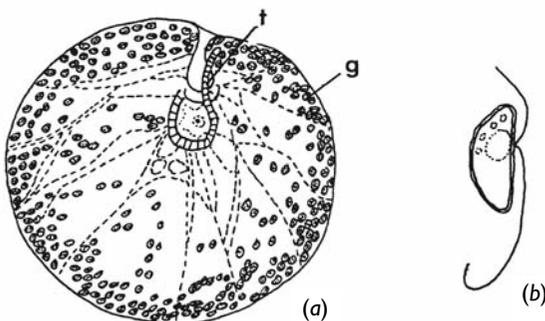


**Fig. 7.53** (a) Cross section of the flatworm *Amphiscolops langerhansii* showing the dinoflagellate *Amphidinium klebsii* (a) in the peripheral parenchyma (pp). (cp) Central parenchyma; (d) dorsal surface; (e) epithelium; (v) ventral surface.  $\times 90$ . (b) Diagrammatic reconstruction of the above association. (a) Accumulation body; (c) chloroplast; (ci) cilia; (cm) circular muscle; (cr) ciliary root; (f) flagellum; (l) lipid; (lm) longitudinal muscle; (n) nucleus; (p) pyrenoid; (pu) pusule; (s) starch; (v) vacuole. (After D. Taylor, 1971.)

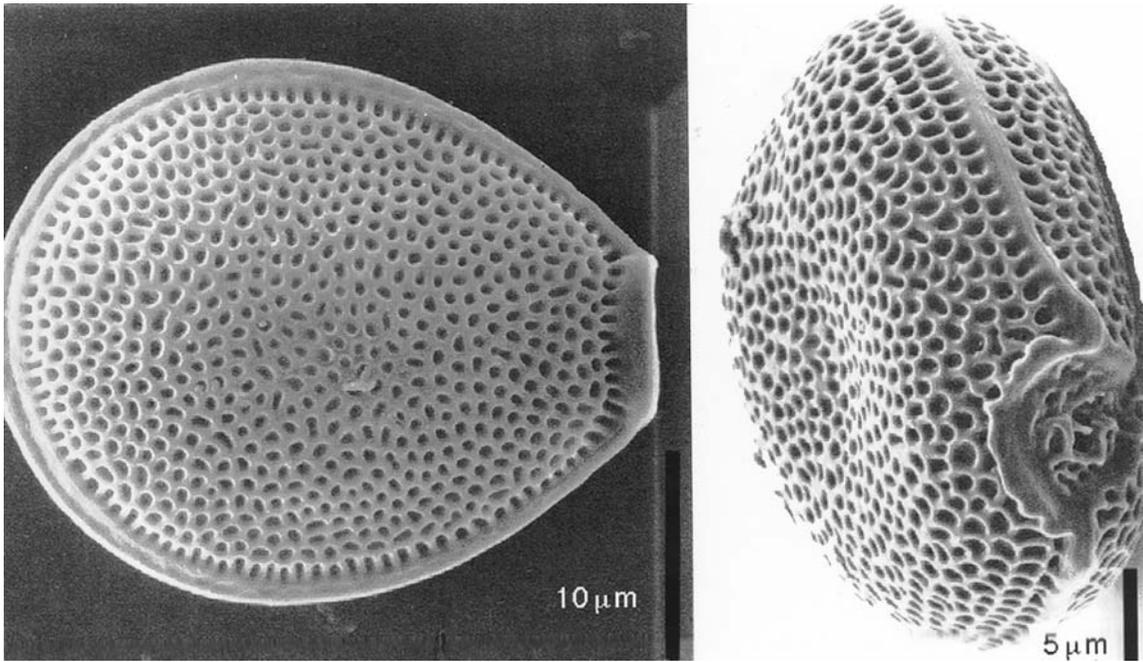
- Order 3 Peridiniales: motile cells with an epicone and hypocone separated by a girdle, relatively thick theca.
- Order 4 Gymnodiniales: motile cells with an epicone and hypocone separated by a girdle; theca thin or reduced to empty vesicles.

### Prorocentrales

These cells have the cell wall divided vertically into two halves, no girdle, and two apically inserted flagella, *Prorocentrum* (Figs. 7.55, 7.56(b), (c)) can be used as an example of the order. The cell is divided vertically into two halves, each half containing a relatively thick thecal plate, the suture joining them running from the anterior to the posterior end. The cell is flattened parallel to the suture so the two halves are like two watch glasses. The two flagella emerge apically through a pore, and there is a single tooth containing cytoplasm. Usually there are two brownish-yellow chloroplasts, apposed to the two valves. Asexual reproduction takes place by longitudinal division, the daughter cells retaining one valve of the parent and forming a new second valve.



**Fig. 7.54** *Noctiluca* containing green algae in its vacuoles. (a) Whole cell. (g) Green algae; (t) tentacle. (b) Green alga symbiont. (After Sweeney, 1971.)



**Fig. 7.55** Scanning electron micrographs of *Prorocentrum hoffmanianum*. (From Faust, 1990.)

## Dinophysiales

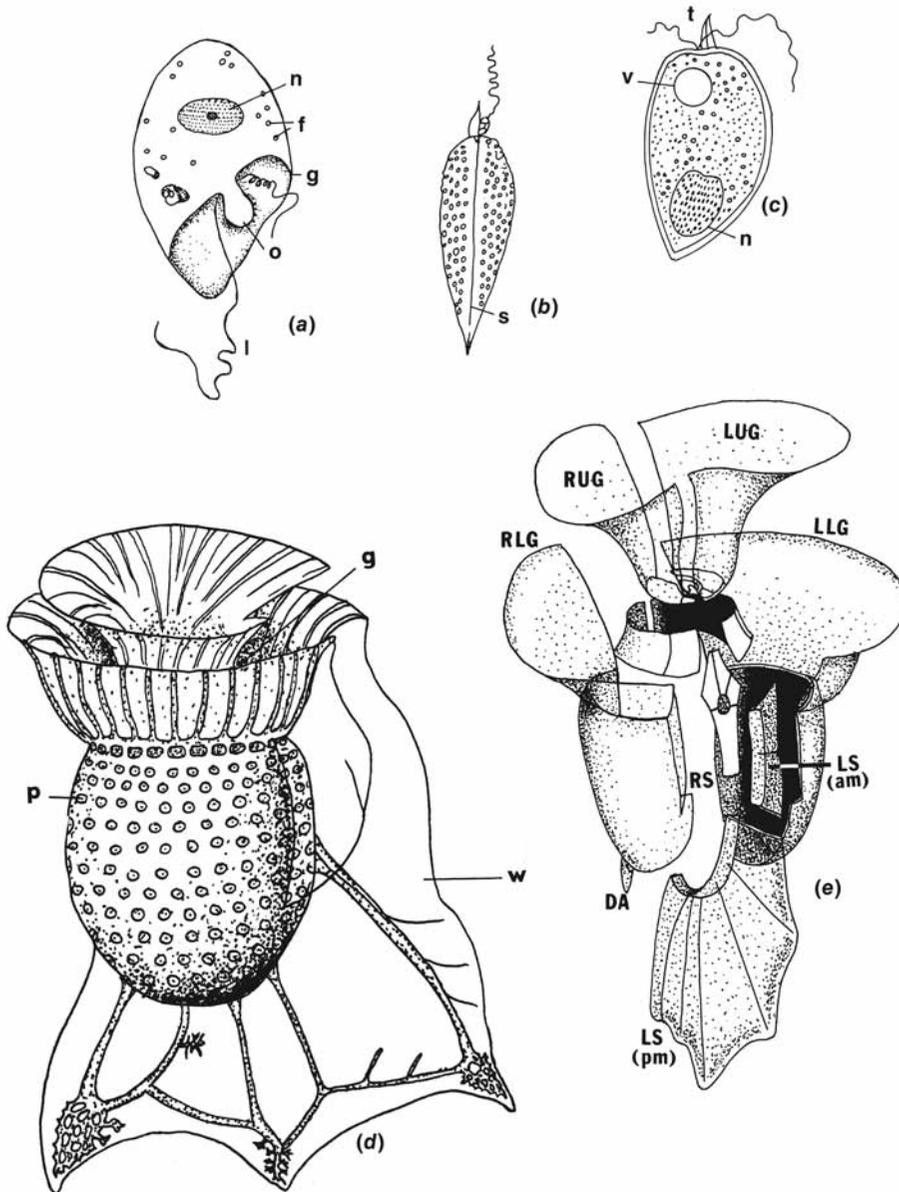
This order consists of morphologically complex organisms that are mainly in tropical seas, with the cells having adaptations to the floating habit such as elaborate wings (*lists*). One of the more complex organisms in this order is *Ornithocercus* (Fig. 7.56(d), (e)). The cell is divided vertically into two halves by an anterior posterior suture. The cells have a girdle and sulcus, with the side having the sulcus being the ventral side and the opposite side being the dorsal side. The respective flagella lie in the girdle and transverse sulcus. The epicone is usually much smaller than the hypocone, and the edges of the thecal plates next to the girdle and sulcus are expanded into the lists. In asexual reproduction the cell is cleaved vertically, with the two halves separating and the missing half being formed by the protoplasm. The oldest fossil of this group is *Nannoceratopsis* from the Lower Jurassic (Loeblich, 1974, 1976).

## Peridinales

The dinoflagellates in this order have relatively thick thecal plates, in contrast to the next order,

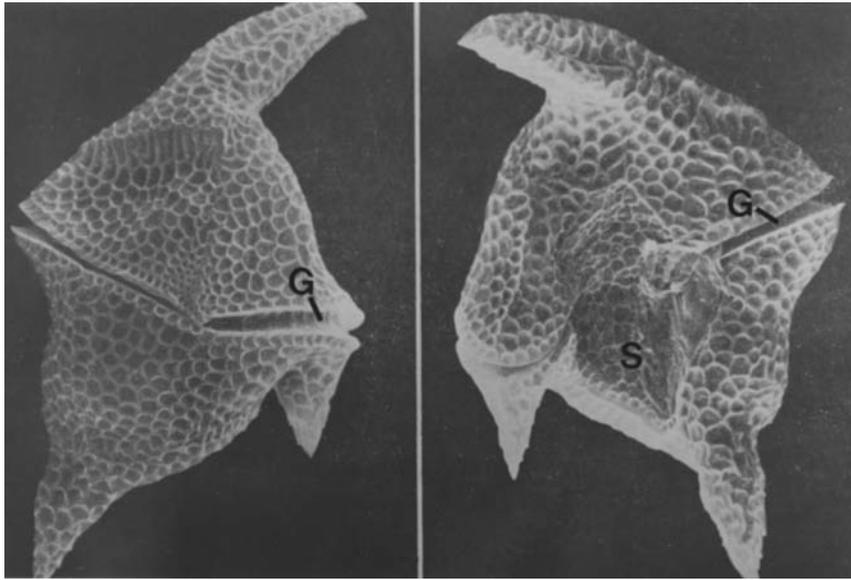
the Gymnodinales, which has no, or thin, thecal plates. The algae in this order have the classic dinoflagellate structure (Figs. 7.1, 7.2) with an epicone and hypocone and two furrows, the transverse girdle and the longitudinal sulcus.

The widely distributed genus *Ceratium* (Figs. 7.12, 7.57, 7.58) is markedly asymmetric, with one apical horn and two to three long antapical horns filled with cytoplasm. The girdle is nearly horizontal and divides the body into two approximately equal, but dissimilar halves. In the middle of the ventral surface is a large rhombic hyaline area, which is probably similar to a sulcus. Like other Dinophyceae and Prymnesiophyceae, *Ceratium* is more common in warmer water than in colder polar waters. There are fewer than ten species common in the colder waters of the North Atlantic, whereas more than 20 species are common in the warmer more southerly waters (Graham and Bronikovsky, 1944). *Ceratium* is one of the members of the phytoplankton that has **shade forms** which show an increase in frequency from the surface to 100 m depth. These shade forms are found only in the relatively sterile warm oceanic waters where the upper layers are usually depleted of nitrogen and phosphorus. The shade forms of *Ceratium* have a survival value in that



**Fig. 7.56** (a) *Oxyrrhis marina*. (f) Fat globule; (g) girdle; (l) longitudinal flagellum; (n) nucleus; (o) tentacle. (b),(c) *Prorocentrum micans*, side (b) and front (c) views. (n) Nucleus; (s) suture; (v) vacuole. (d),(e) *Ornithoceros magnificus*, a right-hand view (d) and an “exploded” view (e) of the theca. (DA) Dorsal accessory moiety of the left sulcal list; (LLG) left lower girdle list; (LS(am)) anterior moiety of the left sulcal list; (LS(pm)) posterior moiety of the left sulcal list; (LUG) left upper girdle list; (RS) right sulcal list; (RLG and RUG) right lower and upper girdle list; (g) girdle; (p) pore; (w) wing. ((d),(e) after F. Taylor, 1971.)

they are able to take advantage of the higher levels of nitrogen and phosphorus deeper in the water and still are able to receive enough light to keep their photosynthetic rate above their respiratory rate. These shade forms adapt themselves to absorb the maximum amount of light by increasing the surface area of the cell by expansion of the cell body and/or horns and packing these extensions with chloroplasts. Long-horned forms are found among surface forms also, but the shade



**Fig. 7.57** Scanning electron micrographs of both sides of a vegetative cell of *Ceratium cornutum*. (G) Girdle; (S) sulcus. (From Happach-Kasan, 1982.)

forms always have their horns crowded with chloroplasts.

*Ceratium horridum* has a life cycle that is more or less similar to the known life cycle of other Dinophyceae. Von Stosch (1972) (Fig. 7.60) has shown that the vegetative cells of *C. horridum* are haploid. The male gametes are smaller than the female gametes; thus fusion in this organism is anisogamous (Fig. 7.58). The male gamete attaches by its ventral side to the ventral side of the female gamete. The cell wall of the male then breaks up into its individual plates, which are taken up into the female cytoplasm. The naked male gamete fuses with the female gamete to produce a zygote. The zygote remains motile (planozygote) through all of the following steps. The planozygote grows for several days, ending up as a large cell with unusually long horns and a thick cell wall. Finally, the nucleus enlarges, and nuclear cycloses (movement) begins. At its highest speed the chromosomal mass circulates once every 30 seconds. After some hours the motion slows down and stops, and approximately 12 hours later meiosis commences. The first meiotic division halves the chromosome number and gives rise to one flagellate with normally sized antapical, and abnormally long apical

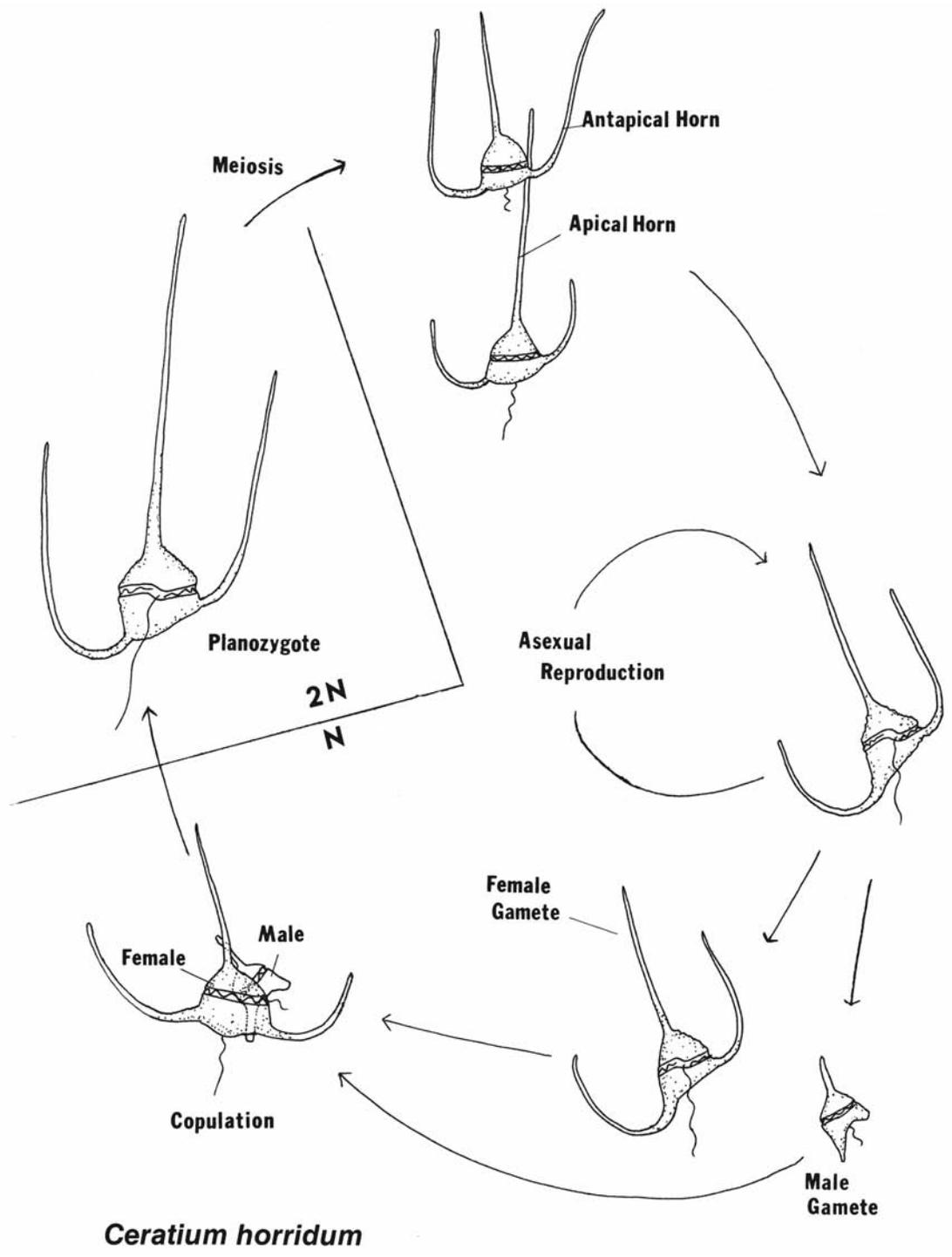
horns and a second flagellate with normally sized apical, and abnormally long antapical horns. After 2 to 3 days the second meiotic division takes place to yield haploid vegetative cells.

### Gymnodiniales

The dinoflagellates in this order are similar to those in the previous order, the Peridiniales, except that they have thin or no thecal plates.

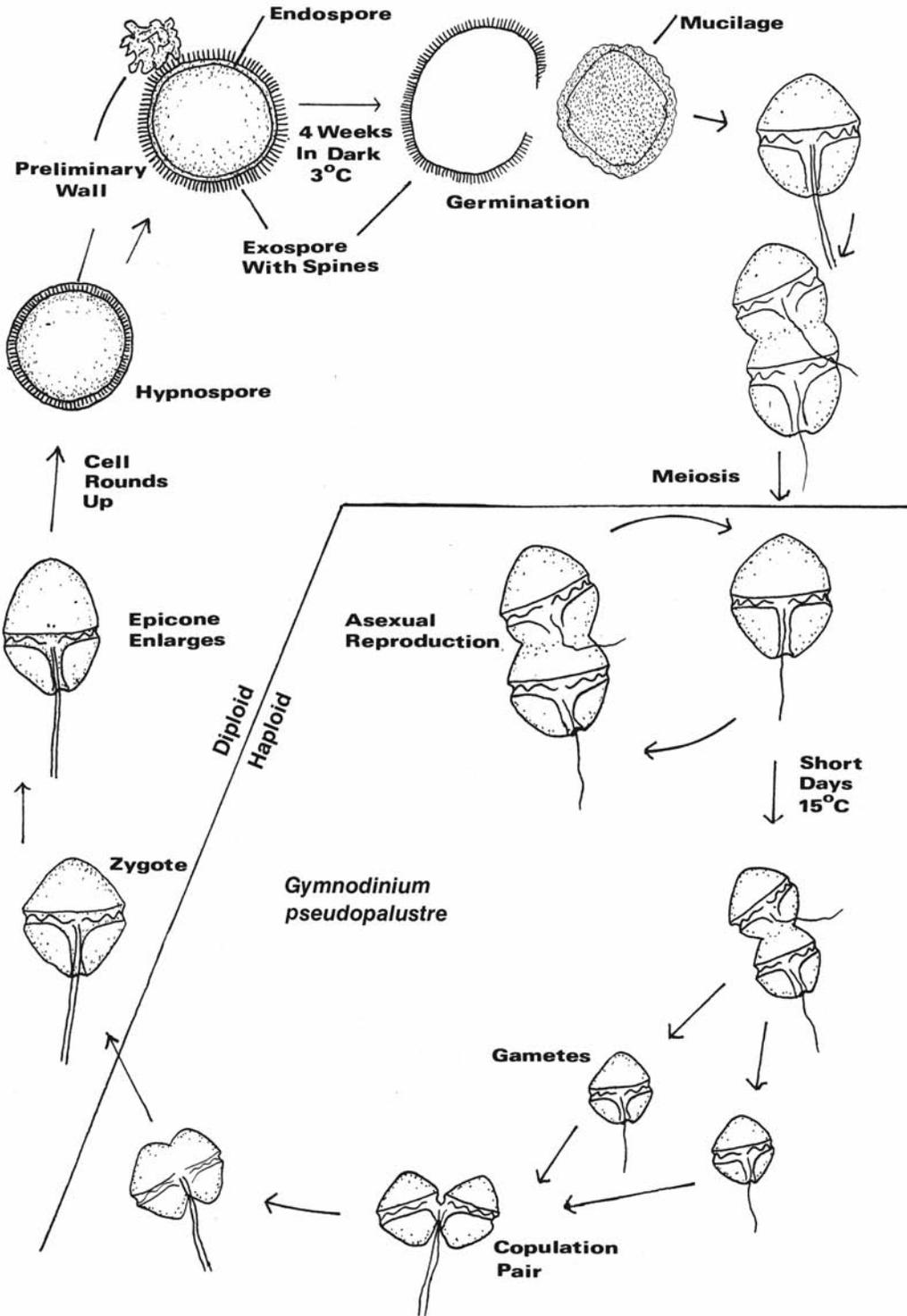
The life cycle of *Gymnodinium pseudopalustre* is a representative of the order. Vegetative multiplication results in daughter cells that remain attached to each other for at least 12 hours (Fig. 7.59). Gametes are formed when an actively growing culture at 21 °C is subjected to a short-day treatment (10 hours light) at 15 °C (von Stosch, 1973). Gamete differentiation occurs by divisions that give rise to cells lower in mass and poorer in plastids and pigments than the vegetative cells. Apart from their smaller size and lighter color, the gametes are not obviously different from vegetative cells. They are incapable of living for long as vegetative cells and die if fusion does not occur. The gametes are homothallic; thus gametes from a single strain will fuse.

Initially, small groups of two to ten gametes swarm around each other in a weaving motion. A copulation pair occurs between two gametes, with the pair swimming rapidly while turning slowly on a common axis. Copulation is isogamous although



***Ceratium horridum***

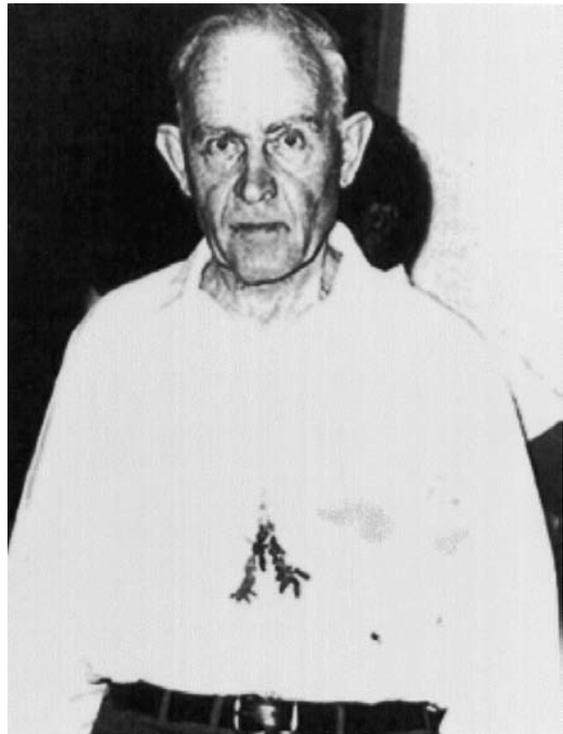
**Fig. 7.58** The life cycle of *Ceratium horridum*. (Adapted from von Stosch, 1972.)



**Fig. 7.59** The life cycle of *Gymnodinium pseudopalustre*.  
(Adapted from von Stosch, 1973.)

there can be a slight difference in the size of the gametes. The cells are joined together by a hyaline globular bridge slightly below the intersection of the transverse and posterior grooves. After 30 minutes the fusion of protoplasts has begun, with the bridge between the two cells enlarging. At the end of fusion a cell similar to a vegetative cell is attained. The flagella and nuclei of each gamete are still distinct. The zygote grows, at first having the shape of a vegetative cell, and later the epicone elongates. One of the transverse flagella is lost during this development, but both of the posterior flagella persist. The non-motile zygote suddenly rounds off and secretes a preliminary wall. This wall is subsequently inflated, giving rise to a hyaline area between the wall and the protoplast surface. An ornamentation of small separated granules now appears on the protoplast wall, which grow out radially to become spines while the hyaline area increases in width. The preliminary wall then bursts and crumples away to one side of the spore. The preliminary wall is necessary for formation of the hypnospore. The duration of the preliminary wall is only about 9 minutes.

During the next 48 hours the hypnospore matures, with the plastids bleaching and becoming inconspicuous, masses of red oil appearing, the starch becoming indistinct, and the two nuclei now fusing. A thick cellulosic endospore is also secreted under the exospore with its spines. The hypnospores germinate after treatment for 4 weeks in the dark at 3 °C before being returned to light and higher temperature. After the cellulosic endospore has been digested away, approaching release of the swarmer is indicated by a slight contraction of the protoplast so that the transverse groove of the prospective flagellate becomes visible. The space between the spore wall and the surface of the swarmer is filled with mucilage. Eventually the wall bursts, and the swarmer escapes, enveloped in mucilage. The swarmer frees itself from the mucilage and swims away. The swarmer is rather plump and of oval shape at first, and, apart from red oil globules, nearly colorless; but later it acquires brown pigment, and its form becomes similar to that of the vegetative cell. Two "skiing track" posterior flagella have reappeared. The swarmer then goes through two meiotic divisions, resulting in four haploid flagellates.



**Fig. 7.60** Hans Adolf von Stosch, 1908–1987. Dr. von Stosch was born in Berlin and studied at the Universities of Kiel, Göttingen, and Munich. Before World War II he worked at the University of Königsberg/Ostpreussen. He became a soldier in 1939, was taken prisoner in Tunisia in 1943, and was released in England in 1947. He obtained a position at the Technical University in Darmstadt, where he began his studies on algae in earnest. In 1955, he moved to the University of Marburg where he stayed until his retirement in 1976. His work on the life histories of dinoflagellates is some of the best work done on the group. (Photo from Garbary and Wynne, 1996.)

## REFERENCES

- Abrahams, M. V., and Townsend, L. D. (1993). Bioluminescence in dinoflagellates: a test of the burglar alarm hypothesis. *Ecology* 74:258–60.
- Adamich, M., Laris, P. C., and Sweeney, B. M. (1976). In vivo evidence for a circadian rhythm in membranes of *Gonyaulax*. *Nature* 261:583–5.
- Baillie, B. K., Belda-Baillie, C. A., and Maruyama, T. (2000). Conspecificity and indo-pacific distribution of *Symbiodinium* genotypes (Dinophyceae) from giant clams. *J. Phycol.* 36:1153–61.

- Balzer, I., and Hardeland, R. (1996). Melatonin in algae and higher plants – possible new roles as a phytochrome and antioxidant. *Bot. Acta* 109:180–3.
- Barlow, S. B., and Triemer, R. E. (1988). The mitotic apparatus of the dinoflagellate *Amphidinium carterae*. *Protoplasma* 145:16–26.
- Berdach, J. T. (1977). In situ preservation of the transverse flagellum of *Peridinium cinctum* (Dinophyceae) for scanning electron microscopy. *J. Phycol.* 13:243–51.
- Bibby, B. T., and Dodge, J. D. (1972). The encystment of a freshwater dinoflagellate: A light and electron-microscopical study. *Br. Phycol. J.* 7:85–100.
- Bouck, G. B., and Sweeney, B. M. (1966). The fine structure and ontogeny of trichocysts in marine dinoflagellates. *Protoplasma* 61:205–23.
- Bricheux, G., Mahoney, D. G., and Gibbs, S. P. (1992). Development of the pellicle and thecal plates following ecdysis in the dinoflagellate *Glenodinium foliaceum*. *Protoplasma* 168:159–71.
- Brooks, B. J., and Anderson, D. M. (1990). Biochemical composition and metabolic activity of *Scrippsiella trochoidea* (Dinophyceae) resting cysts. *J. Phycol.* 26:289–98.
- Burkholder, J. M., and Glasgow, H. B. (1997). Trophic controls on stage transformation of a toxic ambush-predator dinoflagellate. *J. Euk. Microbiol.* 44:200–5.
- Buskey, E. J., and Swift, E. (1983). Behavioral responses of *Acartia hudsonica* to simulated dinoflagellate bioluminescence. *J. Exp. Mar. Biol. Ecol.* 77:43–58.
- Cembella, A. D. (2003). Chemical ecology of eukaryotic microalgae in marine ecosystems. *Phycologia* 42:420–47.
- Chapman, D. V., Dodge, J. D., and Heaney, S. J. (1982). Cyst formation in the freshwater dinoflagellate *Ceratium hirundinella*. *J. Phycol.* 18:121–9.
- Chatton, E. (1952). Classe des dinoflagelles ou peridiniens. In *Traité de Zoologie*, ed. P-P. Grassé, pp. 304–406. Paris: Masson.
- Chinain, M., Germain, M., Sako, Y., Pauillac, S., and Legrand, A-M. (1997). Intraspecific variation in the dinoflagellate *Gambierdiscus toxicus* (Dinophyceae). I. Isoenzyme analysis. *J. Phycol.* 33:36–43.
- Clarke, K. J., and Pennick, N. C. (1976). The occurrence of body scales in *Oxyrrhis marina* Dujardin. *Br. Phycol. J.* 11:345–8.
- Crawford, R. M., Dodge, J. D., and Happey, C. M. (1970). The dinoflagellate genus *Woloszynskia*. I. Fine structure and ecology of *W. tenuissima* from Abbot's Pool, Somerset. *Nova Hedwigia* 19:825–40.
- Daugbjerg, N., Hansen, G., Larsen, J., and Moestrup, O. (2000). Phylogeny of some major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. *Phycologia* 39:302–17.
- Destombe, C., and Cembella, A. (1990). Mating-type determination, gamete recognition and reproductive success in *Alexandrium excavatum* (Gonyaulacales, Dinophyta), a toxic red-tide dinoflagellate. *Phycologia* 29:315–25.
- Dodge, J. D. (1971). Fine structure of the Pyrrophyta. *Bot. Rev.* 37:481–508.
- Dodge, J. D., and Crawford, R. M. (1968). Fine structure of the dinoflagellate *Amphidinium carteri* Hullbert. *Protistologica* 4:231–42.
- Dodge, J. D., and Crawford, R. M. (1969). Observations of the fine structure of the eyespot and associated structures in the dinoflagellate *Glenodinium foliaceum*. *J. Cell Sci.* 5:479–93.
- Dodge, J. D., and Crawford, R. M. (1970). A survey of thecal fine structure in the Dinophyceae. *J. Linn. Soc. Bot.* 63:53–67.
- Downie, C. (1956). Microplankton from the Kimmeridge Clay. *Q. J. Geol. Soc. Lond.* 112:413–34.
- Dunlap, J. C., and Hastings, J. W. (1981). Biochemistry of dinoflagellate bioluminescence: Purification and characterization of dinoflagellate luciferin from *Pyrocystis lunula*. *Biochemistry* 20:983–9.
- Ellegaard, M., Christensen, N. F., and Moestrup, O. (1994). Dinoflagellate cysts from recent Danish marine sediments. *Eur. J. Phycol.* 29:183–94.
- Eppley, R. W., Holm-Hansen, O., and Strickland, J. D. H. (1968). Some observations on the vertical migration of dinoflagellates. *J. Phycol.* 4:333–40.
- Faust, M. A. (1990). Morphological details of six benthic species of *Prorocentrum* (Pyrrophyta) from a mangrove island, Twin Cays, Belize, including two new species. *J. Phycol.* 26:548–58.
- Faust, M. A. (1995). Observation of sand-dwelling toxic dinoflagellates (Dinophyceae) from widely differing sites, including two new species. *J. Phycol.* 31:996–1003.
- Fenchel, T. (2001). How dinoflagellates swim. *Protist* 152:329–38.
- Fritz, L., Milos, P., Morse, D., and Hastings, J. W. (1991). *In situ* hybridization of luciferase-binding protein anti-sense RNA to thin sections of the bioluminescent dinoflagellate *Gonyaulax polyedra*. *J. Phycol.* 27:436–41.
- Gaines, G., and Taylor, F. J. R. (1984). Extracellular digestion in marine dinoflagellates. *J. Plank. Res.* 6:1057–61.

- Gaines, G., and Taylor, F. J. R. (1985). Form and function of the dinoflagellate transverse flagellum. *J. Protozool.* 32:290–6.
- Gallois, R. W. (1976). Coccolith blooms in the Kimmeridge Clay and origin of the North Sea oil. *Nature* 259:473–5.
- Garbary, D. J., and Wynne, M. J. (1996). *Prominent Phycologists of the 20th Century*. Hantsport, Nova Scotia: Lancelot Press.
- Gattuso, J.-P., Reynaud-Vaganay, S., Furla, P., Romaine-Lioud, S., and Jaubert, J. (2000). Calcification does not stimulate photosynthesis in the zooxanthellate scleractinian coral *Stylophora pistillata*. *Limnol. Oceanogr.* 45:246–50.
- Gao, X.-P., and Li, J.-Y. (1986). Nuclear division in the marine dinoflagellate *Oxyrrhis marina*. *J. Cell Sci.* 85:161–75.
- Giner, J.-L., Faraldos, J. A., and Boyer, G. L. (2003). Novel sterols of the toxic dinoflagellate *Karenia brevis* (Dinophyceae): a defensive function for unusual marine sterols. *J. Phycol.* 39:315–19.
- Graham, H. W., and Bronikovsky, N. (1944). The genus *Ceratium* in the Pacific and North Atlantic oceans. *Carnegie Inst. Washington Publ.* 565:1–209.
- Green, B. R. (2004). The chloroplast genome of dinoflagellates: a reduced instruction set? *Protist* 155:23–31.
- Grindley, J. R., and Nel, E. A. (1970). Red water and mussel poisoning at Elands Bay, December 1966. *Fish Bull., S. Afr.* 6:36–55.
- Grindley, J. R., and Sapeika, N. (1969). The cause of mussel poisoning in South Africa. *S. Afr. Med. J.* 43:275–9.
- Gruet, C. (1965). Structure fine de l'ocelle d'*Erythrospira pavillardii* Hetwig, Péridinien Warnowiidae Lindemann. *C. R. Séances Acad. Sci., Paris* 261:1904–7.
- Guisande, C., Frangopulos, M., Carolenuto, Y., Maneiro, I., Riveiro, I., and Vergara, A.R. (2002). Fate of paralytic shellfish poisoning toxins ingested by the copepod *Acartia clausi*. *Mar. Ecol. Progr. Ser.* 240:105–15.
- Hackett, J. D., Anderson, D. M., Erdner, D. L., and Bhattacharya, D. (2004). Dinoflagellates: a remarkable evolutionary experiment. *Amer. J. Bot.* 91:1523–34.
- Hallegraeff, G. M. (1993). A review of harmful algal blooms and their apparent global increase. *Phycologia* 32:79–99.
- Hansen, G. (1989). Ultrastructure and morphogenesis of scales in *Katodinium rotundatum* (Lohmann) Loeblich (Dinophyceae). *Phycologia* 28:385–94.
- Hansen, G. (1993). Light and electron microscopical observation of the dinoflagellate *Actiniscus pentasterias* (Dinophyceae). *J. Phycol.* 29:486–99.
- Hansen, P. J., and Calado, A. J. (1999). Phagotrophic mechanisms and prey selection in free-living dinoflagellates. *J. Eukary. Microbiol.* 46:382–9.
- Hansen, P. J., Miranda, L., and Azanza, R. (2004). Green *Noctiluca scintillans*: a dinoflagellate with its own greenhouse. *Mar. Ecol. Progr. Ser.* 275:79–87.
- Happach-Kasan, C. (1982). Beobachtungen zum Bau der Theka von *Ceratium cornutum*, (Ehrenb.) Clap. et Lachm. (Dinophyta). *Arch. Protistenk.* 125:181–207.
- Harvey, E. N. (1952). *Bioluminescence*. New York: Academic Press.
- Hastings, J. W. (1983). Biological diversity, chemical mechanisms, and the evolutionary origins of bioluminescent systems. *J. Mol. Evol.* 19:309–21.
- Hastings, J. W. (1986). Bioluminescence in bacteria and dinoflagellates. In *Light Emission in Plants and Bacteria*, pp. 363–98. New York: Academic Press.
- Hastings, J. W., and Krasnow, R. (1981). Temporal regulation in the individual *Gonyaulax* cell. In *International Cell Biology 1980–1981*, Proc. 2nd Int. Cong. on Cell Biology, pp. 815–823. Berlin: Springer-Verlag.
- Haywood, A. J., Steidinger, K. A., Truby, E. W., et al. (2004). Comparative morphology and molecular phylogenetic analysis of three new species of the genus *Karenia* (Dinophyceae) from New Zealand. *J. Phycol.* 40:165–79.
- Höhfeld, I., and Melkonian, M. (1992). Amphiesmal ultrastructure of dinoflagellates, A reevaluation of pellicle formation. *J. Phycol.* 28:82–9.
- Höhfeld, I., and Melkonian, M. (1998). Lifting the curtain? The microtubular cytoskeleton of *Oxyrrhis marina* (Dinophyceae) and its rearrangement during phagocytosis. *Protist* 149:75–88.
- Höhfeld, I., Otten, J., and Melkonian, M. (1988). Contractile eukaryotic flagella: Centrin is involved. *Protoplasma* 147:16–24.
- Horiguchi, T., and Pienaar, R. N. (1988). Ultrastructure of a new sand-dwelling dinoflagellate *Scrippsiella arenicola* sp. nov. *J. Phycol.* 24:426–38.
- Horiguchi, T., Kawai, H., Kubota, M., Takahasdi, T., and Watanabe, M. (1999). Phototactic responses of four marine dinoflagellates with different types of eyespot and chloroplast. *Phycol. Res.* 47:101–7.
- Hu, T., Burton, I., Curtis, J. M., et al. (1999). Oxidative transformation of a naturally occurring okadaic acid diol ester by the diatom *Thalassiosira weissflogii*. *Tetrahedron Lett.* 40:3981–4.

- Igarashi, T., Aritake, S., and Yasumoto, T. (1999). Mechanisms underlying the hemolytic and ichthyotoxic activities of maitotoxin. *Nat. Toxins* 7:71–9.
- Ishida, K., and Green, B. R. (2002). Second- and third-hand chloroplasts in dinoflagellates: phylogeny of oxygen-evolving enhancer 1 (PsbO) protein reveals replacement of a nuclear-encoded plastid gene by that of a haptophyte tertiary endosymbiosis. *Proc. Natl. Acad. Sci., USA* 99:9294–9.
- Iwataki, M., Hansen, G., Sawaguchii, T., Hiroishi, S., and Fukuyo, Y. (2004). Investigations of body scales in twelve *Heterocapsa* species (Peridinales, Dinophyceae), including a new species *H. pseudotriquetra* sp. nov. *Phycologia* 43:394–403.
- Jacobsen, D. M. (1999). A brief history of dinoflagellate feeding research. *J. Eukary. Microbiol.* 46:376–81.
- Jacobsen, D. M., and Anderson, D. M. (1986). Thecate heterotrophic dinoflagellates: Feeding behavior and mechanisms. *J. Phycol.* 22:249–58.
- Janofske, B. (2000). *Scrippsiella trochoidea* and *Scrippsiella regalis*, nov. comb. (Peridinales, Dinophyceae): a comparison. *J. Phycol.* 36:178–89.
- John, E. H., and Flynn, K. J. (2002). Modelling changes in paralytic shellfish toxin content of dinoflagellates in response to nitrogen and phosphorus supply. *Mar. Ecol. Progr. Ser.* 225:147–60.
- Johnson, C. H., and Hastings, J. W. (1986). The elusive mechanism of the circadian clock. *Am. Sci.* 74:29–36.
- Johnson, C. H., Inoué, S., Flint, A., and Hastings, J. W. (1985). Compartmentalization of algal bioluminescence: Autofluorescence of bioluminescent particles in the dinoflagellate *Gonyaulax* as studied with image-intensified video microscopy and flow cytometry. *J. Cell Biol.* 100:1435–46.
- Juhl, A. R., and Latz, M. J. (2002). Mechanisms of fluid shear-induced inhibition of population growth in a red-tide dinoflagellate. *J. Phycol.* 38:683–94.
- Keeling, P. J. (2004). Diversity and evolutionary history of plastids and their hosts. *Amer. J. Bot.* 91:1481–93.
- Kennaway, G. M., and Lewis, J. M. (2004). An ultrastructural study of hypnospores of *Alexandrium* species (Dinophyceae). *Phycologia* 43:353–63.
- Klut, M. E., Bisalputra, T., and Antia, N. J. (1985). Some cytochemical studies on the cell surface of *Amphidinium carteri* (Dinophyceae). *Protoplasma* 129:93–9.
- Kokinos, J. P., Eglinton, T. I., Goni, M., Boon, J. J., Martoglio, P. A., and Anderson, D. A. (1998). Characterization of a highly resistant biomacromolecular material in the cell wall of a marine dinoflagellate resting cyst. *Org. Geochem.* 28:265–88.
- Kremp, A. (2001). Effects of cyst resuspension on germination and seeding of two bloom-forming dinoflagellates in the Baltic Sea. *Mar. Ecol. Progr. Ser.* 216:57–66.
- Kubai, D. F., and Ris, H. (1969). Division in the dinoflagellate *Gyrodinium cohnii* (Schiller). A new type of nuclear reproduction. *J. Cell Biol.* 40:508–28.
- Leadbeater, B. S. C., and Dodge, J. D. (1967a). Fine structure of the dinoflagellate transverse flagellum. *Nature* 213:421–2.
- Leadbeater, B. S. C., and Dodge, J. D. (1967b). An electron microscope study of nuclear and cell division in a dinoflagellate. *Arch. Mikrobiol.* 57:239–54.
- Leblond, J. D., and Chapman, P. J. (2002). A survey of the sterol composition of the marine dinoflagellates *Karenia brevis*, *Karenia mikimoto*, and *Karlodinium micrum*: distribution of sterols within other members of the class Dinophyceae. *J. Phycol.* 38:670–82.
- Lee, R. E. (1977). Saprophytic and phagocytic isolates of the colorless heterotrophic dinoflagellate *Gyrodinium lebouriae* Herdman. *J. Mar. Biol. Assoc. UK* 57:303–15.
- Lewitus, A. J., Glasgow, H. B., and Burkholder, J. M. (1999). Kleptoplastidy in the toxic dinoflagellate *Pfiesteria piscicida* (Dinophyceae). *J. Phycol.* 35:305–12.
- Loeblich, A. R. (1968). A new marine dinoflagellate genus, *Cachonia*, in axenic culture from the Salton Sea, California with remarks on the genus *Peridinium*. *Proc. Biol. Soc. Wash.* 81:91–6.
- Loeblich, A. R. (1974). Protistan phylogeny as indicated by the fossil record. *Taxon* 23:277–90.
- Loeblich, A. R. (1976). Dinoflagellate evolution: Speculation and evidence. *J. Protozool.* 23:13–28.
- Lombard, E. H., and Capon, B. (1971). Observations on the tide pool behaviour of *Peridinium gregarium*. *J. Phycol.* 7:188–94.
- MacRae, R. A., Fensome, R. A., and Williams, G. L. (1996). Fossil dinoflagellate diversity, originations, and extinctions and their significance. *Can. J. Bot.* 74:1687–94.
- Maruyama, T. (1982). Fine structure of the longitudinal flagellum in *Ceratium tripos*, a marine dinoflagellate. *J. Cell Sci.* 58:109–23.
- Maruyama, T. (1985). Ionic control of the longitudinal flagellum in *Ceratium tripos* (Dinoflagellida). *J. Protozool.* 3:106–10.
- Matsuoka, K., Cho, H.-J., and Jacobsen, D. M. (2000). Observations of the feeding behavior and growth rates of the heterotrophic dinoflagellate *Polykrikos kofoidii* (Polykrikaceae, Dinophyceae). *Phycologia* 39:82–6.

- Meksumpun, S., Montani, S., and Uematsu, M. (1994). Elemental composition of cell walls of three marine phytoflagellates, *Chattonella antiqua* (Raphidophyceae), *Alexandrium catenella* and *Scrippsiella trochoidea* (Dinophyceae). *Phycologia* 33:275–80.
- Messer, G., and Ben-Shaul, Y. (1969). Fine structure of *Peridinium westii* Lemm., a freshwater dinoflagellate. *J. Protozool.* 16:272–80.
- Montresor, M., Janofske, D., and Willems, H. (1997). The cyst-theca relationship in *Calciodinellum operosum* emend. (Peridinales, Dinophyceae) and a new approach for the study of calcareous cysts. *J. Phycol.* 33:122–31.
- Moronin, L., and Francis, D. (1967). The fine structure of *Nematodinium armatum*, a naked dinoflagellate. *J. Microscopie* 6:759–72.
- Morrill, L. C., and Loeblich, A. R. (1983a). Ultrastructure of the dinoflagellate amphisema. *Int. Rev. Cytol.* 82:151–80.
- Morrill, L. C., and Loeblich, A. R. (1983b). Formation and release of body scales in the dinoflagellate genus *Heterocapsa*. *J. Mar. Biol. Assoc. UK* 63:905–13.
- Morton, S. L., and Tindall, D. R. (1995). Morphological and biochemical variability of the toxic dinoflagellate *Prorocentrum lima* isolated from three locations at Heron Island, Australia. *J. Phycol.* 31:914–21.
- Nagai, S., Matsuyama, Y., Takayama, H., and Kotani, Y. (2002). Morphology of *Polykrikos kofoidii* and *P. schwartzii* (Dinophyceae, Polykrikaceae) cysts obtained in culture. *Phycologia* 41:319–27.
- Nagai, S., Itakura, S., Matsuyama, Y., and Kotani, Y. (2003). Encystment under laboratory conditions of the toxic dinoflagellate *Alexandrium tamiyavanichii* (Dinophyceae) isolated from Seto Island Sea, Japan. *Phycologia* 42:646–53.
- Naustvoll, L.-J. (1998). Growth and grazing by the thecate heterotrophic dinoflagellate *Diplopsalis lenticula* (Diplopsalidaceae, Dinophyceae). *Phycologia* 37:1–9.
- Nawata, T., and Sibaoka, T. (1983). Experimental induction of feeding behavior in *Noctiluca miliaris*. *Protoplasma* 115:34–42.
- Nawata, T., and Sibaoka, T. (1987). Local ion currents controlling the localized cytoplasmic movement associated with feeding initiation of *Noctiluca*. *Protoplasma* 137:125–33.
- Nicolas, M. T., Morse, D., Bassot, J.-M. and Hastings, J. W. (1991). Colocalization of luciferin binding protein and luciferase to the scintillons of *Gonyaulax polyedra* revealed by double immunolabeling after fast-freeze fixation. *Protoplasma* 160:159–66.
- Perez, C. C., Roy, S., Levasseur, M., and Andersen, D. M. (1998). Control of germination of *Alexandrium tamarensis* (Dinophyceae) cysts from the lower St. Lawrence estuary (Canada). *J. Phycol.* 34:242–9.
- Pöggeler, B., Balzer, I., Hardeland, R., and Lerchl, A. (1991). Pineal hormone melatonin oscillates also in the dinoflagellate *Gonyaulax polyedra*. *Naturwissenschaften* 78:268–9.
- Preisig, H. R. (1994). Siliceous structures and silicification in flagellated protists. *Protoplasma* 181:1–28.
- Prézilin, B. B. (1976). The role of peridinin-chlorophyll *a*-proteins in the photosynthetic light adaptation of the marine dinoflagellate, *Glenodinium* sp. *Planta* 130:225–33.
- Prézilin, B. B., and Haxo, F. T. (1976). Purification and characterization of peridinin-chlorophyll *a*-proteins from the marine dinoflagellates *Glenodinium* sp. and *Gonyaulax polyedra*. *Planta* 128:133–41.
- Raven, J. A., and Richardson, K. (1984). Dinophyte flagella: A cost-benefit analysis. *New Phytol.* 98:259–76.
- Ris, H., and Kubai, D. F. (1974). An unusual mitotic mechanism in the parasitic protozoan *Syndinium* sp. *J. Cell Biol.* 60:702–20.
- Rizzo, P. J. (1991). The enigma of the dinoflagellate chromosome. *J. Protozool.* 38:246–52.
- Roberts, K. R., and Roberts, J. E. (1991). The flagellar apparatus and cytoskeleton of the dinoflagellate. *Protoplasma* 164:105–22.
- Robinson, N., Eglinton, G., Brassell, S. C., and Cranwell, P. A. (1984). Dinoflagellate origin for sedimentary 4 $\alpha$ -methylsteroids and 5 $\alpha$ (H)-stanols. *Nature* 308:439–42.
- Rodriguez-Lanetty, M., Krupp, D. A., and Weis, V. M. (2004). Distinct ITS types of *Symbiodinium* in Clade C correlate with cnidarian/dinoflagellate specificity during onset of symbiosis. *Mar. Ecol. Progr. Ser.* 275:97–102.
- Roenneberg, T., and Deng, T.-S. (1997). Photobiology of the *Gonyaulax* circadian system. I. Different phase response curves for red and blue light. *Planta* 202:494–501.
- Sakamoto, B., Hokama, Y., Horgen, F. D., Scheuer, P. J., Kan, Y., and Nagai, H. (2000). Isolation of a sulfoquinovosyl monoacylglycerol from *Bryopsis* sp. (Chlorophyta): identification of a factor causing a possible species specific ecdysis response in *Gamierdiscus toxicus* (Dinophyceae). *J. Phycol.* 36:924–31.
- Samuelsson, G., Sweeney, B. M., Matlock, H. A., and Prézilin, B. B. (1983). Changes in photosystem II account for the circadian rhythm in photosynthesis in *Gonyaulax polyedra*. *Plant Physiol.* 73:329–31.

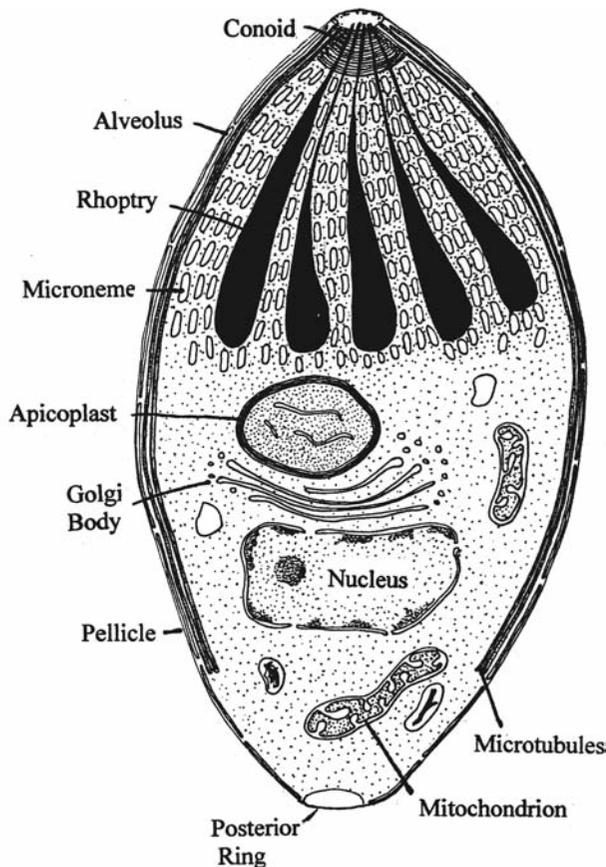
- Schiller, J. (1933). Dinoflagellatae. In *Dr L. Rabenhorst's Kryptogamen-Flora*, Vol. 10. pp. 1–617. Leutershausen: Strauss and Cramer.
- Schmidt, R. J., Gooch, V. D., Loeblich, A. R., and Hastings, J. W. (1978). Comparative study of luminescent and nonluminescent strains of *Gonyaulax excavata* (Pyrrophyta). *J. Phycol.* 14:5–9.
- Schütt, F. (1895). Die Peridineen der Plankton-Expedition. *Ergeb. Plankton Exped. Humboldt-Stiftung.* 4; M.a. A:1–170.
- Sekida, S., Horiguchi, T., and Okuda, K. (2004). Development of thecal plates and pellicle in the dinoflagellate *Scrippsiella hexapraeicingula* (Peridinales, Dinophyceae) elucidated by changes in stainability of the associated membranes. *Eur. J. Phycol.* 39:105–14.
- Seo, K. S., and Fritz, L. (2000). Cell ultrastructural changes correlate with circadian rhythms in *Pyrocystis lunula* (Pyrrophyta). *J. Phycol.* 36:351–8.
- Sgroso, S., Esposito, F., and Montresor, M. (2001). Temperature and daylength regulate encystment in calcareous cyst-forming dinoflagellates. *Mar. Ecol. Progr. Ser.* 211:77–87.
- Shilo, M. (1967). Information and mode of action of algal toxins. *Bacteriol. Rev.* 31:180–93.
- Sigee, D. C. (1984). Structural DNA and genetically active DNA in dinoflagellate chromosomes. *BioSystems* 16:302–10.
- Smalley, G. W., Coats, D. W., and Stoecker, D. K. (2003). Feeding in the mixotrophic dinoflagellate *Ceratium furca* is influenced by intracellular nutrient concentrations. *Mar. Biol. Progr. Ser.* 262:137–51.
- Spero, H. J. (1982). Phagotrophy in *Gymnodinium fungiforme* (Pyrrophyta): The peduncle as an organelle of ingestion. *J. Phycol.* 18:356–60.
- Spero, H. J. (1985). Chemosensory capabilities in the phagotrophic dinoflagellate *Gymnodinium fungiforme*. *J. Phycol.* 21:181–4.
- Spero, H. J., and Moree, M. D. (1981). Phagotrophic feeding and its importance to the life cycle of the holozoic dinoflagellate, *Gymnodinium fungiforme*. *J. Phycol.* 17:43–57.
- Sullivan, J. M., and Swift, E. (2003). Effects of small-scale turbulence on net growth rate and size of ten species of marine dinoflagellates. *J. Phycol.* 39:83–94.
- Sulzman, F. N., Krieger, N. R., Gooch, V. D., and Hastings, J. W. (1978). A circadian rhythm of the luciferin binding protein from *Gonyaulax polyedra*. *J. Comp. Physiol.* 128:251–7.
- Suzuki, L., and Johnson, C. H. (2001). Algae know the time of day: circadian and photoperiodic programs. *J. Phycol.* 37:933–42.
- Suzuki, T., Mitsuya, T., Imai, M., and Yamasaki, M. (1997). DSP toxin contents in *Dinophysis fortii* and scallops collected at Mutsu Bay, Japan. *J. Applied Phycol.* 8:509–15.
- Sweeney, B. M. (1969). *Rhythmic Phenomena in Plants*. London and New York: Academic Press.
- Sweeney, B. M. (1971). Laboratory studies of a green *Noctiluca* from New Guinea. *J. Phycol.* 7:53–8.
- Sweeney, B. M. (1978). Ultrastructure of *Noctiluca miliaris* (Pyrrophyta) with green flagellate symbionts. *J. Phycol.* 14:116–20.
- Sweeney, B. M. (1979). The bioluminescence of dinoflagellates. In *Biochemistry and Physiology of Protozoa*, ed. M. Levandowsky, and S. H. Hutner, Vol. 1, pp. 287–306. New York: Academic Press.
- Sweeney, B. M. (1980). Intracellular source of bioluminescence. *Int. Rev. Cytol.* 68:173–95.
- Swift, E., Biggley, W. H., and Seliger, H. H. (1973). Species of oceanic dinoflagellates in the genera *Dissodinium* and *Pyrocystis*: Interclonal and intraspecific comparisons of color and photon yield of bioluminescence. *J. Phycol.* 9:420–6.
- Takishita, K., Ishida, K.-I., Ishikura, M., and Maruyama, T. (2005). Phylogeny of the *psbC* gene, coding a photosystem II component CP 43, suggests separate origins for the peridinin- and fucoxanthin derivative-containing plastids of dinoflagellates. *Phycologia* 44:26–34.
- Taylor, D. L. (1969). On the regulation and maintenance of algal numbers in zooxanthellae-coelenterate symbiosis, with a note on the nutritional relationship in *Anemonia sulcata*. *J. Mar. Biol. Assoc. UK* 49:1057–65.
- Taylor, D. L. (1971). On the symbiosis between *Amphidinium klebsii* (Dinophyceae) and *Amphiscolops langerhansi* (Turbellaria: Acoela). *J. Mar. Biol. Assoc. UK* 51:301–13.
- Taylor, F. J. R. (1971). Scanning electron microscopy of thecae of the dinoflagellate genus *Ornithocercus*. *J. Phycol.* 7:249–58.
- Taylor, F. J. R. (1999). Morphology (tabulation) and molecular evidence for dinoflagellate phylogeny reinforce each other. *J. Phycol.* 35:1–3.
- Trench, R. K. (1993). Microalgal–invertebrate symbioses: a review. *Endocytobios Cell Res.* 9:135–75.
- Triemer, R. E. (1982). A unique mitotic variation in the marine dinoflagellate *Oxyrrhis marina* (Pyrrophyta). *J. Phycol.* 18:399–411.
- Vogel, K., and Meeuse, B. J. D. (1968). Characterization of the reserve granules from the dinoflagellate *Thecadinium inclination* Balech. *J. Phycol.* 4:317–18.

- von Stosch, H. A. (1972). La signification cytologique de la "cyclose nucléaire" dans le cycle de vie des Dinoflagellés. *Soc. Bot. Fr., Memoires*, pp. 201–12.
- von Stosch, H. A. (1973). Observations on vegetative reproduction and sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* Schiller and *Woloszynskia apiculata* sp. nov. *Br. Phycol. J.* 8:105–34.
- Wang, J-T., and Douglas, A. E. (1997). Nutrients signals and photosynthate release by symbiotic algae. *Plant Physiol.* 114:631–6.
- Wilcox, L. W., and Wedemeyer, G. J. (1991). Phagotrophy in the freshwater photosynthetic dinoflagellate *Amphidinium cryophilum*. *J. Phycol.* 27:600–9.
- Windust, A. J., Hu, T., Wright, J. L. C., Quilliam, M. A., and McLachlan, J. L. (2000). Oxidative metabolism by *Thalassiosira weissflogii* (Bacillariophyceae) of a diol-ester of okadaic acid, the diarrhetic shellfish poisoning. *J. Phycol.* 36:342–50.
- Withers, N. (1982). Ciguatera fish poisoning. *Annu. Rev. Med.* 33:97–111.
- Yoon, H. S., Hackett, J. D., and Bhattacharya, D. (2002). A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. *Proc. Natl. Acad. Sci., USA* 99:11724–9.
- Zardoya, R., Costas, E., Lopez-Rodas, V., Garrido-Pertierra, A., and Bautista, J. M. (1995). Revised dinoflagellate phylogeny inferred from molecular analysis of large-subunit ribosomal RNA sequences. *J. Mol. Evol.* 41:637–45.
- Zhou, J., and Fritz, L. (1994). Okadaic acid localizes to chloroplasts in the DSP-toxin-producing dinoflagellates *Prorocentrum lima* and *Prorocentrum maculosum*. *Phycologia* 33:455–61.

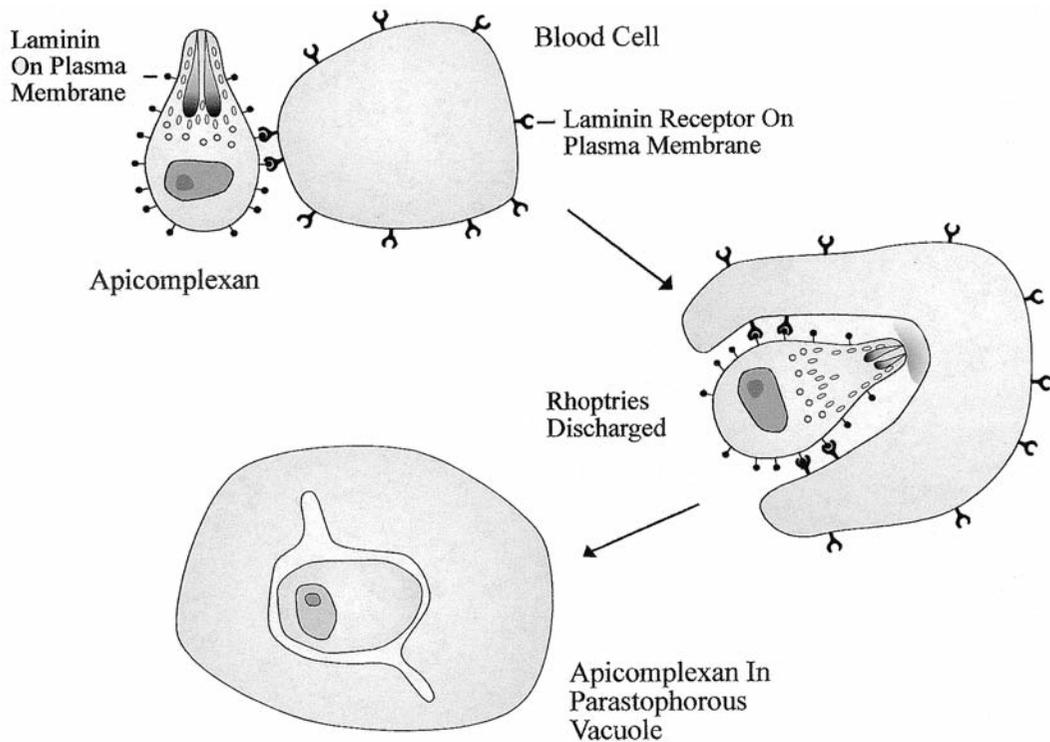
## Apicomplexa

Algae are organisms that have plastids, or organisms that are derived from cells whose ancestors possessed plastids. Until 1994, it was thought that the apicomplexa did not have plastids (and consequently were not covered in phycology textbooks). Then it was shown that a known organelle in many apicomplexa was actually a reduced

colorless plastid called an apicoplast (Fig. 8.1) (Wilson, 1993; Wilson et al., 1994). Molecular studies have shown that the apicoplast and dinoflagellate plastids originated from red algae by a single endosymbiotic event that occurred relatively early in eukaryotic evolution (Fast et al., 2001).



**Fig. 8.1** Drawing of the basic cytology of an apicomplexan cell.



**Fig. 8.2** General scheme by which an apicomplexan infects a blood cell. The apicomplexan has a characteristic laminin polysaccharide on the surface of the plasma membrane that binds with laminin receptors on the blood cell. This forms a tight junction between the apicomplexan and blood cell. The apicomplexan discharges its rhoptries. The blood cell phagocytoses the apicomplex into a parasitophorous vacuole. (Modified from Sam-Yellowe, 1996.)

The discovery of the apicoplast generated considerable interest since most apicomplexans are unicellular endoparasites that cause some of the most significant tropical diseases (Foth and McFadden, 2003). Malaria in humans is produced by the apicomplexan *Plasmodium*. About 300 million people are infected with malaria, leading to one million deaths annually (Ralph et al., 2004). Apicomplexans cause other serious diseases in livestock and humans, such as cryptosporidiosis, babesiosis (Texas cattle fever), theileriosis (East Coast fever), and toxoplasmosis. The realization that these endoparasites were once algae raised hopes that the apicoplast might be a drug target for two reasons. The first is that the apicoplast is essential for the survival of *Plasmodium* and

*Toxoplasma*. The second is that drugs effective against prokaryotic organisms might be effective against the apicoplast since all plastids originally evolved from endosymbiotic prokaryotic cyanobacteria. Apicomplexans are absolutely dependent on the apicoplast, which has led to speculation that this curious organelle is a potential “Achilles heel” of parasites, such as *Plasmodium*.

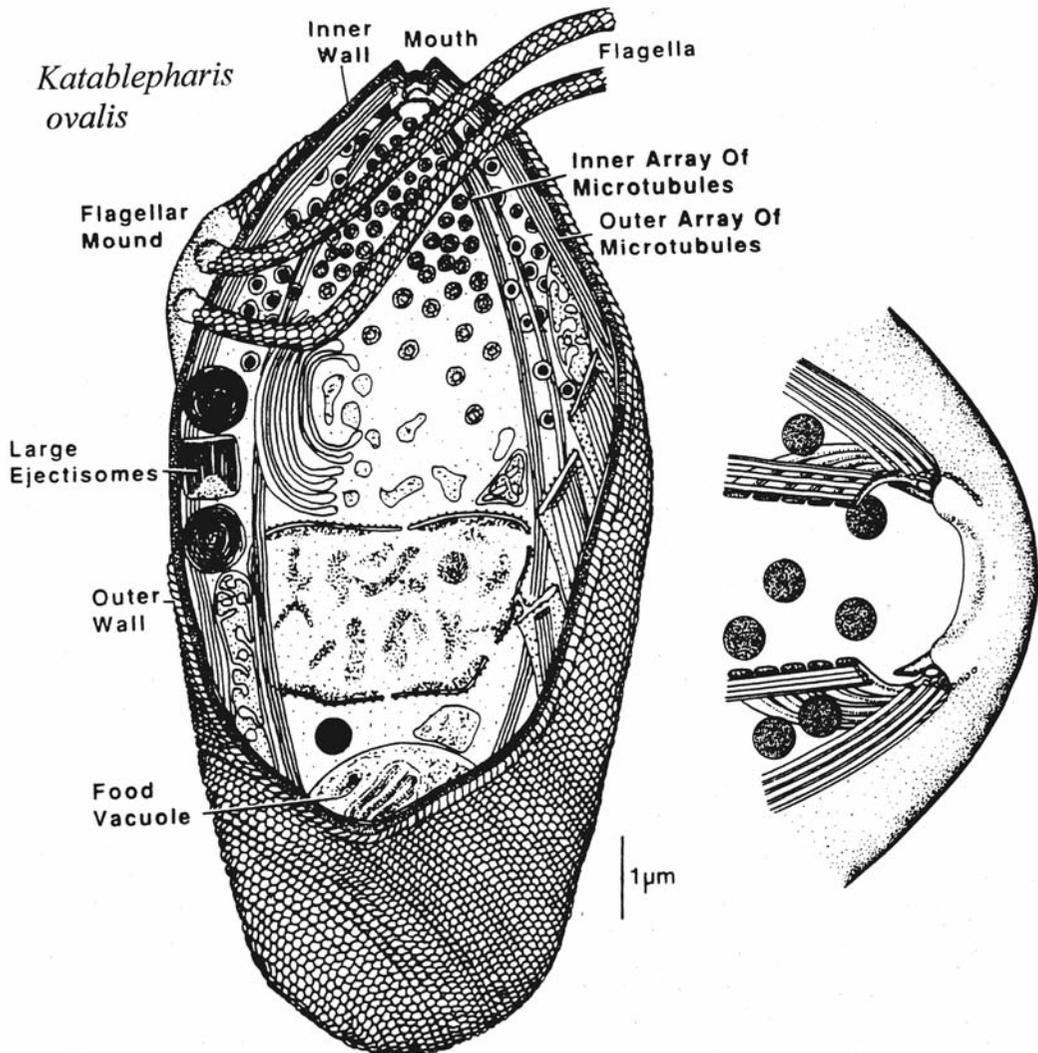
The typical apicomplexan vegetative cell (merozoite) (Fig. 8.1) has an **apicoplast** surrounded by four membranes. The inner two membranes are the inner and outer plastid membranes while the outer membranes are derived from the vacuolar membrane and the plasma membrane of the endosymbiotic red alga.

The **apical complex** consists of a **polar ring** and a **conoid** formed of spirally coiled microtubules (Fig. 8.1). The apicomplexan has laminin polysaccharide on its surface while the host cell has a laminin receptor (Fig. 8.2). The apicomplexan parasite attaches to the host cell with the conoid protruding to produce a **stylet** that forms a tight junction between the apicomplexan parasite and host cell. The apicomplexan cell is taken

up into the host cell in the **parasitophorous vacuole**. The contents of the **rhoptries** and **micronemes** are emptied into the space between the apicomplexan plasma membrane and the parasitophorous vacuole membrane.

Apicomplexans have a layer of flattened membranous sacs or **alveoli** (Fig. 8.1) beneath the plasma membrane that comprise the subpellicular membrane complex, similar to that found in the dinoflagellates.

*Katablepharis* (Fig. 8.3) is a heterotrophic unicellular flagellate that lacks a plastid. *Katablepharis* cells have ejectionosomes and was classified with the Cryptophyceae. However, ultrastructural studies (Lee and Kugrens, 1991; Lee et al., 1991) revealed the presence of an anterior conoid apparatus involved in phagocytosis of prey. The conoid apparatus is very similar to those of the apicomplexans and it is likely that *Katablepharis* should be classified as an apicomplexan.



**Fig. 8.3** Drawings of *Katablepharis ovalis*. Left: whole cell. Right: anterior part of cell. (From Lee and Kugrens, 1991; Lee et al., 1991.)

## REFERENCES

- Fast, N. M., Kissinger, J. C., Roos, D. S., and Keeling, P. J. (2001). Nuclear-encoded, plastid-targeted genes suggest a single common origin for apicomplexan and dinoflagellate plastids. *Mol. Biol. Evol.* 18:418–26.
- Foth, B. J., and McFadden, G. I. (2003). The apicoplast: a plastid in *Plasmodium falciparum* and other apicomplexan parasites. *Int. Rev. Cytol.* 224:57–110.
- Lee, R. E., and Kugrens, P. (1991). *Katablepharis ovalis*, a colorless flagellate with interesting cytological characteristics. *J. Phycol.* 27:505–15.
- Lee, R. E., Kugrens, P., and Mylnikov, A. P. (1991). Feeding apparatus of the colorless flagellate *Katablepharis* (Cryptophyceae). *J. Phycol.* 27:725–33.
- Ralph, S. A., van Dooren, G. G., Waller, R. F., et al. (2004). Metabolic maps and functions of the *Plasmodium falciparum* apicoplast. *Nat. Rev./Microbio.* 2:203–16.
- Sam-Yellowe, T. Y. (1996). Rhoptry organelles of the Apicomplexa: their role in host cell invasion and intracellular survival. *Parasitol. Today* 12:308–16.
- Wilson, R. J. (1993). Plastids better red than dead. *Nature* 366:638.
- Wilson, R. J., Williamson, D. H., and Preiser, P. (1994). Malaria and other apicomplexans: the “plant” connection. *Infect. Agents Dis.* 3:29–37.



# Part V

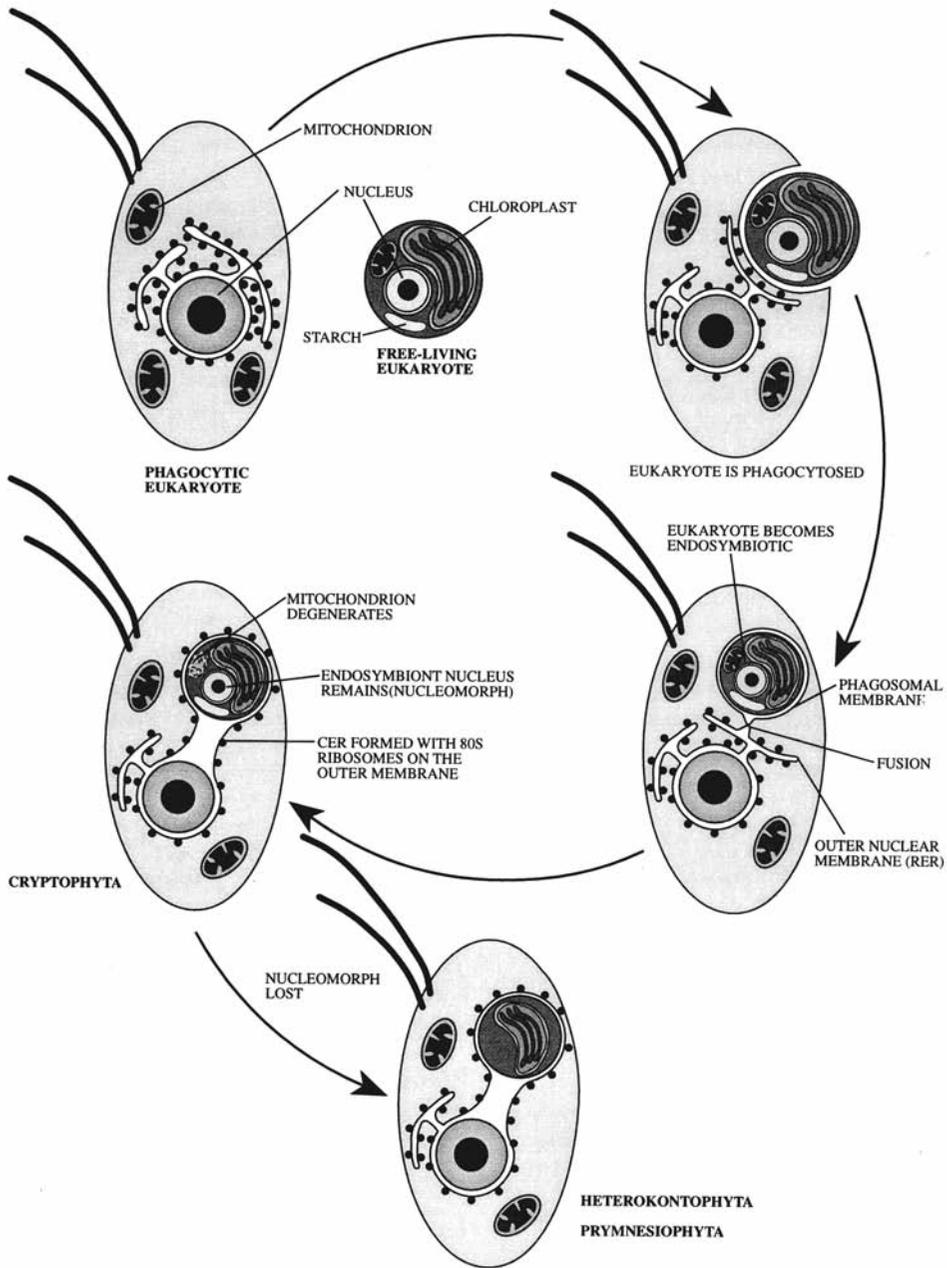
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## Evolution of two membranes of chloroplast endoplasmic reticulum and the Chlorarachniophyta

Algae with two membranes of chloroplast endoplasmic reticulum (chloroplast E.R.) have the inner membrane of chloroplast E.R. surrounding the chloroplast envelope. The outer membrane of chloroplast E.R. is continuous with the outer membrane of the nuclear envelope and has ribosomes on the outer surface (Fig. V.1).

The algae with two membranes of chloroplast E.R. evolved by a secondary endosymbiosis (Fig. V.1) (Lee, 1977) when a phagocytic protozoan took up a eukaryotic photosynthetic alga into a food vesicle. Instead of being phagocytosed by the protozoan, the photosynthetic alga became established as an endosymbiont within the food vesicle of the protozoan. The endosymbiotic photosynthetic alga benefited from the acidic environment in the food vesicle that kept much of the inorganic carbon in the form of carbon dioxide, the form needed by ribulose biphosphate/carboxylase for carbon fixation (see Part IV for further explanation). The host benefited by receiving some of the photosynthate from the endosymbiotic alga. The food vesicle membrane eventually fused with the endoplasmic reticulum of the host protozoan, resulting in ribosomes on the outer surface of this membrane, which became the outer membrane of the chloroplast E.R. Through evolution, ATP production and other functions of the endosymbiont's mitochondrion were taken over by the mitochondria of the protozoan host, and the mitochondria of the endosymbiont were lost. The host nucleus also took over some of the genetic control of the endosymbiont, with a reduction in the size and function of the nucleus of the endosymbiont. The resulting cytology is characteristic of the extant algae in the Chlorarachniophyta and Cryptophyta, which have a nucleomorph representing the degraded endosymbiotic nucleus, as well as storage product produced in what remains of the endosymbiont cytoplasm.

The type of chloroplast E.R. that exists in the Heterokontophyta and the Prymnesiophyta resulted from further reduction. The nucleomorph



**Fig. V.1** The sequence of events that led to the evolution of algae with two membranes of chloroplast endoplasmic reticulum. (Drawing by Brec Clay.)

was completely lost and storage product formation was taken over by the host. The resulting cell had two membranes of chloroplast envelope surrounding the chloroplast. Outside of this was the inner membrane of chloroplast E.R. that was the remains of the plasma membrane of the endosymbiont. Outside of this was the outer membrane of

chloroplast E.R. which was the remains of the food vesicle membrane of the host.

Although the above evolutionary scheme is discussed in one sequence, it is probable that two membranes of chloroplast E.R. evolved at least three times, with one line leading to the Chlorarachniophyta, a second to the Cryptophyta, and the third (or more) leading to the Heterokontophyta and Prymnesiophyta.

The algae with two membranes of chloroplast E.R. are:

**Chlorarachniophyta:** chloroplast derived from a green alga, chlorophyll *a* and *b* present, nucleomorph between inner and outer membrane of chloroplast E.R.

**Cryptophyta:** Chlorophyll *a* and *c*, phycobiliproteins, nucleomorph between inner and outer membranes of chloroplast E.R., starch in grains between inner membrane of chloroplast E.R. and chloroplast envelope, periplast inside plasma membrane, tripartite hairs on flagella.

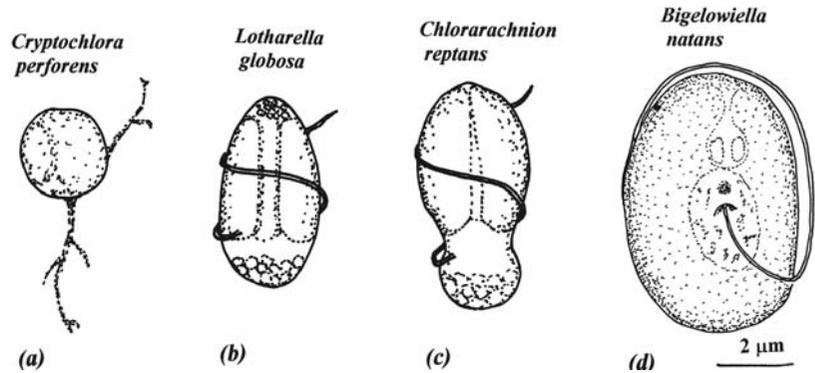
**Heterokontophyta:** tripartite hairs on anterior tinsel flagellum, posterior whiplash flagellum, chlorophyll *a* and *c*, fucoxanthin, storage product usually chrysolaminarin in vesicles in cytoplasm.

**Prymnesiophyta** (haptophytes): two whiplash flagella, haptonema present, chlorophyll *a* and *c*, fucoxanthin, scales common outside cell, storage product usually chrysolaminarin in vesicles in cytoplasm.

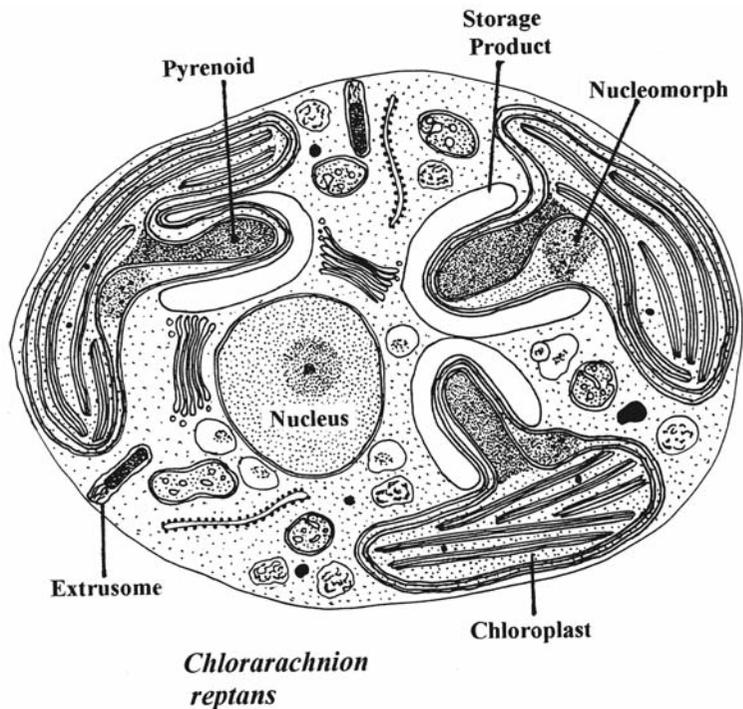
## Chlorarachniophyta

These algae (Fig. V.2) represent an intermediate stage in the evolution of two membranes of chloroplast endoplasmic reticulum. This group has a small number of green amoebae that have ingested green algal cells in the past, with the green algal cells evolving into endosymbionts within the amoeba host (Fig. V.3) (Hibberd and Norris, 1984). A **nucleomorph** or reduced nucleus occurs in the green algal symbiont. The reduced nature of the nucleomorph implies that some of the functions originally coded by the DNA of the endosymbiont nucleus have been taken over by the nucleus of the host amoeba. The chloroplast (e.g., endosymbiont chloroplast) contains chlorophyll *a* and *b* and is surrounded by four membranes. The innermost two membranes are those of the chloroplast envelope of the endosymbiont. The next membrane is the plasma membrane of the endosymbiont and the outer membrane represents the food-vacuole membrane of the amoeba host. Thus, the algae in the Chlorarachniophyta represent an intermediate stage in the evolution of the chloroplasts of some of the algae in the Heterokontophyta.

*Chlorarachnion reptans* is a marine amoeba that forms large plasmodia with the individual cells linked by a network of reticulopodia (Geitler, 1930; Hibberd and Norris, 1984). The cells are naked and contain a number of lobed chloroplasts, each with a central pyrenoid (Fig. V.3). Four

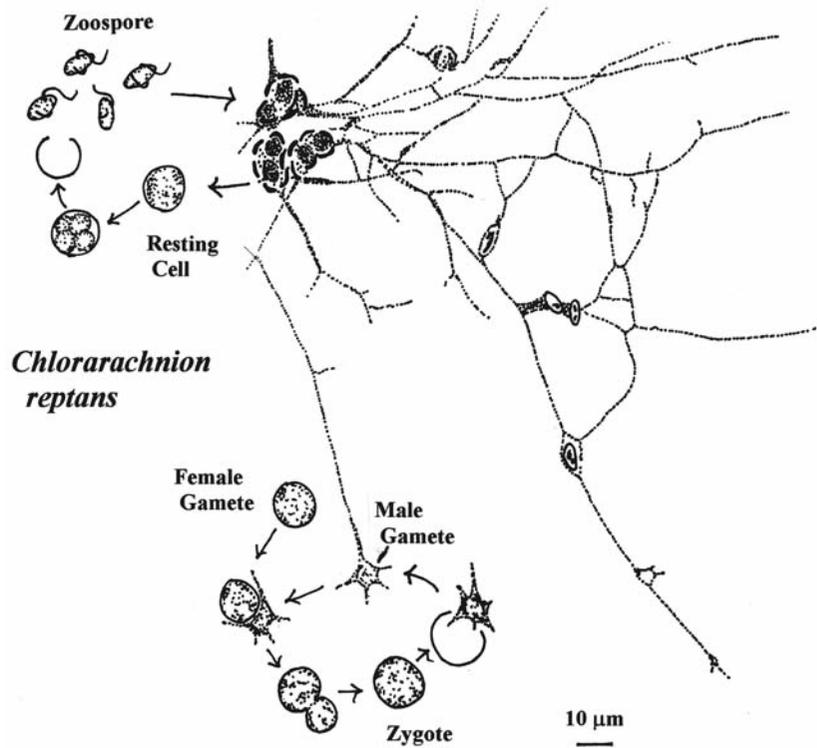


**Fig. V.2** Examples of algae in the Chlorarachniophyta. ((a) adapted from Calderon-Saenz and Schnettner, 1989; (b) adapted from Ishida et al., 1996; (c) adapted from Hibberd and Norris, 1984; (d) adapted from Moestrup and Sengco, 2001.)



**Fig. V.3** Semidiagrammatic drawing of the cell structure of *Chlorarachnion reptans*. (Adapted from Hibberd and Norris, 1984.)

membranes surround the chloroplast, which has a pyrenoid and nucleomorph. A vesicle containing the storage product caps the pyrenoid. *Chlorarachnion* means “green spider” for the web-like network of reticulopodia (pseudopodia) in which are embedded the green amoeboid cells.



**Fig. V.4** *Chlorarachnion reptans*. (Adapted from Hibberd and Norris, 1984; Grell, 1990.)

The cells move over the reticulopodia and ingest other algal cells and bacteria as a food source.

Under nutrient deprivation, the star-shaped vegetative cells become resting cells by retracting their reticulopodia, rounding up and secreting a thin cell wall (Grell, 1990). The resting cells apparently rely principally on photosynthate from the chloroplasts as a food source. The resting cells germinate to star-shaped vegetative cells under favorable conditions. Zoosporogenesis occurs by a resting cell dividing twice to produce four zoospores, each with a single flagellum wrapped around the cell body (Fig. V.1(c) and V.4). The zoospores settle to produce the star-shaped vegetative cells. Sexual reproduction occurs when a non-motile female gamete is approached by a motile, star-shaped, male gamete. The gametes fuse producing a zygote that germinates into a star-shaped vegetative cell (Grell, 1990).

## REFERENCES

- Calderon-Saenz, E., and Schnetter, R. (1989). Morphology, biology, and systematics of *Cryptochlora perforans* (Chloroarachniophyta), a phagotrophic marine alga. *Pl. Syst. Evol.* 163:165-76.

- Geitler, L. (1930). Ein grünes Filarplasmaodium und andere neue Protisten. *Arch. Protistenkd.* 69:615–36.
- Grell, K. G. (1990). Some light microscope observations on *Chlorarachnion reptans* Geitler. *Arch. Protistenkd.* 138:271–90.
- Hibberd, D. J., and Norris, R. E. (1984). Cytology and ultrastructure of *Chlorarachnion reptans* (Chlorarachniophyta division nova, Chlorarachniophyceae classis nova). *J. Phycol.* 20:310–30.
- Ishida, K., Nakayama, T., and Hara, Y. (1996). Taxonomic studies on the Chlorarachniophyta. II. Generic delimitation of the chlorarachniophytes and description of *Gymnochlora syellata* gen. et sp. nov. and *Lotharella* gen. nov. *Phycol. Res.* 44:37–45.
- Lee, R. E. (1977). Evolution of algal flagellates with chloroplast endoplasmic reticulum from the ciliates. *South African J. Sci.* 73:179–82.
- Moestrup, Ø., and Sengco, M. (2001). Ultrastructural studies on *Bigelowiella natans*, gen. et sp. nov., a chlorarachniophyte flagellate. *J. Phycol.* 37:624–6.

# Cryptophyta

## CRYPTOPHYCEAE

This group is composed primarily of flagellates that occur in both marine and freshwater environments. The cells contain chlorophylls *a* and *c*<sub>2</sub> and phycobiliproteins that occur inside the thylakoids of the chloroplast. The cell body is asymmetric with a clearly defined dorsi-ventral/right-left sides (Figs. 9.1, 9.9, 9.10). The asymmetric cell shape results in a peculiar swaying motion during swimming. Most cryptophytes have a single lobed chloroplast with a central pyrenoid.

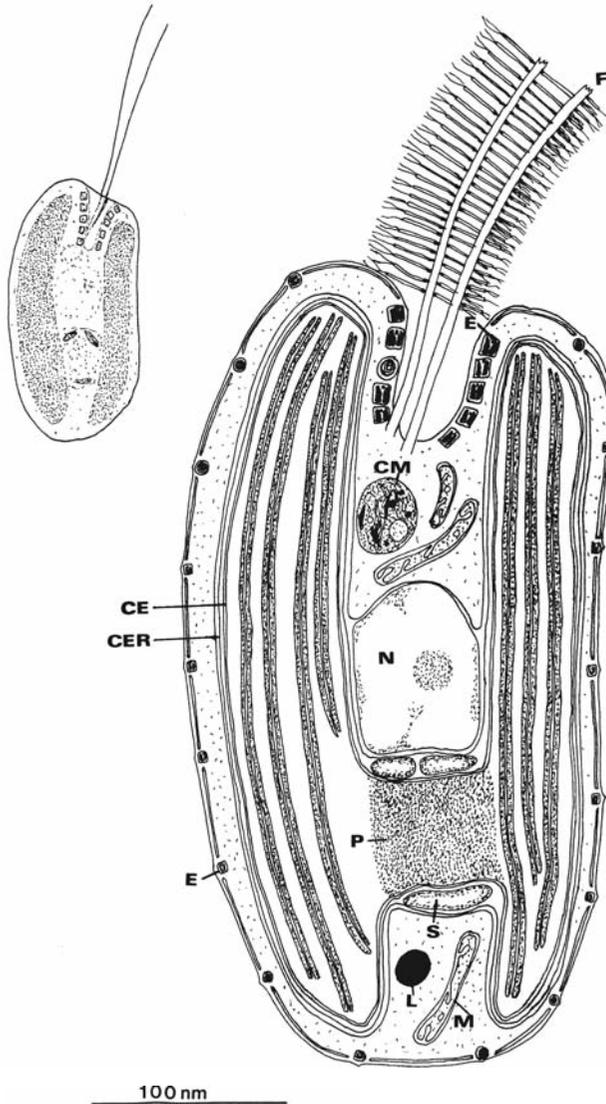
### Cell structure

There are two apically or laterally attached flagella at the base of a depression. Each flagellum is approximately the same length as the body of the cell (Figs. 9.1, 9.8, 9.9, 9.10). Depending on the species, there are one or two rows of microtubular hairs attached to the flagellum. In *Cryptomonas* sp., the hairs on one flagellum are 2.5 μm long and in two rows whereas the hairs on the other flagellum are only 1 μm long and arranged in a single row (Heath et al., 1970; Kugrens et al., 1987). Small, 150-nm-diameter organic scales (Fig. 9.2) are common on the flagellar surface and sometimes on the cell body (Lee and Kugrens, 1986).

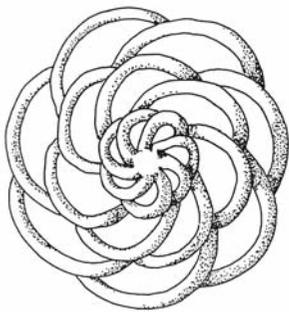
The outer portion of the cell, or **periplast** (Gantt, 1971), is composed of the plasma membrane and a plate, or series of plates, directly under the plasma membrane (Figs. 9.1, 9.10) (Kugrens

and Lee, 1987). The number and shape of these plates are used to characterize genera taking into consideration that the haploid and diploid phases of a single genus can have different plates (Hoef-Emden and Melkonian, 2003). New periplast plates are added in an area adjacent to the vestibulum (Brett and Wetherbee, 1996). Sulfated fucose-rich polysaccharides can be excreted outside of the cell (Giroldo and Vieira, 2002).

The chloroplast most likely evolved from a symbiosis between an organism similar to the phagocytic cryptomonad *Goniomonas* and a red alga (Kugrens and Lee, 1991; Liaud et al., 1997; McFadden et al., 1994). The chloroplast is surrounded by two membranes of chloroplast endoplasmic reticulum and the two membranes of the chloroplast envelope (Fig. 9.1). Between the outer membrane and the inner membrane of the chloroplast endoplasmic reticulum are starch grains and a nucleomorph (Figs. 9.1, 9.4). The nucleomorph contains three minute paired-chromosomes with 531 genes (humans have at least 31 000 genes) that encode 30 proteins targeted into the chloroplast (Douglas et al., 2001; Cavalier-Smith, 2002). The nucleomorph is probably the remnant of the nucleus of the endosymbiont in the event that led to chloroplast E.R. The nucleomorph is surrounded by an envelope that has pores similar to those in a nuclear envelope. The nucleomorph exhibits a rudimentary type of division utilizing microtubules (Morrall and Greenwood, 1982). The nucleomorph divides in preprophase of the main nucleus following basal body replication, but before division of the chloroplast and the chloroplast endoplasmic reticulum (McKerracher



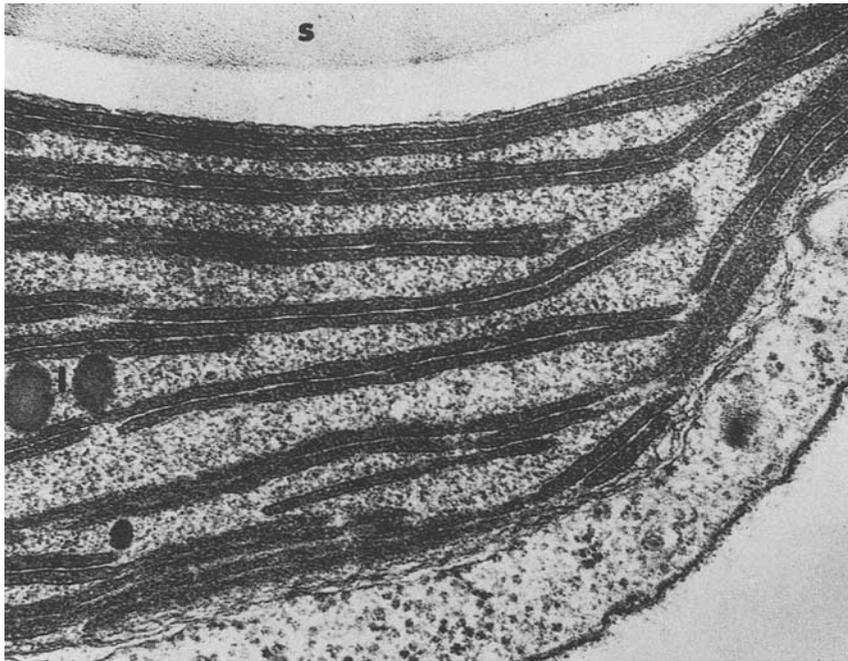
**Fig. 9.1** Drawing of a cell of the Cryptophyceae as seen in the light and electron microscope. (CE) Chloroplast envelope; (CER) chloroplast endoplasmic reticulum; (CM) Corps de Maupas; (D) dorsal; (E) ejectisome; (L) lipid; (M) mitochondrion; (N) nucleus; (NM) nucleomorph; (P) pyrenoid; (PP) periplast plate; (S) starch; (V) ventral.



**Fig. 9.2** Drawing of the most common type of flagellar scale found in freshwater cryptophytes. (From Lee and Kugrens, 1986.)

and Gibbs, 1982). The only cryptophyte that is known to lack a nucleomorph is *Goniomonas* (Figs. 9.8, 9.9(c)), a colorless cryptophyte that lacks a plastid. A second colorless cryptophyte, *Chilomonas* (Fig. 9.9(b)), is a reduced form of a photosynthetic cryptophyte and contains a leucoplast and a nucleomorph (McKerracher and Gibbs, 1982).

In the chloroplast, the thylakoids are grouped in pairs (Fig. 9.3), and there are no connections between adjacent thylakoids. The Cryptophyta is the only group to have this arrangement of thylakoids. Chlorophylls *a* and *c*<sub>2</sub> are present. The major carotenoid present is  $\alpha$ -carotene, and the major xanthophyll, diatoxanthin. There are three spectral types of phycoerythrin and three spectral



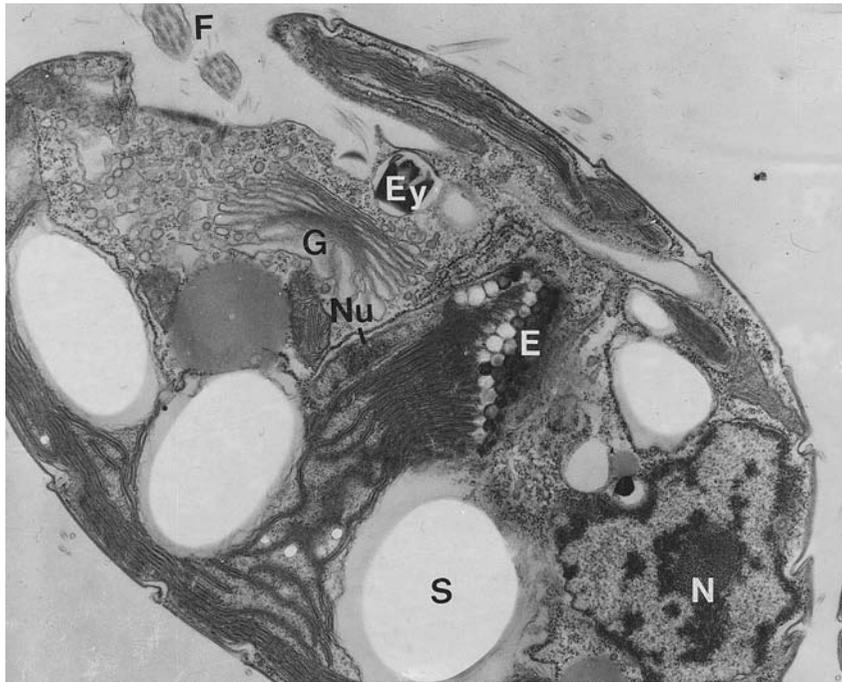
**Fig. 9.3** Transmission electron micrograph of part of a chloroplast of *Chroomonas mesostigmatica*. The thylakoids are grouped in pairs. The dense contents of the thylakoids represent the phycobilisomes. Also present are lipid droplets (l) and a large starch grain (s).  $\times 50\,000$ . (From Dodge, 1969.)

types of phycocyanin, all of which are different from the phycobiliproteins found in the cyanobacteria and red algae (Hill and Rowan, 1989). The phycobiliproteins are in the intrathylakoid space (inside the thylakoids (Fig. 9.3) (Gantt et al., 1971; Spear-Bernstein and Miller, 1984), and are not on the stromal side of the thylakoids in phycobilisomes as occurs in the cyanobacteria and red algae. Each photosynthetic cryptophyte has only one species of phycobiliprotein – either a phycoerythrin or a phycocyanin – but never both. No allophycocyanin is present (Gantt, 1979). Allophycocyanin acts as a bridge in the transfer of light energy from phycoerythrin and phycocyanin to chlorophyll *a* of the reaction center in red algae and cyanobacteria. The presence of allophycocyanin may not be necessary in the cryptophytes because the greater absorption range of cryptophycean phycobiliproteins in conjunction with chlorophyll *c* overlaps the chlorophyll *a* absorption spectrum. There is a variation in

the amount of pigments under different light-intensity conditions. Cells of *Cryptomonas* grown under low light-intensity conditions ( $10\ \mu\text{E m}^{-2}\ \text{s}^{-1}$ ) contain twice as much of chlorophylls *a* and *c*, and six times as much phycoerythrin per cell, as those grown under high light-intensity conditions ( $260\ \mu\text{E m}^{-2}\ \text{s}^{-1}$ ) (Thin, 1983). Under low light-intensity conditions there is a higher concentration of phycoerythrin and the thylakoids are thicker.

The reserve product (similar in appearance to starch grains) is appressed to the pyrenoid area outside of the chloroplast envelope but inside the chloroplast E.R. The cryptophytes are the only algae that form their storage product in this area. The starch is an  $\alpha$ -1,4-glucan composed of about 30% amylose and amylopectin. Cryptophycean starch is similar to potato starch and starch found in the green algae and dinoflagellates (Antia et al., 1979).

Some of the Cryptophyceae have eyespots. The eyespots that have been reported consist of lipid granules inside the chloroplast envelope. In *Chroomonas mesostigmatica*, the red eyespot is in the center of the cell (Fig. 9.4) and is an extension of the chloroplast beyond the pyrenoid (Dodge, 1969). In *Cryptomonas rostella*, the eyespot is



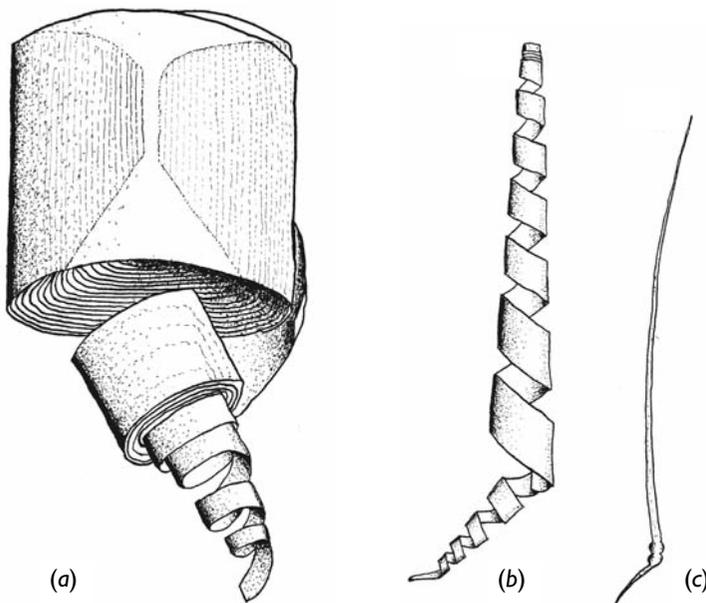
**Fig. 9.4** Transmission electron micrograph of a cell of *Chroomonas mesostigmatica* showing the eyespot (E) present in the chloroplast. (Ey) Ejectisome; (F) flagellum; (G) Golgi; (N) nucleus; (Nu) nucleomorph; (S) starch. (Micrograph provided by Paul Kugrens.)

beneath the chloroplast membrane near the depression. Some of the Cryptophyceae exhibit positive phototaxis, with maximum sensitivity of the colorless *Chilomonas* being in the blue at 366 nm (Halldal, 1958).

The Cryptophyceae have projectiles called **ejectisomes**, which are of different structure from the trichocysts of the Dinophyceae and which are probably closely related to the R-bodies of the kappa particles of the ciliates (Hovasse et al., 1967; Kugrens et al., 1994). Within a cell there are usually large ejectisomes near the anterior depression and smaller ejectisomes around the cell periphery (Figs. 9.1, 9.4, 9.8). Both sizes of ejectisomes have the same structure; they are made up of two unequal-sized bodies enclosed within a single membrane (Fig. 9.5). Each of these bodies is a long tape curled up on a very tight spiral. The tape is tapered, with the greatest width being on the outside of the ejectisome. The

smaller body is joined to the first and sits at an angle within the V-shaped portion of the larger body. The two bodies actually constitute one long tape with two spirals. The bodies are always arranged so that the smaller body is near the surface. The ejectisomes discharge when the organism is irritated (Fig. 9.5), the discharged ejectisome being a long tubular structure with a short portion at an angle to the long portion. The discharged small ejectisome from the cell periphery is 4  $\mu\text{m}$  long, whereas that of a larger ejectisome from under the anterior depression is about 20  $\mu\text{m}$  long. The discharge of the ejectisome results in a movement of the organism in the opposite direction. The discharge of the ejectisome could function as an escape mechanism, or it could be a direct defense mechanism causing damage to an offending organism. Ejectisomes originate in vesicles in the area of Golgi bodies.

The **Corps de Maupas** is a large vesicular structure in the anterior portion of the cell (Fig. 9.1). Its main function is probably that of disposing of unwanted protoplasmic structures by digestion (Lucas, 1970a,b).



**Fig. 9.5** (a) General organization of an ejectosome showing the two subparts. (b) A model of an ejectosome being fired outside of the cell. (c) A drawing of a discharged ejectosome. (After Hovasse et al., 1967.)

## Ecology

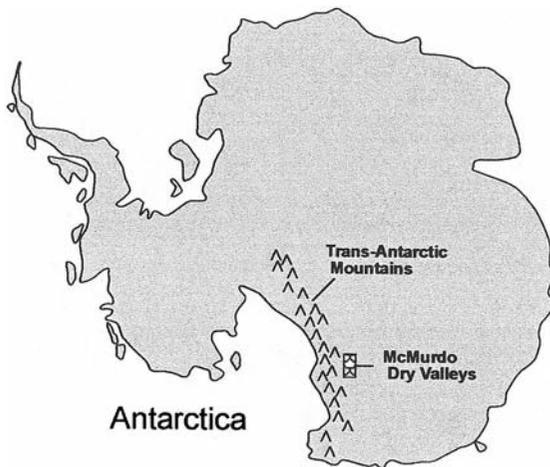
In comparison with other algal groups, the Cryptophyta appear to be especially light sensitive, often forming the deepest living populations in clear oligotrophic lakes (Nauwerk, 1968). In higher mountain and north temperate lakes, cryptomonads and other flagellates are present in the water column throughout the winter. Because of the low light intensity under snow and ice cover, these algae concentrate in surface waters to receive sufficient light from net photosynthesis (Wright, 1964; Pechlauer, 1971). Survival at these extremely low light levels depends not only on a highly efficient photosynthetic system, but also on slow rates of cell respiration at low water temperatures and reduced winter zooplankton grazing. In spring, with the disappearance of snow and resulting sudden increase in light in Arctic and mountain lakes, cryptomonads suffer considerable light stress, such that the biomass maximum moves to deeper waters (Kalff and Welch, 1974).

Cryptophytes will often undergo diel vertical migrations with an amplitude less than 5 meters. In small humic forest lakes, species of *Cryptomonas* are positively phototactic in the morning, moving into the phosphorus-depleted upper layer. Later in

the day the cells move away from the uppermost water layer, avoiding high levels of irradiance, and move into the phosphorus-rich hypolimnion (Knapp et al., 2003). A further advantage of this cycle is the reduction of grazing pressure by zooplankton (for which cryptophytes are a preferred food) (Loret et al., 2000) which often migrate in the reverse direction.

Cryptophyte algae are mixotrophic, capable of phototrophy and phagotrophy. Phagocytotic ingestion of bacteria is thought primarily to provide a source of phosphorus and nitrogen in nutrient-limiting conditions (Urabe et al., 2000). These algae are also chemotactic, swimming in a straight line until they reach a nutrient patch, at which time the cells stop and tumble in the volume of high-nutrient concentration (Lee et al., 1999).

Cryptophytes are the dominant algae in the freshwater lakes of Antarctica. The best studied lakes are those in the McMurdo Dry Valleys (Fig. 9.6) (Roberts and Laybourn-Parry, 1999; McKnight et al., 2000). The McMurdo Dry Valleys are the largest ice-free areas in Antarctica (about 4000 km<sup>2</sup>) and constitute a polar desert with temperatures ranging from  $-45^{\circ}\text{C}$  to  $5^{\circ}\text{C}$ . These valleys remain ice free because the Trans-Antarctic Mountains not only block the flow of the ice sheet, but also block the flow of moisture, the valleys



**Fig. 9.6** The location of the McMurdo Dry Valleys in Antarctica.

receive only about 10 cm of snow a year. The lakes are fed by glacial melt streams that flow for 6–10 weeks during the brief austral (southern) summer. The lakes are perennially covered by debris-containing ice caps up to 5 m thick that reduce light penetration. In addition, the sun does not arise above the horizon for a number of months during the austral winter. These lakes are highly stratified because of a lack of forces that could generate turnover of the water column (e.g., wind, water temperature changes). Cryptophytes dominate the lower stratified levels where they live heterotrophically during winter months, taking up about one bacterium per hour by phagocytosis (Roberts and Laybourn-Parry, 1999). During the summer months, the cryptophytes are mixotrophic (combining heterotrophy and autotrophy by photosynthesis). A key to the survival of cryptophytes in this environment is maintaining the population in the vegetative state, rather than entering a resting state. The cryptophyte population can respond quickly when “good” conditions return in the short Antarctic summer.

## Symbiotic associations

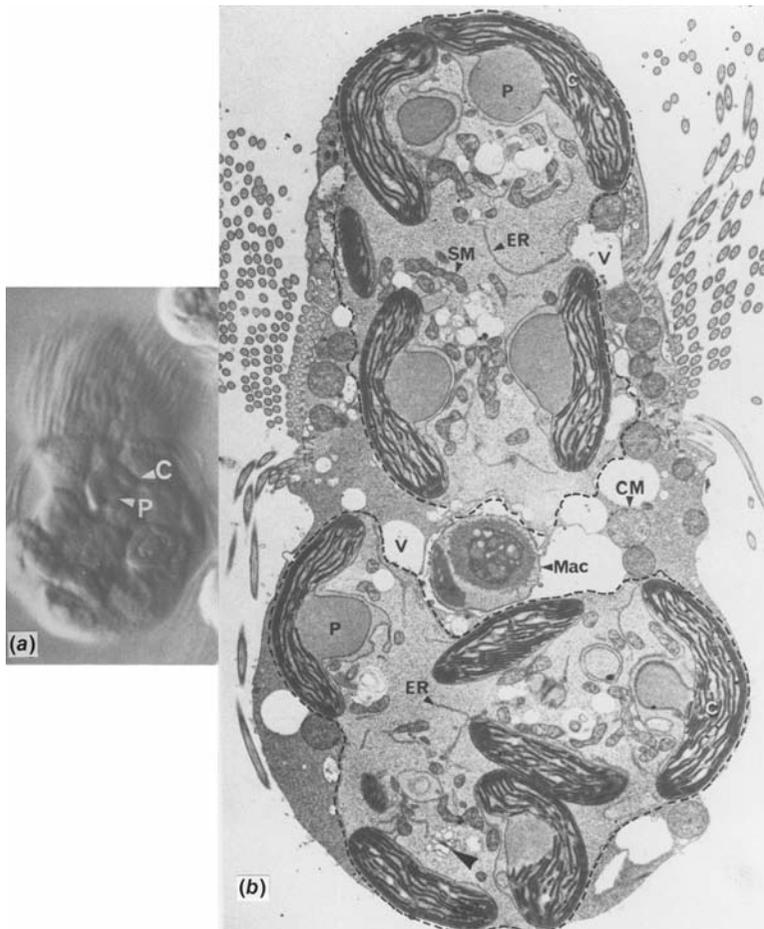
*Mesodinium rubrum* is a marine planktonic holotrich ciliate of extremely wide geographical

distribution that colors the water in which it is growing red. It has been recorded from neritic locations such as bays and fjords; away from the coast it is usually associated with regions of upwelling and in such conditions the blooms have been recorded as extending over areas as large as 100 square miles. The color of the ciliate (Fig. 9.7) is due to numerous reddish-brown chloroplasts, which belong to a single cryptophycean alga that lives symbiotically inside the ciliate (Gustafson et al., 2000). The cryptophyte is surrounded by a single membrane, and has a nucleus and the normal cytology and pigments of the Cryptophyceae. The endosymbiotic cryptophyte is able to fix  $^{14}\text{C}$  in the light, evolve oxygen in photosynthesis, and assimilate  $^{32}\text{P}$ , indicating that it is a functioning autotroph. The association is probably similar to that of symbiotes in other classes, with the endosymbiont providing the host with photosynthate and the host providing the endosymbiont with a protected environment. Blooms of *Mesodinium rubrum* are a regular feature of upwelling ecosystems. The organism has three characteristics that enable it to compete effectively with other autotrophic plankton (Smith and Barber, 1979). (1) It is motile, swimming at rates of 2.0 to 7.2  $\text{m h}^{-1}$ , an order of magnitude greater than the maximum swimming speeds attained by dinoflagellates. (2) It has strong phototropisms, being positively phototactic in an increasing light regime in the morning and negatively phototactic in decreasing light and in nutrient-depleted waters. (3) It has extremely high photosynthetic rates (1000 to 2000  $\text{mg C m}^{-3} \text{h}^{-1}$ ), equaling the highest ever observed for oceanic plankton. Conventional dinoflagellate or diatom blooms typically have only 60 to 70  $\text{mg C m}^{-3} \text{h}^{-1}$ .

## Classification

There are three recognizable groups within the Cryptophyceae (Marin et al., 1998; Deane et al., 2002):

- Order 1 Goniomonadales: colorless cells with no plastids.
- Order 2 Cryptomonadales: cells usually reddish in color with chloroplasts containing the phycobiliprotein Cr-phycoerythrin.



**Fig. 9.7** *Mesodinium rubrum* with its cryptomonad symbiont. (a) Light micrograph of the ciliate showing the chloroplast (C) and pyrenoid (P) of the cryptomonad endosymbiont. (b) Transmission electron micrograph. The dotted lines indicate the boundary between the cytoplasm of the ciliate and the cryptomonad symbiont; the difference in density of the two cells is particularly clear. The symbiont nucleus, one of the macronuclei, and the micronucleus of the ciliate are out of the plane of the section. (CM) Ciliate mitochondrion; (ER) endoplasmic reticulum; (Mac) macronucleus; (P) pyrenoid; (SM) symbiont mitochondrion; (V) vacuole. The large arrowhead indicates a possible region of Golgi activity.  $\times 4500$ . (From Hibberd, 1977.)

Order 3 Chromonadales: the remainder of the cryptophyte algae, often blue-green in color due to chloroplasts containing the phycobiliprotein Cr-phycoyanin.

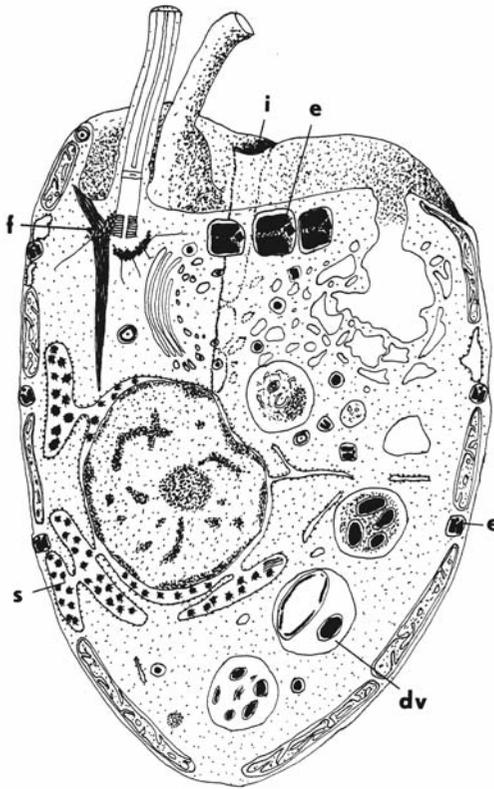
### Goniomonadales

*Goniomonas* (Figs. 9.8, 9.9(c)), a colorless alga with freshwater and marine species, is the sole alga in the order. *Goniomonas* is colorless and does not contain a plastid. Food organisms are taken up by an anterior tubular invagination, the infundibulum, and digested in food vacuoles in the cytoplasm. Storage granules occur inside an extension of the outer membrane of the nuclear envelope. Large ejectisomes occur under the anterior plasma membrane and small ejectisomes occur between the periplast plates.

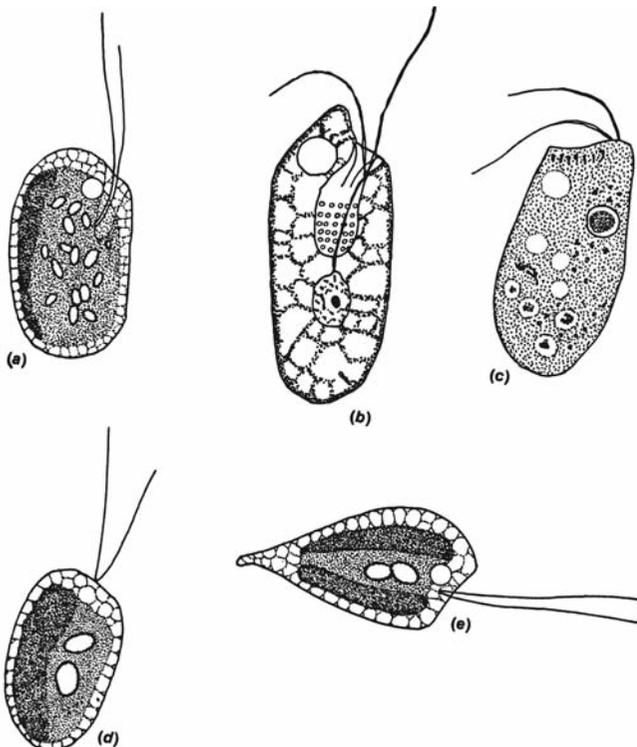
### Cryptomonadales

*Cryptomonas* (Figs. 9.9(a), 9.10, 9.11) and *Chilomonas* (Fig. 9.9(b)) are the only two genera in the order. *Cryptomonas* spp. are reddish in color due to the presence of the phycobiliprotein Cr-phycoerythrin in a bilobed chloroplast joined in the center by a pyrenoid. *Chilomonas* is a reduced form of *Cryptomonas* (Hoef-Emden and Melkonian, 2003) containing a leucoplast without photosynthetic pigments.

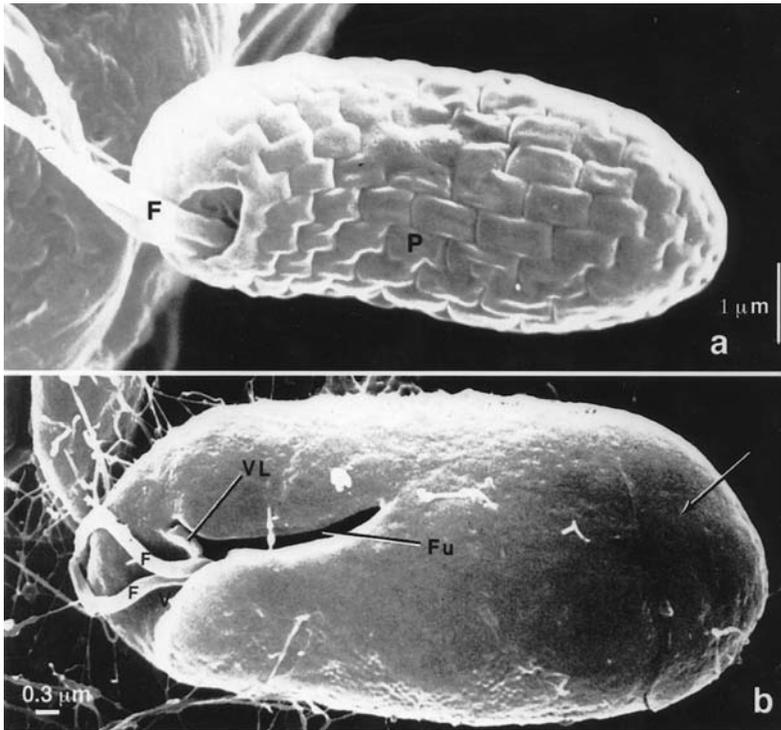
*Cryptomonas* has an asymmetric shape, which can be attributed, in part, to a subapical depression called the **vestibulum** which may extend internally to form a **gullet** or progress along the ventral surface into a **furrow** (Fig. 9.10(b)) (Kugrens and Lee, 1991). Large ejectosomes occur in rows under the furrow. Sexual reproduction occurs in *Cryptomonas* (Fig. 9.11) (Kugrens and Lee, 1988).



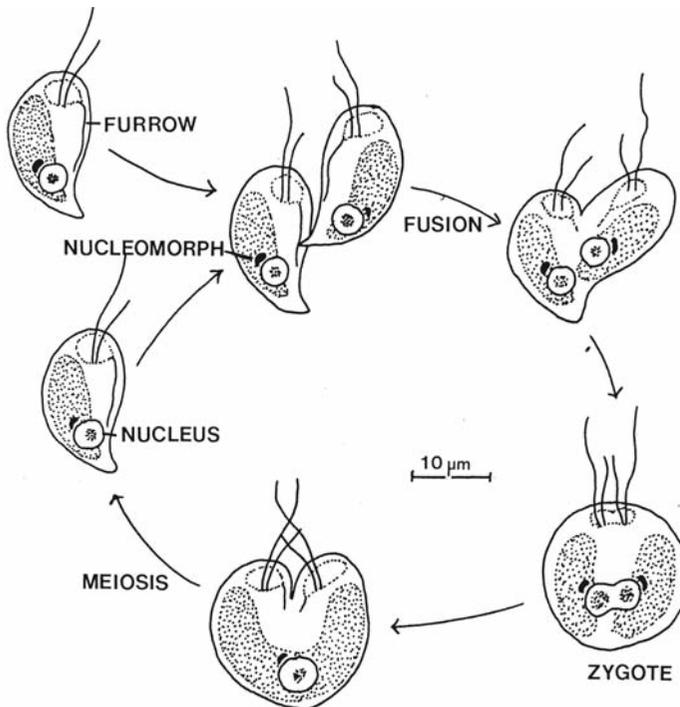
**Fig. 9.8** Reconstruction of a cell of *Goniomonas truncata*. (f) Flagellar roots; (s) storage granules; (e) ejectisome; (dv) digestive vacuole; (i) infundibulum. (After Mignot, 1965.)



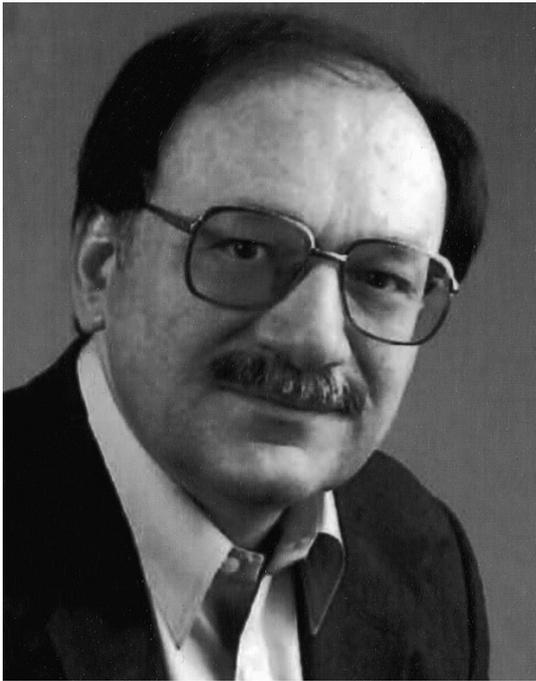
**Fig. 9.9** (a) *Cryptomonas erosa*.  
(b) *Chilomonas paramecium*.  
(c) *Goniomonas truncata*.  
(d) *Rhodomonas lacustris*.  
(e) *Chroomonas nordstedtii*.



**Fig. 9.10** Scanning electron micrographs of *Chroomonas oblonga* (a) and *Cryptomonas* sp. (b). *Chroomonas oblonga* has multiple periplast plates (P) under the plasma membrane, no furrow is present, and the flagella (F) arise from an anterior vestibular depression. *Cryptomonas* sp. has a smooth surface that is produced by a single periplast plate under the plasma membrane. The furrow (Fu) is an extension of the anterior vestibulum. A vestibular ligule (VL) overlaps the vestibulum. (From Kugrens et al., 1986.)



**Fig. 9.11** The life cycle of *Cryptomonas* sp. (Adapted from Kugrens and Lee, 1988.)



**Fig. 9.12 Michael Melkonian** Born October 4, 1948.

Dr. Melkonian received a Diploma in Biology (1974) from the University of Hamburg. From 1978 to 1983, he was Wissenschaftlicher Assistent at the Botanisches Institut, University of Münster, and in 1983 Habilitation (Venia Legendi) in Botany and Privatdozent at the Botanisches Institut. From 1986 to 1988 he was a Heisenberg Fellow, German Research Foundation (DFG), at Münster. In 1988 he moved to the Institute of Botany at the University of Cologne where he is a full professor.

## Chroomonadales

The remaining cyptophyte algae are placed in this order. Many, though not all, of these algae are blue-green in color due to the presence of the phycobiliproteins Cr-phycocyanin in the chloroplasts. *Chroomonas* (Fig. 9.9(e)) has an anterior vestibulum in the chloroplast. *Chroomonas* (Figs. 9.9(e), 9.10(a)) has an anterior vestibulum through which the two flagella emerge.

## REFERENCES

- Antia, N. J., Cheng, J. Y., Foyle, R. A. J., and Percival, E. (1979). Marine cryptomonad starch from autolysis of glycerol-grown *Chroomonas salina*. *J. Phycol.* 15:57–62.
- Brett, S. J., and Wetherbee, R. (1996). Periplast development in Cryptophyceae. II. Development of the inner periplast component in *Rhinomonas pauea*, *Proteomonas sulcata* [haplomorph], *Rhodomonas baltica*, and *Cryptomonas ovata*. *Protoplasma* 192:40–8.
- Cavalier-Smith, T. (2002). Nucleomorphs: enslaved algal nuclei. *Curr. Opin. Microbiol.* 5:612–9.
- Deane, J. A., Strachan, I. M., Saunders, G. W., Hill, D. R. A., and McFadden, G. I. (2002). Cryptomonad evolution: nuclear 18S rDNA phylogeny versus cell morphology and pigmentation. *J. Phycol.* 38:1236–44.
- Dodge, J. D. (1969). The ultrastructure of *Chroomonas mesostigmatica* Butcher (Cryptophyceae). *Arch. Mikrobiol.* 69:266–80.
- Douglas, S., Zauner, S., Fraunholz, M., et al. (2001). The highly reduced genome of an enslaved algal nucleus. *Nature* 410:1091–6.
- Gantt, E. (1971). Micromorphology of the periplast of *Chroomonas* sp. (Cryptophyceae). *J. Phycol.* 7:177–84.
- Gantt, E. (1979). Phycobiliproteins of Cryptophyceae. In *Biochemistry of Protozoa*, ed. N. Levandowsky, and S. A. Hutner, pp. 121–37. New York: Academic Press.
- Gantt, E., Edwards, M. R., and Provasoli, L. (1971). Chloroplast structure of the Cryptophyceae. Evidence for phycobiliproteins within the intrathylakoidal spaces. *J. Cell Biol.* 48:280–90.
- Giroldo, D., and Vieira, A. A. H. (2002). An extracellular surface fucose-rich polysaccharide produced by a tropical strain of *Cryptomonas obovata* (Cryptophyceae). *J. Appl. Phycol.* 14:185–91.
- Gustafson, D. E., Stoecker, D. K., Johnson, M. D., van Heukelem, W. F., and Sneider, K. (2000). Cryptophyte algae are robbed of their organelles by the marine ciliate *Mesodinium rubrum*. *Nature* 405:1049–52.
- Halldal, P. (1958). Action spectra of phototaxis and related problems in Volvocales: *Ulva*-gametes and Dinophyceae. *Physiol. Plant.* 11:118–53.
- Heath, I. B., Greenwood, A. D., and Griffiths, H. B. (1970). The origin of flimmer in *Saprolegnia*, *Dictyuchus*, *Synura* and *Cryptomonas*. *J. Cell Sci.* 7:445–61.
- Hibberd, D. J. (1977). Observations on the ultrastructure of the cryptomonad endosymbiont of the red water ciliate *Mesodinium rubrum*. *J. Mar. Biol. Assoc. UK* 57:45–61.
- Hill, D. R. A., and Rowan, K. S. (1989). The biliproteins of the Cryptophyceae. *Phycologia* 28:455–3.
- Hoef-Emden, K., and Melkonian, M. (2003). Revision of the genus *Cryptomonas* (Cryptophyceae): a combination of molecular phylogeny and morphology provides insights into a long-hidden dimorphism. *Protist* 154:371–409.

- Hovasse, R., Mignot, J. P., and Joyon, L. (1967). Nouvelles observations sur les trichocystes des Cryptomonadines et les "R bodies" des particules kappa de *Paramecium aurelia* Killer. *Protoplastologia* 3:241-55.
- Kalff, J., and Welch, H. E. (1974). Phytoplankton production in Char Lake, a natural polar lake, Cornwallis Is., Northwest Territories. *J. Fish. Res. Board Can.* 31:621-36.
- Knapp, C. W., deNoyelles, F., Graham, D. W., and Bergin, S. (2003). Physical and chemical conditions surrounding the diurnal vertical migration of *Cryptomonas* spp. (Cryptophyceae) in a seasonally stratified mid-western reservoir (USA). *J. Phycol.* 39:855-61.
- Kugrens, P., and Lee, R. E. (1987). An ultrastructural survey of cryptomonad periplasts using quick-freezing freeze-fracture techniques. *J. Phycol.* 23:365-76.
- Kugrens, P., and Lee, R. E. (1988). Ultrastructure of fertilization in a cryptomonad. *J. Phycol.* 24:385-93.
- Kugrens, P., and Lee, R. E. (1991). Organization of cryptomonads. In *The Biology of Free-living Heterotrophic Flagellates*, ed. P. J. Patterson, and J. Larsen, pp. 219-33. Oxford: Clarendon Press.
- Kugrens, P., Lee, R. E., and Kugrens, P. (1986). The occurrence and structure of flagellar scales in some freshwater cryptophytes. *J. Phycol.* 22:549-52.
- Kugrens, P., Lee, R. E., and Andersen, R. A. (1987). Ultrastructural variations in cryptomonad flagella. *J. Phycol.* 23:511-18.
- Kugrens, P., Lee, R. E., and Corliss, J. O. (1994). Ultrastructure, biogenesis, and functions of extrusive organelles in selected non-ciliate protists. *Protoplasma* 181:164-90.
- Lee, E. S., Lewitus, A. J., and Zimmer, R. K. (1999). Chemoreception in a marine cryptophyte: behavioral plasticity in response to amino acids and nitrate. *Limnol. Oceanogr.* 44:1571-4.
- Lee, R. E., and Corliss, J. O. (1994). Ultrastructure, biogenesis, and functions of extrusive organelles in selected non-ciliate protists. *Protoplasma* 181:164-90.
- Lee, R. E., and Kugrens, P. (1986). The occurrence and structure of flagellar scales in some freshwater cryptophytes. *J. Phycol.* 22:549-52.
- Liaud, M-F., Brandt, U., Scherzinger, M., and Cerff, R. (1997). Evolutionary origin of cryptomonad microalgae. Two novel chloroplast/cytosol-specific GAPDH genes as potential markers of ancestral endosymbiont and host cell components. *J. Mol. Evol.* 44 (Suppl. 1): 528-37.
- Loret, P., Pastoureaud, A., Bacher, C., and Delesalle, B. (2000). Phytoplankton composition and selective feeding of the pearl oyster *Pinctada margaritifera* in the Takapoto lagoon (Tuamotu Archipelago, French Polynesia): *in situ* study using optical microscopy and HPLC pigment analysis. *Mar. Ecol. Progr. Ser.* 199:55-67.
- Lucas, I. A. N. (1970a). Observations on the fine structure of the Cryptophyceae. I. The genus *Cryptomonas*. *J. Phycol.* 6:30-8.
- Lucas, I. A. N. (1970b). Observations on the ultrastructure of representatives of the genera *Hemiselmis* and *Chroomonas* (Cryptophyceae) *Br. Phycol. J.* 5:29-37.
- McFadden, G. I., Gilson, P. R., and Hill, D. R. A. (1994). *Goniomonas*: rRNA sequences indicate that this phagotrophic flagellate is a close relative of the host component of cryptomonads. *Eur. J. Phycol.* 29:29-32.
- McKerracher, L., and Gibbs, S. P. (1982). Cell of nucleomorph division in the alga *Cryptomonas*. *Can. J. Bot.* 60:2440-52.
- McKnight, D. M., Howes, B. L., Taylor, C. D., and Goehringer, D. D. (2000). Phytoplankton dynamics in a stably stratified Antarctic lake during winter conditions. *J. Phycol.* 36:852-61.
- Maier, U-G., Hofmann, C. J. B., Eschbach, S., Wolters, J., and Igloi, G. (1991). Demonstration of nucleomorph-encoded small subunit ribosomal RNA in cryptomonads. *Mol. Gen. Genet.* 230:155-60.
- Marin, B., Klingberg, M., and Melkonian, N. (1998). Phylogenetic relationships among the Cryptophyta: analyses of nuclear-encoded SSU rRNA sequences support the monophyly of extant plastid-containing lineages. *Protist* 149:265-76.
- Mignot, J. P. (1965). Etrude ultrastructurale de *Cyathomonas truncata* From. (Flagellé Cryptomonadine). *J. Microscopie* 4:239-52.
- Morrall, S., and Greenwood, A. D. (1982). Ultrastructure of nucleomorph division in species of Cryptophyceae and its evolutionary implications. *J. Cell Sci.* 54:311-28.
- Nauwerk, A. (1968). Das Phytoplankton des Latnjajaure 1954-55. *Schweiz. Z. Hydrol.* 30:188-216.
- Pechlauer, R. (1971). Factors that control the production rate and biomass of phytoplankton in high-mountain lakes. *Mitt. Int. Ver. Theor. Angew. Limnol.* 19:124-5.
- Roberts, E. C., and Laybourn-Parry, J. (1999). Mixotrophic cryptophytes and their predators in the Dry Valley lakes of Antarctica. *Freshwater Biol.* 41:737-46.
- Smith, W. O., and Barber, R. T. (1979). A carbon budget for the autotrophic ciliate *Mesodinium rubrum*. *J. Phycol.* 15:27-33.

- Spear-Bernstein, L., and Miller, K. R. (1984). Unique localization of the phycobiliprotein light-harvesting pigment in the Cryptophyceae. *J. Phycol.* 25:412-19.
- Thin, L-V. (1983). Effect of irradiance on the physiology and ultrastructure of the marine cryptomonad, *Cryptomonas* strain Lis (Cryptophyceae). *Phycologia* 22:7-11.
- Urabe, J., Gurung, T. B., Yoshida, T., et al. (2000). Diel changes in phagotrophy by *Cryptomonas* in Lake Biwa. *Limnol. Oceanogr.* 45:1558-63.
- Wright, R. T. (1964). Dynamics of a phytoplankton community in an ice-covered lake. *Limnol. Oceanogr.* 9:163-78.

# Heterokontophyta

The algae in the Heterokontophyta usually have cells with an anterior tinsel and posterior whiplash flagellum (Fig. 10.1). The plastids contain chlorophylls *a* and *c* along with fucoxanthin. The storage product is usually chrysolaminarin in cytoplasmic vesicles.

The following classes are commonly recognized (Andersen, 2004):

- Chrysophyceae (golden-brown algae)  
(Chapter 10)
- Synurophyceae (Chapter 11)
- Eustigmatophyceae (Chapter 12)
- Pinguiophyceae (Chapter 13)
- Dictyochophyceae (silicoflagellates)  
(Chapter 14)
- Phaeophyceae (Chapter 15)
- Bolidophyceae (Chapter 16)
- Bacillariophyceae (diatoms) (Chapter 17)
- Raphidophyceae (chloromonads) (Chapter 18)
- Xanthophyceae (yellow-green algae)  
(Chapter 19)
- Phaeothamniophyceae (Chapter 20)
- Phaeophyceae (brown algae) (Chapter 21)

## CHRYSOPHYCEAE

The Chrysophyceae are distinguished chemically by having chlorophylls *a*, *c*<sub>1</sub>, and *c*<sub>2</sub> (Andersen and Mulkey, 1983) and structurally by two flagella inserted into the cell perpendicular to each other, one photoreceptor on the short flagellum that is usually shaded by an eyespot in the anterior portion of the chloroplast, contractile vacuoles in the

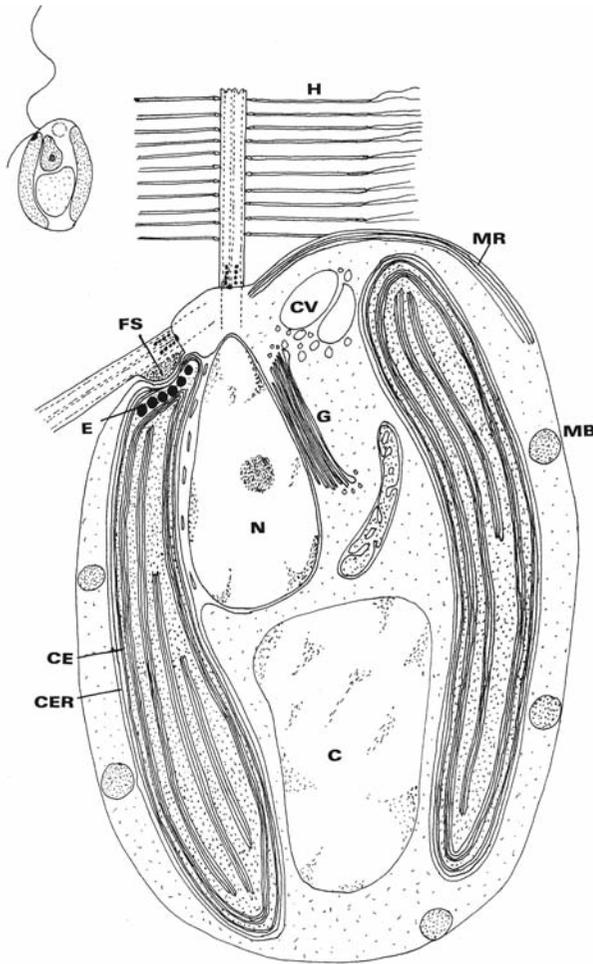
anterior portion of the cell, chloroplast endoplasmic reticulum, and radially or biradially symmetrical silica scales (if they are present). The storage product is chrysolaminarin. Many members of the class produce statospores enclosed in a silicified wall with a terminal pore.

Most of the species in the Chrysophyceae are freshwater and occur in soft waters (low in calcium). Many of the freshwater species are in the plankton of lakes where they are present in abundance. The coccoid and filamentous genera are found mostly in cold springs and brooks, where they occur as gelatinous or crustous growths on stones and woodwork. Most of the Chrysophyceae are sensitive to changes in the environment and survive the unfavorable periods as statospores.

## Cell structure

### Flagella and eyespot

Many of the Chrysophyceae have a tinsel flagellum that is inserted at the anterior end of the cell parallel to the cell axis (Fig. 10.1) and a whiplash flagellum that is inserted approximately perpendicular to the tinsel flagellum. The whiplash flagellum is often reduced to a short stub. The hairs on the tinsel flagellum are usually tripartite microtubular hairs (Hill and Outka, 1974), although tripartite and fibrillar hairs have been reported in *Ochromonas* (Bouck, 1971) (Fig. 10.2). Flagellar scales have been seen in a couple of genera (Andersen, 1982). The posterior whiplash flagellum is usually the shorter flagellum and has a swelling at its base on the side toward the cell



**Fig. 10.1** Semidiagrammatic drawing of a light and electron microscopical view of the basic organization of a cell of the Chrysophyceae. (C) Chrysolaminarin vesicle; (CE) chloroplast envelope; (CER) chloroplast endoplasmic reticulum; (CV) contractile vacuole; (E) eyespot; (FS) flagellar swelling; (G) Golgi body; (H) hair of the anterior flagellum; (MB) muciferous body; (MR) microtubular root of flagellum; (N) nucleus. (Adapted from Hibberd, 1976.)

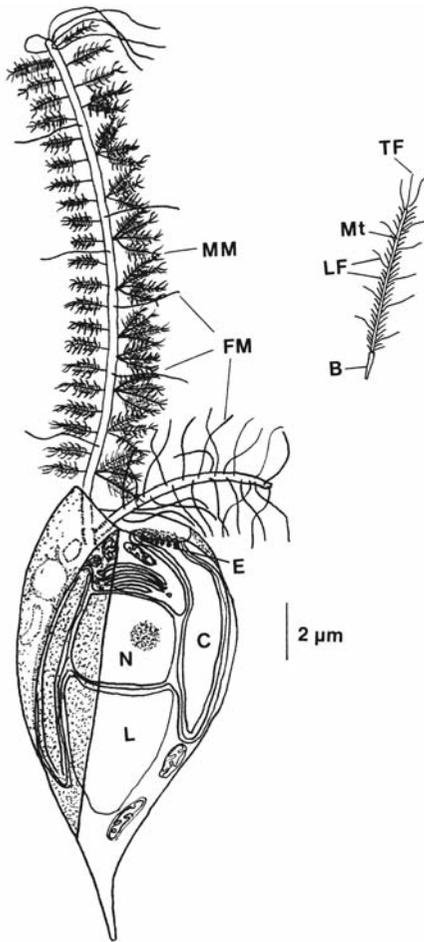
(Fig. 10.1). This flagellar swelling contains an electron-dense area referred to as the photoreceptor. The flagellar swelling contains **retinal**, the chromophore of rhodopsin-like proteins, suggesting that a rhodopsin-like protein is the photoreceptor in the Chrysophyceae (Walne et al., 1995). The flagellar swelling fits into a depression of the cell immediately beneath which, inside the chloroplast, is the eyespot. The eyespot consists of lipid globules inside the anterior portion of the chloroplast, between the chloroplast envelope and the first band of thylakoids.

In *Ochromonas*, the long tinsel flagellum beats in one plane and pulls the cell forward, whereas the shorter flagellum is flexed over the anterior eyespot and appears to play little role. During forward movement the cell rotates because of the shape of the body.

### Internal organelles

The chloroplasts are parietal and usually only a few in number, often only one or two. Chlorophylls *a*, *c*<sub>1</sub>, and *c*<sub>2</sub> are present, with the main carotenoid being fucoxanthin. The chloroplasts are surrounded by two membranes of chloroplast E.R., the outer membrane of which is usually continuous with the outer membrane of the nuclear envelope (Fig. 10.1). The thylakoids are usually grouped three to a band.

The chloroplast of *Ochromonas danica* will form a small proplastid if the cells are grown in the dark (Gibbs, 1962). The proplastid contains a single thylakoid, a few small vesicles, and a large number of dense granules. The chlorophyll *a* content of the proplastid is about 1.2% of the mature chloroplast. On exposure of the proplastid to light, vesicles appear that fuse to form the thylakoids. After 2 days



**Fig. 10.2** *Ochromonas danica*. (B) Basal attachment region; (C) chloroplast; (E) eyespot; (FM) fibrillar hair; (L) leucosin; (LF) lateral filament; (MM) microtubular hair; (Mt) microtubule; (N) nucleus; (TF) terminal filament. (After Bouck, 1971.)

in the light, the chloroplasts have reached their mature form, although it takes 8 days for them to acquire their full complement of chlorophyll.

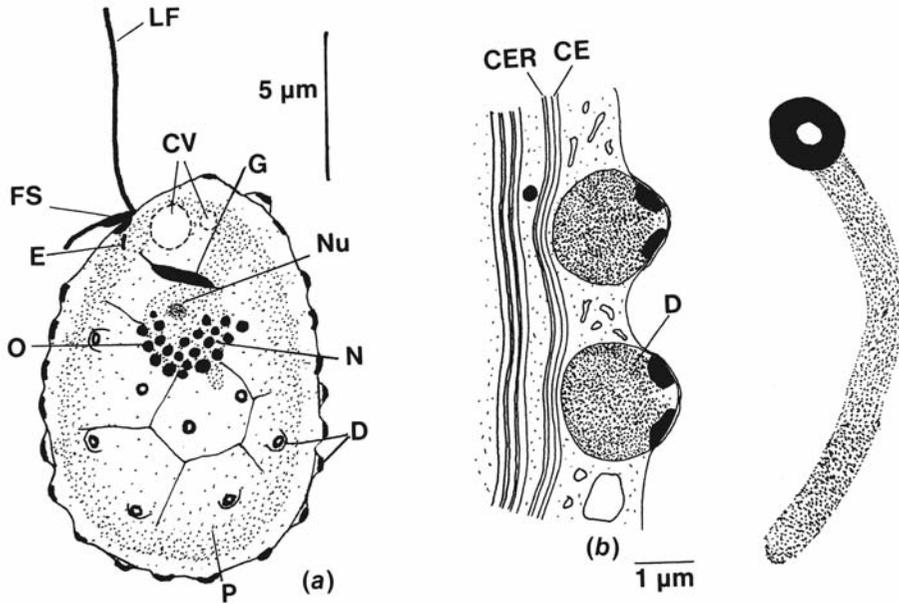
Pyrenoids are common in chloroplasts of the Chrysophyceae. They consist of a granular area that is different in appearance from the stroma. Few, if any, thylakoids traverse the pyrenoid area.

The storage product is **chrysolaminarin (leucosin)**, a  $\beta$ -1,3 linked glucan, supposedly found in a posterior vesicle (Fig. 10.1). The actual function of the so-called chrysolaminarin vesicle may be more complex than supposed with the discovery of microorganisms in the chrysolaminarin vesicle

of *Ochromonas* (Daley et al., 1973; Dubowsky, 1974). The chrysolaminarin vesicle is much larger in organisms grown in the dark on a synthetic medium than in cells grown in the light. The opposite would be expected if the vesicle stored chrysolaminarin, the accumulation product of photosynthesis in the light. It may be that the structure may also function as a digestive vesicle, breaking down material taken up by the cell into building blocks for metabolism and growth.

The single nucleus is pear-shaped (pyriform), with its narrow anterior end extended in the direction of the basal bodies (Fig. 10.1). There is a single, large Golgi body which lies against the nucleus in the anterior part of the cell, often in a concavity in the nuclear envelope. Contractile vacuoles are common, usually occurring in the anterior part of the cell next to the Golgi apparatus. There is often a complex system of vesicles associated with the contractile vacuoles similar to the pusule system in the Dinophyceae. Lipid bodies can also be found in the protoplasm. In young cells there are usually few lipid bodies; however, as the cell ages, the lipid bodies become larger and more numerous until they fill the protoplasm.

Two different types of projectiles occur in the Chrysophyceae, muciferous bodies and discobolocysts, the former like the muciferous bodies in the Prymnesiophyceae, Raphidophyceae, and Dinophyceae. The muciferous bodies (Fig. 10.1) contain granular material and are bonded by a single membrane. On discharge the contents of the vesicle often form a fibrous network outside the cell. The **discobolocysts** are similar to the muciferous bodies and have been described in the most detail by Hibberd (1970), in *Ochromonas tuberculatus* (Fig. 10.3). The discobolocysts are in the outer layer of cytoplasm and consist of a single membrane-bounded vesicle with a hollow disc in the outward-facing part of the vesicles. The discharge of the discobolocyst is explosive, taking place by the expansion of the projectile into a thin thread 6 to 11  $\mu$ m long, the disc being at the tip of the mucilage. As the discharge occurs, the cell jerks violently under the recoil to a distance of 5  $\mu$ m. After the discharge of either muciferous bodies or discobolocysts, the protoplast recovers without any deleterious effects. Both of the projectiles originate in the area of the Golgi apparatus.



**Fig. 10.3** (a) *Ochromonas tuberculatus*. (b) Charged and discharged discobolocysts. (CE) Chloroplast envelope; (CER) chloroplast endoplasmic reticulum; (CV) contractile vacuole; (D) discobolocyst; (E) eyespot; (FS) flagellar swelling; (G) Golgi body; (LF) long flagellum; (N) nucleus; (Nu) nucleolus; (O) oil; (P) plastid. (After Hibberd, 1970.)

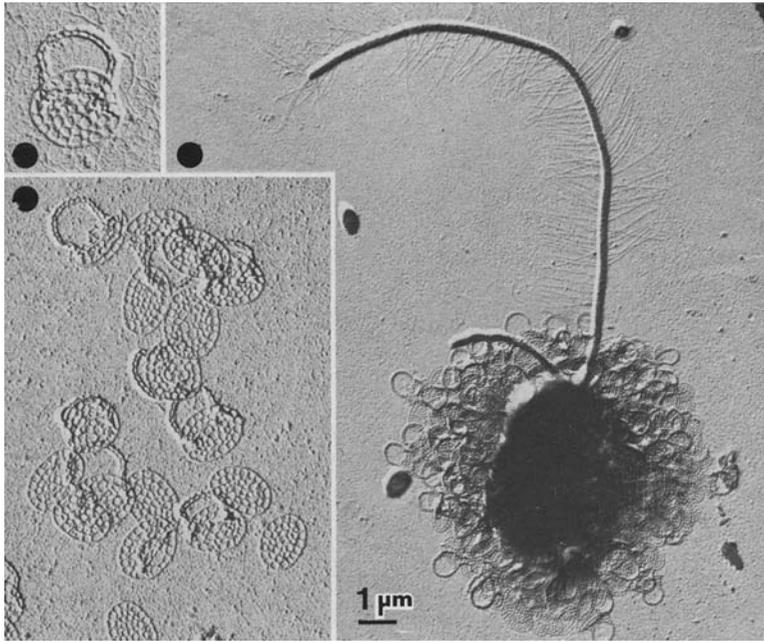
### Extracellular deposits

Cell walls composed of cellulose (Herth and Zugenmaier, 1979), loricas, and silicified scales and walls, occur in some of the Chrysophyceae (Preisig, 1994). Silica scales, such as those in *Paraphysomonas* (Fig. 10.4), are radially or biradially symmetrical. The scales are arranged loosely outside the plasma membrane without any clearly defined pattern. Like the Bacillariophyceae and Synurophyceae, the scales of the Chrysophyceae are formed inside a silica deposition vesicle that is derived from endoplasmic reticulum (Schnepf and Deichgraber, 1969). This arrangement differs from that of the calcified scales of the Prymnesiophyceae and the Chlorophyta, which are formed by the Golgi apparatus. Scale formation is also similar to frustule formation in the diatoms in that the addition of germanium (a competitive inhibitor of silicon utilization) to the growth medium results in inhibition of growth (Lee, 1978). Organic scales without mineralization also occur in the Chrysophyceae. In *Chromulina placentula*, there is a single layer of

organic scales covering the posterior portion of the cell, the scales being very similar to those found in the Prymnesiophyceae (Thronsdon, 1971).

In one of the orders (Parmales), silicified walls occur which are composed of five or eight parts (Figs. 10.14, 10.15) (Booth and Marchant, 1987). Loricas, such as those in *Pseudokephyrion pseudospirale* and *Kephyrion rubri claustris*, can also be mineralized. Manganese can occur in loricas as needle-like structures, whereas iron occurs as granular deposits (Dunlap et al., 1987).

Some of the species have a lorica (an envelope around the protoplast, but not generally attached to the protoplast as a wall is). In *Dinobryon* (Fig. 10.11), the lorica is composed of an interwoven system of microfibrils (Kristiansen, 1969). The formation of the lorica begins when a small funnel-shaped piece arises from the cell. The protoplast then rotates on its axis following a spiral course and secreting the remainder of the lorica. When the lorica is complete, the protoplast withdraws to its base. Several strains of *Ochromonas malhamensis* and *O. sociabilis* produce a delicate lorica consisting of a 10- to 20-μm hollow stalk and a cup-shaped envelope that encloses a long protoplasmic filament and the basal half of the cell, respectively. The lorica of *O. malhamensis* is composed of the polysaccharide chitin (Herth et al., 1977). The lorica can be mineralized as is the case with



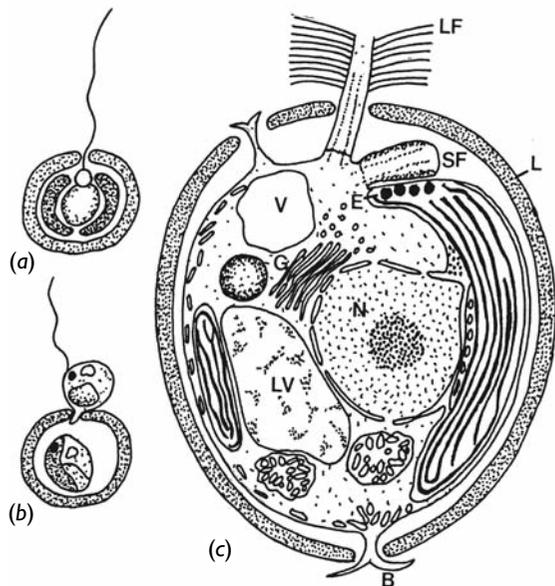
**Fig. 10.4** Transmission electron micrographs of *Paraphysomonas sigillifera* showing a whole cell with a long tinsel flagellum and a short whiplash flagellum. Also included are higher-magnification micrographs of the scales. (From Thomsen et al., 1981.)

*Chrysooccus rufescens* (Fig. 10.5). This alga has a basically mucilaginous lorica surrounding the cell (Belcher, 1969). In young cells the lorica is very transparent, probably consisting just of mucilage. At a later stage, dark needle-shaped crystals appear, which are primarily oriented parallel to the cell surface. The crystals ultimately unite to form a felty mass outside the cell.

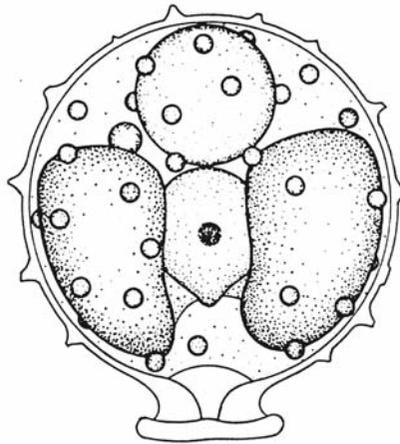
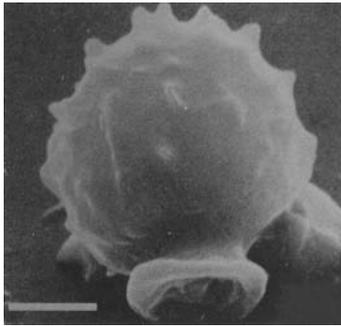
*Anthophysa vegetans* (Fig. 10.10(b), (c)) is a freshwater colonial alga that produces a mineralized stalk that can contain salts of calcium, iron, or manganese. The minerals in the environment determine color and the mineral composition of the stalk (Lee and Kugrens, 1989).

## Statospores

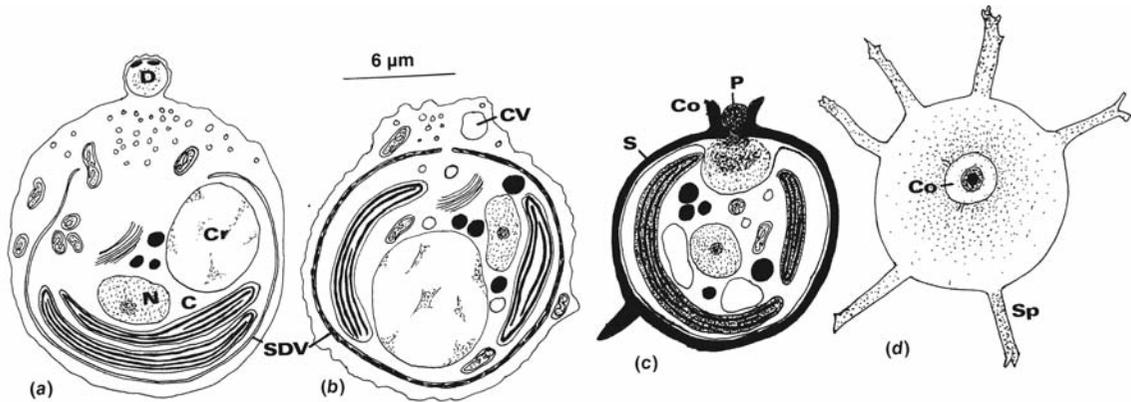
The formation of a **cyst** or **statospore** or **resting spore** is one character by which a member of the Chrysophyceae or Synurophyceae may be unequivocally recognized. **Statospores** are mostly



**Fig. 10.5** *Chrysooccus rufescens*. (a) Whole cell. (b) Cell undergoing reproduction. (c) Ultrastructure of vegetative cell. (B) Branched cytoplasmic process; (E) eyespot; (G) Golgi; (L) lorica; (LV) leucosin vesicle; (LF) long flagellum; (N) nucleus; (SF) short flagellum; (V) contractile vacuole. ((c) after Belcher, 1969.)



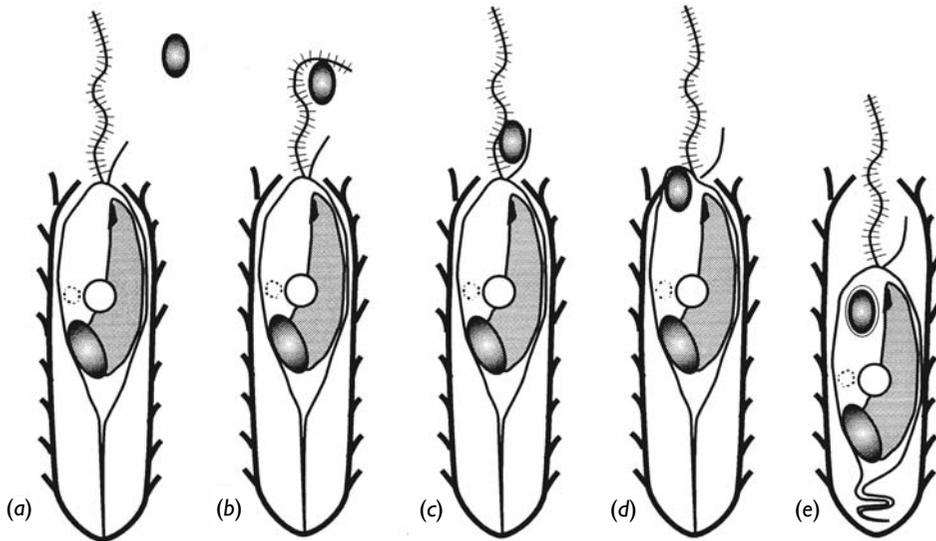
**Fig. 10.6** Statospore of *Ochromonas sphaerocystis*. Bar = 5  $\mu\text{m}$ . (From Andersen, 1982.)



**Fig. 10.7** Formation of a statospore or cyst in *Ochromonas tuberculata*. (a)–(c) The formation of the statospore. (d) A mature statospore as seen from the collar end. (C) Chloroplast; (Co) collar of statospore; (Cr) chrysolaminarin vesicle; (CV) contractile vacuole; (D) discobolocyst; (N) nucleus; (P) plug in pore of statospore; (S) statospore wall; (SDV) silica deposition vesicle; (Sp) spine. (Adapted from Hibberd, 1977.)

spherical, ellipsoidal, or ovate in shape, and the outer surface may be smooth or variously ornamented with warts, spines, or arms (Figs. 10.6, 10.7). Wall ornamentation is species specific. The statospore has a pore with a collar that is closed by a plug. A vegetative cell forms a statospore internally. In the formation of a statospore the cells become non-motile, any projectiles are discharged, and there is considerable contractile vacuole activity (Sheath et al., 1975; Hibberd, 1977). A nearly spherical vesicle called the silica deposition vesicle is formed in the cytoplasm, and silica is deposited

inside the vesicle (Fig. 10.7) (Preisig, 1994). A complete sphere of silica is formed, interrupted only by the developing pore and collar. The nucleus, chloroplast, flagellar basal bodies, mitochondria, Golgi body, chrysolaminarin vesicle, and ribosomes are segregated to the inside of the silica deposition vesicle, whereas outside there are mitochondria, ribosomes, contractile vacuoles, and small vesicles. After the spines, pore, and collar have been formed in the silica deposition vesicle, a plug is formed by the cytoplasm in the pore area. With the formation of the unsilicified plug, contact is lost between the protoplasm inside the statospore and that outside, with the inner membrane of the silica deposition vesicle becoming the new plasmalemma. When a statospore germinates, there is a dissolution of the plug or separation of it from the spore wall. The protoplast then moves out of the statospore by amoeboid motion, forming flagella as it moves out.



**Fig. 10.8** Phagotrophy in *Epipyxix pulchra*. The cell has a posterior stalk by which it is attached to a lorica. (a) The long tinsel flagellum beats in such a way that water and suspended particles are drawn to the cell. (b) A particle is seized by the long tinsel flagellum. (c) The particle is maneuvered between the two flagella. (d) A feeding cap from the cell envelopes the particle. (e) The particle is enclosed within a food vacuole within the cytoplasm. The stalk has pulled the cell into the lorica. (Modified from Wetherbee and Andersen, 1992.)

## Nutrition

Nutrition in the Chrysophyceae can be either phototrophic, phagotrophic, or mixotrophic (photosynthetic organism capable of taking up particles and molecules from the medium) (Zhang and Watanabe, 2001). *Dinobryon* (Fig. 10.11) is a mixotrophic chrysophyte that can have half of the carbon in the cells derived from ingestion of microorganisms (Caron et al., 1993). *Dinobryon* has the ability to compete with crustaceans, rotifers, and ciliates in capturing microorganisms. Under low-light conditions, *Dinobryon* can ingest an average of three bacterial cells every five minutes (Bird and Kalff, 1986). Chrysophytes have the ability to ingest prey up to 30 times larger than themselves. The ability to take up microorganisms appears to be about the same for pigmented and non-pigmented chrysophytes (Zhang et al., 1996).

Like other mixotrophic chrysophytes, *Epipyxix pulchra* phagocytizes food particles that are both

living (bacteria, small algae, or even cells of its own kind) and non-living (detritus, fecal material) (Wetherbee and Andersen, 1992). During prey gathering, the long flagellum, which is adorned with stiff hairs, beats rapidly to direct a strong current towards the cell while the short, smooth flagellum moves very little. When a potential food particle is drawn by current to contact the flagellar surface, the long flagellum stops beating and positions itself, in concert with the short flagellum, to seize the prey between the two flagella (Fig. 10.8). Both flagella briefly rotate the prey before selecting or rejecting it. If selected as food, the prey is held in place until a complex collecting cup emanates out and engulfs the prey. The cup plus the prey, now a food vacuole, is then retracted back into the cell proper.

Isofloridoside, a product of photosynthesis in *Ochromonas malhamensis*, is used to adapt the cells to changes in the dissolved substances (osmotic pressure) of the medium. As the osmotic pressure of the medium increases, *O. malhamensis* responds by increasing the concentration of isofloridoside inside the cell, preventing water loss to the medium (Kauss, 1967).

## Ecology

Fauré-Fremiet (1950) has shown that *Chromulina psammobia* moves up to the surface of mud flats at

low tide, and as the tide comes in, the flagellate moves down in the mud so as not to be washed away. The basis for the migration is a reversal in the phototactic response, the cells being positively phototactic at low tide and negatively phototactic at high tide. The presence of mud is not necessary for the changes in phototaxis, which will continue for 6 to 7 days in the laboratory, more or less synchronized with the tide. After this the phototaxis changes gradually until the cells are always positively phototactic.

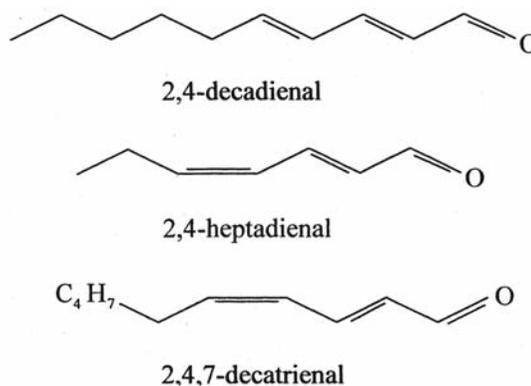
*Dinobryon* (Fig. 10.11) is a freshwater alga that is almost never found in waters with a high concentration of phosphorus. This observation led some investigators to conclude that high concentrations of phosphorus are inhibitory to growth of the alga. More detailed work (Lehman, 1976) showed this not to be true, that *Dinobryon* in culture grows well at high concentrations of phosphorus. What happens in nature is that other algae grow faster and outcompete *Dinobryon* at higher concentrations of phosphorus; only when **vernal** (spring) **blooms** of diatoms or other phytoplankton have reduced phosphorus to a level that inhibits their own growth will *Dinobryon* compete effectively. This demonstrates a basic rule among algae (Eppley et al., 1969; Fuhs et al., 1972), which is *algae that have efficient uptake of nutrients (are able to utilize low levels of a nutrient) usually have lower maximum intrinsic growth rates than algae that are less efficient in taking up nutrients*. These differences represent two variations of an adaptive scheme for nutrient utilization. Some species exploit a resource-laden environment rapidly, whereas others display measured efficiency in utilizing an energy source. Energy trade-offs among competing intracellular processes might preclude organisms from excelling at both simultaneously.

*Dinobryon* uses phosphate not only as inorganic phosphate but also as organically bound phosphate in moderate-sized molecules (glycerophosphate, uridylic acid, adenylic acid) (Lehman, 1976). It can also use nitrogen either from inorganic sources or from organic molecules (urea, glycine, uridylic acid, adenylic acid). Its capacity to utilize these organic sources and its phagocytosis of whole particles imply that *Dinobryon* is suited to occupy the water column when death and decline of previous bloom-forming organisms release products of cell

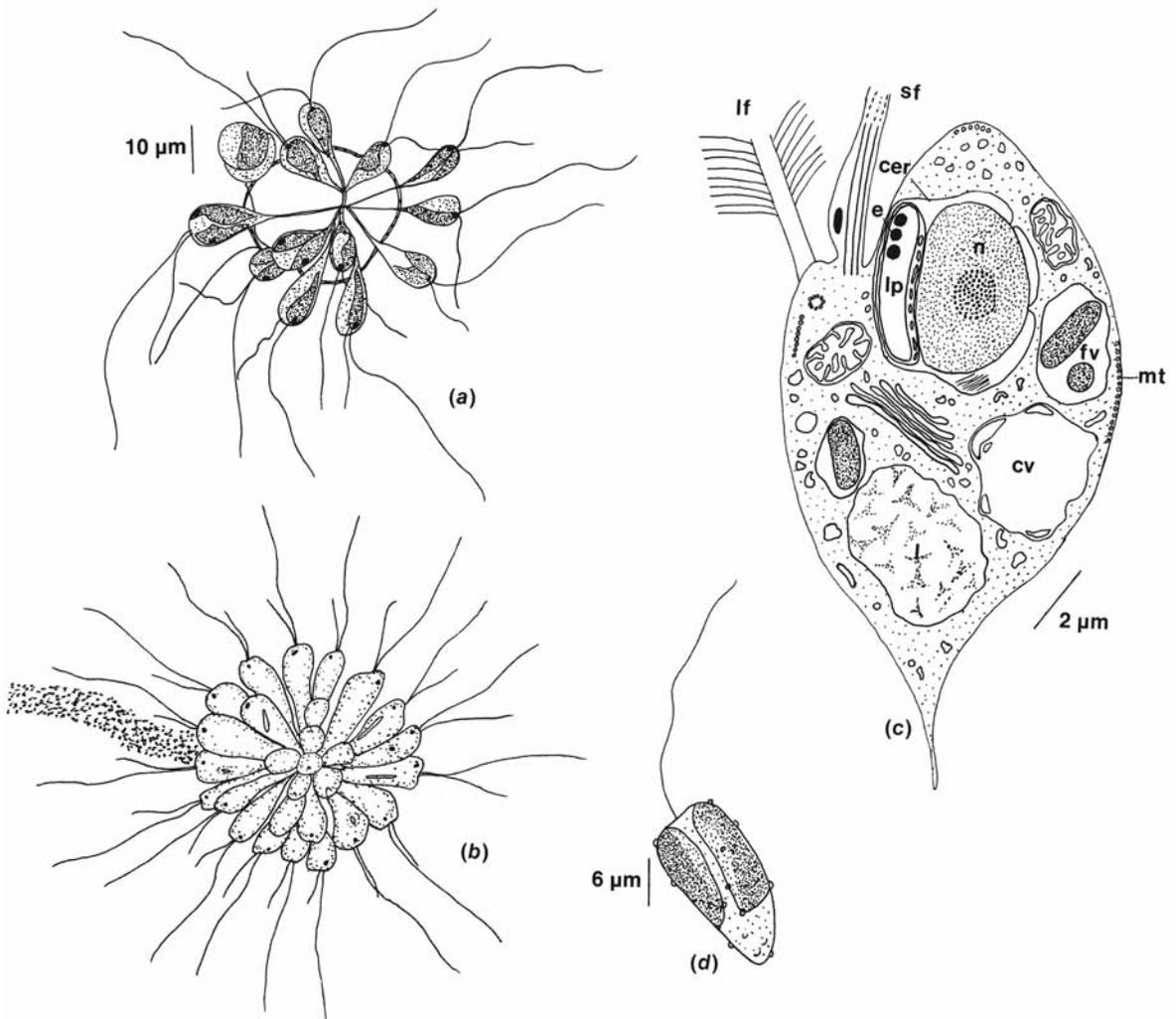
breakdown to solution. It is also at these times, when ambient levels of dissolved inorganic nutrients have become somewhat depleted, that the potentials of the cells for effective uptake of nutrients show their advantage.

Although high concentrations of phosphorus do not inhibit the growth of *Dinobryon*, high concentrations of potassium do (Lehman, 1976). The concentrations necessary for inhibition are commonly found in many lakes. As an example, in one Swedish lake, *D. sertularia* appears in June a few weeks after the ice breaks, with the alga persisting in the cool water until early autumn (Willén, 1961). Its period of abundance matches exactly with a summertime decrease in potassium caused partly by the precipitation of potassium by clay colloids and partly by the uptake by littoral vegetation (Ahl, 1966). The disappearance of *Dinobryon* corresponds to increased concentration of potassium as the element is released from dying vegetation.

Chrysophytes are notorious for their production of fishy or rancid smells, reflecting release of unsaturated aldehydes derived from the high cell content of polyunsaturated acids. *Uroglena* (Fig. 10.10(a)) and *Dinobryon* (Fig. 10.11) produce unsaturated fatty-acid derivatives (Fig. 10.9) that contribute to these smells (Watson and Satchwill, 2003). These chemicals are classified as **algal volatile organic compounds (AVOCs)**.



**Fig. 10.9** Three unsaturated fatty-acid derivatives produced by chrysophytes that result in rancid or fishy odors.



**Fig. 10.10** (a) *Uroglena conradii*. (b), (c) *Anthophysa vegetans*.

(d) *Chromulina conica*. (cer) Chloroplast endoplasmic reticulum; (cv) contractile vacuole; (e) eyespot; (fv) food vacuole; (l) leucosin; (lf) long flagellum; (lp) leucoplast; (mt) microtubules; (n) nucleus; (sf) short flagellum. ((a), (d) after Schiller, 1929; (b) after Pringsheim, 1946; (c) after Belcher and Swale, 1972.)

Order 2 Parmales: cells with siliceous walls composed of five or eight plates.

Order 3 Chrysomeridales: motile naked cells with laterally inserted flagella and an eyespot, motile cells similar to those in the brown algae.

## Classification

Three of the orders of the Chrysophyceae (Preisig, 1995) will be considered here:

Order 1 Chromulinales: cells with the flagella inserted into the anterior portion of the cell.

## Chromulinales

All of the organisms in this order have a unicell with two apically inserted flagella somewhere in their life history. One of the flagella is tinsel with mastigonemes and directed forward, while the second flagellum is whiplash (lacking mastigonemes) and is inserted at approximately 90° to the tinsel flagellum (e.g., *Ochromonas*,

Figs. 10.2, 10.3). In some of the genera (e.g., *Chromulina*, Fig. 10.10(d)) the whiplash flagellum is reduced to a stub. There are usually two parietal chloroplasts, a central nucleus, and a large posterior chrysolaminarin vesicle.

Within the order there is a progression from the unicellular to the colonial form (Fig. 10.10) as exemplified by *Uroglena* (Fig. 10.10(a)) and *Anthophysa* (Fig. 10.10(b), (c)). Some of the genera, such as *Dinobryon* (Fig. 10.11), have cells surrounded by a lorica. In *Chrysococcus* (Fig. 10.5), the cell is surrounded by a lorica that has pores in it.

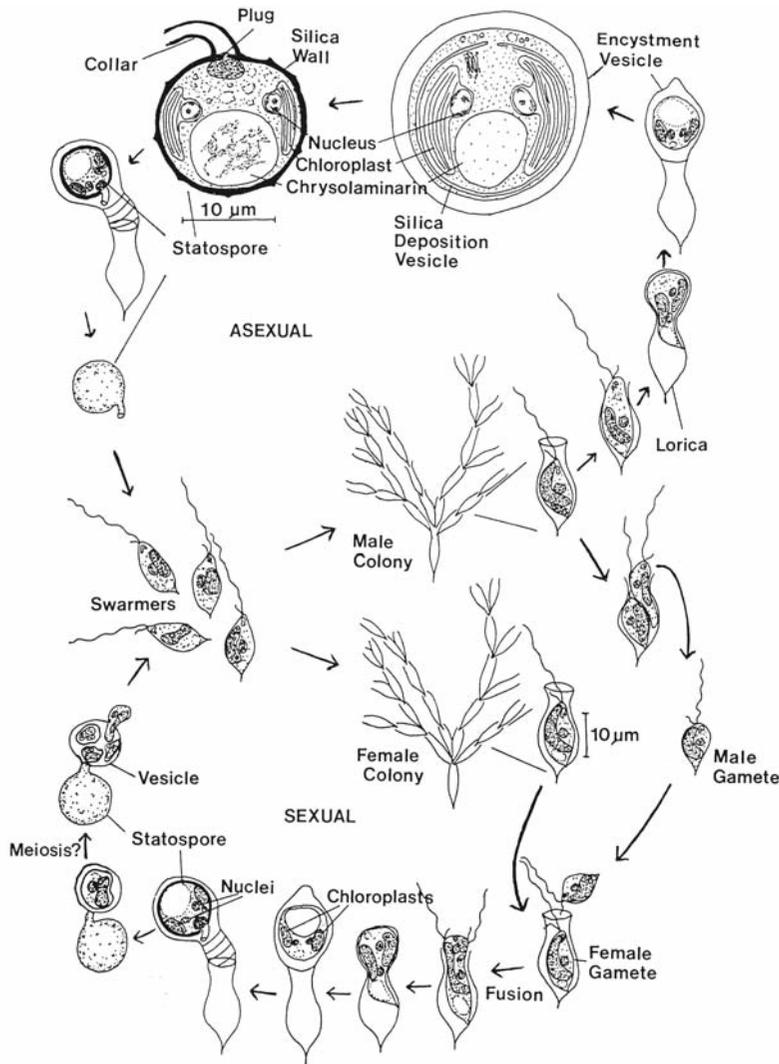
In the Chromulinales there is a frequent tendency toward loss of photosynthetic activity and adaptation to various forms of phagotrophy and chemo-organotrophy. Associated with this is a diminution of the chloroplast as in *Anthophysa* (Fig. 10.10(b), (c)), which has a leucoplast with a pigmented eyespot. The end of this reduction is represented by *Paraphysomonas* (Fig. 10.4), which has no trace of an eyespot or plastid. Along with the reduction in the chloroplast have arisen adaptations for feeding, such as in *Anthophysa*, which beats its long flagellum, creating a water current toward the cells, which brings with it bacteria from as far away as 200  $\mu\text{m}$ . The bacteria strike the anterior end of the cells and are ingested at the point where they touch. The bacteria sink into the cytoplasm, which closes behind them, and are completely in the cell within 2 to 3 seconds of contact (Belcher and Swale, 1972).

In *Dinobryon cylindricum*, sexual reproduction is heterothallic and dioecious, and morphologically and physiologically anisogamous (Fig. 10.11) (Sandgren, 1981). Cells of the second or third tier of the colony (the basal cell being the oldest) are the best potential gamete-producing cells. Gametes are produced mainly in exponentially growing populations. Female cells release a chemical **erogen** that causes male cells to divide once. One of the male cells remains in the lorica, whereas the other swims away as a naked male gamete, instead of attaching to the lorica mouth and building a new lorica as a vegetative cell would. The non-loricated male gamete (which is structurally similar to an *Ochromonas* cell) swims to the female cell in its lorica, the flag-

ella become oriented parallel to each other, and fusion occurs at the anterior end of each cell. Plasmogamy results in a quadriflagellate planozygote that fills the lorica. Nuclear fusion does not occur. After 30 minutes, the flagella have been lost and the zygote creeps to the lorica mouth by amoeboid movement. A thin cellulose encystment vesicle is formed as a lorica extension, the zygote rounds up, and a binucleate statospore (Fig. 10.12) is formed. In *D. divergens*, most statospores settle to the lake bottom and germinate early the next year (Sheath et al., 1975). Statospore germination occurs by the formation of a cellulosic vesicle at the statospore pore, followed by a presumably meiotic cleavage into four daughter cells that migrate into the vesicle. The *Ochromonas*-like swimmers escape to form new vegetative cells.

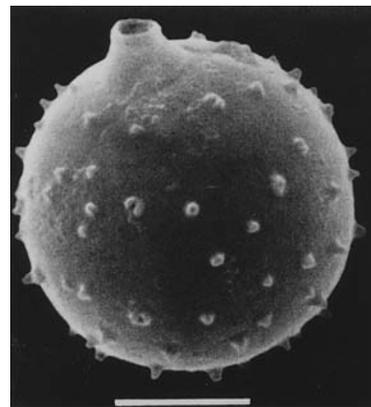
Asexually produced statospores of *Dinobryon cylindricum* may occur in exponential or stationary-phase populations, depending upon the clones involved (Sandgren, 1980, 1981). Asexual statospores (cysts) are formed at a much lower frequency (0.05% or less) than sexual statospores unless the population is placed in a nitrogen-depleted environment where asexual encystment can reach 4%. The continual production of a low number of the resistant statospores by a *Dinobryon* population acts as a hedge against a rapid unfavorable change in environmental conditions that would kill the vegetative cells.

The first event in statospore formation is vegetative cell enlargement so that the cell begins to bulge out of the lorica. The Golgi form vesicles that fuse to form a continuous silica deposition vesicle in the peripheral cytoplasm. The silica deposition vesicle has a pore that eventually will be the statospore pore. Precipitation of silica proceeds in the silica deposition vesicle to form the silicified statospore wall. The portion of the cytoplasm that is exterior to the wall retracts through the pore in the cyst wall into the statospore interior. The small amount of cytoplasm outside the wall forms the collar around the pore. A plug is formed in the cyst wall, sealing the statospore protoplasm. The mature statospore has two nuclei, two plastids, and a rich supply of energy reserves in the form of oil and chrysolaminarin.

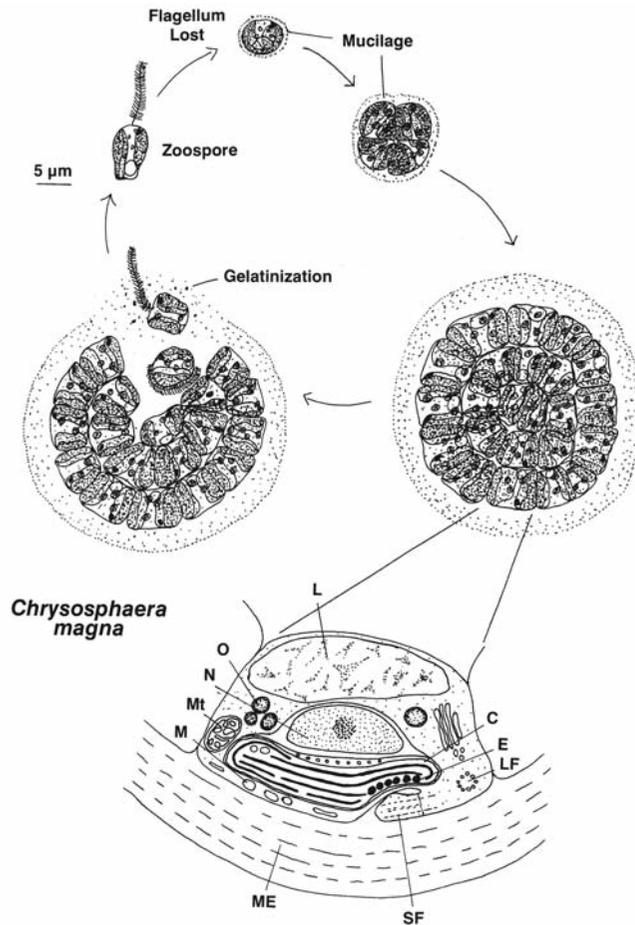


**Fig. 10.11** The life cycle of *Dinobryon*. (Adapted from Sheath et al., 1975; Sandgren, 1980, 1981.)

*Chrysosphaera* (Fig. 10.13) is an example of a colonial alga in the order. This organism has a dominant spherical non-motile stage with cells adhering to each other and the substrate in irregular clusters. The smallest individuals are unicellular, but individuals with up to 256 cells within a common mucilaginous envelope are not uncommon. All the cells within the envelope have a long tinsel flagellum and a short stub of the second flagellum (Belcher, 1974). When a colony is placed in distilled water, it begins to swell within



**Fig. 10.12** Scanning electron micrograph of a cyst of *Dinobryon cylindricum*. Bar = 5 µm. (From Sandgren, 1983.)



**Fig. 10.13** The life cycle of *Chrysoisphaera magna*. (C) Chloroplast; (E) eyespot; (L) leucosin vesicle; (LF) basal body of long flagellum; (M) muciferous body; (Mt) mitochondria; (ME) mucilaginous envelope; (N) nucleus; (O) oil; (SF) short flagellum. (After Belcher, 1974.)

15 minutes, and the mucilage gelatinizes on one side. The zoospores are released a few minutes later and swim for about 2 minutes before they shed their flagella, settle down, form a mucilaginous envelope, and become identical in appearance to the youngest coccoid stages.

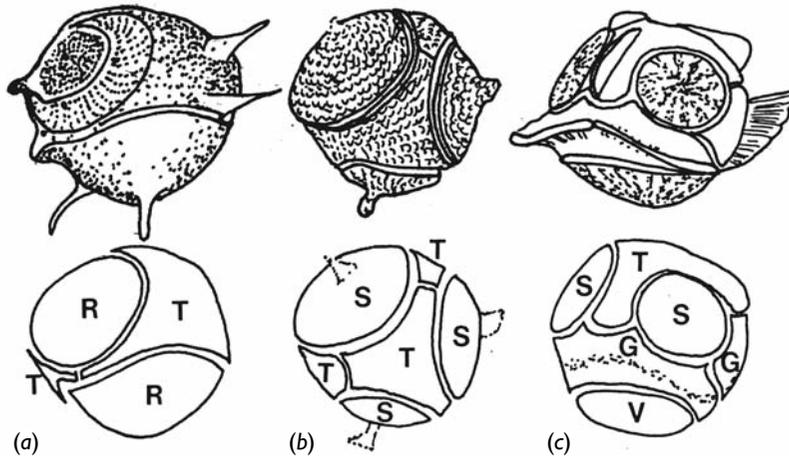
### Parmales

The Parmales consist of small cells, generally 2 to 5  $\mu\text{m}$  in diameter, each with a chloroplast (Marchant and McEldowney, 1986) and a silicified cell wall composed of five to eight plates (Figs. 10.14, 10.15). In the Pentalaminaeae there are five wall plates whereas in the Octolaminaeae there are eight wall plates (Booth and Marchant, 1987). Members of the Parmales occur at concentrations of  $10^5$  cells per liter in polar and subpolar marine waters, making them one of the more abundant groups in these waters.

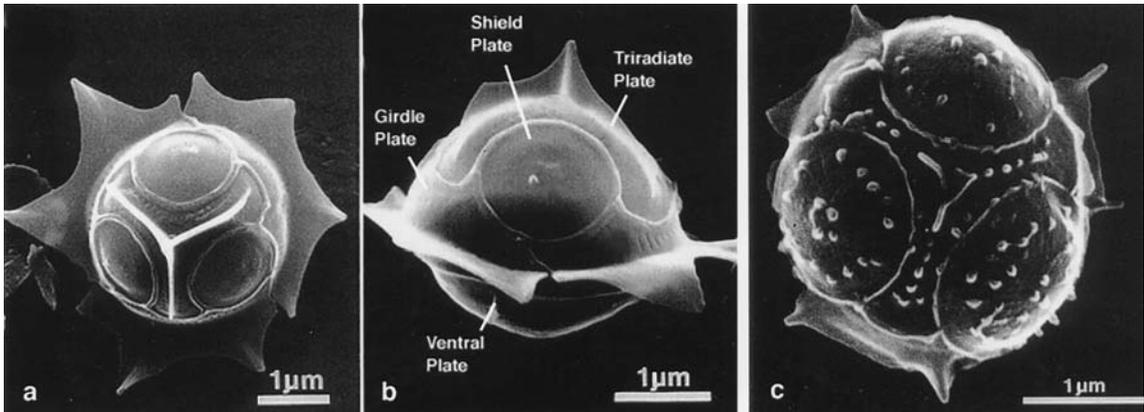
Four different shapes of plates occur around the cells (Figs. 10.14, 10.15) (Booth and Marchant, 1987). A **shield plate** or **round plate** is a circular plate with or without a central knob or process. A **triradiate plate** has three arms equally spaced, each arm fitting between two shield plates. A **ventral plate** is a round plate of greater diameter than that of shield plates; it is found in *Triparma* on the opposite side of the sphere from the triradiate plate. **Girdle plates** are three oblong plates in *Triparma*, juxtaposed end to end to form a ring around the ventral plate between it and the other four plates. Ornamentation of various types (papillae, wings, spines, keels) occurs on the plates.

### Chrysoisomeridales

These algae have zoospores with the flagella more or less laterally inserted into the cell body. There



**Fig. 10.14** Three genera in the Parmales. (a) *Pentalamina*. (b) *Tetraparma*. (c) *Triparma*. (G) Girdle plate; (R) round plate; (S) shield plate; (T) triradiate plate; (V) ventral plate. (After Booth and Marchant, 1987.)



**Fig. 10.15** (a), (b) *Triparma laevis*. (c) *Triparma strigata*. (From Kosman et al., 1993.)

is an eyespot in the chloroplast and the accessory pigment violaxanthin is present. The similarities in the zoospores of the algae in this order have led to the speculation that the Phaeophyceae (brown algae) probably evolved from an alga similar to *Giraudyopsis* in this order (Fig. 10.16) (O’Kelly, 1989; Saunders et al., 1997). The algae in the Chrysomeridales, however, lack the unilocular and plurilocular sporangia, as well as the plasmodesmata and alginates characteristic of the Phaeophyceae.



**Fig. 10.16** *Giraudyopsis stelliger*. (After Dangeard, 1966.)



*Adolph Pascher*

**Fig. 10.17 Adolph Pascher** Born May 31, 1881 in Tusset, Bohmerwalde, Province of Krummau, Austria-Hungary. Died by his own hand May 1945. Dr. Pascher was brought up in the Bohmerwalde, a snowy mountainous area, where he attended small schools until he went to the University of Prague. Here he was able to use his first microscope, a tool he was to use for the rest of his life. He graduated from the University of Prague in 1909. From 1909 to 1912, at a time when he suffered from a chronic illness, he studied pharmaceutical botany. From 1912 to 1927, he worked as a lecturer at different universities including the German Technical University in Prague. During this time he performed phycological research at a hydrobiological station in Hirschberg, Germany, and at another hydrobiological station that he founded in Upper Bohemia in Czechoslovakia. In 1927, he became director of the Botanical Institute and Gardens at the University of Prague. In the early 1930s, Dr. Pascher became involved in the Nazi Brown Shirt movement, which resulted in a hiatus of his numerous publications from 1933 to 1938. In 1939, he started publishing again while remaining active in the Nazi administration. With the fall of the Third Reich, he shot himself in May 1945. Dr. Pascher could be considered as the most prominent phycologist of his time. As stated by Prescott (1951) "His work exerted a greater influence than that of any other phycologist in clarifying modern concepts of algal taxonomy and phylogeny . . . it was his erudite interpretation of facts, nevertheless, which placed the studies of Pascher in a corner stone position."

## REFERENCES

- Ahl, T. (1966). Chemical conditions in Ösbysjön, Djursholm. 2. The major constituents. *Oikos* 17:175-6.
- Andersen, R. A. (1982). A light and electron microscopical investigation of *Ochromonas sphaerocystis* Matvienko (Chrysophyceae): The statospore, vegetative cell and its peripheral vesicles. *Phycologia* 21:390-8.
- Andersen, R. A. (2004). Biology and systematic of heterokont and haptophyte algae. *Amer. J. Bot.* 91:1508-22.
- Anderson, R. A., and Mulkey, T. J. (1983). The occurrence of chlorophylls  $c_1$  and  $c_2$  in the Chrysophyceae. *J. Phycol.* 19:289-94.
- Belcher, J. H. (1969). A morphological study of the phytoflagellate *Chrysococcus rufescens* Klebs in culture. *Br. Phycol. J.* 4:105-17.
- Belcher, J. H. (1974). *Chrysosphaera magna* sp. nov., a new coccoid member of the Chrysophyceae. *Br. Phycol. J.* 9:139-44.
- Belcher, J. H., and Swale, E. M. F. (1972). The morphology and fine structure of the colourless colonial flagellate *Anthophysa vegetans* (O. F. Müller) Stein. *Br. Phycol. J.* 7:335-46.
- Bird, D. F., and Kalff, J. (1986). Bacterial grazing by planktonic lake algae. *Science* 231:493-4.
- Booth, B. C., and Marchant, H. J. (1987). Parmales, a new order of marine chrysophytes, with descriptions of three new genera and seven new species. *J. Phycol.* 23:245-60.
- Bouck, G. B. (1971). The structure, origin, isolation, and composition of the tubular mastigonemes of the *Ochromonas* flagellum. *J. Cell Biol.* 50:362-84.
- Caron, D. A., Sanders, R. W., Lim, E-L., Marrasé, C., Amaral, L. A., Whitney, S., Aoki, R. B., and Porter, K. G. (1993). Light-dependent phagotrophy in the freshwater mixotrophic chrysophyte *Dinobryon cylindricum*. *Microbial Ecol.* 25:93-111.
- Daley, R. J., Morris, G. P., and Brown, S. R. (1973). Phagotrophic ingestion of a blue-green alga by *Ochromonas*. *J. Protozool.* 20:58-61.
- Dangeard, P. (1966). Sur le nouveau genre *Giraudyopsis* P.D. *Le Botantiste* 49:99-108.
- Dubowsky, N. (1974). Selectivity of ingestion and digestion in the chryomonad flagellate *Ochromonas malhamensis*. *J. Protozool.* 21:295-8.
- Dunlap, J. R., Walne, P. L., and Preisig, H. R. (1987). Manganese mineralization in chrysophycean loricas. *Phycologia* 26:394-6.
- Eppley, R. W., Rogers, J. N., and McCarthy, J. J. (1969). Half-saturation constants for uptake of nitrate and

- ammonium by marine phytoplankton. *Limnol. Oceanogr.* 14:912–20.
- Fauré-Fremiet, E. (1950). Rythme de marée d'une *Chromulina psammophile*. *Bull. Biol. Fr. Belg.* 84:207–14.
- Fuhs, G. W., Demmerle, S. D., Canelli, E., and Chen, M. (1972). Characterization of phosphorus-limited algae (with reflections on the limiting nutrient concept). *Am. Soc. Limnol. Oceanogr. Spec. Symp.* 1:113–32.
- Gibbs, S. P. (1962). Nuclear envelope–chloroplast relationships in algae. *J. Cell Biol.* 14:433–44.
- Herth, W., and Zugenmaier, P. (1979). The lorica of *Dinobryon*. *J. Ultrastruct. Res.* 69:262–72.
- Herth, W., Kuppel, A., and Schnepf, E. (1977). Chitinous fibrils in the lorica of the flagellate chrysophyte *Poteriochromonas stipitata* (Syn. *Ochromonas malhamensis*). *J. Cell Biol.* 73:311–21.
- Hibberd, D. J. (1970). Observations on the cytology and ultrastructure of *Ochromonas tuberculatus* sp. nov. (Chrysophyceae), with special reference to the discobolocysts. *Br. Phycol. J.* 5:119–43.
- Hibberd, D. J. (1976). The ultrastructure and taxonomy of the Chrysophyceae and Prymnesiophyceae (Haptophyceae): A survey with some new observations on the ultrastructure of the Chrysophyceae. *Bot. J. Linn. Soc.* 72:55–80.
- Hibberd, D. J. (1977). Ultrastructure of cyst formation in *Ochromonas tuberculata* (Chrysophyceae). *J. Phycol.* 13:309–20.
- Hill, F. G., and Outka, D. E. (1974). The structure and origin of mastigonemes in *Ochromonas minute* and *Monas* sp. *J. Protozool.* 21:299–312.
- Kauss, H. (1967). Metabolism of isofluoridose (*O*- $\alpha$ -D-galactopyranosyl-[1  $\rightarrow$  1]-glycerol) and osmotic balance in the freshwater alga *Ochromonas*. *Nature* 214:1129–30.
- Kosman, C. A., Thomsen, H. A., and Ostergaard, J. B. (1993). Parmales (Chrysophyceae) from Mexican, Californian, Baltic, Arctic and Antarctic waters with a description of a new subspecies and several new forms. *Phycologia* 32:116–28.
- Kristiansen, J. (1969). Lorica structure in *Chrysolynos* (Chrysophyceae). *Bot. Tidsskr.* 64:162–8.
- Lee, R. E. (1978). Formation of scales in *Paraphysomonas vestita* and the inhibition of growth by germanium dioxide. *J. Protozool.* 25:163–6.
- Lee, R. E., and Kugrens, P. (1989). Biomineralization in *Anthophysa vegetans* (Chrysophyceae). *J. Phycol.* 25:591–6.
- Lehman, J. T. (1976). Ecological and nutritional studies on *Dinobryon* Ehrenb: Seasonal periodicity and the phosphate toxicity problem. *Limnol. Oceanogr.* 21:646–64.
- Marchant, H. J., and McEldowney, A. (1986). Nanoplankton siliceous cysts from Antarctica are algae. *Mar. Biol. (Berl.)* 92:53–7.
- O'Kelly, C. J. (1989). The evolutionary origin of the brown algae: information from the studies of motile cell ultrastructure. In *The Chrysophyte Algae: Problems and Perspectives*, ed. J. C. Green, B. S. C. Leadbeater, and W. L. Diver, pp. 255–78. Oxford: Clarendon Press.
- Preisig, H. R. (1994). Siliceous structures and silicification in flagellated protists, *Protoplasma* 181:29–42.
- Preisig, H. R. (1995). A modern concept of chrysophyte classification. In *Chrysophyte Algae*, ed. C. D. Sandgren, J. R. Smol, and J. Kristiansen, pp. 46–74. Cambridge: Cambridge University Press.
- Prescott, G. W. (1951). History of phycology. In *Manual of Phycology*, ed. G. M. Smith, pp. 1–12. New York: The Ronald Press Co.
- Pringsheim, E. G. (1946). On iron flagellates. *Philos. Trans. R. Soc. [B]* 232:311–42.
- Sandgren, C. D. (1980). An ultrastructural investigation of resting cyst formation in *Dinobryon cylindricum* Imhof (Chrysophyceae, Chrysophycophyta). *Protistologica* 16:259–76.
- Sandgren, C. D. (1981). Characteristics of sexual and asexual resting cyst (stato-spore) formation in *Dinobryon cylindricum* Imhof (Chrysophyta). *J. Phycol.* 17:199–210.
- Sandgren, C. D. (1983). Morphological variability in populations of chrysophycean resting cysts. I. Genetic (interclonal) and encystment temperature effects on morphology. *J. Phycol.* 19:64–70.
- Saunders, G. W., Potter, D., and Andersen, R. A. (1997). Phylogenetic affinities of the Sarcinochrysidales and Chrysomeridales (Heterokonta) based on analyses of molecular and combined data. *J. Phycol.* 33:310–18.
- Schiller, J. (1929). Neue Chryso- and Cryptomonaden aus Altwässern der Donau bei Wien. *Arch. Protistenk.* 66:436–58.
- Schnepf, E., and Deichgraber, G. (1969). Über die Feinstruktur von *Synura petersenii* unter besonderer Berücksichtigung der Morphogenese ihrer Kieselschuppen. *Protoplasma* 68:85–106.
- Sheath, R. G., Hellebust, J. A., and Sawa, T. (1975). The stato-spore of *Dinobryon divergens* Imhoff: Formation and germination in a subarctic lake. *J. Phycol.* 11:131–8.
- Thomsen, H. A., Zimmerman, B., Moestrup, Ø., and Kristiansen, J. (1981). Some new freshwater species of *Paraphysomonas* (Chrysophyceae). *Nord. J. Bot.* 1:559–81.

- Thronsdén, J. (1971). *Apedinella* gen. nov. and the fine structure of *A. spinifera* (Thronsdén) comb. nov. *Norw. J. Bot.* 18:47–64.
- Walne, P. L., Passarelli, V., Lenzi, P., Barsanti, L., and Gualtieri, P. (1995). Isolation of the flagellar swelling and identification of retinal in the phototactic flagellate, *Ochromonas danica* (Chrysophyceae). *J. Euk. Microbiol.* 42:7–11.
- Watson, S. B., and Satchwill, T. (2003). Chrysophyte odour production: resource-mediated changes at the cell and population levels. *Phycologia* 42:393–405.
- Wetherbee, R., and Andersen, R. A. (1992). Flagella of a chrysophycean alga play an active role in prey capture and selection. Direction observations on *Epipyxis pulchra* using image enhanced video microscopy. *Protoplasma* 166:1–7.
- Willén, T. (1961). The phytoplankton of Ösbysjön Djursholm. 1. Seasonal and vertical distribution of the species. *Oikos* 12:36–79.
- Zhang, X., and Watanabe, M. M. (2001). Grazing and growth of the mixotrophic chrysomonad *Poterioochromonas malhamensis* (Chrysophyceae) feeding on algae. *J. Phycol.* 37:738–43.
- Zhang, X., Watanabe, M. M., and Inouye, I. (1996). Light and electron microscopy of grazing by *Poterioochromonas malhamensis* (Chrysophyceae) on a range of phytoplankton taxa. *J. Phycol.* 32:37–46.

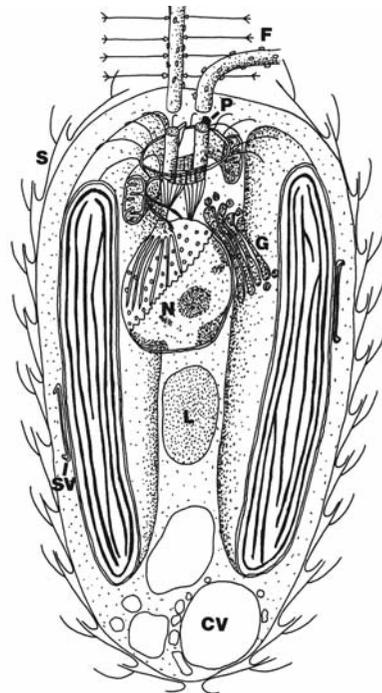
## Heterokontophyta

### SYNUROPHYCEAE

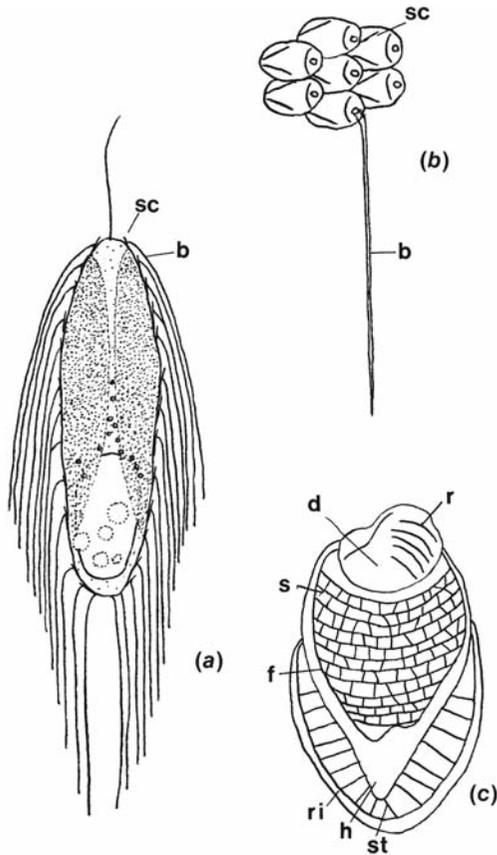
The Synurophyceae are closely related to the Chrysophyceae (Ariztia et al., 1991). The Synurophyceae differ, however, from the Chrysophyceae in the following: the Synurophyceae have chlorophylls  $a$  and  $c_1$  (Andersen and Mulkey, 1983), the flagella are inserted into the cell approximately parallel to one another (Fig. 11.1), there is a photoreceptor near the base of each flagellum, there is no eyespot, and the contractile vacuole is in the posterior portion of the cell (Lavau et al., 1997; Andersen et al., 1999). Chloroplast endoplasmic reticulum is present in a few species, but absent in most. The cells usually are covered by bilaterally symmetrical scales.

In the Synurophyceae, scales composed of silica commonly occur outside the cell (Figs. 11.1, 11.2). The scales are bilaterally symmetrical and are formed in a silica deposition vesicle. The membrane of the silica deposition vesicle (the **silicalemma**) controls the shape of the scale along with proteins and glycoproteins that adhere the developing scale to the silicalemma (Schultz et al., 2001). The presence of germanium in the medium results in inhibition of scale formation (Klaveness and Guillard, 1975). The scales are carried in the scale vesicle to the plasma membrane where the plasma membrane and the scale vesicle fuse, releasing the scales outside the cell (Beech et al., 1990). The scales are held next to the cell in an organic envelope (Ludwig et al., 1996), which is either hyaline or yellow-brown, the latter appearance being due to the impregnation of iron salts. The scales of the Synurophyceae are commonly

composed of a number of parts, such as the dome, shield, and bristle of *Mallomonas* (Lavau and Wetherbee, 1994) (Fig. 11.2). The scales of the Synurophyceae are overlapped precisely so that the anterior end of one scale overlaps the right margin of the scale to its left (Leadbeater, 1990).



**Fig. 11.1** Semidiagrammatic drawing of the cytology of *Synura*, showing the characteristic cytology of the Synurophyceae. (CV) Contractile vacuole; (F) flagella; (G) Golgi; (L) chrysolaminarin vesicle; (N) nucleus; (P) photoreceptor; (S) scale; (SV) scale vesicle. (Adapted from Andersen, 1985.)



**Fig. 11.2** *Mallomonas zellensis*. (a) Whole cell. (b) Scales. (c) A scale with the bristle removed. (b) Bristle; (d) dome; (f) flange; (h) hood; (r) ribs; (s) shield; (sc) scale; (st) strut. (After Fott, 1962.)

The scales are cemented together to form a scale case by the organic envelope (Fig. 11.4). This precise arrangement of scales differs from the loosely arranged scales of the Chrysophyceae.

*Tessellaria volvocina* (Fig. 11.3) is a colonial member of the class that has a large number of cells encased within a multilayer covering of siliceous scales. The individual cells of *T. volvocina* do not have a covering of scales (Tyler et al., 1989; Pipes and Leedale, 1992).

Analysis of lake sediments often reveals the presence of the silicified scales of the Synurophyceae as well as the silicified frustules of diatoms (Smol et al., 1984; Dixit et al., 1999). Analysis of the species that produced these silicified deposits can often result in a history of

environmental conditions in the lake. Diatoms usually do not grow in waters below a pH of 5.8 to 6, whereas several members of the Synurophyceae thrive in acidic lakes (Saxby-Rouen et al., 1997). As environmental concerns over the acidification of lakes by acid rains increase, these species will probably be more widely used as indicators of lake acidification.

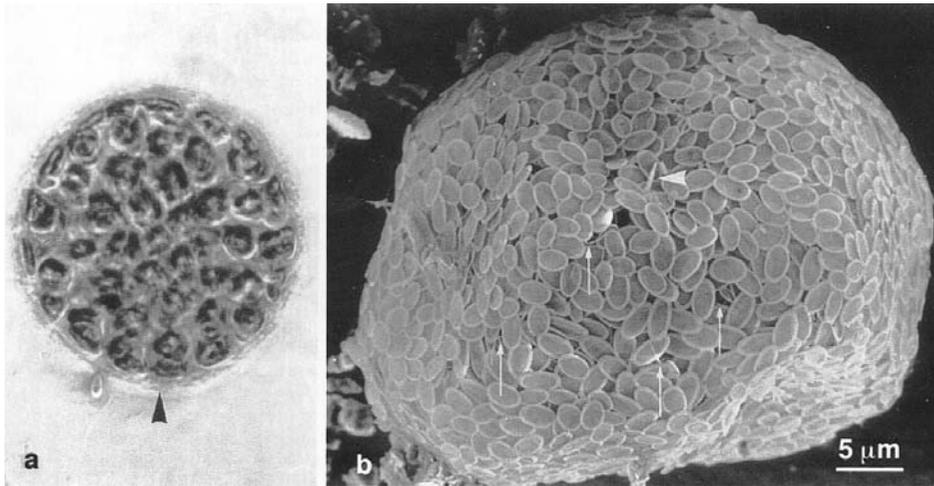
Unlike the Chrysophyceae, the Synurophyceae are not phagocytotic, probably because the fixed scale-case is solidly formed and bacteria and food particles cannot easily reach the cell membrane. The cysts (statozooids or stromatocysts) of the Synurophyceae are usually more elaborate than those of the Chrysophyceae.

## Classification

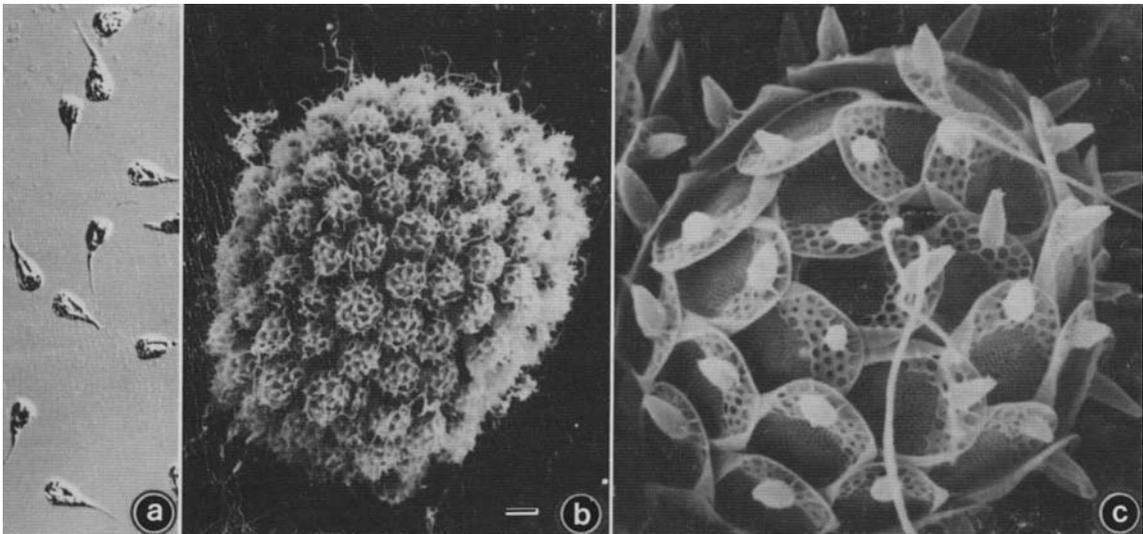
The Synurophyceae share affinities with the Bacillariophyceae, Chrysophyceae, and Phaeophyceae (Lavau et al., 1997; Andersen et al., 1999). Because of their silicified outer covering, they have been called “flagellated diatoms”; yet they have many of the characteristics of the Chrysophyceae such as statozooids.

There is a single class, the Synurales. All of the organisms are flagellates. Siphonaceous forms do not exist, as true filaments cannot be formed by organisms with a silica scale-case, and coccoid forms would be similar to diatoms. The diatoms *Corethron*, *Rhizosolenia*, and *Attheya* are similar in appearance to coccoid Synurophyceae (Andersen, 1987).

*Synura* (Fig. 11.4) is a widely distributed colonial member of the class. The life cycle of *Synura petersenii* (Figs. 11.5(a), 11.6) has been described by Sandgren and Flanagan (1986). Vegetative colonies of *S. petersenii* normally consist of a number of biflagellate cells joined at their posterior end. The cells are covered with silicified scales (Leadbeater, 1990). Each cell has two parietal chloroplasts, a central nucleus, and a large posterior chrysolaminarin vacuole. Sexual reproduction in *S. petersenii* is isogamous and heterothallic. Gametes appear when two compatible clones are mixed. No special culture conditions are required to induce sexual behavior. Actively growing cell populations are continually



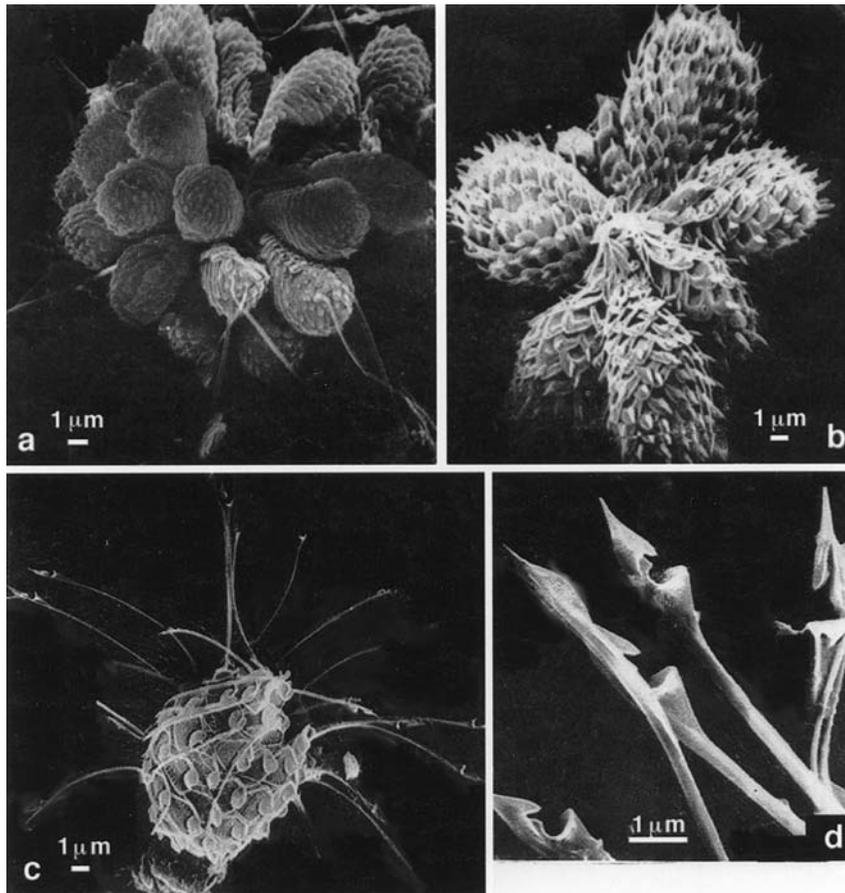
**Fig. 11.3** *Tessellaria volvocina*. (a) Light micrograph. Arrowhead points to a scale case. (b) Scanning electron micrograph showing overlapping of plate scales and rare spine scales (arrowhead). (From Tyler et al., 1989.)



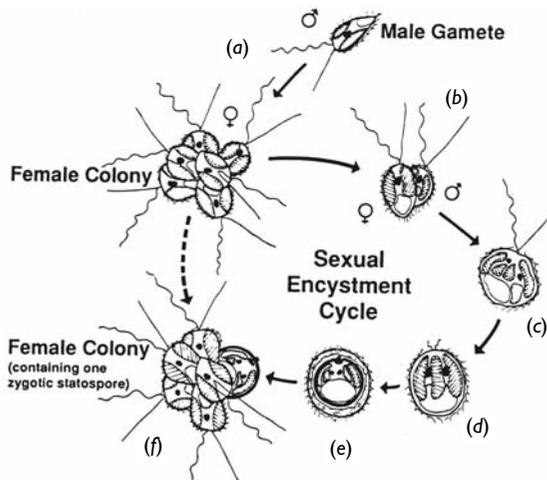
**Fig. 11.4** *Synura uvella*. (a) Light micrograph. (b) Scanning electron micrograph of a colony of cells. (c) Scanning electron micrograph of a single cell showing the two flagella emerging from an apical pore in the scale case. Bar = 10  $\mu\text{m}$ . (From Andersen, 1985.)

receptive to mating when mixed with a sufficient number of cells from a compatible clone. Male gametes are solitary biflagellate cells that appear similar to vegetative cells. The solitary male

gametes are probably released from the colonies of the male clone. The female gametes occur in colonies or as solitary cells, and are also similar in appearance to vegetative cells. Male and female gametes initially contact each other anteriorly; this is followed by lateral fusion. The silica scale layers mingle so that the resulting fusion cell maintains a complete scale layer. The biflagellate planozygote undergoes minor shape changes



**Fig. 11.5** Scanning electron micrographs. (a) *Synura petersenii*, whole colony. (b) *S. echinulata*. (c) *Mallomonas acaroides*, whole cell. (d) *M. acaroides*, distal end of helmet bristles. Bar = 1  $\mu\text{m}$ . (From Dürschmidt, 1984.)



**Fig. 11.6** Process of sexual copulation and zygotic encystment in *Synura petersenii*. (a) Initial contact of a solitary male gamete with a female gamete within a colony. (b) An early stage in plasmogamy characterized by the lateral fusion of cells, each with active flagellar pairs. (c) A biflagellate planozygote stage with the fusion cell containing four plastids, two nuclei, and two chrysolaminarin vesicles. Note that the planozygote is completely covered with scales. (d) An early stage in zygotic encystment during which the remaining flagella are lost and significant rearrangement of cytoplasmic organelles occurs to establish a symmetrical orientation. Fusion of chrysolaminarin vesicles occurs, and the nuclei become associated with the inner margins of the plastids. (e) A mature zygotic statospore as it ultimately appears taking the place of the female gamete in the colony. (f) Germination of statospore. (From Sandgren and Flanagin, 1986.)

and either oscillates in place or swims sluggishly for at least 4 to 8 hours. During this time, the four chloroplasts assume a symmetrical orientation around the flagellar bases as is typical of vegetative *Synura* cells, and the two large chrysolaminarin vesicles fuse to occupy the cell posterior. The flagella are lost, and the zygote becomes spherical as cyst wall deposition begins. After 6 to 8 hours, a morphologically mature zygote statospore is produced. Mature zygospores are observed as solitary objects if the female gametes are solitary. If the female gametes are in a colony, then the zygospores take the place of the female gametes in the female colonies. Female colonies can have as many as eight statospores, apparently as the result of numerous cells serving as female gametes. The cyst is an unornamented sphere, 13 to 16  $\mu\text{m}$  in diameter, and has a single, small recessed pore that lacks a surrounding collar. Presumably, statospores germinate meiotically to produce haploid cells. The number of statospores produced is in the range of 1% to 20% of the vegetative cell density. In addition, some statospores are produced asexually and can be seen in the clones before they are mixed.

## REFERENCES

- Andersen, R. A. (1985). The flagellar apparatus of the golden alga *Synura uvella*: Four absolute orientations. *Protoplasma* 128:94–106.
- Andersen, R. A. (1987). Synurophyceae classis nov., a new class of algae. *Am. J. Bot.* 74:337–53.
- Andersen, R. A., and Mulkey, T. J. (1983). The occurrence of chlorophylls  $c_1$  and  $c_2$  in the Chrysophyceae. *J. Phycol.* 19:289–94.
- Andersen, R. A., van de Peer, Y., Potter, D., Sexton, J. P., Kawachi, M., and Lajeunesse, T. (1999). Phylogenetic analysis of the SSU rRNA from members of the Chrysophyceae. *Protist* 150:71–84.
- Beech, P. L., Wetherbee, R., and Pickett-Heaps, J. D. (1990). Secretion and development of bristles in *Mallomonas splendens* (Synurophyceae). *J. Phycol.* 26:112–22.
- Dixit, S. S., Dixit, A. S., and Smol, J. P. (1999). Lake sediment chrysophyte scales from the northeastern U.S.A. and their relationship to environmental variables. *J. Phycol.* 35:903–18.
- Dürschmidt, M. (1984). Studies on scale-bearing Chrysophyceae from the Giessen area, Federal Republic of Germany. *Nord. J. Bot.* 4:123–43.
- Fott, B. (1962). Taxonomy of *Mallomonas* based on electron micrographs of scales. *Preslia* 34:69–84.
- Klavness, D., and Guillard, R. R. L. (1975). The requirement for silicon in *Synura petersenii* (Chrysophyceae). *J. Phycol.* 11:349–55.
- Lavau, S., and Wetherbee, R. (1994). Structure and development of the scale case of *Mallomonas adamas* (Synurophyceae). *Protoplasma* 181:259–68.
- Lavau, S., Saunders, G. W., and Wetherbee, R. (1997). A phylogenetic analysis of the Synurophyceae using molecular data and scale case morphology. *J. Phycol.* 33:135–51.
- Leadbeater, B. S. C. (1990). Ultrastructure and assembly of the scale case in *Synura* (Synurophyceae Andersen). *Br. Phycol. J.* 25:117–32.
- Ludwig, M., Lind, J. L., Miller, E. A., and Wetherbee, R. (1996). High molecular mass glycoprotein associated with the siliceous cell scales and bristles of *Mallomonas splendens* (Synurophyceae) may be involved in cell surface development and maintenance. *Planta* 199:219–28.
- Pipes, L. D., and Leedale, G. F. (1992). Scale formation in *Tessellaria volvocina* (Synurophyceae). *Br. Phycol. J.* 27:11–19.
- Sandgren, C. D., and Flanagan, J. (1986). Heterothallic sexuality and density dependent encystment in the chrysophycean alga *Synura petersenii* Korsch. *J. Phycol.* 22:206–16.
- Saxby-Rouen, K. J., Leadbeater, B. S. C., and Reynolds, C. S. (1997). The growth response of *Synura petersenii* (Synurophyceae) to photon flux density, temperature, and pH. *Phycologia* 36:233–43.
- Schultz, T. F., Egerton-Warburton, L., Crawford, S. A., and Wetherbee, R. (2001). Identification of a 41kDa protein embedded in the biosilica of scales and bristles isolated from *Mallomonas splendens* (Synurophyceae, Ochrophyta). *Protist* 152:315–27.
- Smol, J. P., Charles, D. F., and Whitehead, D. R. (1984). Mallomonadacean microfossils provide evidence of recent lake acidification. *Nature* 307:628–30.
- Tyler, P. A., Pipes, L. D., Croome, R. L., and Leedale, G. F. (1989). *Tessellaria volvocina* rediscovered. *Br. Phycol. J.* 24:329–37.

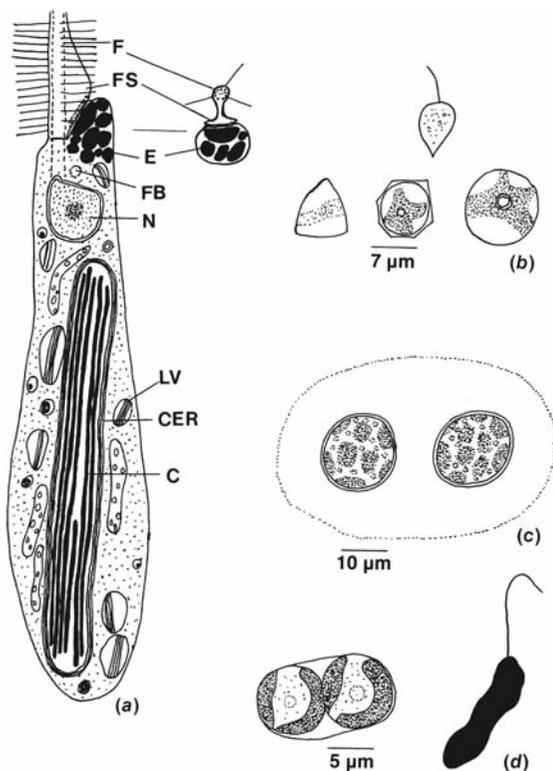
# Heterokontophyta

## EUSTIGMATOPHYCEAE

Eustigmatophytes are yellow-green unicells that occur in freshwater, brackish water, and seawater as well as in the soil. The cells are similar to those in the Xanthophyceae, but differ in having an eyespot outside the chloroplast (Fig. 12.1) (the eyespot in the Xanthophyceae is in the chloroplast)

(Hibberd and Leedale, 1970). Other characteristics of the class include a basal swelling of the tinsel flagellum adjacent to the eyespot, only chlorophyll *a*, chloroplasts without girdle lamellae and no peripheral ring of DNA, and chloroplast endoplasmic reticulum not connected to the nuclear envelope (Schnepf et al., 1996).

The eyespot (Figs. 12.1, 12.2) is a large orange-red body at the anterior of the motile cell and



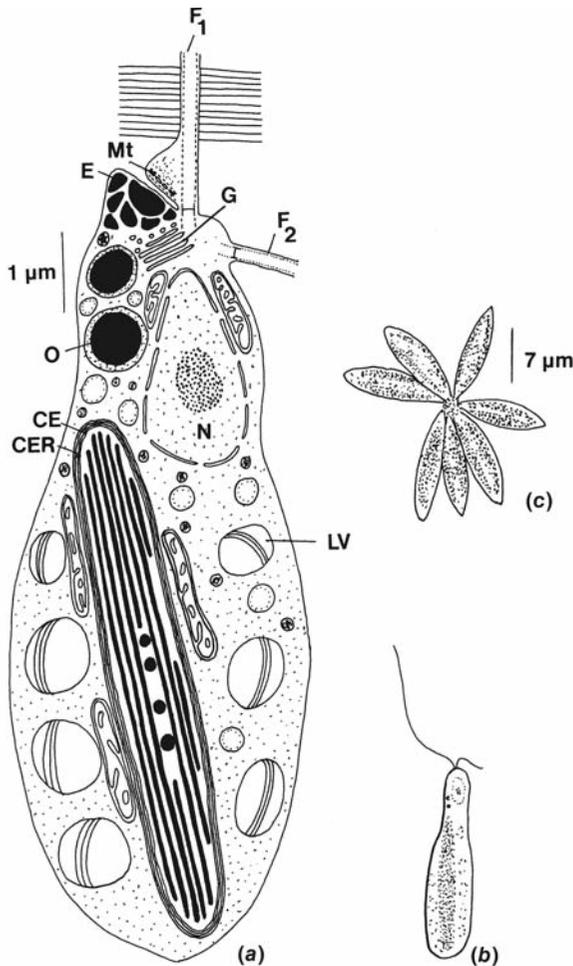
**Fig. 12.1** (a) Diagrammatic representation of the basic morphology of a zoospore of the Eustigmatophyceae. (C) Chloroplast; (CER) chloroplast endoplasmic reticulum; (E) eyespot; (F) long flagellum; (FB) basal body of short flagellum; (FS) flagellar swelling; (LV) lamellate vesicles; (N) nucleus. (b) *Polyhedriella helvetica*, zoospore and vegetative cells. (c) *Chlorobotrys regularis*. (d) *Pleurochloris magna*, vegetative cell and zoospore. ((a) after Hibberd and Leedale, 1972; (b) after Fritsch and John, 1942; (c) after Smith, 1950; (d) after Boye Petersen, 1932.)

is completely independent of the chloroplast. It consists of an irregular group of droplets with no membrane around the whole complex of droplets. The flagellar sheath is extended to form a T-shaped flagellar swelling at the base of the tinsel flagellum (Figs. 12.1, 12.2). This swelling is always closely appressed to the plasmalemma in the region of the eyespot. In turn, in the eyespot there is a large droplet closely applied to the plasmalemma in the area of the flagellar swelling.

The chloroplasts of the Eustigmatophyceae have chlorophyll *a* and  $\beta$ -carotene, with the two major xanthophylls being violaxanthin and vaucherianxanthin (Whittle and Casselton, 1969; Antia and Cheng, 1982), the only difference in pigments compared to the Xanthophyceae being the presence of violaxanthin and the absence

of antheraxanthin. Violaxanthin is the major light-harvesting pigment in the Eustigmatophyceae (Owens et al., 1987).

The Eustigmatophyceae is a monophyletic group (Andersen et al., 1998). Most of the species produce zoospores with only a single emergent flagellum (*Pleurochloris magna*, Fig. 12.1(d); *Polyedriella helvetica*, Fig. 12.1(b), Hibberd and Leedale, 1972), but there is a second basal body present, indicating that the cells had a biflagellate ancestor. The emergent flagellum is tinsel with microtubular hairs, and the flagellum is inserted subapically. Two of the algae in the class, *Ellipsoidion acuminatum* and *Pseudocharaciopsis texensis* (Fig. 12.2) (Lee and Bold, 1973), have zoospores with a long forward tinsel flagellum and a short posteriorly directed smooth



**Fig. 12.2** *Pseudocharaciopsis texensis*: (a), (b) zoospores; (c) vegetative cells. (CE) Chloroplast envelope; (CER) chloroplast endoplasmic reticulum; (E) eyespot; (F<sub>1</sub>) long flagellum; (F<sub>2</sub>) short flagellum; (G) Golgi; (LV) lamellate vacuoles; (Mt) microtubules; (N) nucleus; (O) oil body. (After Lee and Bold, 1973.)

flagellum. One of the organisms, *Chlorobotrys regularis* (Fig. 12.1(c)), does not form zoospores (Hibberd, 1974).

## REFERENCES

- Andersen, R. A., Brett, R. W., Potter, D., and Sexton, J. P. (1998). Phylogeny of the Eustigmatophyceae based upon 18S rDNA, with emphasis on *Nannochloropsis*. *Protist* 149:61–74.
- Antia, N. J., and Cheng, J. Y. (1982). The keto-carotenoids of two marine coccoid members of the Eustigmatophyceae. *Br. Phycol. J.* 17:39–50.
- Boye Petersen, J. (1932). Einige neue Erdalgen. *Arch. Protistenk.* 76:395–408.
- Fritsch, F. E., and John, R. P. (1942). An ecological and taxonomic study of the algae of British soils. II. Consideration of the species observed. I. Chlorophyceae. *Ann. Bot. N.S.* 6:371–95.
- Hibberd, D. J. (1974). Observations on the cytology and ultrastructure of *Chlorobotrys regularis* (West) Bohlin with special reference to its taxonomic position in the Eustigmatophyceae. *Br. Phycol. J.* 9:37–46.
- Hibberd, D. J., and Leedale, G. F. (1970). Eustigmatophyceae – a new algal class with unique organization of the motile cell. *Nature* 225:758–60.
- Hibberd, D. J., and Leedale, G. F. (1972). Observations on the cytology and ultrastructure of the new algal class Eustigmatophyceae. *Ann. Bot. N.S.* 36:49–71.
- Lee, K. W., and Bold, H. C. (1973). *Pseudocharaciopsis texensis* gen. et sp. nov., a new member of the Eustigmatophyceae. *Br. Phycol. J.* 8:31–7.
- Owens, T. G., Gallagher, J. C., and Alberte, R. S. (1987). Photosynthetic light-harvesting function of violaxanthin in *Nannochloropsis* spp. (Eustigmatophyceae). *J. Phycol.* 23:79–85.
- Schnepf, E., Niemann, A., and Wilhelm, C. (1996). *Pseudostaurastrum limneticum*, a Eustigmatophycean alga with astigmatic zoospores: morphogenesis, fine structure, pigment composition and taxonomy. *Arch. Protistenk.* 146:237–49.
- Smith, G. M. (1950). *The Freshwater Algae of the United States*, 2nd edn. New York and London: McGraw-Hill.
- Whittle, S. J., and Casselton, P. J. (1969). The chloroplast pigments of some green and yellow-green algae. *Br. Phycol. J.* 4:55–64.

# Heterokontophyta

## PINGUIOPHYCEAE

The Pinguiphyceae is a class of marine planktonic algae that were previously classed as “chrysophytes.” Analysis of nuclear-encoded 18S rRNA and chloroplast-encoded *rbcL* gene sequences uncovered the close relationship of the organisms in this class (Kawachi et al., 2002). The class is characterized by unusually high concentrations of polyunsaturated acids, especially 20:5 (n-3) (EPA-eicosapentaenoic acid) in the cells (Kawachi et al., 1996) (Fig. 13.1). These fatty acids are the basis for choosing the latin noun “Pingue” (meaning fat,

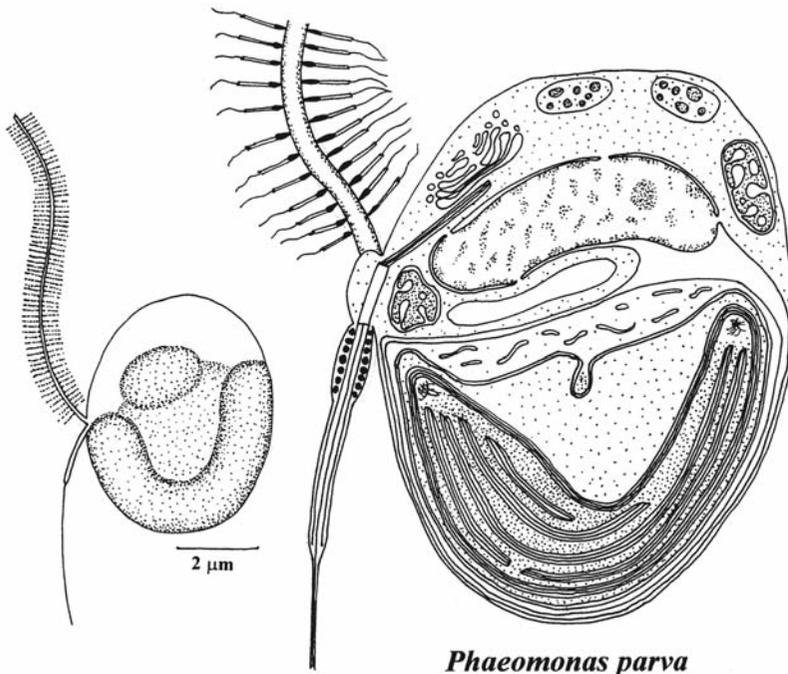


Eicosapentaenoic Acid

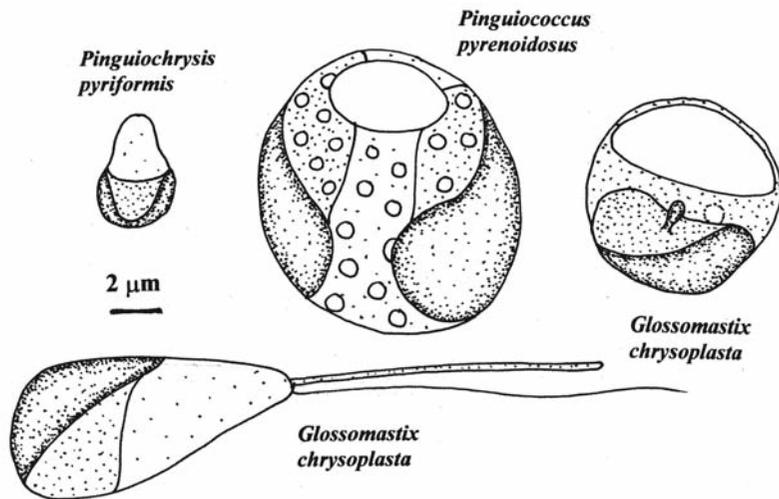
**Fig. 13.1** The structure of eicosapentaenoic acid, a polyunsaturated fatty acid found in high concentrations in algae in the Pinguiphyceae.

grease) as the root of the class name. The high percentage of unsaturated fatty acids, and the lack of a cell wall, make these algae desirable as a source of unsaturated fatty acids and of animal feed.

The cells (Figs. 13.2, 13.3) (Honda and Inouye,



**Fig. 13.2** Ultrastructural characteristics of an alga in the Pinguiphyceae.



**Fig. 13.3** Algae classified in the Pinguiphyceae.

2002) are derived from a typical heterokont ancestor with an anterior tinsel flagellum with tripartite hairs and a posterior smooth whiplash flagellum (although many of the genera have lost one or both flagella through evolution). The chloroplasts have two membranes of chloroplast endoplasmic reticulum, the outer membrane continuous with the nuclear envelope. A girdle band of thylakoids occurs under the chloroplast envelope. Pyrenoids occur in the chloroplast and the mitochondria have tubular cristae. Chlorophyll *a* and chlorophyll *c*-related pigments as well as fucoxanthin, violaxanthin, zeaxanthin, and  $\beta$ -carotene are present.

## REFERENCES

- Honda, D., and Inouye, I. (2002). Ultrastructure and taxonomy of a marine photosynthetic stramenopile *Phaeomonas parva* gen. et sp. nov. (Pinguiphyceae) with emphasis on the flagellar apparatus architecture. *Phycol. Res.* 50:75–89.
- Kawachi, M., Kato, M., Ikemoto, H., and Miiyachi, S. (1996). Fatty acid composition of a new marine picoplankton species of the Chromophyta. *J. Appl. Phycol.* 8:397–401.
- Kawachi, M., Inouye, I., Honda, D., et al. (2002). The Pinguiphyceae *classis nova*, a new class of photosynthetic stramenopiles whose members produce large amounts of omega-3 fatty acids. *Phycol. Res.* 50:31–47.

# Heterokontophyta

## DICTYOCHOPHYCEAE

These golden-brown algae are characterized by tentacles or rhizopodia on basically amoeboid vegetative cells (Moestrup, 1995; Preisig, 1995). Amoeboid cells are relatively rare among the algae, being mostly restricted to the Dictyochophyceae and the Xanthophyceae (Hibberd and Chretiennot-Dinet, 1979). The algae in the Dictyochophyceae have been previously classified in the Chrysophyceae, although molecular evidence shows them to be most closely related to the Pelagophyceae (Cavalier-Smith et al., 1995) or Eustigmatophyceae (Daugbjerg and Andersen, 1997).

## Classification

The Dictyochophyceae can be divided into three orders (Preisig, 1995):

- Order 1 Rhizochromulinales: marine and freshwater unicells with tentacles.
- Order 2 Pedinellales: unicells with a long anterior flagellum and a second flagellum reduced to a basal body, usually three to six chloroplasts (if chloroplasts are present), marine and freshwater.
- Order 3 Dictyocales: marine unicells with an external silicified skeleton.

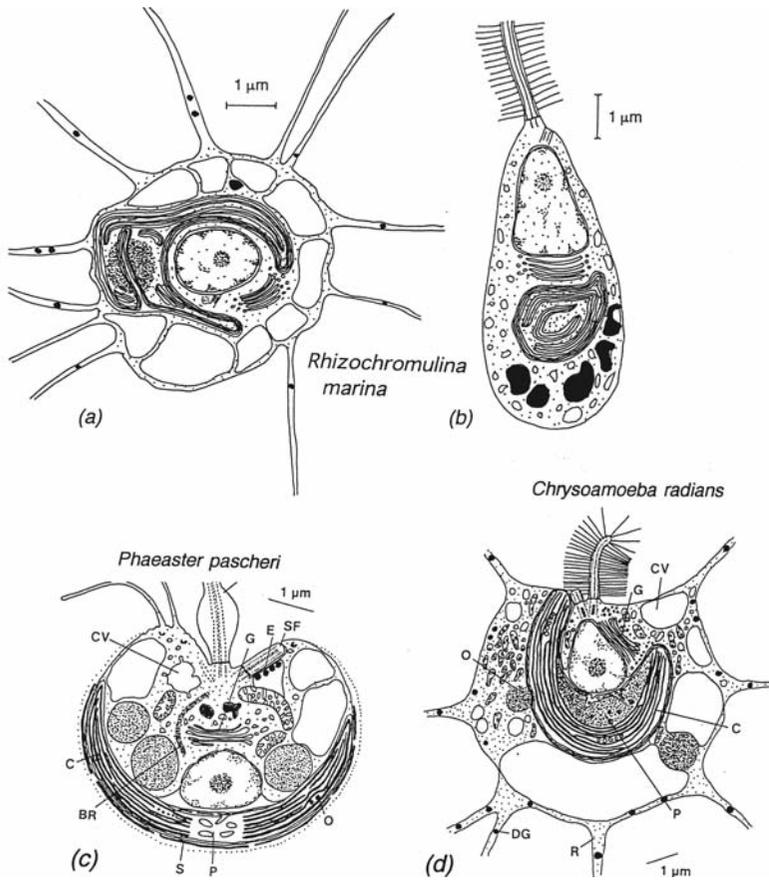
### Rhizochromulinales

This order contains the more primitive organisms in the order (O'Kelly and Wujek, 1995). *Rhizochromulina* (Fig. 14.1(a), (b)) has amoeboid

non-flagellated vegetative cells with many fine beaded-filipodia and a single golden-brown chloroplast (Hibberd and Chretiennot-Dinet, 1979). The fusiform zoospore has a single tinsel flagellum with a second basal body in the protoplasm (Fig. 14.1(b)). *Chrysoamoeba* (Fig. 14.1(d)) lives as a solitary amoeba for the greater part of its life cycle, transforming into swimming cells with a single long flagellum only for short periods. In *Phaeaster* (Fig. 14.1(c)), the anterior portion of the cell is drawn out into rhizopodia.

### Pedinellales

The pedinellids are unique in containing genera that are phototrophic (*Apedinella* (Fig. 14.2(c)), *Pseudopedinella*), mixotrophic (able to photosynthesize and take up organic compounds) (*Pedinella*) (Figs. 14.2(a), (b), 14.3, 14.4), and phagotrophic (*Pteridomonas*, *Ciliophrys*). The phagotrophic genera have vestigial chloroplasts and evolved from genera with chloroplasts (Sekiguchi et al., 2002). The organisms in this order have three interconnected microtubules (triads) that course from the nuclear envelope through tentacles (if they are present) to the plasma membrane (Fig. 14.2(a)) (Daugbjerg, 1996). A long apical flagellum is extended into a lateral wing by a paraxonemal rod (Figs. 14.2, 14.3(b)). The apical flagellum is inserted in a pit and there is a second flagellum that is reduced to a basal body. The basal bodies are at a slight angle to each other. The cells are radially symmetrical with a large central nucleus and a posterior Golgi apparatus. There are usually three to six chloroplasts present



**Fig. 14.1** (a), (b) *Rhizochromulina marina*. Vegetative cell (a) and zoospore (b). (c) *Phaeaster pascheri*. (d) *Chrysoamoeba radians*. (BR) Basal root; (C) chloroplast; (CV) contractile vacuole; (DG) dense granule; (E) eyespot; (G) Golgi body; (LF) long flagellum; (O) oil; (P) pyrenoid; (R) rhizopodium; (S) scale; (SF) short flagellum. ((a), (b) Redrawn from Hibberd and Chretiennot-Dinet, 1979; (c) redrawn from Belcher and Swale, 1971; (d) redrawn from Hibberd, 1971.)

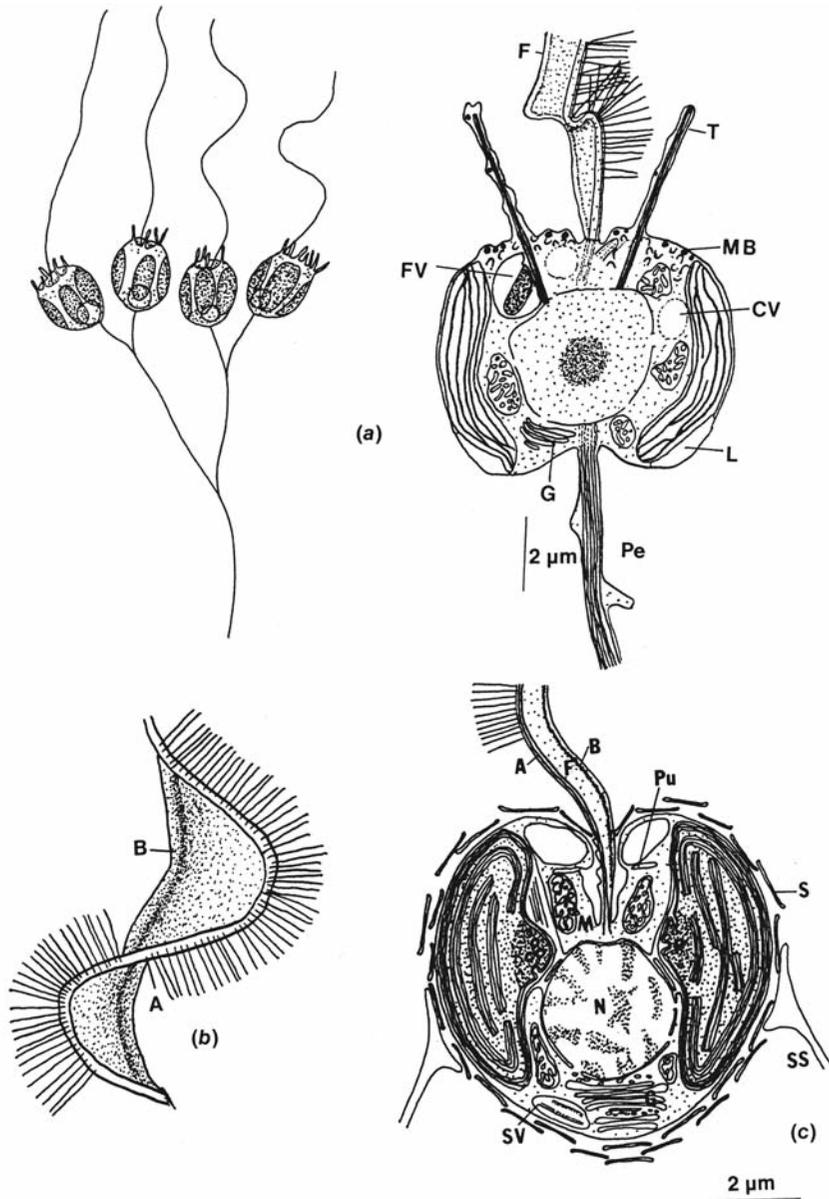
if the organism is phototrophic (whereas in the Chrysophyceae there are usually only one or two chloroplasts). Some genera, such as *Pedinella* (Fig. 14.4(d), (e)) and *Apedinella* (Fig. 14.2(c)), have scales attached to the plasma membrane by microligaments (Koutoulis et al., 1988).

A posterior trailing stalk, associated with a system of vacuoles, can be present. *Pedinella* (Figs. 14.2(a), 14.4) cells rotate while swimming, trailing the stalk behind. The stalk appears to be sticky, and swimming cells will often adhere to a substrate by means of the stalk. *Pedinella* can undergo phagotrophy and ingest other small cells. Bacteria are passed down the flagellum and adhere to the plasma membrane, just outside the row of tentacles. Secretions of the muciferous bodies in this region provide an adhesive that sticks the cells to the plasmalemma. Usually within a minute, the bacteria become enveloped in a sheet of cytoplasm that is extruded from the cell.

## Dictyocales

The Dictyocales or silicoflagellates are a group of cosmopolitan marine flagellates, presently represented by only one extant genus *Dictyocha*, although an abundance of taxa have been described from fossil siliceous skeletons (Henriksen et al., 1993).

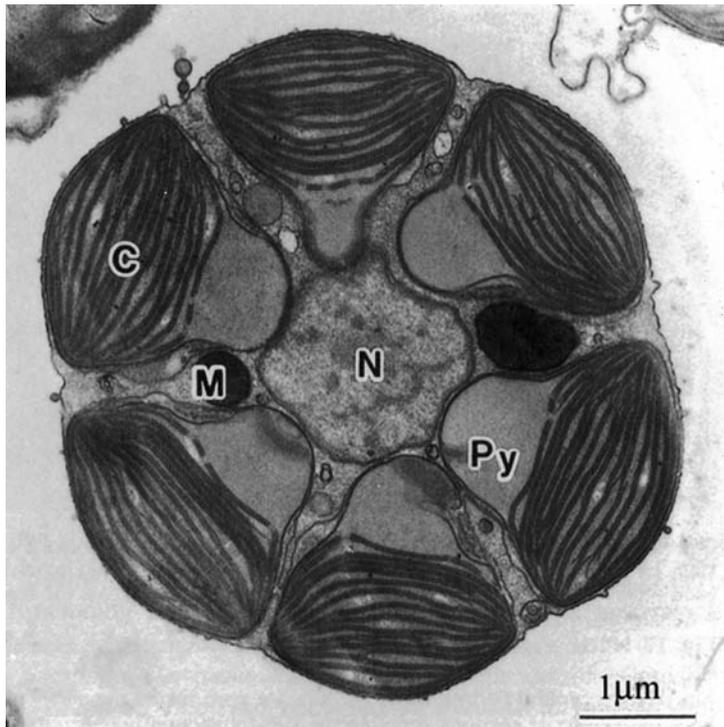
Silicoflagellate cells have one emergent flagellum and a skeleton of hollow siliceous rods outside of the protoplasm. The skeleton can be a simple ring, an ellipse, or a triangle, but it is often much more complete. In *Dictyocha fibula* (Fig. 14.5), the skeleton is composed of a series of peripheral polygons surrounding a central hexagon. The nucleus is in the center of the protoplasm with a number of cytoplasmic processes extending from the central mass. The chloroplasts are usually in the cytoplasmic processes (van Valkenburg, 1971a, b). Most species have chloroplasts that are derived from secondary endosymbioses, although



**Fig. 14.2** (a) *Pedinella hexacostata* in the light and electron microscope. (b) Structure of the winged flagellum of *P. hexacostata*. (c) *Apedinella spinifera*. (A) Axoneme of flagellum; (B) paraxonemal rod of flagellum; (CV) contractile vacuole; (F) flagellum with hairs; (FV) food vacuole; (G) Golgi body; (L) leucosin; (M) mitochondrion; (MB) muciferous body; (N) nucleus; (Pe) peduncle; (Pu) pusule; (S) scale; (SS) spined scale; (SV) scale vesicle; (T) tentacle. ((a), (b) after Swale, 1969; (c) after Thronsdén, 1971.)

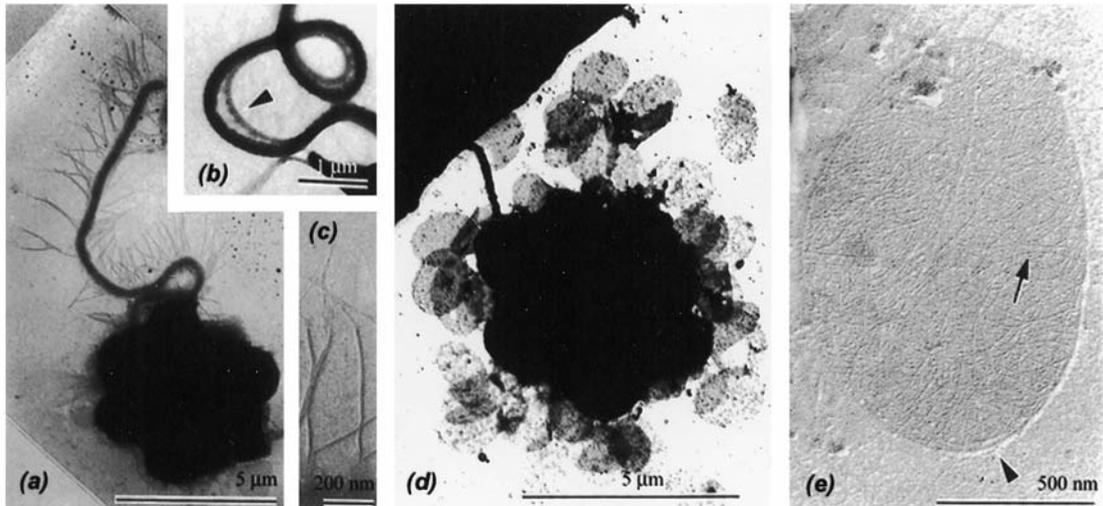
there has been a report of chloroplasts derived from haptophytes through tertiary endosymbioses (Daugbjerg and Henriksen, 2001).

In *Dictyocha speculum*, the skeleton-bearing cells multiply vegetatively by mitotic division (Fig. 14.6) (Henriksen et al., 1993). Cells connected by bridges develop and give rise to large spherical cells without skeletons that become multinucleate. Uninucleate swimmers with a single flagellum develop in the large spherical cells. The swimmers are released and grow into large multinucleate

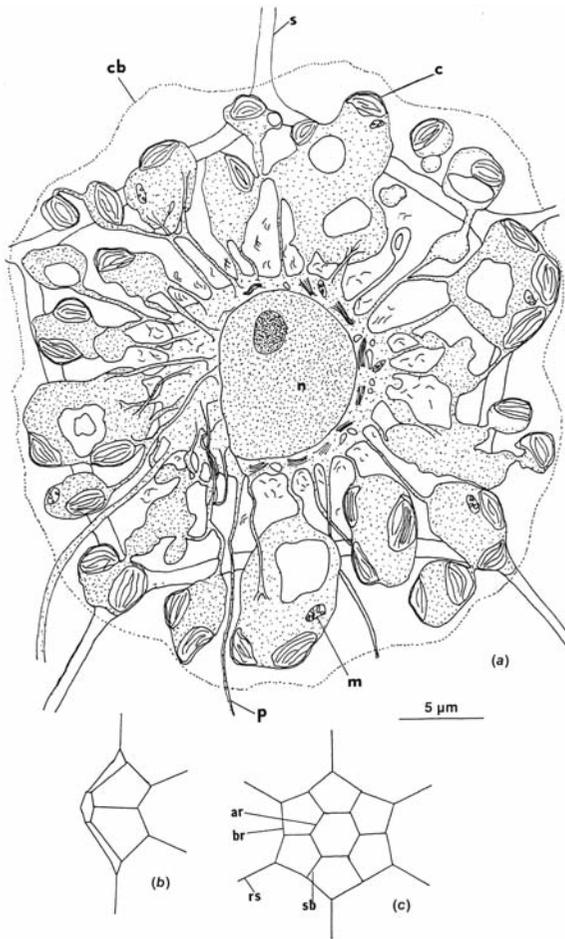


**Fig. 14.3** *Pedinella squamata*.

Transmission electron micrograph. (C) Chloroplast; (M) mitochondrion; (N) nucleus; (Py) pyrenoid. (From Sekiguchi et al., 2003.)

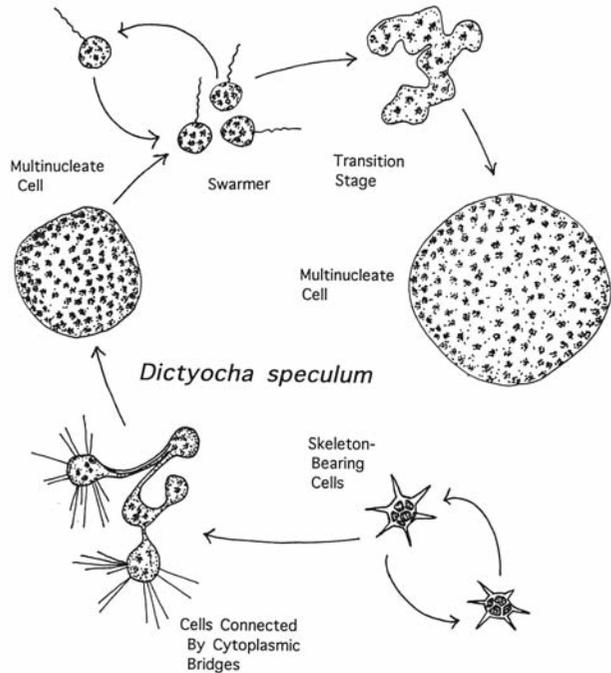


**Fig. 14.4** *Pedinella squamata*. Transmission electron micrographs. (a) Whole mount of a cell showing the flagellum and mastigonemes. (b) Higher magnification of a flagellum showing a paraxonemal rod (arrowhead). (c) Higher magnification of mastigonemes with no lateral filaments on the shafts. (d) Plate scales scattered about a whole mount of a cell. (e) A single plate scale with fibers (arrow) and the marginal rim (arrowhead). (From Sekiguchi et al., 2003.)



**Fig. 14.5** (a) A drawing of the fine structure of *Dictyocha fibula*. (b), (c) Side and front views of the skeleton of *Dictyocha*. (ar) Apical ring; (br) basal ring; (c) chloroplast; (cb) cell boundary; (m) mitochondrion; (n) nucleus; (p) pseudopodium; (rs) radial spine; (s) silica skeleton; (sb) supporting bar. (After van Valkenburg, 1971a,b.)

**Fig. 14.6** Growth stages of the silicoflagellate *Dictyocha speculum*. (Adapted from Henriksen et al., 1993.)



cells, which are probably a form of resting cell. All of the cells are of the same ploidy level and sexual reproduction is not known.

The silicoflagellates originated in the Cretaceous, with the earlier forms having a simpler skeleton than those existing today. Fossils of silicoflagellates are common in calcareous chalks along with members of the Prymnesiophyceae. Silicoflagellates constitute a prominent part of the phytoplankton in the colder seas of today.

## REFERENCES

- Belcher, J. H., and Swale, E. M. F. (1971). The microanatomy of *Phaeaster pascheri* Scherffel (Chrysophyceae). *Br. Phycol. J.* 6:157–69.
- Cavalier-Smith, T., Chao, E. E., and Allsopp, T. E. P. (1995). Ribosomal RNA evidence for chloroplast loss within Heterokonta: pedinellid relationships and a revised classification of ochristan algae. *Arch. Protistenkd.* 145:209–20.
- Daugbjerg, N. (1996). *Mesopedinella arctica* gen. et sp. nov. (Pedinelles, Dictyochophyceae). I. Fine structure of a new marine phytoflagellate from Arctic Canada. *Phycologia* 35:435–45.
- Daugbjerg, N., and Andersen, R. A. (1997). A molecular phylogeny of heterokont algae based on analyses of chloroplast-encoded *rbcL* sequence data. *J. Phycol.* 33:1031–41.
- Daugbjerg, N., and Henriksen, P. (2001). Pigment composition and *rbcL* sequence data from the silicoflagellate *Dictyocha speculum*: a heterokont alga with pigments similar to some haptophytes. *J. Phycol.* 37:1110–20.
- Henriksen, P., Knipschildt, F., Moestrup, Ø., and Thomsen, H. A. (1993). Autecology, life history and toxicology of the silicoflagellate *Dictyocha speculum* (Silicoflagellata, Dictyochophyceae). *Phycologia* 32:29–39.
- Hibberd, D. J. (1971). Observations on the cytology and ultrastructure of *Chrysoamoeba radians* Klebs (Chrysophyceae). *Br. Phycol. J.* 6:207–23.
- Hibberd, D. J., and Chretiennot-Dinet, M.-J. (1979). The ultrastructure and taxonomy of *Rhizochromulina marina* gen. et sp. nov., an amoeboid marine chrysophyte. *J. Mar. Biol. Assn., UK* 59:179–93.
- Koutoulis, A., McFadden, G. I., and Wetherbee, R. (1988). Spine-scale reorientation in *Apedinella radians* (Pedinelles, Chrysophyceae): the microarchitecture and immunocytochemistry of the associated cytoskeleton. *Protoplasma* 147:25–41.
- Moestrup, Ø. (1995). Current status of chrysophyte ‘splinter groups’: synurophytes, pedinellids, silicoflagellates. In *Chrysophyte Algae*, ed. C. D. Sandgren, J. R. Smol, and J. Kristiansen, pp. 75–91. Cambridge: Cambridge University Press.
- Preisig, H. R. (1995). A modern concept of chrysophyte classification. In *Chrysophyte Algae*, ed. C. D. Sandgren, J. R. Smol, and J. Kristiansen, pp. 46–74. Cambridge: Cambridge University Press.
- Sekiguchi, H., Moriya, M., Nakayama, T., and Inouye, I. (2002). Vestigial chloroplasts in heterotrophic stramenopiles *Pteridomonas danica* and *Ciliophrys infusionum* (Dictyochophyceae). *Protist* 153:157–67.
- Sekiguchi, H., Kawachi, M., Nakayama, T., and Inouye, I. (2003). A taxonomic re-evaluation of the Pedinellales (Dictyophyceae) based on morphological, behavioural and molecular data. *Phycologia* 42:165–82.
- Swale, E. M. F. (1969). A study of the nanoplankton flagellate *Pedinella hexacostata* Vysotshii by light and electron microscopy. *Br. Phycol. J.* 4:65–86.
- Thronsdon, J. (1971). *Apedinella* gen. nov. and the fine structure of *A. spinifera* (Thronsdon) comb. nov. *Norw. J. Bot.* 18:47–64.
- van Valkenburg, S. D. (1971a). Observations on the fine structure of *Dictyocha fibula* Ehrenberg. I. The skeleton. *J. Phycol.* 7:113–18.
- van Valkenburg (1971b). Observations on the fine structure of *Dictyocha fibula* Ehrenberg. II. The protoplast. *J. Phycol.* 7:118–32.

# Heterokontophyta

## PELAGOPHYCEAE

The Pelagophyceae are a group of basically unicellular algae that are cytologically similar to the Chrysophyceae, in which they were previously classified (Andersen et al., 1993). The cells are very small (3–5  $\mu\text{m}$ ) members of the ultraplankton and appear as small spheres with indistinct protoplasm under the light microscope. Recent studies on the sequences of small-subunit RNA nucleotides in these algae have shown them to be closely related to each other and distinct from other members of the Heterokontophyta (Saunders et al., 1997). While these algae have been shown to be distinct from other members of the Heterokontophyta based on molecular data, they do not have cytological or morphological characters which are very different from other members of the phylum.

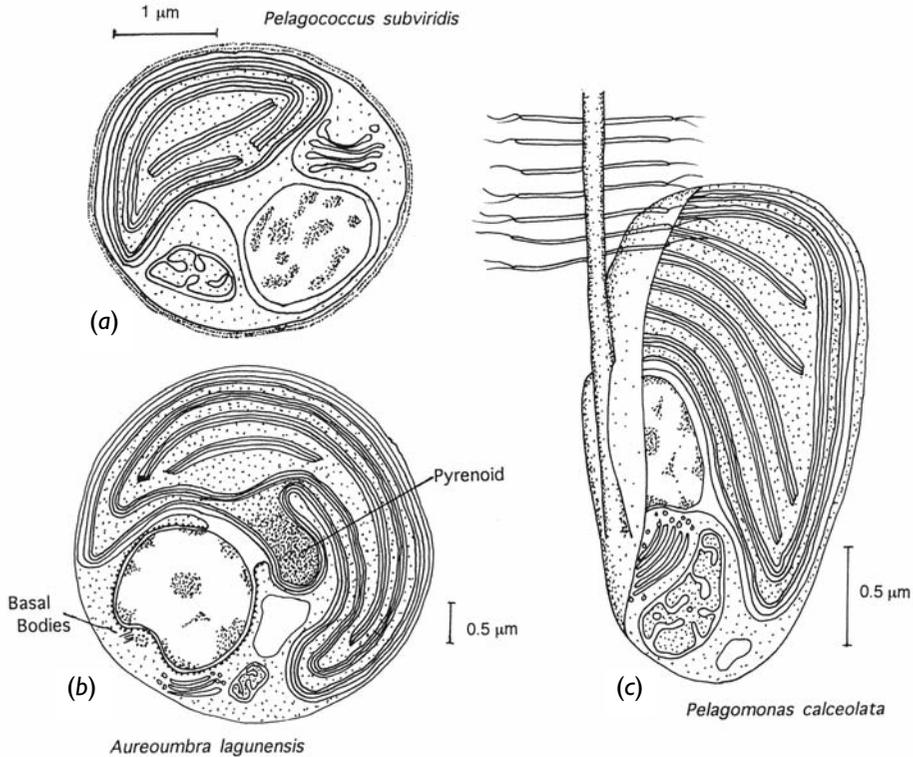
*Pelagomonas calceolata* is a very small (1.5  $\mu\text{m} \times 3 \mu\text{m}$ ) ultraplanktonic marine alga with a single tinsel flagellum and basal body, and a single chloroplast and mitochondrion (Fig. 15.1(c)) (Andersen et al., 1993). Another member of the marine ultraplankton is *Pelagococcus subviridis*, a green-gold spherical non-motile cell (2.5–3.0  $\mu\text{m}$ ) with a single chloroplast, mitochondrion, and nucleus (Fig. 15.1(a)) (Vesk and Jeffery, 1987).

Members of the class are economically important because some of the algae produce “brown tides.” *Aureoumbra lagunensis* (Fig. 15.1(b)) is the causative agent of brown tides in Texas (DeYoe

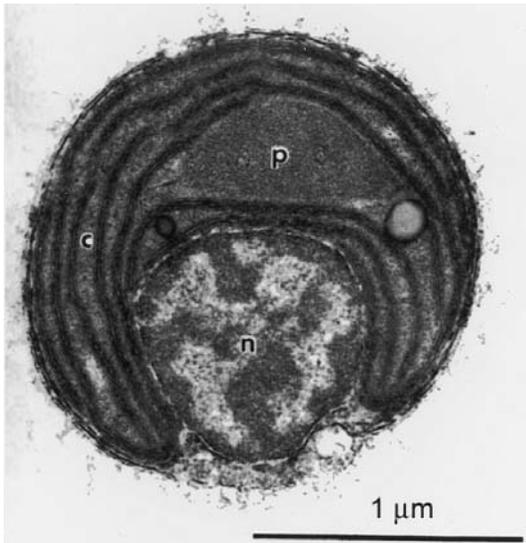
et al., 1997), while *Aureococcus anophagefferens* forms brown tides along the coasts of New Jersey, New York, and Rhode Island. The numbers of cells in brown tides can be so large that they can exclude light from the benthic eelgrass (*Zostera marina*), resulting in elimination of the eelgrass. The larvae of the bay scallop feed off eelgrass and the bay scallop industry was virtually wiped out for a number of years after a brown tide in the waters off the northeast United States (Nicholls, 1995).

*Aureoumbra lagunensis* (Fig. 15.1(b)) is able to grow at its maximum rate at salinities as high as 70 PSU (**practical salinity units**; seawater is about 35 PSU). Few algae are able to survive these hypersaline conditions. In addition, the surface of the cells are covered with a slime layer that reduces predation (Liu and Buskey, 2000). A combination of these advantages enables *A. lagunensis* to out-compete other algae.

*Aureococcus anophagefferens* (Fig. 15.2) is a psychrophilic alga that is able to grow at low temperatures and survive extended periods of darkness. This explains its ability to form algal blooms (Popels and Hutchins, 2002). Dense cell populations of 100 000 cells  $\text{ml}^{-1}$  were recorded before the ice formed in Great Bay, Long Island, New York. Two months later, after the ice had melted the cell populations were still as high as 60 000 cells  $\text{ml}^{-1}$ , concentrations that still were at bloom levels (Gobler et al., 2002). It has been shown that filter feeders grow at a reduced rate when cells of *Aureococcus* are present (Greenfield et al., 2004).



**Fig. 15.1** (a) *Pelagococcus subviridis* showing the single chloroplast, nucleus, and mitochondrion. (b) *Aureoumbra lagunensis* with the characteristic stalked pyrenoid and two basal bodies near the nucleus. (c) *Pelagomonas calceolata* showing the single flagellum fitting into a groove in the cell. ((a) adapted from Vesik and Jeffery, 1987; (b) adapted from DeYoe et al., 1997; (c) adapted from Andersen et al., 1993.)



## REFERENCES

- Andersen, R. A., Saunders, G. W., Paskind, M. P., and Sexton, J. P. (1993). Ultrastructure and 18S rRNA gene sequence for *Pelagomonas calceolata* gen. et sp. nov. and the description of a new algal class, the Pelagophyceae classis nov. *J. Phycol.* 29:701–15.
- DeYoe, H. R., Stockwell, D. A., Bidigare, R. R., Latasa, M., Johnson, P. W., Hargraves, P. E., and Suttle, C. A. (1997). Description and characterization of the algal species *Aureoumbra lagunensis* gen. et sp. nov. and referral of *Aureoumbra* and *Aureococcus* to the Pelagophyceae. *J. Phycol.* 33:1042–8.
- Gobler, C. J., Renaghan, M. J., and Buck, N. J. (2002). Impacts of nutrients and grazing mortality on the abundance of *Aureococcus anophagefferens* during a New York brown tide bloom. *Limnol. Oceanogr.* 47:129–41.
- Greenfield, D. I., Lonsdale, D. J., Cerrato, R. M., and Lopez, G. R. (2004). Effects of background

**Fig. 15.2** Transmission electron micrograph of a section of a cell of *Aureococcus anophagefferens*. (c) Chloroplast; (n) nucleus; (p) pyrenoid. (From Sieburth et al., 1988.)

- concentrations of *Aureococcus anophagefferens* (brown tide) on growth and feeding in the bivalve *Mercenaria mercenaria*. *Mar. Biol. Progr. Ser.* 274:171–81.
- Liu, H., and Buskey, E. J. (2000). The exopolymer secretions (EPS) layer surrounding *Aureoumbra lagunensis* cells affects growth, grazing, and behavior of protozoa. *Limnol. Oceanogr.* 45:1187–91.
- Nicholls, K. H. (1995). Chrysophyte blooms in the plankton and neuston of marine and freshwater systems. In *Chrysophyte Algae, Ecology, Phylogeny and Development*, ed. C. D. Sandgren, J. P. Smol, and J. Kristiansen, pp. 181–213. Cambridge: Cambridge University Press.
- Popels, L. C., and Hutchins, D. A. (2002). Factors affecting dark survival of the brown tide alga *Aureococcus anophagefferens* (Pelagophyceae). *J. Phycol.* 38:738–44.
- Saunders, G. W., Potter, D., and Andersen, R. A. (1997). Phylogenetic affinities of the Sarcinochrysidales and Chrysomeridales (Heterokonta) based on analysis of molecular and combined data. *J. Phycol.* 33:310–18.
- Sieburth, J. M., Johnson, P. W., and Hargraves, P. E. (1988). Ultrastructure and ecology of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyceae): the dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, summer 1985. *J. Phycol.* 24:416–25.
- Vesk, M., and Jeffrey, S. W. (1987). Ultrastructure and pigments of two strains of the picoplanktonic alga *Pelagococcus subviridis* (Chrysophyceae). *J. Phycol.* 23:322–36.

## Heterokontophyta

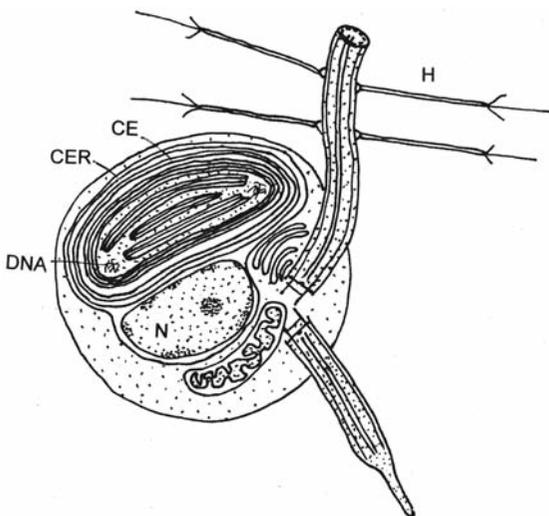
### BOLIDOPHYCEAE

This is a small order of flagellated marine picophytoplankton that is related to the diatoms (Bacillariophyceae) (Guillou et al., 1999). The cells contain chlorophylls *a*, *c*<sub>1</sub>, *c*<sub>2</sub>, *c*<sub>3</sub>,  $\beta$ -carotene, diatoxanthin, and fucoxanthin, as do the diatoms. It is probable that the diatoms originated from a motile ancestor similar to those in this class (although there is no report of silicification in the Bolidophyceae).

*Bolidomonas* is a unicell with a long tinsel flagella bearing tripartite tubular hairs and a shorter smooth flagellum (Fig. 16.1). The cell is only 1.2  $\mu\text{m}$  in diameter and has a simple internal organization. There is one plastid, mitochondrion, and Golgi apparatus. There are two membranes of chloroplast endoplasmic reticulum, with the outer membrane continuous with the outer membrane of the nuclear envelope. The DNA is contained within a ring-shaped genophore and there is no eyespot.

### REFERENCE

Guillou, L., Chretiennot-Dinet, M.-J., Medlin, L. K., Claustre, H., Loiseaux-de Goer, S., and Vaultot, D. (1999). *Bolidomonas*: a new genus with two species belonging to a new algal class, the Bolidophyceae (Heterokonta). *J. Phycol.* 35:368–81.



**Fig. 16.1** Semidiagrammatic drawing of the cytology of *Bolidomonas*. (CE) Chloroplast envelope; (CER) chloroplast endoplasmic reticulum; (H) tripartite flagellar hair; (N) nucleus. (Adapted from Guillou et al., 1999.)

# Heterokontophyta

## BACILLARIOPHYCEAE

The Bacillariophyceae or the diatoms probably evolved from a scaly member of the Chrysophyceae (similar to the organisms in the Parmales) or Bolidophyceae (Guillou et al., 1999). The diatoms are unicellular, sometimes colonial algae found in almost every aquatic habitat as free-living photosynthetic autotrophs, colorless heterotrophs, or photosynthetic symbiotes (Schmaljohann and Röttger, 1978). They may occur as plankton or periphyton, with most brownish-green films on substrates such as rocks or aquatic plants being composed of attached diatoms. The cells are surrounded by a rigid two-part box-like cell wall composed of silica, called the **frustule**. The chloroplasts contain chlorophylls *a*, *c*<sub>1</sub>, and *c*<sub>2</sub> with the major carotenoid being the golden-brown fucoxanthin, which gives the cells their characteristic color.

In discussing diatoms and silica, there is often confusion over terminology in regard to silicon. **Silicon** is the element. **Silica** is a short convenient designation for **silicon dioxide** (SiO<sub>2</sub>) in all of its crystalline, amorphous, and hydrated or hydroxylated forms. **Silicate** is any of the ionized forms of monosilicic acid [Si(OH)<sub>4</sub>] (Iler, 1979).

### Cell structure

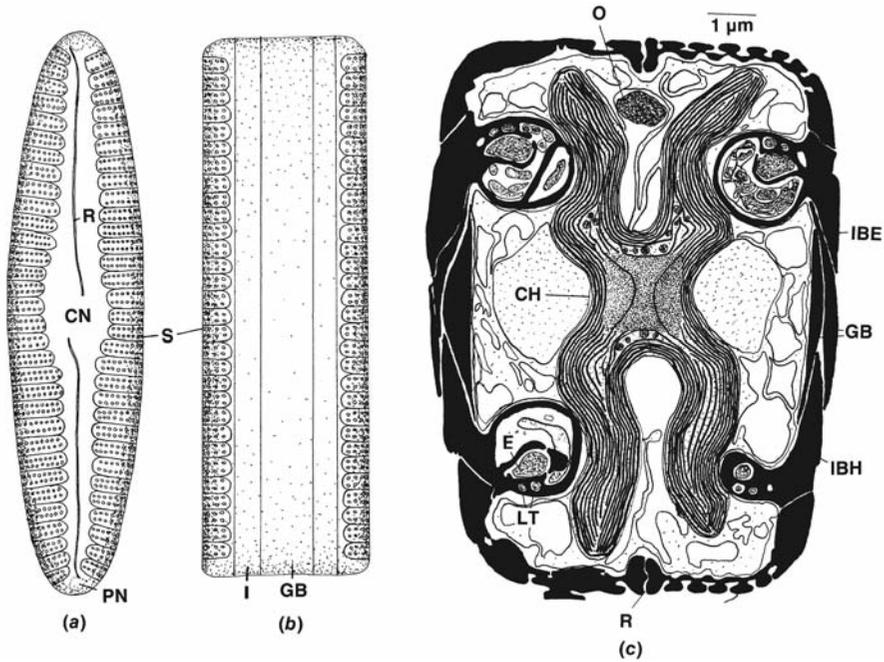
The two-part frustule surrounds protoplasm that has a more or less central nucleus suspended in a system of protoplasmic threads. The chloroplasts occupy most of the cell (Figs. 17.17, 17.46) usually

as two parietal plastids although sometimes as numerous discoid plastids. The storage product, chrysolaminarin, occurs in vesicles in the protoplasm.

### Cell wall

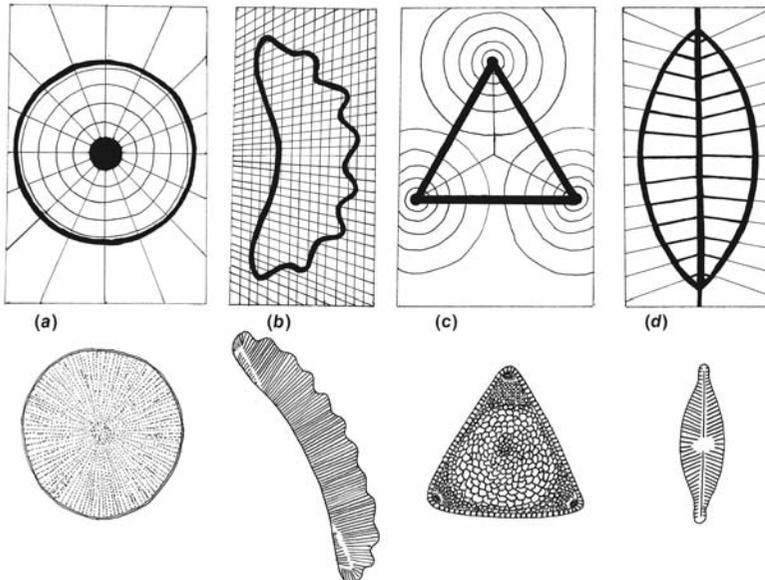
The characteristic feature of the Bacillariophyceae is their ability to secrete an external wall composed of silica, the frustule. It is constructed of two almost equal halves, the smaller fitting into the larger like a Petri dish (Figs. 17.1, 17.9, 17.10, 17.41). The outer of the two half-walls is the **epitheca** and the inner the **hypotheca**. Each theca is composed of two parts, the **valve**, a more or less flattened plate, and the **connecting band**, attached to the edge of the valve. The two connecting bands, one attached to each valve, are called the **girdle** (von Stosch, 1975). Sometimes the connecting bands themselves are called **girdle bands** (Fig. 17.41). Occasionally there are one or more additional bands between the valve and the girdle, which are called **intercalary bands**. When an appreciable part of the edge of the valve is bent inward, this portion is called the **mantle** or **valve-jacket**. The girdle bands, often furnished with minute teeth, hold the valves together by their edges. The valve margin thus butts onto the end of the girdle band and is usually connected to it by a pectinaceous film. If this film is destroyed, the valve and girdle bands separate.

The siliceous material of the frustule is laid down in certain regular patterns that leave the wall ornamented. According to Hendey (1964), the ornamentation of diatoms can be divided into four basic types: (1) **centric** and radial, where the



**Fig. 17.1** Light microscopical drawing of valve (a) and girdle (b) views of the diatom *Mastogloia*. (c) Drawing of a transverse section of *M. grevillei* in the transmission electron microscope. (Ch) Chloroplast; (CN) central nodule; (E) elongate chamber of a septum; (GB) girdle band; (I) intercalary band; (IBE) intercalary band of the epitheca; (IBH) intercalary band of the hypotheca; (LT) locule tubule; (O) oil; (R) raphe; (S) stria. ((c) adapted from Stoermer et al., 1965.)

structure is arranged according to a central point, for example, *Coscinodiscus* (Fig. 17.2(a)); (2) **trellisoid**, where the structure is arranged uniformly over the surface without reference to a point or line, for example, *Eunotia* (Fig. 17.2(b)); (3) **gonoid**, where the structure is dominated by angles, for example, *Triceratium* (Fig. 17.2(c)); (4) **pennate**,

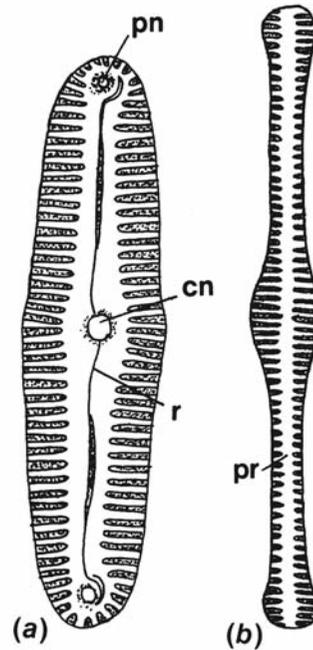


**Fig. 17.2** The basic patterns of ornamentation in the Bacillariophyceae. (a) Centric and radial (example *Coscinodiscus*). (b) Trellisoid, with structure arranged margin to margin (example *Eunotia*). (c) Gonoid, with structure supported by angles (example *Triceratium*). (d) Pennate, symmetrical about an apical line (example *Navicula*). (After Hendey, 1964.)

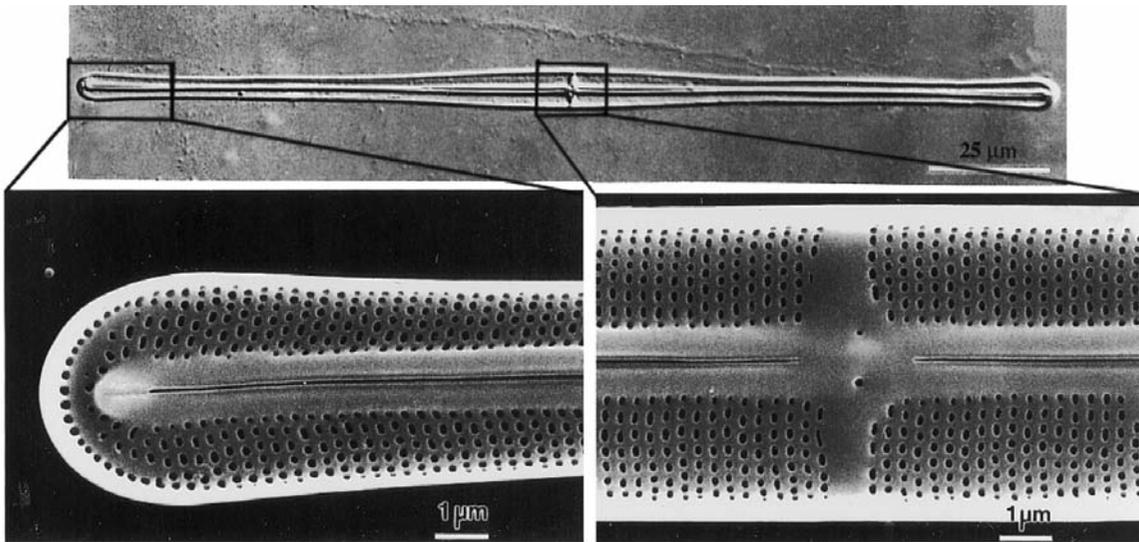
where the structure is symmetrically arranged upon either side of a central line – for example, *Navicula* (Fig. 17.2(d)).

Some pennate diatoms have a raphe system composed of the **raphe** (a longitudinal slot in the theca), divided into two parts by the **central nodule** (Figs. 17.3, 17.4, 17.5). Each half of the raphe terminates in a swelling of the wall called the **polar nodule**. The ornamentation in the pennate diatoms is **bilaterally symmetrical** around the raphe. In those pennate diatom valves that do not have a raphe system, there is instead an unornamented area running down the center of the valve, which is called the **pseudoraphe** (Fig. 17.3). The raphe is not a simple cleft in the wall but is instead an S-shaped slit that is wider at the outer and inner tissue, and thinner in the middle partition region (Fig. 17.5).

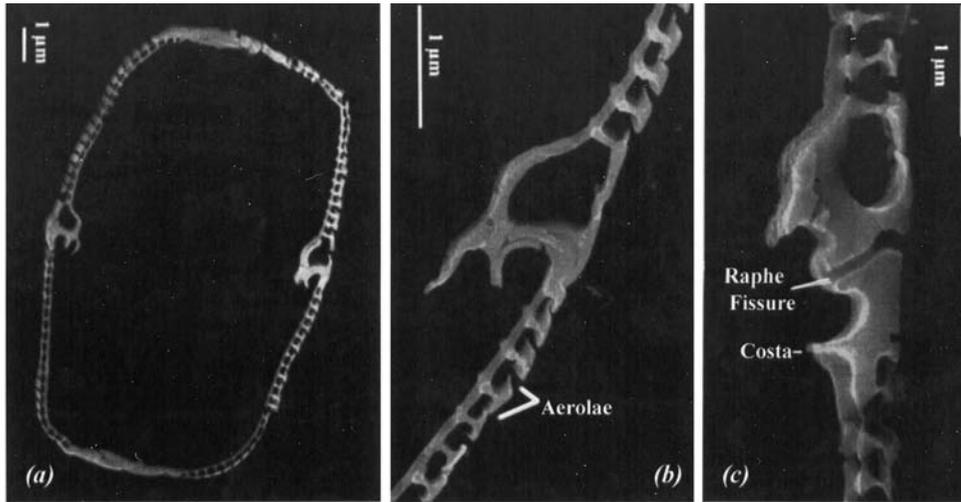
Besides the raphe, there are basically two types of wall perforations within the Bacillariophyceae: the simple **pore** or hole, and the more complex **loculus** or **areola** (Figs. 17.6, 17.12) (Hendey, 1964; Ross and Sims, 1972). The pore consists of a simple hole within a usually homogeneous silicified wall



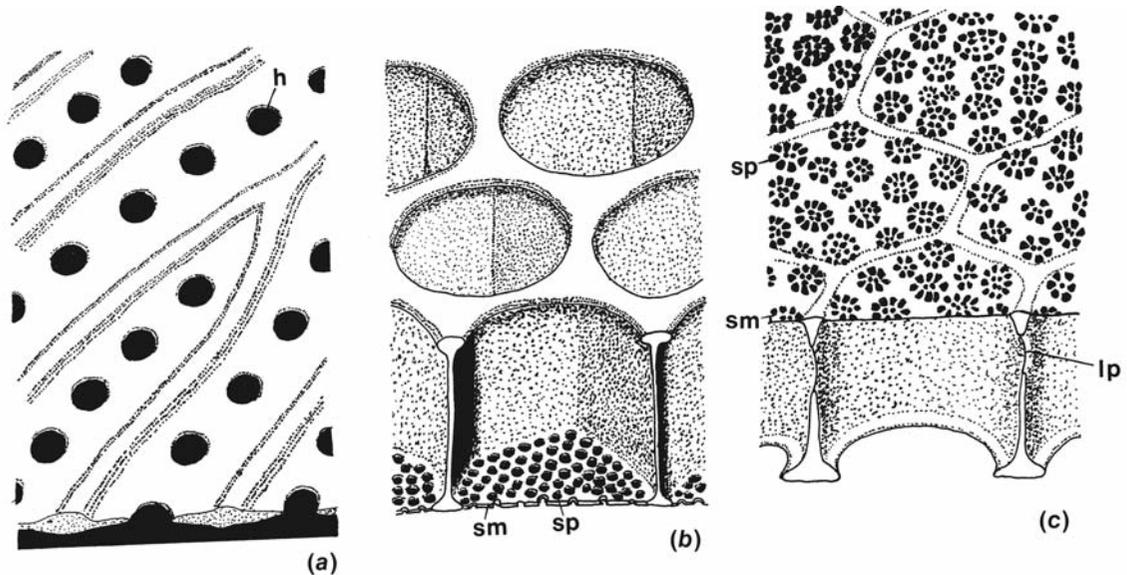
**Fig. 17.3** (a) A cell with a raphe system (*Pinnularia viridis*). (cn) Central nodule; (pn) polar nodule; (r) raphe. (b) A cell with a pseudoraphe (pr) (*Tabellaria fenestrata*).



**Fig. 17.4** *Climaconeis colemaniae*. Light and scanning electron micrographs of the frustule. The valve contains linear striae, each with 6–8 poroid areolae. The valve contains a raphe opening. Two pores occur in the area of the central nodule. (From Prasad et al., 2000.)



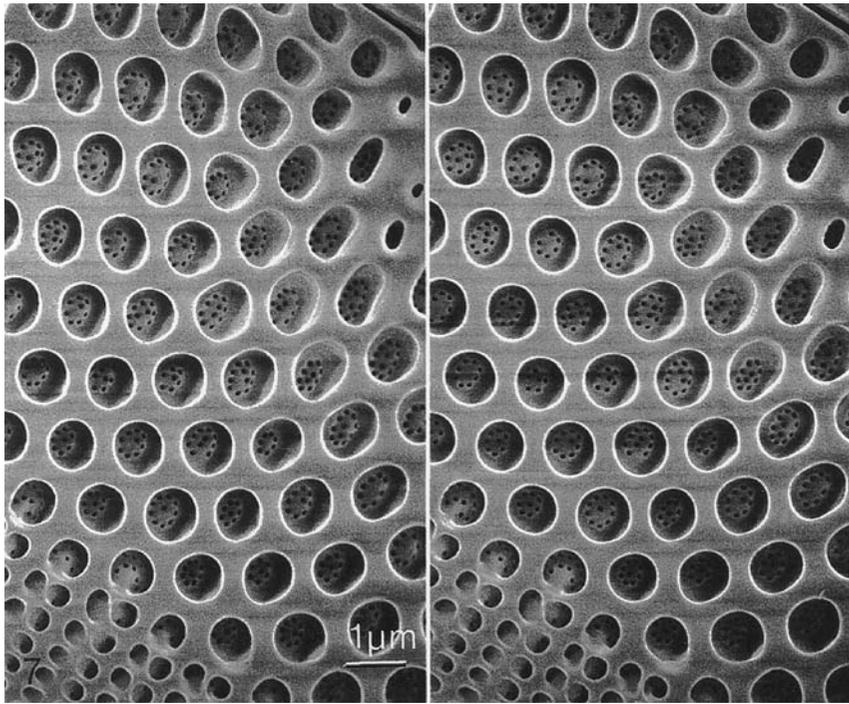
**Fig. 17.5** Scanning electron micrographs of sections of the frustule of *Haslea nipkowii*. (From Poulin et al., 2004.)



**Fig. 17.6** The types of openings in frustule walls. (a) Hole or pore (*Chaetoceros didymos* var. *anglica*). (b) Loculus opening outward (*Coccinodiscus linatus*). (c) Loculus opening inward (*Thalassiosira wailesii*). (h) Hole; (lp) lateral pore or pass pore; (sm) sieve membrane; (sp) sieve pore. (After Hendey, 1971.)

that is frequently strengthened by **ribs** and **costae** (Figs. 17.5, 17.27). If the pore is occluded by a plate, then it is called a **poroid**. The loculus consists of a usually hexagonal chamber in the wall that is separated from other loculi by vertical spacers, which

often have pores in them to allow for communication between loculi (Fig. 17.6(b), (c)). At one end of the loculus is a **sieve membrane (pore membrane, velum, cribrum)** (Figs. 17.6, 17.7). The sieve membrane can be on the outside (an inward-opening loculus) or on the inside (an outward-opening loculus) (Fig. 17.6(b), (c)). The structure of the valve wall with loculi thus resembles a honeycomb. Pores or loculi (**punctae**) in a single row are referred to as **stria** (plural **striae**) (Figs. 17.1, 17.4).



**Fig. 17.7** Stereopair illustrating details of the external areolar pattern and structure in *Mastogloia angulata*. If a stereo viewer is not available, bring the photograph about 6 inches (15 cm) from your eyes and cross your eyes to view in three dimensions the loculi, each with a sieve membrane at the bottom of the loculi. (From Navarro, 1993.)

The girdle bands in some diatoms have the same sculpturing as the valve, but in many others sculpturing on the girdle and valve is different. In some cases, the girdle bands have no sculpturing.

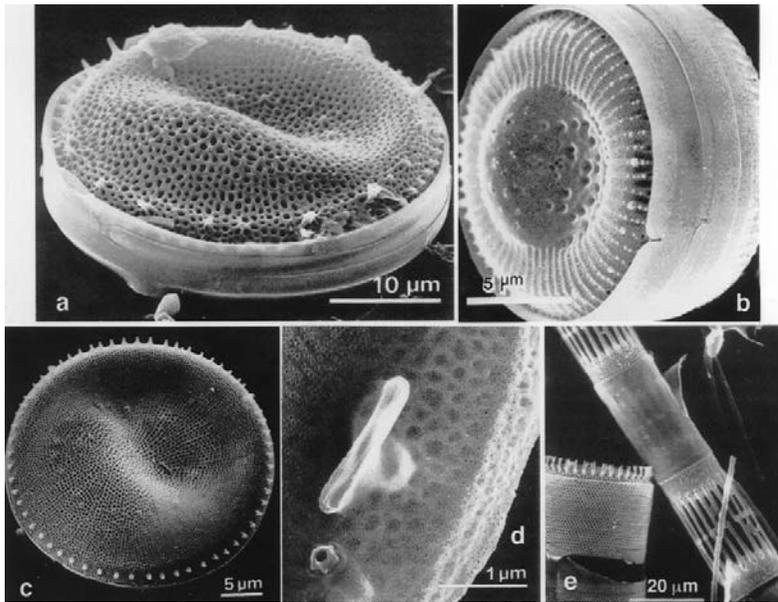
Special pores (mucilage or slime pores) (Fig. 17.18) through which mucilage is secreted are known in many diatoms. In the pennate diatoms, these pores usually occur singly near one or both poles of the valve and generally occupy thickenings in the walls.

The valve surface can have extensions, called **processes**, whose main function appears to be to maintain contact between contiguous cells and to assist colony formation. These processes are given different names: **Cornutate** processes are horn-like; **strutted** processes are ones that have been reduced to a boss at the apex of a valve (Figs. 17.8, 17.38(c), (d)); **spinulae** are very small processes; **awns** or **setae** are hollow and elongated (Figs. 17.32, 17.44).

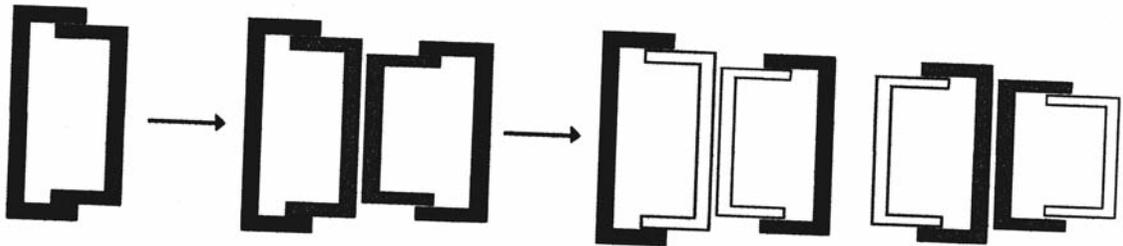
The frustule is composed of **quartzite** or hydrated amorphous silica that may also have small amounts of aluminum, magnesium, iron, and titanium mixed with it (Mehta et al., 1961; Lewin, 1962). Diatom frustules from marine plankton contain 96.5%  $\text{SiO}_2$  and 1.5%  $\text{Al}_2\text{O}_3$  or  $\text{Fe}_2\text{O}_3$  (Rogall, 1939). The inorganic component of the frustule is enveloped by an organic component or “skin” (Reimann et al., 1965), the latter composed of amino acids and sugars (Coombs and Volcani, 1968; Hecky et al., 1973) with the amino acid hydroxyproline and collagen present (Nakajima and Volcani, 1969).

### Cell division and the formation of the new wall

The normal asexual method of reproduction is by division of one cell into two, the valves of the parent cell becoming the epithecas of the daughter cells with each daughter cell producing a new hypotheca (Figs. 17.9, 17.10). As a result of cell division, one of the daughter cells is of the same size as the parent cell, and the other is smaller. As the size of the cell decreases, so does the relative width to height and the morphology of the cell; in other words, the smaller cells are



**Fig. 17.8** Scanning electron micrographs of centric diatoms. (a) *Thalassiosira lacustris*. Undulated hypovalve with strutted processes and part of epitheca showing distal pattern of band openings. (b) *Cyclotella striata*. A frustule showing the epitheca, intercalary, and girdle bands. (c), (d) *Thalassiosira gessneri*. Valve showing undulation, aerolation, and subcentral and marginal rings of strutted processes (c). Higher magnification of labiate and strutted processes (d). (e) *Skeletonema costatum* showing the marginal ring of strutted processes used in chain formation. ((a), (c), (d) from Hasle and Lange, 1989; (b) from Prasad et al., 1990; (e) from Medlin et al., 1993.)

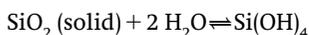


**Fig. 17.9** Diagrammatic representation of a diatom cell in girdle view showing the reduction in size through two divisions. (After Hendey, 1964.)

not geometrically proportional to the larger ones from which they arise.

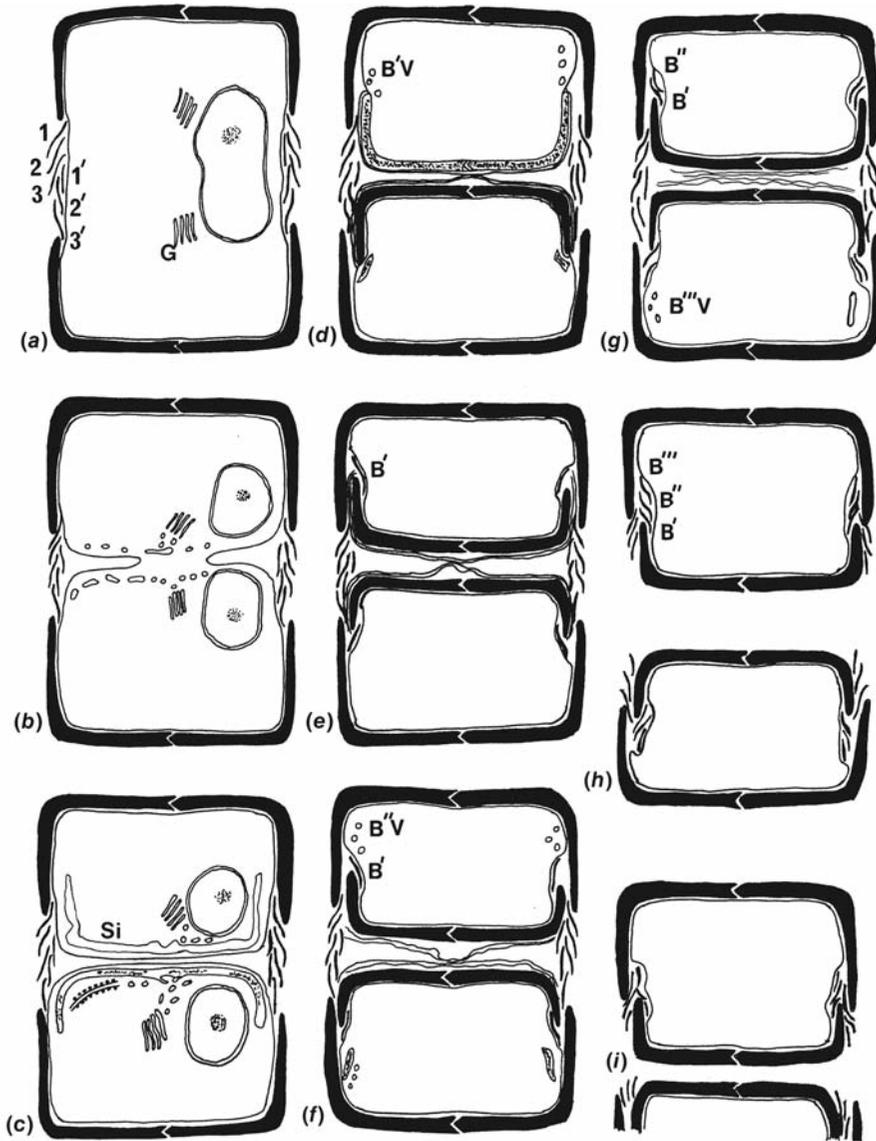
Uptake of silica is confined to a period of the cell cycle following cytokinesis and prior to the separation of the two daughter cells (Sullivan, 1977). Cellular energy for silicification and transport comes from aerobic respiration without any direct involvement of photosynthetic energy (Martin-Jezequel et al., 2000).

Diatoms have an absolute requirement for silicon if cell division is to take place. In water, solid silica dissociates to produce undissociated silicic acid  $\text{Si}(\text{OH})_4$ :



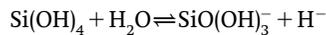
By increasing the concentration in solution with a pH less than 9, or decreasing the pH of a saturated solution, silicic acid will autopolymerize to form amorphous silica. Amorphous silica is the form of silicon in diatom cell walls. Although silicon is the second most abundant element in the Earth's crust, its availability is limited by its solubility in water. The growth of marine diatoms can so deplete surface waters of silicon that their further growth is prevented (Hildebrand, 2004).

The  $\text{Si}(\text{OH})_4$  in marine waters is about 6 ppm. In the global ocean, about 97% of the dissolved Si is present as  $\text{Si}(\text{OH})_4$ , with the pH of seawater buffered at about 8.0 by the  $\text{CO}_2$ -carbonate system (Del Amo and Brzezinski, 1999). However, in freshwater lakes where pH values reach up to 10,  $\text{Si}(\text{OH})_4$  is only about 23% of the total dissolved silicon, most of the rest being present as ionized



**Fig. 17.10** Diagrammatic representation of the stages of frustule formation, including girdle band formation, in *Gomphonema parvulum*. (a) The nucleus divides, and the cell elongates. (b) There is invagination of the plasma membrane. (c) Formation of the silicalemma occurs by the fusion of Golgi-derived vesicles. (d) Silica is deposited within the silicalemma to form the valves; vesicles accumulate in the area of the first girdle band. (e) The first girdle band is formed within a vesicle and secreted to the outside. The internal membrane of the silicalemma becomes the new plasmalemma, and the old plasmalemma and the outer membrane of the silicalemma are lost. (f)–(i) The second and third girdle bands are formed. (After Dawson, 1973.)

silicic acid  $\text{SiO}(\text{OH})_3^-$  according to the following formula:



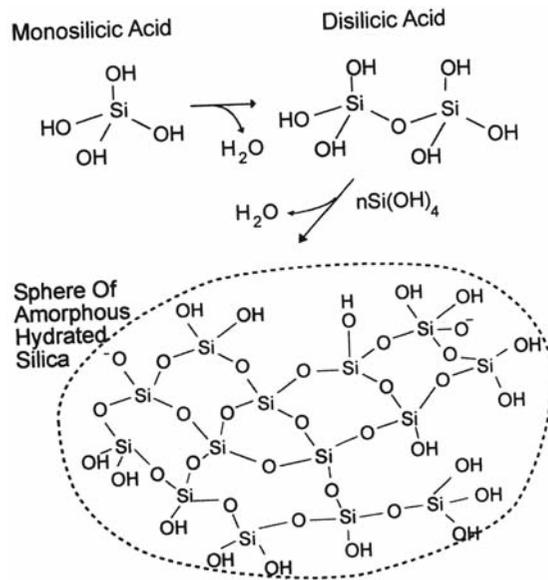
Silica is taken up into the diatom cell as  $\text{Si}(\text{OH})_4$  by a protein as an active silicon transporter requiring metabolic energy (Hildebrand et al., 2004; Wetherbee et al., 2004). Silicic acid transport is coupled to sodium in marine diatoms and sodium and perhaps potassium in freshwater diatoms. The transport in marine species has the characteristics of a sodium/silicic acid symporter

with a  $\text{Si(OH)}_4:\text{Na}^+$  ratio of 1:1. At least five types of silicic acid transporter (*SIT*) genes have been isolated. Germanium (germanic acid) and silicic acid are competitive inhibitors of each other but germanium can not be incorporated into the diatom cell wall.

Prior to cell division, the cell elongates, pushing the epitheca away from the hypotheca, and the nucleus divides. After the protoplasm has divided (Fig. 17.10) into two by the invagination of the plasmalemma, the Golgi bodies produce translucent vesicles which collect beneath the plasmalemma. These vesicles fuse to form the silicalemma or membrane of the silica deposition vesicle (Li et al., 1989). The vesicle gradually expands and assumes the shape of a new valve (Schmid and Volcani, 1983). Two silica deposition vesicles are formed per cell with silica deposited in each to form two new hypovalves (Brzezinski and Conley, 1994). The silicon is probably packaged by Golgi into vesicles that are transported to the silica deposition vesicle by microfilaments in the cytoplasm. At the silica deposition vesicle, the small, silica-laden vesicles fuse with the silicalemma, adding membrane material to the silicalemma, and releasing silica to the interior of the silica deposition vesicle (Fig. 17.10). The silica deposition vesicle determines the ultimate form of the silicified frustule (Fig. 17.15). Silica is deposited as amorphous silica in the form of spheres 30–50 nm in diameter (Figs. 17.11, 17.12) (Crawford et al., 2001).

The silica deposition vesicles are acidic, the low pH facilitating fast nucleation and aggregation of silica particles (Vrieling et al., 1999). The acidic environment also protects the newly formed valves from dissolution before coverage by an organic casing prior to secretion.

The silica deposition vesicles contain peptides called **silaffins** (Fig. 17.13) that cause the precipitation of silicic acid into silica nanospheres (Figs. 17.12, 17.14) (Kroger et al., 1999, 2000; Kroger and Sumper, 2004). Different diatom species have different silaffins that, in turn, have different polyamine chains (Fig. 17.13) attached to the silaffins. Different silaffins produce different sized silica nanospheres and it may be that the specific silaffin controls the frustule ornamentation, a characteristic of the individual diatom species.



**Fig. 17.11** The formation of a sphere of hydrated amorphous silica from molecules of monosilicic acid.

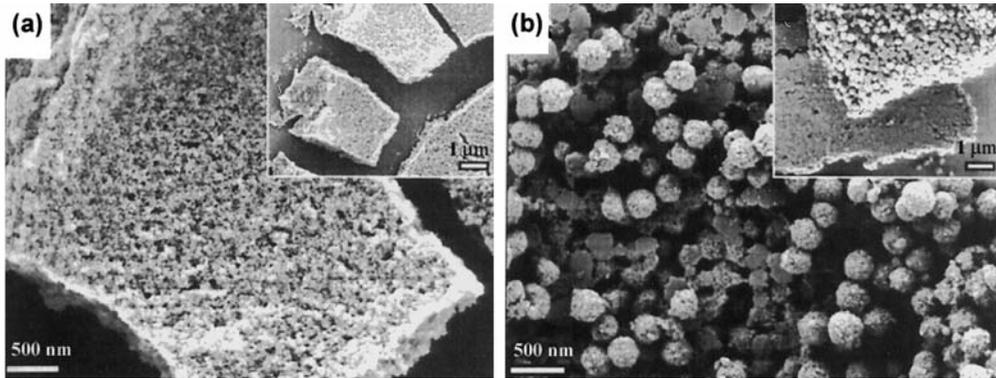
The discovery of silaffins has generated considerable commercial interest since silica-based materials such as resins, molecular sieves, and catalysts are widely used in industry. The production of the manufactured silica-based materials currently requires extremes of temperature, pressure, and pH. In contrast, biosilicification with silaffins proceeds at ambient temperatures and pressures.

A class of glycoproteins, called **frustulins**, is also associated with the silica deposition vesicle. The frustulins are associated with deposition of the organic casing around the silicified frustule (Perry and Keeling-Tucker, 2000).

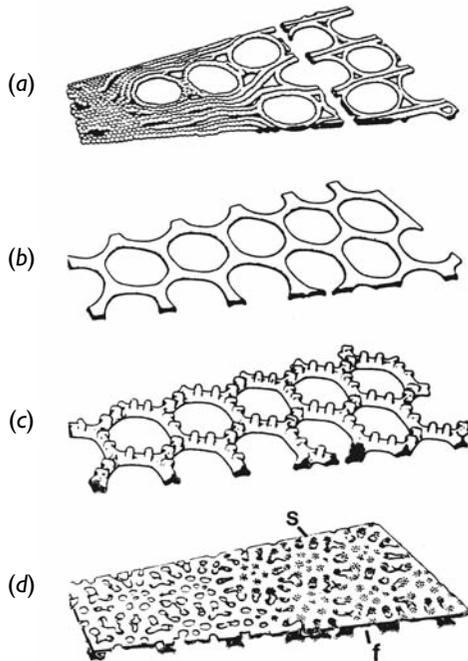
When the deposition of silica is complete, the inner membrane of the silicalemma becomes the plasmalemma of the daughter cell, and original plasmalemma and the external membrane of the silicalemma are lost (Fig. 17.10). After the epitheca and the hypotheca are formed, Golgi vesicles now collect and fuse to form the silicalemma of the girdle band. The girdle band is deposited outside the cell in the area between the hypovalve and epivalve when silica deposition is complete. If there are further girdle bands, they are formed in the same manner (Dawson, 1973).

Although amorphous silica is slowly soluble at the pH of natural waters, the silica frustule of





**Fig. 17.14** Scanning electron micrographs of silica spheres precipitated out of solution using two different silaffins and polyamines. The silica spheres in (a) are much smaller than the silica spheres in (b). The size of the silica spheres is controlled by the type of silaffin and/or polyamine in the silica deposition vesicle of the diatom. (From Kroger et al., 2000.)



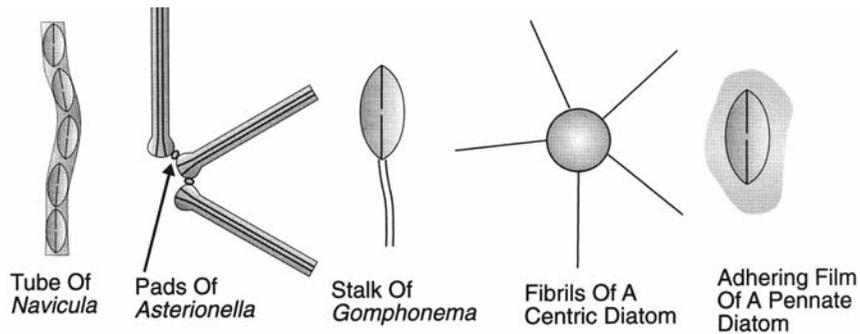
**Fig. 17.15** Drawings of the formation of the frustule of the centric diatom *Coscinodiscus wailesii*. Initially silica is deposited in the part of the frustule that will be next to the plasma membrane in the mature frustule (a) and (b). Subsequently the exterior portion of the frustule is deposited in (c) and (d). The mature frustule has a sieve membrane (s) on the exterior and a foramen (f) on the interior of the loculus. (From Schmid and Volcani, 1983.)

reducing the recreational suitability of the beaches. Extracellular mucilage production by diatoms is stimulated when phosphorus is limited, leading to marine snow blooms. Bacteria growing in the aggregates produce gas bubbles that give the marine snow positive buoyancy. The organisms in the aggregates benefit from reduced predation by grazers.

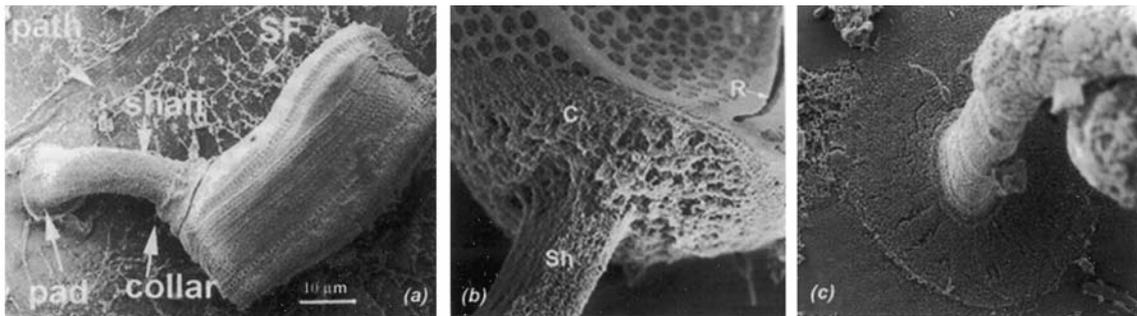
Diatoms are ubiquitous fouling microorganisms, attaching to submerged structures by secreting insoluble mucilages. *Achnanthes longipes* is a common marine fouling diatom that is highly resistant to toxic antifouling coatings (Johnson et al., 1995). It produces a stalk that elevates it above the toxic coatings on ship bottoms (Fig. 17.17). Fouling of ship bottoms increases the frictional drag, leading to excess fuel consumption. Cleaning ship hulls costs millions of dollars each year, leading to an additional loss of revenue (Chiovitti et al., 2003).

## Motility

Some diatoms are able to glide over the surface of a substrate, leaving a mucilaginous trail in their wake. **Gliding** is restricted to those pennate diatoms with a raphe (Figs. 17.3, 17.4, 17.5) and those centric diatoms with labiate processes (Figs. 17.19, 17.20, 17.32). The gliding movement is characterized by large fluctuations in the velocity of movement, with great changes in speed occurring within tenths of seconds (Edgar, 1979). In pennate diatoms, the path of the diatom seems to be essentially dependent on the shape of the raphe.



**Fig. 17.16** Forms of extracellular mucilage in diatoms.  
(Modified from Hoagland et al., 1993.)

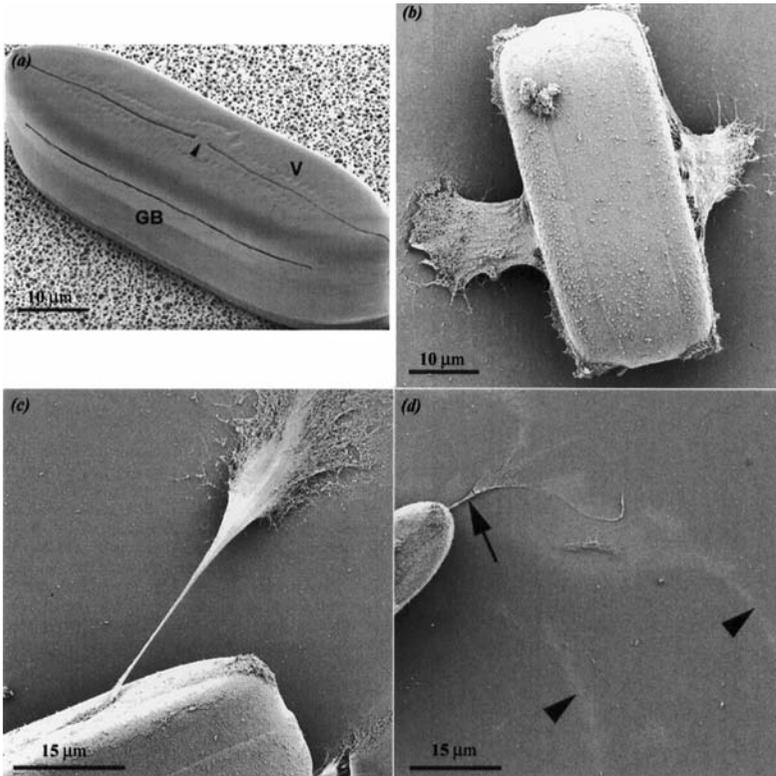


**Fig. 17.17** Scanning electron micrographs of *Achnanthes longipes*. (a) Whole cell showing the pad, shaft, and collar of the mucilaginous stalk. Also shown is a path of mucilage left by the gliding diatom and surface film (SF) of mucilage left on the substratum. (b) The attachment of the collar (C) of the stalk (Sh) on the cell. (R) Raphe. (c) The attachment of the pad to the substratum. (From Wang et al., 2000.)

Nultsch (1956) distinguished at least three types: (1) the *Navicula* type, with a straight movement; (2) the *Amphora* type, in which the path is usually curved; and (3) the *Nitzschia* type, which always exhibits curved pathways with two different radii. The observed rates of gliding in diatoms vary from 2 to 14  $\mu\text{m s}^{-1}$  at room temperature (Cohn and Weitzell, 1996). *Nitzschia palea* is able to penetrate into 2% agar and to move within the medium. The less solid the substrate is, the slower the movement of the diatom; the rate of movement of *Nitzschia putrida* was found to be 2.7  $\mu\text{m s}^{-1}$  on glass but only 0.8  $\mu\text{m s}^{-1}$  on agar (Wagner, 1934). Many of the diatoms exhibit backward and forward movements in which the direction may alternate at intervals of a minute. According to

von Denffer (1949), the motility of *N. palea* is dependent on light. In liquid cultures the cells tend to agglutinate into spherical clumps, loosely held together by mucilage. When they are transferred to a glass slide, the cells move away from one another. These movements do not take place in darkness.

Diatoms can glide only when the valve containing a raphe is in contact with the surface. If the diatom cell settles with the girdle contacting the substrate, the diatom secretes a mucilaginous tether from the portion of the raphe near the central nodule (Fig. 17.18). The tether attaches to the substratum and the cell pulls itself onto a valve containing a raphe using the tether. Sometimes tethers are produced from raphes on opposite sides of the cell. This results in the cell being tethered on both sides of the cell. Withdrawal of the tethers causes the cell to rock back and forth until one of the tethers breaks and the cell rights itself on the substratum. The mucilage that comprises the tether is different from the mucilage involved in gliding (Higgins et al., 2003).



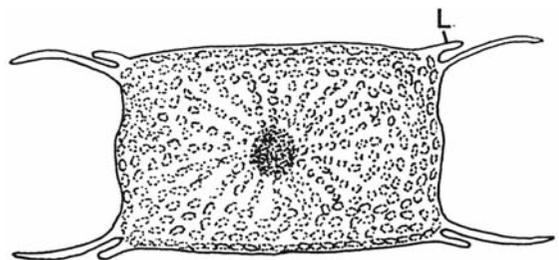
**Fig. 17.18** Scanning electron micrographs of *Pinnularia viridis*. (a) Cell showing raphe (longitudinal slit) in the middle of the valve (V). The girdle bands (GB) are located around the periphery of the cell. Arrowhead points to the central nodule. (b) A cell that has settled on the girdle bands. Tether mucilage is secreted from the raphes of each valve. The tether mucilage is attached to the substrate. (c) Tether mucilage extending from the raphe in the central nodule area to the substrate. (d) Tether mucilage connected to fine curved threads (arrowheads). (From Higgins et al., 2003.)

Those pennate diatoms that glide have bundles of actin microfilaments running parallel to the raphe (Pickett-Heaps et al., 1979a,b). The microfilament bundles may serve to orient crystalloid bodies containing mucilaginous material in the cytoplasm immediately below the raphe. On an appropriate stimulus, the mucilaginous material is released into the raphe system from the area of the central or terminal pore (Fig. 17.4) (Drum and Hopkins, 1966). The mucilaginous material then streams in the raphe in one direction until it strikes an object to which it adheres. If the object is fixed, then the streaming in the raphe forces the diatom to move in the opposite direction. Nearly all motile diatoms must adhere to the substratum in the area of their raphe in order for movement to occur.

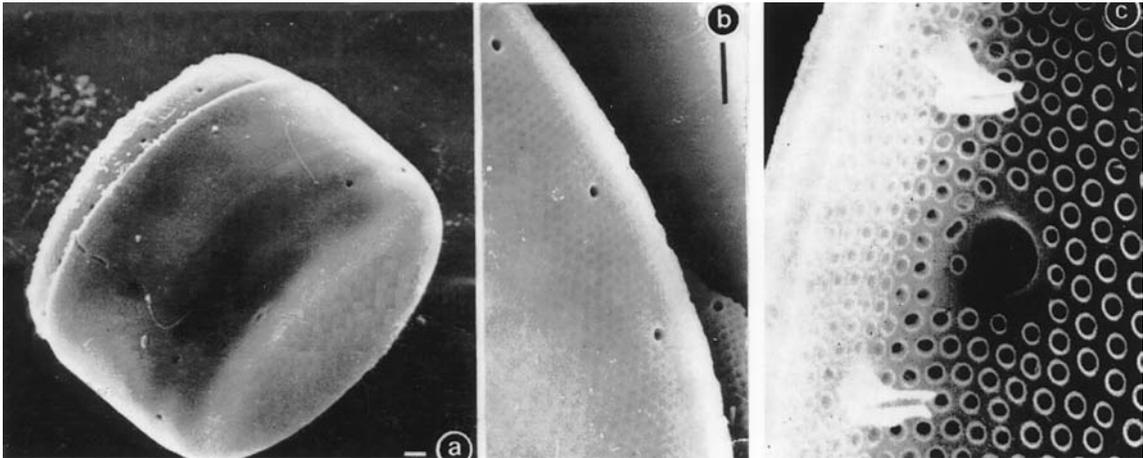
Some of the benthic centric diatoms with labiate processes (Figs. 17.19, 17.20, 17.32) are capable of gliding when attached to a substrate. The labiate processes have a pore in the center, and the mucilage is secreted through the pore. In *Actinocyclus subtilis* (Fig. 17.20), secretion of the

mucilage through the labiate process causes the diatom to move forward while rotating at the same time (Medlin et al., 1986).

Diatoms that are attached to a substrate and are motile on the substrate have the advantages of (1) being held in position in moving water; (2) avoiding burial by moving up and over sediments; (3) moving to colonize vacant areas; and (4) moving to areas with more light and/or nutrients (Medlin et al., 1986).



**Fig. 17.19** Drawing of a side view of *Odontella sinensis* showing the location of the labiate processes (L). (After Pickett-Heaps et al., 1986.)



**Fig. 17.20** Scanning electron micrographs of *Actinocyclus subtilis*. (a) Whole cell. (b) Detail of the labiate processes, showing how they open to the outside by a simple hole. (c) Internal view of an acid-cleaned valve showing structure of two labiate processes. Internally, each labiate process consists of a short tube that projects from the valve mantle to a slit-like opening. Bar = 1  $\mu\text{m}$ . (From Andersen et al., 1986.)

### Plastids and storage products

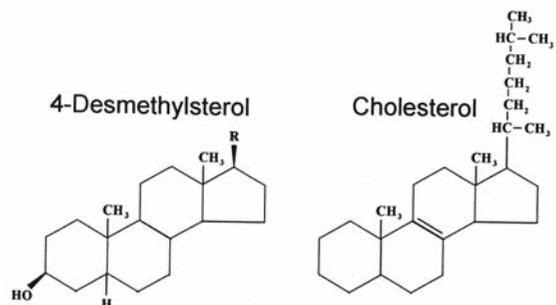
The chloroplasts are surrounded by two membranes of the chloroplast envelope, outside of which are the two membranes of the chloroplast E.R., the outer membrane being continuous with the outer membrane of the nuclear envelope. The thylakoids within the chloroplast are grouped three to a band, and in most chloroplasts there is a more or less central pyrenoid (Fig. 17.1). The pyrenoid is usually crossed by widely spaced bands of thylakoids, which in some cases are reduced from the normal three thylakoids per band to two. The chloroplasts contain chlorophylls  $a$ ,  $c_1$ , and  $c_2$ , with an  $a:c$  ratio of 4:1 in *Phaeodactylum tricornerutum* (Mann and Myers, 1968). Fucoxanthin is the principal carotenoid, giving the cells their golden-brown color. Fucoxanthin is an efficient carotenoid in the transfer of energy to chlorophyll  $a$  and is part of photosystem II of photosynthesis.

There are also colorless or **apochlorotic** diatoms that live on decaying marine vegetation and the mucilages of large seaweeds (Lewin and Lewin, 1967). With the light microscope these

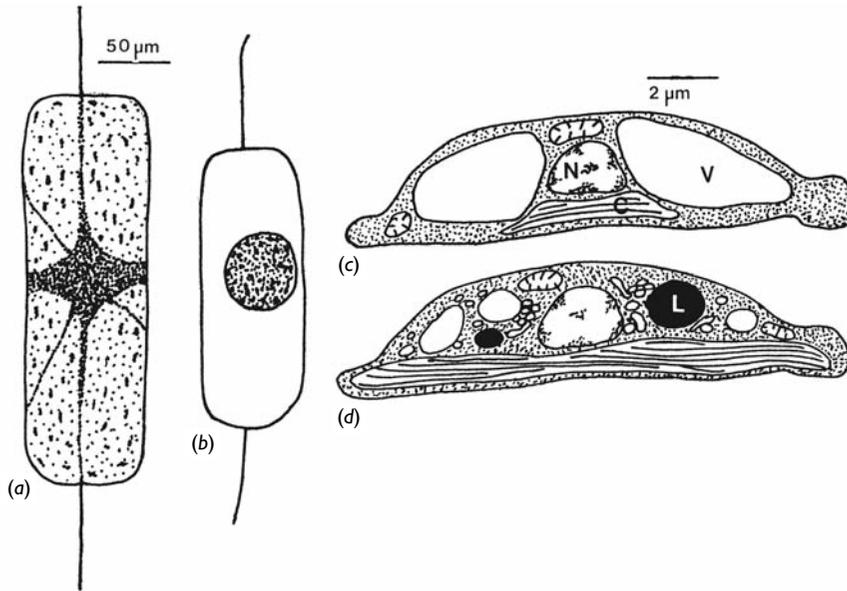
diatoms have no apparent plastids, although when examined at the fine-structural level they can be seen to have proplastids with little if any internal membrane system. The proplastids have no detectable chlorophylls or carotenoids (Lauritis et al., 1968).

The storage product in the Bacillariophyceae is chrysolaminarin, which is located in vesicles in the cell (Granum and Myklestad, 2001). Chrysolaminarin differs from the laminarin found in the Phaeophyceae (Fig. 1.28) by lacking a terminal mannitol residue at the reducing end of the polysaccharide (Chiovitti et al., 2004).

Diatoms contain unique  $4\alpha$ -methyl sterols, such as 4-desmethylsterol and cholesterol (Fig. 17.21), that are useful as diatom markers in the water column and in organic sediments (Mansour et al., 1999).



**Fig. 17.21** Two  $4\alpha$ -methyl sterols that occur in diatoms.



**Fig. 17.22** (a), (b) *Ditylum brightwelli*. (a) Vegetative cell. (b) Resting spore. (c), (d) *Amphora coffaeiformis*. Drawings of the ultrastructure of a vegetative cell (c) and a resting cell (d). (C) Chloroplast; (L) lipid; (N) nucleus; (V) vacuole. ((a), (b) after Gross, 1940; (c), (d) after Anderson, 1975.)

## Resting spores and resting cells

Some diatom cells form thick, ornamented walls at different times in their life cycle and become **resting spores** (Fig. 17.22) (McQuoid and Hobson, 1996). If such cells are planktonic, they fall to the bottom where, presumably, they await more favorable conditions. In *Ditylum brightwelli* (Gross, 1940), the resting spore is formed by the protoplasm shrinking and the plasmalemma drawing away from the cell wall (Fig. 17.22(a), (b)). The resting spore then formed has a much smaller volume than the original cell, the main decrease being due to the loss of vacuoles and their contents. In germination, the resting spore produces a number of fine protoplasmic strands, which radiate in all directions. Chloroplasts are gradually released from the central mass and move along the processes while the whole protoplast expands. After 2 days, the protoplast has assumed a spherical shape, resembling an auxospore. This protoplast elongates and becomes cylindrical, with a valve appearing at one end within 24 hours and a

second valve appearing at the other end after 48 hours. The new cells are much wider than the parent cells. As noted above, the normal method of reproduction, by binary fission, results in a decreased size in the daughter cells. In some species a certain size is reached when sexually or asexually produced auxospores may reestablish the maximum cell size (Nagai et al., 1995). It is, however, known that auxospore formation is generally infrequent, probably taking place once in two or more years in some species. Resting spore formation is therefore a much more frequent method of reestablishment of cell size (Fig. 17.44).

Resting spores are formed after the diatom cells have been subjected to a stress shock. In the ocean, light, temperature, and salinity are relatively stable, with sudden changes rarely occurring, even though there are seasonal changes. In the marine environment, it is usually stress shocks from nutrient depletion that trigger resting spore formation (Davis et al., 1980). Because of the exponential nature of phytoplankton growth, the limiting nutrient is reduced from a considerable level to near zero during the last doubling of the phytoplankton at the end of a bloom (in 1 day or less for many diatoms). Thus a sudden nutrient shock is a typical feature at the end of a diatom bloom.

Marine centric diatoms commonly form morphologically different resting spores, whereas

there are only a few pennate diatoms that do. *Eunotia soleirolii* is one of the few freshwater pennate diatoms that form resting spores (von Stosch and Fecher, 1979). In the freshwater environment, diatoms are subjected to more environmental shocks than in the marine environment. *Eunotia soleirolii* will form resting spores when subjected to (1) high temperatures (24 °C); (2) high or low pH; or (3) iron, silica, phosphate, or nitrate deficiencies. In many other diatoms, resting spores germinate when placed in fresh medium. However, the resting spores of *E. soleirolii* require dormancy to be broken by a dark period at -2 °C to 15 °C for a minimum of 4 to 5 weeks. Thus *E. soleirolii* thrives in colder seasons in northern Europe and is induced to form resting spores by rising temperatures and perhaps concomitant nutrient deficiencies. The resting spores are viable for up to 3 years, but would normally last the summer before dormancy would be broken by the cold temperature of autumn, resulting in germination of the resting spores.

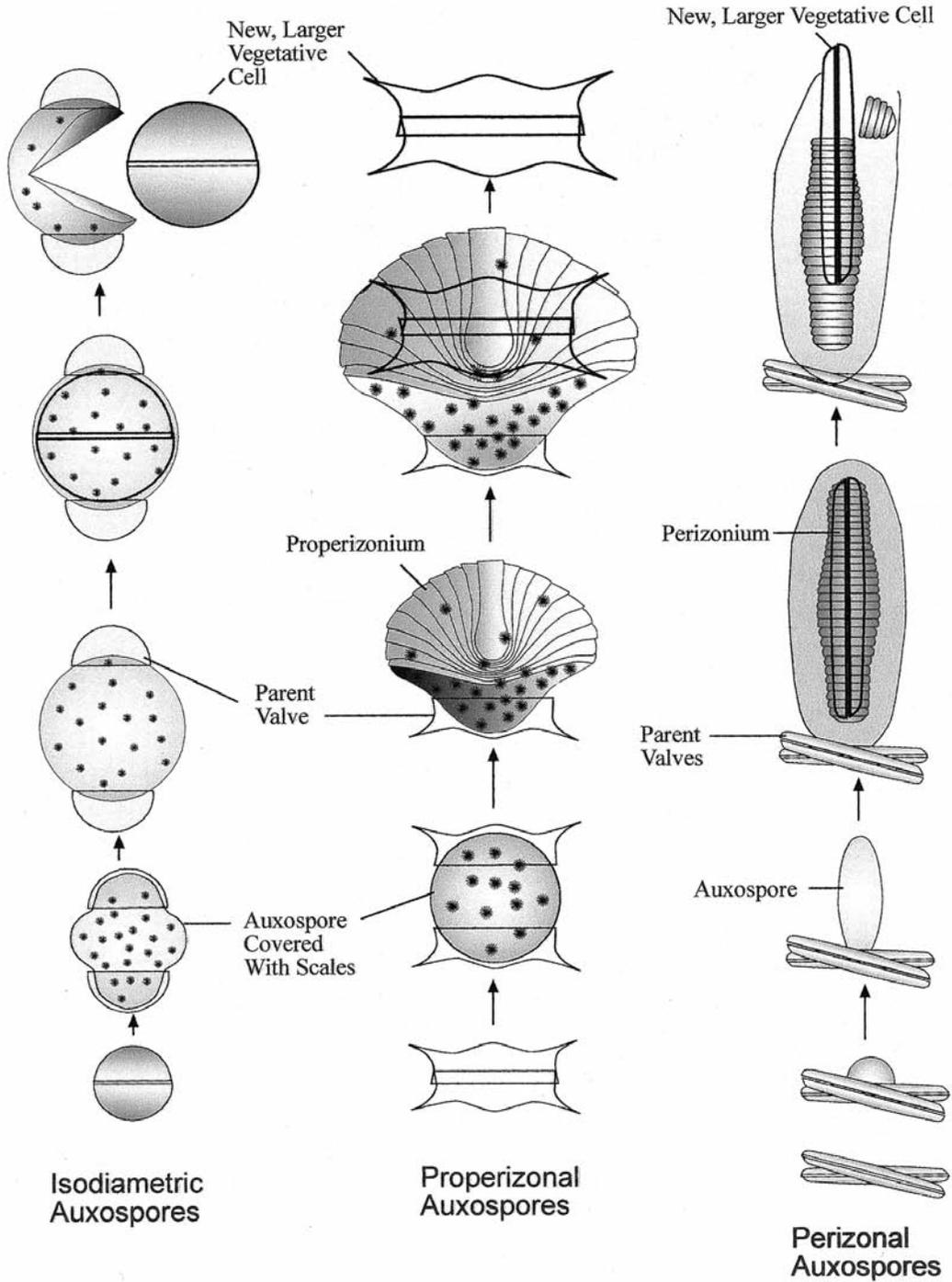
**Resting cells** have the same morphology as vegetative cells and do not form a protective layer, thereby differing from resting spores. Growing vegetative cells of *Amphora coffaeiformis* require 4 weeks in the dark to form resting cells (Anderson, 1975). Formation of resting cells initially involves autophagic activity, with the breakdown of existing structures. Large vacuoles decrease in size, and many small ones develop; the mitochondria become fewer; and large lipid bodies are formed (Fig. 17.22). The resting cells contain as much chlorophyll as the vegetative cells, and the whole cell appears to be a parsimonious assemblage of organelles prepared to resume metabolism and growth upon return to favorable conditions. During the summer, diatom cells sink into deep water below the euphotic zone, where they form resting cells and become dormant. Such cells remain viable for at least 2 months in such an environment (Anderson, 1976). Their respiratory rate is 20% that of normal cells, and their photosynthetic capability is very low. Vertical mixing brings these cells and nutrient-rich water to the surface, and within 2 days the cells become active and begin to reproduce. Viable resting cells of diatoms have been collected at 6150-m water depth in the North Atlantic.

## Auxospores

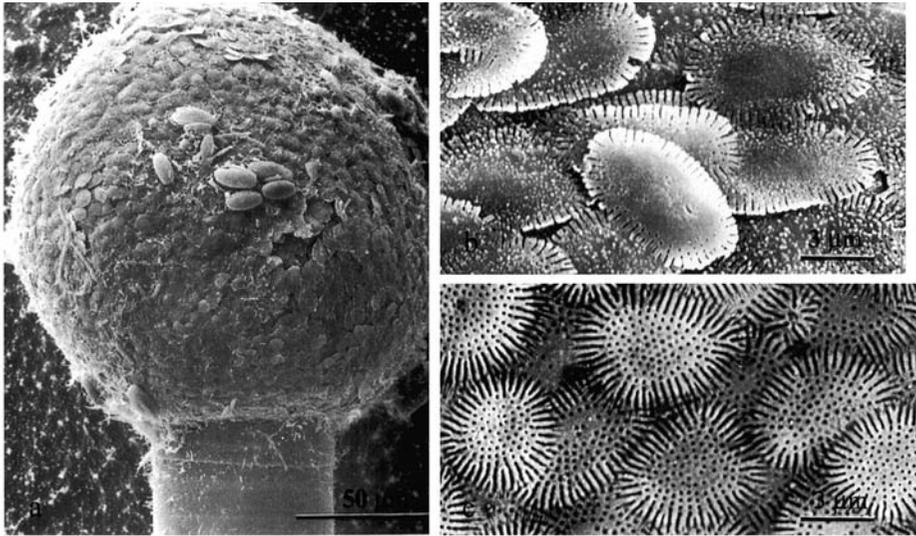
**Auxospore** formation is a second mechanism (in addition to resting spores) for reestablishing the original size of the cell. The auxospores are formed by the fusion of two gametes. In the centric and gonoid diatoms, the male gamete is motile (Fig. 17.45), whereas the female gamete (egg) is non-motile (Figs. 17.42, 17.44). In the pennate and trelloid diatoms, both gametes are non-flagellated (Figs. 17.29, 17.46) (Chepurnov et al., 2004).

Depending on the species, auxospores develop in one of three different ways (Fig. 17.23) (von Stosch, 1982; Medlin and Kaczmarska, 2004).

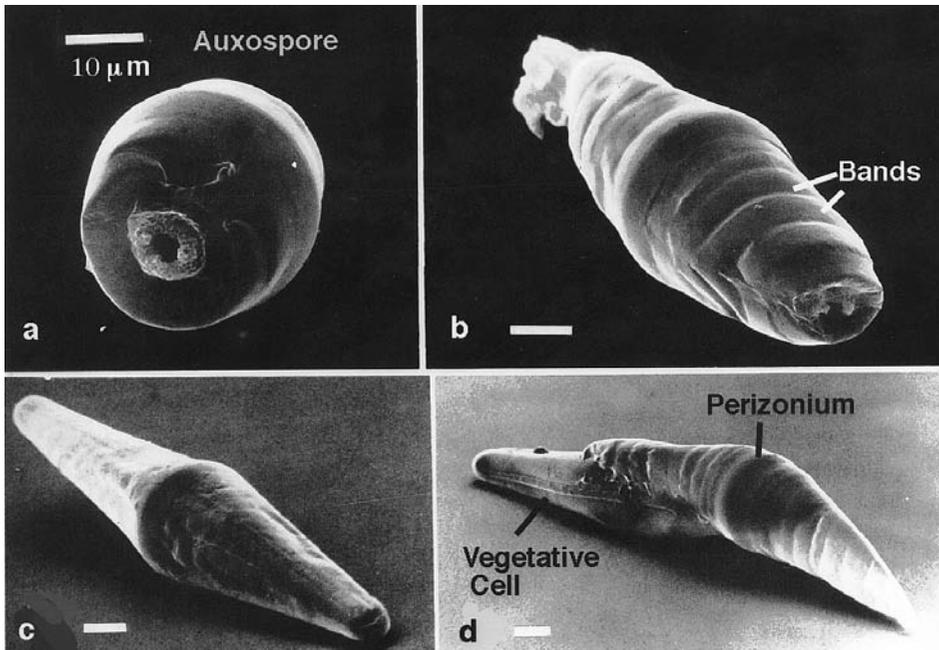
- 1 **Isodiametric auxospores.** Centric diatoms such as *Melosira* (Figs. 17.35, 17.40, 17.41, 17.42, 17.43), *Coscinodiscus* (Figs. 17.2(a), 17.6(b), 17.15, 17.38(a), (b)), and *Stephanopyxis* (Figs. 17.33(a), 17.38(c), (d)) produce this type of auxospore. The globular auxospore more or less maintains its shape during development. The auxospore usually is attached to the parent valves during maturation. The auxospore has a wall with embedded scales (Fig. 17.24), inside of which are produced the roughly dome-shaped valves of the mature vegetative cells.
- 2 **Properizonial auxospores.** Centric diatoms such as *Chaetoceros* (Figs. 17.6(a), 17.32, 17.44, 17.45) produce non-isodiametric (non-spherical) mature auxospores. The immature globular auxospore splits and bands are produced from an asymmetrical auxospore. The bands are collectively called the properizonium. In *Chaetoceros*, a fan-like assortment of bands is formed on the dorsal surface of the auxospore while the ventral surface is composed of a primary envelope containing scales. The mature auxospore produces a flattened frustule similar in shape to the vegetative cell.
- 3 **Perizonial auxospores.** Pennate diatoms such as *Navicula* (Figs. 17.2(d), 17.16, 17.25, 17.26(a), 17.33(b), 17.35) and *Pseudo-nitzschia* (Figs. 17.27, 17.29) form this type of non-isodiametric mature auxospore (Sato et al., 2004). The immature globular auxospore has scales embedded in a primary wall. The primary auxospore wall splits and separates into two



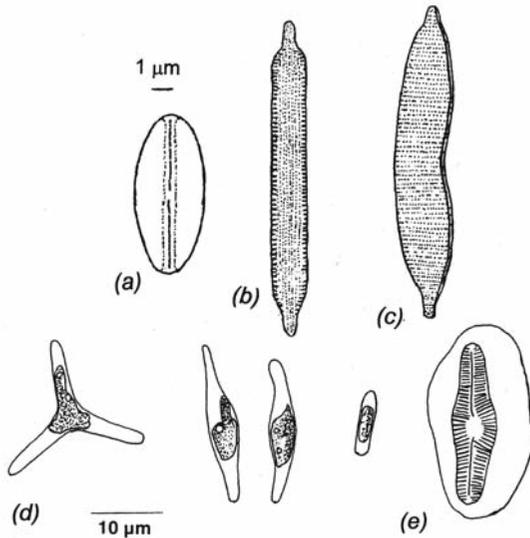
**Fig. 17.23** Diagrammatic summary of the three types of sexual auxospores in diatoms.



**Fig. 17.24** *Ellerbeckia arenaria*. Scanning electron micrographs of auxospores. (Left) Scale-covered auxospore perched in the epitheca. (Top right) External view of scales. (Bottom right) Internal view of scales. (From Schmid and Crawford, 2001.)



**Fig 17.25** Scanning electron micrographs of sexual stages of *Navicula cuspidata*. (a) Immature auxospore. (b) Auxospore midway through elongation. The underlying perizonal bands are evident. (c) Mature auxospore showing biconical shape of the perizonium. (d) Vegetative cell emerging from the perizonium. (From Cohn et al., 1989.)



**Fig. 17.26** (a) *Navicula pelliculosa*. (b) *Nitzschia palea*. (c) *Hantzschia amphioxys*. (d), (e) *Phaeodactylum tricornutum*. (d) Three forms, triradiate, fusiform, and oval. The alga is pleomorphic, ovate cells form on agar or solutions low in calcium while fusiform and triradiate cells occur in liquid medium. (e) An oval cell embedding in mucilage with a single valve. (After Wilson, 1946.)

equal halves. The auxospore produces bands that mold the cell into the tubular perizonium. The new pennate frustule is formed within the perizonium of the mature auxospore.

Sexual reproduction in diatoms can occur only after two general conditions have been met (Edlund and Stoermer, 1997). First, cells must reach a minimum size range, typically 30–40% of their maximum size. Second, there must be the presence of correct environmental conditions. These include combinations of temperature, light, nutrients, trace metals, organic growth factors, and osmolarity (Potapova and Snoeijs, 1997). Contrary to most other algal groups, sexuality is primarily a means of size restoration, and is not normally a factor in dormancy or dispersal (Edlund and Stoermer, 1997).

## Rhythmic phenomena

It is possible to synchronize the division of diatom cells in a culture in a couple of different ways. Lewin (1966) has shown that removal of silicon from cultures of *Navicula pelliculosa* (Fig. 17.26(a)) stops growth of the cells at a stage prior to cytokinesis. When silica is added to the culture,

all of the cells then divide synchronously. Another way of obtaining synchronized cell divisions is by keeping the diatoms in the dark for a long period followed by exposure to light. In *Nitzschia palea* (Fig. 17.26(b)) the shortest time that can be obtained between cell divisions is 16 hours (von Denffer, 1949). If the cells are grown on an 8 hour light : 8 hour dark cycle, synchronously dividing cells are obtained. If the cycle is shortened to 6 hours light:6 hours dark, then cell division occurs every second dark period because there is insufficient time for the diatom to prepare itself for the next division.

Fauré-Fremiet (1951) was the first to observe that the diatom *Hantzschia amphioxys* (Fig. 17.26(c)) accumulates on mud flats in certain areas during low tides, causing brown spots on the mud. The cells can glide rapidly at a rate of half their length in a second and can reverse their phototactic response. During low tide the cells are positively phototactic, so that they come to the surface of the mud, and at high tide they are negatively phototactic, so that they descend into the mud. At high tide they also exude a mucilaginous material, which causes them to stick to one another and to sand grains. This rhythmic phenomenon prevents the cells from being washed away by the tide.

## Physiology

For growth of *Navicula pelliculosa* (Fig. 17.26(a)), silicon cannot be replaced by any of the elements similar to it in physical and chemical properties or in atomic radius, such as Ge, C, Sn, Pb, As, P, B, Al, Mg, or Fe (Lewin, 1962). Lewin (1966) has shown that with a mixture of algae and other organisms, concentrations of germanium dioxide ( $\text{GeO}_2$ ) above  $1.5 \text{ mg liter}^{-1}$  will specifically suppress the growth of diatoms. In cultures of many types of algae, diatom contaminants frequently grow so fast that they obscure the growth of the algae the investigator is working with. The finding that  $\text{GeO}_2$  specifically inhibits diatom growth was a welcome one for phycologists working on algal cultures. In experiments with 14 pure cultures of diatoms,  $1 \text{ mg liter}^{-1}$  of  $\text{GeO}_2$  reduced the growth rate significantly;  $10 \text{ mg liter}^{-1}$  was even more inhibitory to growth and in a few cases killed the cells. *Phaeodactylum tricornutum* (Fig. 17.46(d)), a diatom with little or no silicified wall, was the least sensitive to  $\text{GeO}_2$  of the diatoms tested. By increasing the amount of  $\text{SiO}_2$  in solution, it is possible to reverse the inhibitory effect of  $\text{GeO}_2$  on growth. The results indicate that  $\text{GeO}_2$  is a specific inhibitor of silicate utilization because concentrations of  $\text{GeO}_2$  as high as  $400 \text{ mg liter}^{-1}$  have no effect on respiration. The minimum inhibitory concentrations of  $\text{GeO}_2$  depends to a certain extent on the pH and concentration of the nutrient medium. Such inhibitory concentrations are rarely, if ever, reached in natural waters, the highest concentration reported being that of a mineral spring in Niigata Prefecture, Japan, which contained only  $0.03 \text{ mg liter}^{-1}$  of Ge, with normal concentrations in seawater being about  $5 \times 10^{-5} \text{ mg liter}^{-1}$ .

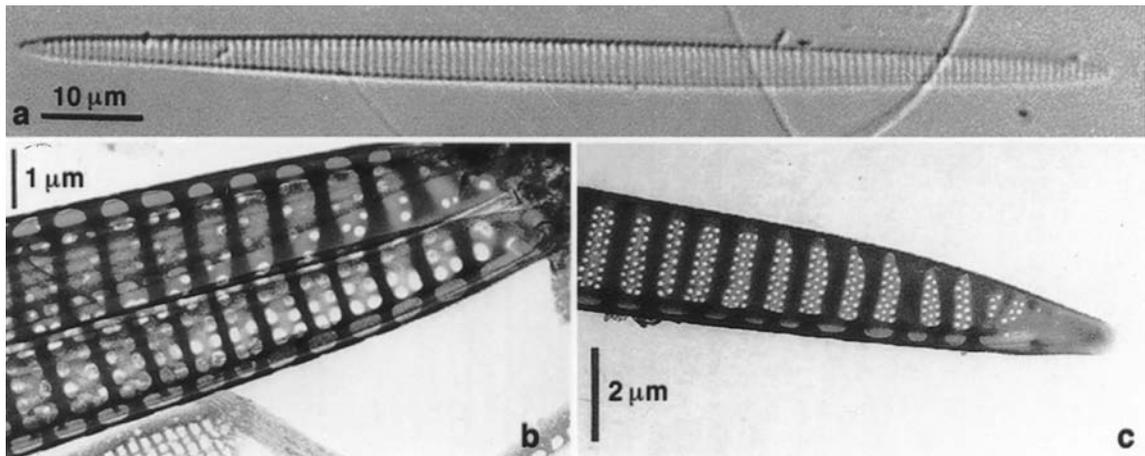
In addition to responding adversely to germanium in solution, diatoms are sensitive to copper. Erickson (1972) has shown that *Thalassiosira pseudonana* has its growth inhibited at concentrations of copper as low as  $5 \mu\text{g liter}^{-1}$ . The presence of detritus affects the toxicity to a certain extent. Concentrations of  $0.25 \text{ ppm}$  copper as  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  are normally used to control algal blooms without affecting fish in freshwater lakes. Copper toxicity and silicon metabolism are linked in

several marine diatoms. *Skeletonema costatum* (Fig. 17.8(e), 17.31(a)) and *Thalassiosira pseudonana* exhibit slower growth in the presence of copper. Inhibition of growth by copper is alleviated by increasing the silicic acid concentration in the media (Morel et al., 1978; Rueter et al., 1981). It is probable that copper interferes with the silicic acid transport site across the plasma membrane and slows uptake of silicic acid (Rueter et al., 1981; Rueter, 1983).

The effects of heavy metals on diatoms can be divided into three groups (Thomas et al., 1980): (1) Cu, Zn, and Ge affect the biochemical pathway of silicon metabolism; (2) Hg, Cd, and Pb interfere with cell division and cause morphologically distorted cells to be produced; and (3) Cr, Ni, Se, and Sb have no effects up to a concentration of  $1 \mu\text{M}$ , well above the concentrations that show effects with other toxic metals.

Some normally photosynthetic diatoms are able to grow under heterotrophic conditions (Kroger, 2001). In two studies (Lewin, 1953; Lewin and Lewin, 1960), a number of cultures of photosynthetic diatoms were found that would grow heterotrophically, usually only with glucose as a carbon source. One of these diatoms, *Cyclotella cryptica* (Hellebust, 1971), can grow in the dark in an organic medium with glucose (but not lactate or tryptone) as the sole carbon source. When the organism is growing in the light, it does not have the mechanism for the utilization of glucose in the medium. It requires about 24 hours in the dark in a glucose medium before it is able to use the glucose as a carbon source. This lag period indicates that the lack of light induces an uptake and/or assimilation system for the glucose. White (1974) suggested that such facultative heterotrophy enables these diatoms to settle into bottom deposits, live heterotrophically for long periods, then rise and begin photosynthesis again. Although the above diatoms still have functional chloroplasts, there are some apochlorotic diatoms lacking functional chloroplasts (Lewin and Lewin, 1967). The latter, which are species of *Nitzschia* (Fig. 17.26(b)), are able to grow with lactate or succinate as the sole organic carbon source.

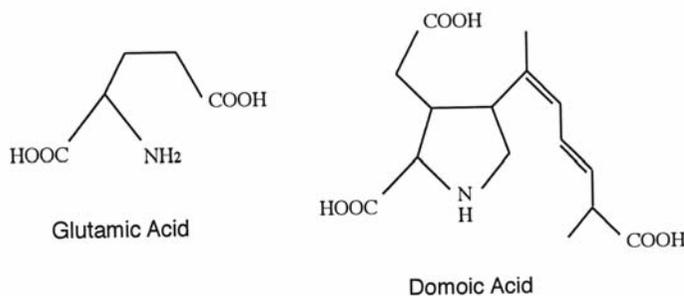
**Amnesic shellfish poisoning** occurs when shellfish filter diatoms from the genera *Nitzschia* (Figs. 17.26(b), 17.35), *Pseudo-nitzschia* (Figs. 17.27,



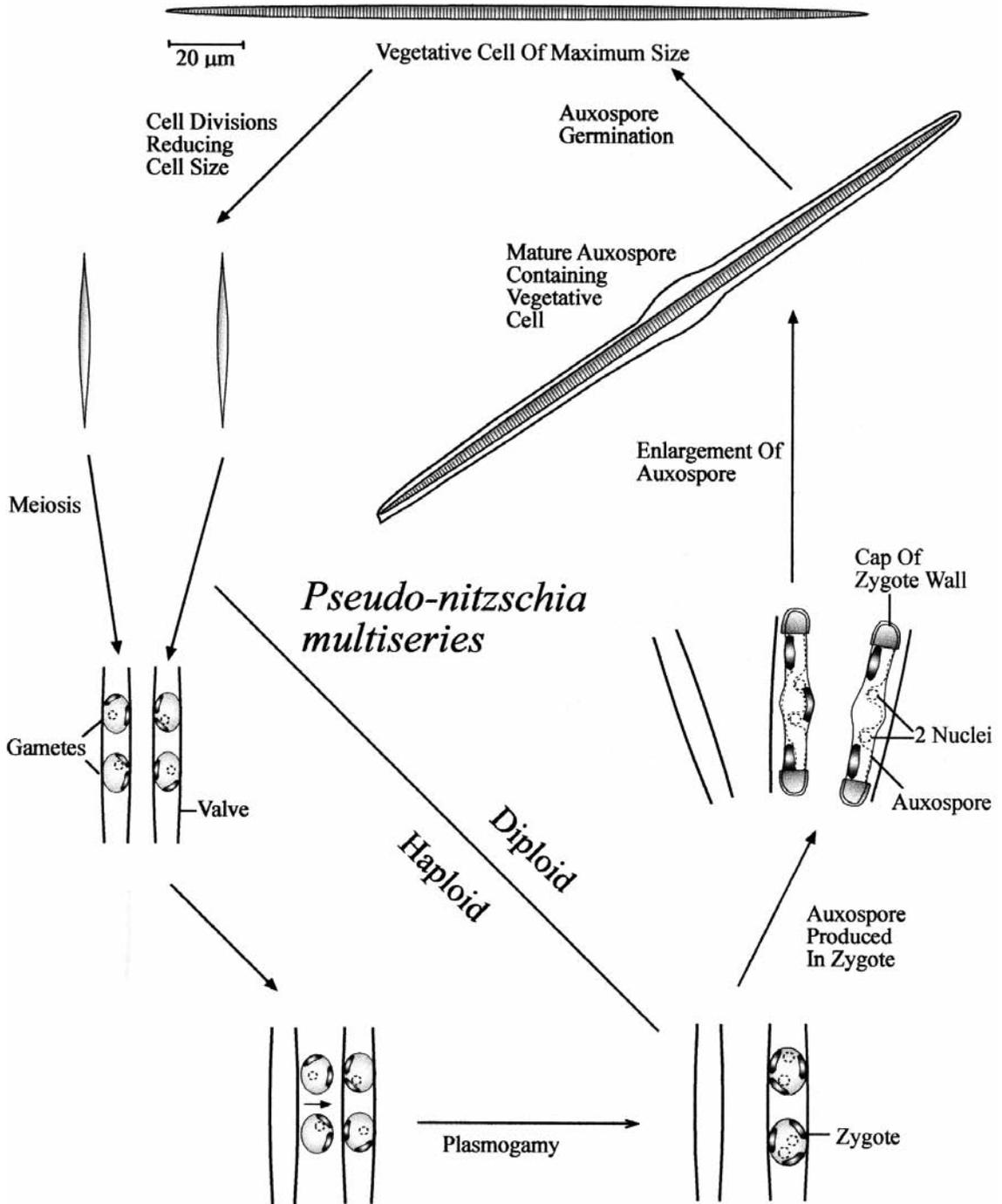
**Fig. 17.27** *Pseudo-nitzschia*, a diatom responsible for amnesic shellfish poisoning. (a) Light micrograph of one valve. (b), (c) Transmission electron micrographs of the ends of cleaned valves showing the ribs and poroids. (From Hasle, 1995.)

17.29), and *Amphora* (Figs. 17.22(c), (d)), 17.35) from marine waters (Bates, 2000; Lundholm and Moestrup, 2000). Subsequent ingestion of the shellfish by man and birds results in memory loss (amnesia), abdominal cramps, vomiting, disorientation, and even death. Amnesic shellfish poisoning was first recognized in 1987 in Prince Edward Island, Canada, where it caused 3 deaths and 105 other affected individuals after blue mussels were eaten (Subba Rao et al., 1988). The diatom produces domoic acid, a derivative of the neuroexcitatory amino acid *L*-glutamic acid (Fig. 17.28). Domoic acid is especially prevalent in moribund cells of the diatom and can be induced by depriving the cells of nutrients, particularly silicate and phosphate (Pan et al., 1996).

Sexual reproduction in *Pseudo-nitzschia multiseries* is on an internal clock controlled by the size of the cells (Davidovich and Bates, 1998). A maximum cell size is attained after sexual reproduction by auxospore germination producing vegetative cells with a length of 120–170 µm (Fig. 17.29). Sexual reproduction is induced in nature about three years later after successive cell divisions have decreased the cell size by 30–40%. Mating in *P. multiseries* begins when cells of different strains contact each other, valve to valve in parallel. Gametogenesis occurs by cells dividing meiotically to form morphologically similar non-flagellated gametes. The valves of the parent frustules separate and gametes from one cell (male) travel by amoeboid movement to the passive gametes in the other cell (female). Within a couple of minutes, the gametes fuse to form a spherical zygote that expands to produce auxospores still attached to one of the parent valves. The fully expanded auxospore still has unfused



**Fig. 17.28** The structure of domoic acid, the causative chemical of amnesic shellfish poisoning, and its analog, *L*-glutamic acid.



**Fig. 17.29** The life cycle of the toxic diatom *Pseudo-nitzschia multiseries*. (Modified from Davidovich and Bates, 1998.)

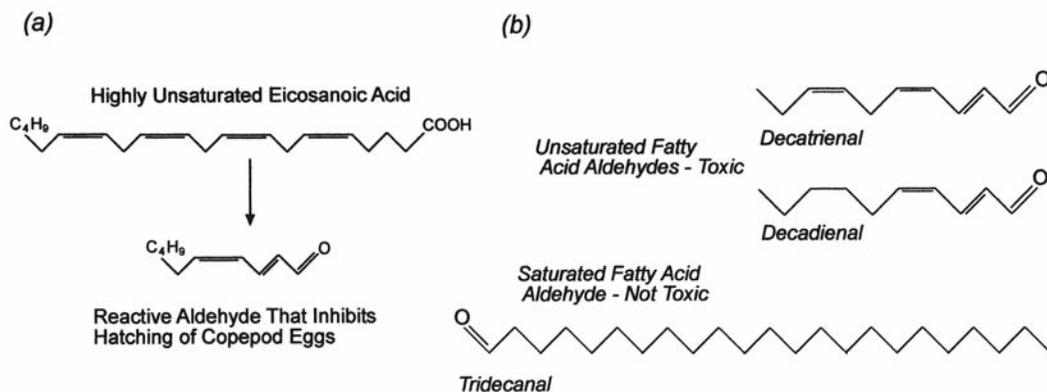
nuclei and chloroplasts from the gametes. Most of the auxospore volume is occupied by a single vacuole. The auxospores germinate to produce vegetative cells of maximal cell size. Physiologically sexual reproduction is isogamous because the cells of one clone are passive (female) remaining within the parent frustule, while the cells of the other clone are active (male), moving to the passive gametes of the other clone.

## Chemical defense against predation

Diatoms are preferred food for invertebrates such as copepods. Some diatoms (e.g., *Phaeodactylum tricornutum*, *Skeletonema pseudocostatum*) have evolved a mechanism to reduce predation by releasing chemicals that reduces the fecundity of the next generations of invertebrates. Diatoms cells contain large quantities of highly unsaturated fatty acids such as eicosanoic acid (Fig. 17.30(a)) in vesicles in the cytoplasm. Death of cells during feeding by invertebrates results in the release of the unsaturated fatty acids into the seawater. Upon release, phospholipases convert the unsaturated fatty acids into the unsaturated short-chain aldehydes 2,4-decadienal and 2,4,7-decatrienal (Fig. 17.30(b))

(Miralto et al., 1999; Pohnert, 2002; Pohnert and Boland, 2002; Pohnert et al., 2002). These short-chain fatty-acid aldehydes are toxic to developmental stages of a range of invertebrates including copepods, sea urchins, polychaetes, and ascidians (although similar saturated fatty acids with a terminal aldehyde group, such as tridecanal (Fig. 17.30(b)), are not toxic to copepod eggs) (Caldwell et al., 2004). They appear to work by affecting cell membrane characteristics and microtubule and microfilament stability (Casotti et al., 2005). Future generations of grazers are sabotaged, encouraging the survivability of diatom populations.

Eicosanoic acid in the diatom cell is not toxic to the diatom cell; the chemical is only toxic after the unsaturated fatty acid is released into the environment and converted into an aldehyde. Thus, the diatom cells are able to invest their metabolic energy into the formation of unsaturated fatty acids utilized in normal cell activities. When the cells are damaged, the unsaturated fatty acids are released and converted into toxic compounds that reduce the numbers of the next generation of grazers (Pohnert, 2002). Interestingly, the released aldehydes also are toxic to diatoms (Casotti et al., 2005). However, the diatoms cells are being destroyed by the grazing and are already out of the gene pool.



**Fig. 17.30** (a) The reaction by which a non-toxic highly unsaturated fatty acid is converted by a phospholipase into a reactive unsaturated fatty-acid aldehyde that is toxic to invertebrates. The reaction is initiated by wounding of the diatom cell. (b) Two unsaturated fatty-acid aldehydes, decatrienal and decadienal, that are toxic to invertebrates and a saturated fatty-acid aldehyde, tridecanal, that is not toxic.

## Ecology

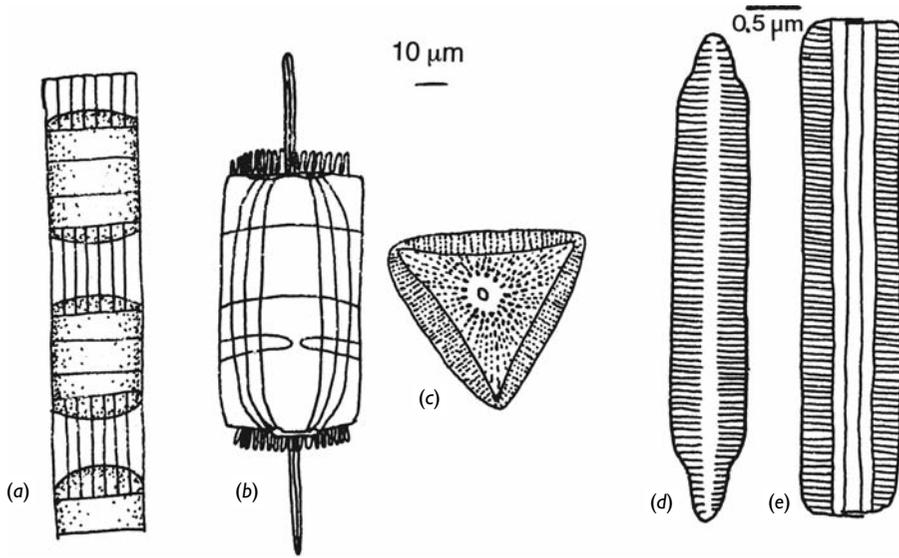
Diatoms comprise the main component of the open-water marine flora and a significant part of the freshwater flora. Attached diatoms can be characterized by the brown scums found on various kinds of substrata, as well as the fluffy brown growths caused by abundant epiphytic diatoms. The pennate diatoms are represented in about equal numbers in the freshwater and marine habitats, whereas the centric and gonoid diatoms are present predominantly in the marine environment. Generally speaking, in the marine environment the colder the water is, the greater the diatom population. The populations of diatoms in the open oceans usually have a large number of species with the total number of organisms being low, in contrast to the diatoms living close to the shore, where the total number of diatoms is very high, but the number of different species within the population is low. This situation is actually fairly typical of oligotrophic, unenriched waters (the open ocean) versus eutrophic, enriched waters (the coastal waters receiving enrichment from the land).

### Marine environment

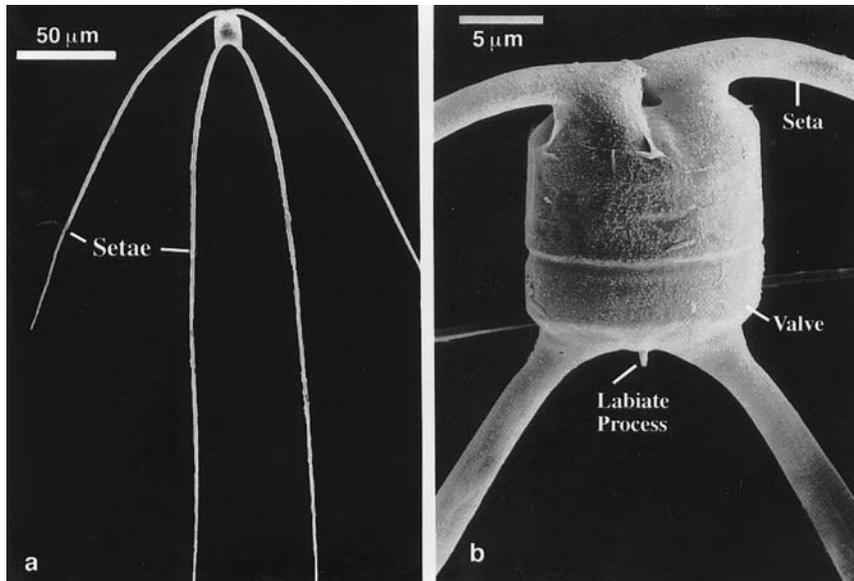
The maintenance of oceanic diatoms in the water column involves some adaptation of the cells to make them buoyant. The silicified wall of diatoms has a density of about 2.600, comprising up to 50% of the dry weight of the cell (see Smayda, 1970, for a review). The density of seawater varies from 1.021 to 1.028; therefore, planktonic diatoms must have cellular components that are lighter than seawater to achieve neutral or positive buoyancy. The density of the cytoplasm in marine organisms is slightly heavier than seawater, varying from 1.030 to 1.100. This leaves only the vacuole as a likely source of positive buoyancy in diatoms. In fact, most planktonic diatoms have a large vacuole whose contents, while being isotonic, are lighter than seawater. For example, the vacuolar sap of the planktonic diatom *Ditylum brightwelli* (Figs. 17.22(a), (b), 17.31(b), (c)) has a density of 1.0202, less than that of seawater. The vacuole of these diatoms contains lighter ions than the surrounding seawater: the concentration of  $\text{Na}^+$  is great relative to  $\text{K}^+$  (the

latter element is heavier by about 40%), there is a relatively high concentration of light  $\text{NH}_4^+$  ions, and divalent relatively heavy ions, especially  $\text{SO}_4^{2-}$ , are excluded. This ionic mechanism of buoyancy is probably important in planktonic diatoms as long as they are above a certain size (20  $\mu\text{m}$  diameter in *Ditylum*), below which the size of the vacuole is too small to provide the necessary lift to the cell. The ionic mechanism of buoyancy cannot be applied to the flotation of freshwater phytoplankton because of the low quantity of salts in the water. In freshwater, most colonial diatoms are encased in a gelatinous sac of low density which aids the flotation of the organisms. Settling in the water column is slowed by increasing the surface area relative to the volume, thereby increasing the drag of the cell in the water. In diatoms this can be accomplished by setae (*Chaetoceros*, Figs. 17.6(a), 17.32, 17.44, 17.45) or by cells shaped as discs (*Coscinodiscus*, Figs. 17.2(a), 17.6(b), 17.15, 17.38(a), (b)), ribbons (*Fragilaria*, Fig. 17.31(d), (e)), or elongate forms (*Rhizosolenia*, Fig. 17.33(c)). The aggregation of diatom cells into chains increases the settling rate of the cells because it decreases the surface area. Increasing the size of diatom cells results in an increase in the ascension rate. The largest diatom cells can ascend up to 8 meters per hour (Moore and Villareal, 1996).

Planktonic diatoms can vary in density during the day, moving up and down in the water column. This causes a constant flow of water over the surface of the diatom, enabling a better absorption of nutrients. *Ditylum brightwelli* (Figs. 17.22(a), (b), 17.31(b), (c)) is a diatom with a variation in sedimentation rates, the greatest rate of settling occurring during the latter half of the light period and the least settling occurring at the end of the dark period (Fisher and Harrison, 1996). The insignificance of fat deposits in buoying up cells can be seen here, where the cells contain the maximum amount of fat during the period of maximum settling (Andersen and Sweeney, 1977). Sedimentation of diatoms in the ocean continues in many cases until an increased density of the water, due to either a thermocline or greater water depth, causes them to remain steady in the water column. In the central north Pacific Ocean, there is a widespread deep (110- to 130-m depth) chlorophyll layer containing pigments of living algae, not detritus. This chlorophyll maximum



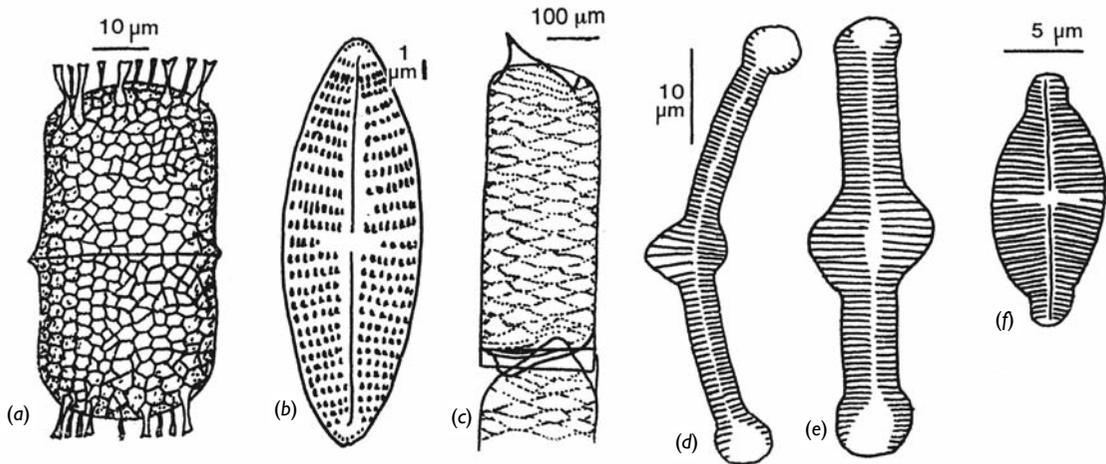
**Fig. 17.31** (a) *Skeletonema costatum*. (b), (c) *Ditylum brightwelli*. (b) Girdle view. (c) Valve view. (d), (e) *Fragilaria virescens*, valve and girdle view.



**Fig. 17.32** Scanning electron micrograph of *Chaetoceros peruvianus* showing the setae and a labiate process. (From Pickett-Heaps et al., 1994.)

contains growing organisms and is not an accumulation of moribund cells (Venrick et al., 1973; Jeffrey, 1976). Diatoms make up a good proportion of these cells and are able to grow there by virtue

of an adaptation of the cells to the decreased light (Jeffrey and Vesik, 1977). The light that penetrates down to this depth is composed principally of low-intensity blue-green light with a maximum at about 480 nm. It has been shown with the marine diatom *Stephanopyxis turris* (Figs. 17.33(a), 17.38(c), (d)) that blue-green light increases the



**Fig. 17.33** (a) *Stephanopyxis turris*. (b) *Navicula glaciei*.  
(c) *Rhizosolenia castracanei*. (d) *Tabellaria fenestrata*.  
(e) *Tabellaria flocculosa*. (f) *Achnanthes exigua*.

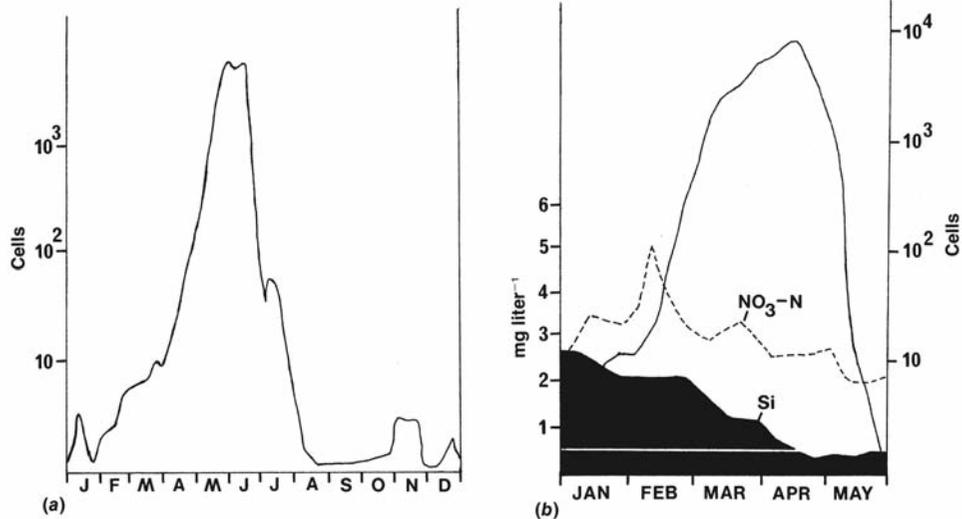
amount of chlorophyll up to 100% when compared to white light, with an appropriate increase in chloroplasts but with no change in the chlorophyll:carotenoid ratio. At the same time, blue-green light enhances the photosynthetic fixation of carbon dioxide. Thus the diatoms living at great water depth receiving only blue-green light “switch on” a mechanism for more efficient photon capture of light and increased fixation of carbon dioxide. They are therefore able to remain viable and grow at a water depth that has a low amount of light.

Symbioses between nitrogen-fixing (diazotrophic) bacteria and cyanobacteria, and the diatom genera *Rhizosolenia* and *Hemiaulus* are common in warm oligotrophic seas (Villareal, 1989). *Rhizosolenia castracanei* (Fig. 17.33(c)) and *R. imbricata* var. *shrubsolei* form free-floating diatom mats in oligotrophic central oceanic regions, such as the Sargasso Sea and the North Pacific Gyre (where *Rhizosolenia* mats make up 98% of all living diatom silicon (Shipe et al., 1999)). In these areas the fixation of nitrogen by the endosymbionts contribute a significant amount of nitrogen to the ecosystem (Martinez et al., 1983). The *Rhizosolenia* cells migrate vertically in the column at a rate of several meters per hour and can be found to a depth of 150 meters (Richardson et al., 1996; Villareal and Carpenter, 1994). Cells that are

deprived of nitrogen are negatively buoyant and sink below the euphotic zone into waters that are relatively high in nutrients. After taking up nutrients, the cells become positively buoyant and move up into the euphotic zone and resume photosynthesis.

### Freshwater environment

Many planktonic diatoms have regular annual fluctuations in growth that can be attributed to environmental conditions. *Asterionella formosa* is one of these diatoms (Fig. 17.16), a common freshwater planktonic diatom that forms large spring growths and smaller autumn ones (Fig. 17.34) (Lund, 1949, 1950). During the winter months, light and temperature limit the growth of the diatoms; lower temperature affects respiration more than photosynthesis so that the compensation point is lowered, and the possibility that photosynthesis will exceed respiration is increased. With the beginning of spring and consequent increase in temperature and light, there is a rapid growth of cells until the middle of spring, when another factor becomes limiting – this time the concentration of dissolved silica in the water; the growth of diatoms then results in much of the dissolved silica being incorporated into the siliceous frustules. Once the concentration of dissolved silica reaches  $0.5 \text{ mg liter}^{-1}$ , most diatoms cease growth. Because the silica is now limiting, the number of diatoms falls off rapidly and increase only to a lesser degree in the fall when more



**Fig. 17.34** (a) Seasonal distribution of live cells of *Asterionella* in Lake Windermere, England, plotted on a logarithmic scale in the 0- to 5-m water column. The horizontal axis represents the months of 1947. (b) Numbers of *Asterionella formosa* (solid line) and the concentrations of dissolved silica and nitrate-nitrogen in mg liter<sup>-1</sup> in the 0- to 5-m water column in Esthwaite Water, England. The white line represents the 0.5 mg liter<sup>-1</sup> concentration of silica necessary for growth of diatoms. ((a) after Lund, 1949; (b) after Lund, 1950.)

dissolved silica is available from the breakdown of the frustules of the spring population and the inflow of water containing silica from streams. There are other factors, such as grazing by invertebrates and fungal parasitism, that also check the growth of *Asterionella*, but they are only of secondary significance.

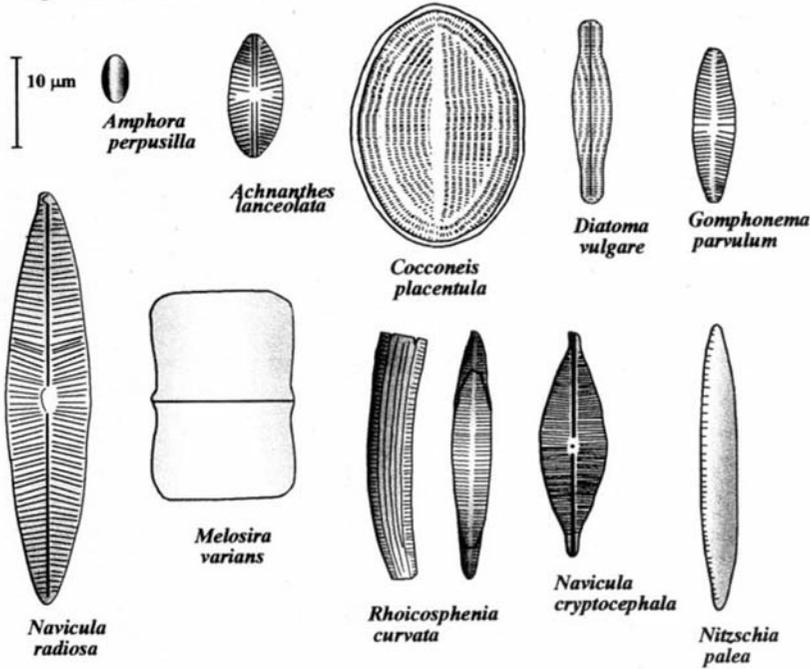
Enrichment of streams by run off from agricultural land, or input from industry, results in the diatom community being dominated by the "agricultural guild" (Fig. 17.35) (Richardson et al., 1996). The appearance of these diatom species in water samples is an indication of the deterioration of water quality.

The diatoms also make up a large part of the periphyton or the attached algae in freshwaters. These attached diatoms in streams have two opposing factors acting on their growth (Reisen and Spencer, 1970): (1) any increase in current retards the attachment of the diatoms to the substrate, and (2) the increase in current results in

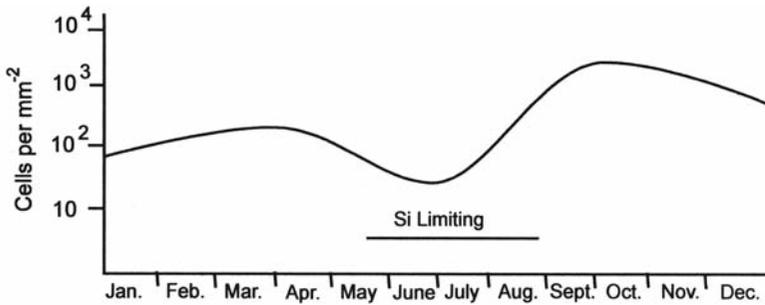
an increase in the growth of the diatoms. This leads to the observation that the diatoms growing in fast-flowing streams usually have a higher total standing crop in the long term than those in slow-flowing streams, although initially the fast-flowing water results in a lower number of diatoms being attached to the substrate. The diatoms in the fast-flowing water are, however, able to grow much faster and soon grow so rapidly that they overcome the disadvantage caused by low initial attachment numbers.

Attached diatoms in standing waters have good growth in spring, as do the planktonic diatoms, but do not show as marked a decrease in growth when the concentration of silica in water reaches 0.5 mg liter<sup>-1</sup> or less. This can be seen by the growth of the diatom *Tabellaria flocculosa* (Fig. 17.33(e), (f)) on stems of the reeds *Phragmites communis* and *Schoenoplectus lacustris* (Fig. 17.36) (Knudson, 1957). In the May-June period, when the dissolved silica in the water is usually less than 0.5 mg liter<sup>-1</sup>, there is a decrease in the number of diatoms, but not as great as that of planktonic diatoms, probably because of leaching of silica from the stems of the host plants. A second difference between planktonic and epiphytic diatom seasonal growth is that in the epiphytic diatoms, maximum growth normally occurs in the winter, possibly owing to the secretion by the host of some organic products that are used by epiphytic diatoms.

**Agricultural Guild**



**Fig. 17.35** Diatoms that are characteristic of eutrophic freshwaters. The diatoms make up the “agricultural guild”, named for waters that are polluted by runoff from agricultural land that have been fertilized with nitrogen, phosphorus, and potash.



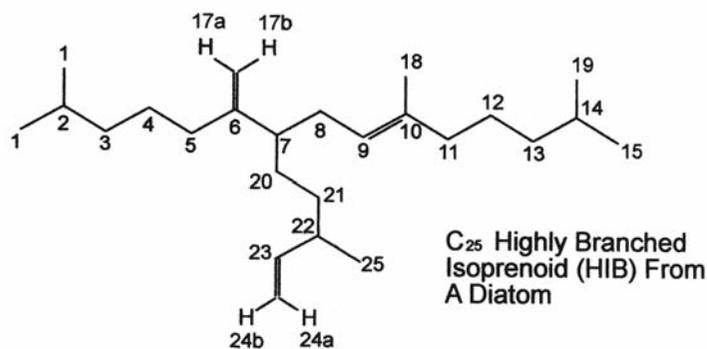
**Fig. 17.36** Seasonal fluctuations of epiphytic *Tabellaria flocculosa* on *Phragmites communis* and *Schoenoplectus lacustris* stems. The straight line represents the period when dissolved silica is less than 0.5 mg liter<sup>-1</sup>. (After Knudson, 1957.)

In freshwater habitats, diatoms often comprise the dominant algal flora in thermal waters between 30 and 40 °C. Fairchild and Sheridan (1974) showed that *Achnanthes exigua* (Fig. 17.33(f)) isolated from a hot spring showed optimum photosynthesis at 42 °C, with maximum and minimum temperatures for growth at 44 and 10 °C, respectively – characteristics of a thermophilic organism. This particular diatom is usually associated with an

algal mat covered by a shallow layer of water in these hot springs.

**Fossil diatoms**

The fossil record of diatoms begins in the Jurassic Period (185 million years ago) with diatoms becoming more common in the mid-Cretaceous



**Fig. 17.37** Chemical structure of a highly branched alkene characteristic of diatoms.

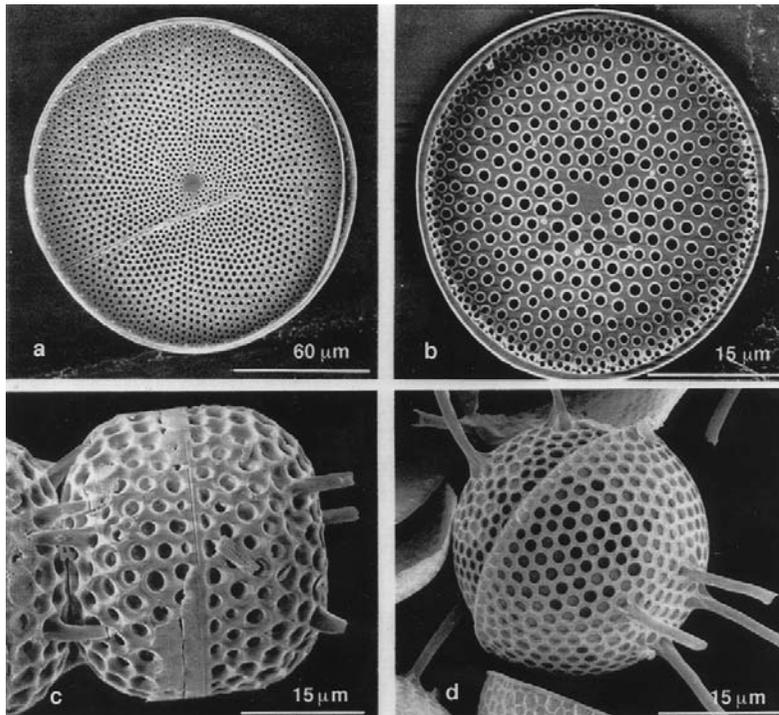
(Medlin et al., 1997). The fossil record indicates that centric diatoms evolved before pinnate diatoms (Round et al., 1990). Another way of dating diatoms is by molecular fossils. Some diatom genera (*Rhizosolenia*, Fig. 17.33(c); *Haslea*, *Navicula*, Figs. 17.2(d), 17.16, 17.26(a), 17.33(b), 17.35; and *Pleurosigma*) contain unique isoprenoids called highly branched isoprenoid (HBI) alkenes that have a T-branch formed by attachment of a C<sub>10</sub> isoprenoid unit to a C<sub>15</sub> isoprenoid unit (Fig. 17.37) (Damste et al., 2004). The occurrence of these alkenes in sediments beginning 90 million years ago parallels the widespread explosion of diatoms in the marine environment at this time.

After death of diatom cells, the frustule usually dissolves after bacteria degrade the organic casing around the frustule (Bidle and Azam, 1999). However, under certain circumstances, the frustules remain intact and accumulate at the bottom of the water (Fig. 17.38). Where conditions are exceptionally favorable and long continued, such accumulations may reach considerable thickness. Deposits of fossil diatoms, known as diatomaceous earth or *kieselguhr*, are found in various parts of the world. None of these deposits originated earlier than the Cretaceous; some diatomaceous earths originated in freshwaters, others in the ocean. However, known deposits of marine species are found inland and above the ocean as a result of geological changes. The best-known and most extensive deposits of marine species are those at Lompoc, California, where the beds are several kilometers in extent and 200 m in thickness (Fig. 17.39). Most of the commercially available diatomaceous earth comes from California, where the deposits are worked as open quar-

ries. In quarrying, the overburden of soil is removed, and the diatomaceous earth is mined. Diatomaceous earth is also obtained from lakes in Florida by dredging with a suction pump and carrying the material through sluiceways to settling tanks. The material from some deposits can be used directly; that from other deposits must be incinerated to remove organic substances.

The industrial uses of diatomaceous earth are varied. One of the first uses was as a mild abrasive in toothpaste and metal polishes. Diatomaceous earth was also used as an absorbent for liquid nitroglycerin to make dynamite that could be transported with comparative safety. The inert medium used in the present-day manufacture of dynamite is wood meal. Probably the most extensive industrial use of diatomaceous earth is in the filtration of liquids, especially those of sugar refineries. Another major use is in the insulation of boilers, blast furnaces, and other places where a high temperature is maintained.

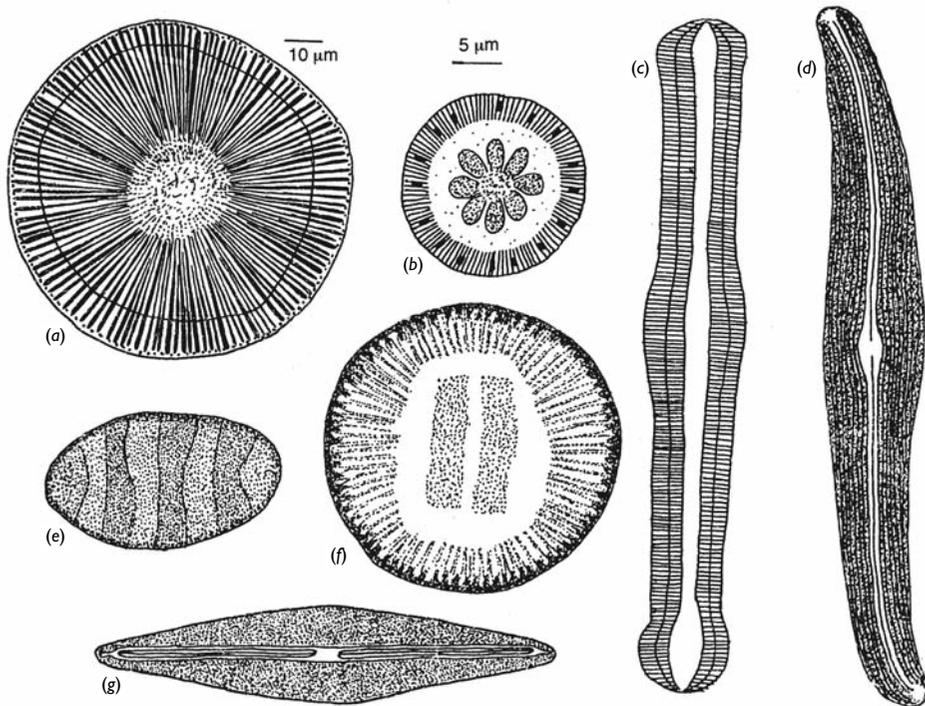
Analysis of sediments containing remains of diatoms can provide information on past environmental conditions of lakes (Reavic et al., 1998; Clavero et al., 2000). This technique has been applied to late glacial and postglacial diatom deposits in the English Lake District (Round, 1957, 1961). During the end of the last glacial period, there were few diatoms present, with the sediments being mostly of inorganic origin, indicating that the lakes initially were very oligotrophic and lacked planktonic diatoms. This period was followed by diatoms characteristic of melt waters under semi-arctic conditions (*Melosira arenaria*, Fig. 17.40(a). *Cyclotella antique*, Fig. 17.40(b); *Gyrosigma*, Fig. 17.40(d); *Cymatopleura*, Fig. 17.40(e);



**Fig. 17.38** Scanning electron micrographs of fossil diatoms compared with frustules of living diatoms from the same genera. (a) *Coscinodiscus radiatus* from Cretaceous sediments. (b) *Coscinodiscus radiatus* from recent plankton. (c) *Stephanopyxis turris* from Cretaceous sediments. (d) *Stephanopyxis broschii* from recent plankton. (From Medlin et al., 1993.)



**Fig. 17.39** Diatomaceous earth mine at Lompoc, California, with deposits of diatomaceous earth in the background. (Courtesy of Johns-Manville Corporation.)



**Fig. 17.40** (a) *Melosira arenaria*. (b) *Cyclotella antiqua*.  
(c) *Rhopalodia gibba*. (d) *Gyrosigma attenuatum*. (e) *Cymatopleura elliptica*. (f) *Campylodiscus clypeus*. (g) *Frustulia rhomboides*.

*Campylodiscus*, Fig. 17.40(f)) indicating leaching of base-rich components from surrounding rocks into the lakes. The next area of sediments showed a decrease in alkaline-water species and an increase in acid-water species (*Eunotia*, Fig. 17.2(b); *Anomooneis*; *Frustulia*, Fig. 17.40(g); *Tabellaria*, Figs. 17.3(b), 17.33(d), (e)), a trend that has continued up to recent times. This trend is related to a decrease in bases being leached from the rocks because no new rock surfaces have been exposed, and to a decline in the stands of birch/pine on the land. At the top of the sediments are remains of *Asterionella formosa*, indicating a recent change of waters to less acidic, more eutrophic conditions, a state related to man's activities. *A. formosa* is a diatom that grows readily in eutrophic waters and shows changes in colony morphology, depending on nutrients in the water. Under optimum growth conditions, the colonies average eight cells, whereas under phosphate limitation the number of cells per colony drops to two, and under silicate limitation the number of cells per colony increases to 20. Under

both P and Si limitations, there is a decrease in growth rate (Tilman et al., 1976).

## Classification

The diatoms probably originated about 200 million years ago in the late Permian (Medlin et al., 1997) from a scaly member of the Chrysophyceae or Bolidophyceae (Guillou et al., 1999). Two scales evolved into valves, while other scales formed the girdle bands. The first diatom had a centric organization with two dome-shaped valves and many scale-like bands (Round et al., 1990). The first recognizable fossils are centric diatoms from the early Cretaceous with pennate diatoms being recorded from the late Cretaceous. The first pennate diatom fossils were araphid (no raphe) with raphid diatoms appearing in the middle Eocene.

The Bacillariophyceae can be divided into two orders as follows:

- Order 1 Biddulphiales: radial or gonoid ornamentation; many chloroplasts; no raphe; resting spores formed; motile spermatozoids with a single tinsel

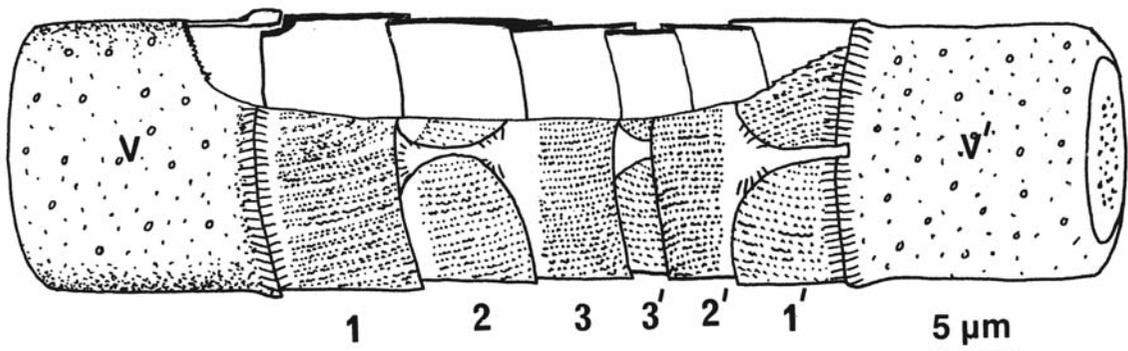
flagellum; oogamous sexual reproduction.

Order 2 Bacillariales: pennate or trellisoid ornamentation; one or two chloroplasts; raphes possibly present with gliding; no flagellated spermatozoids; sexual reproduction by conjugation.

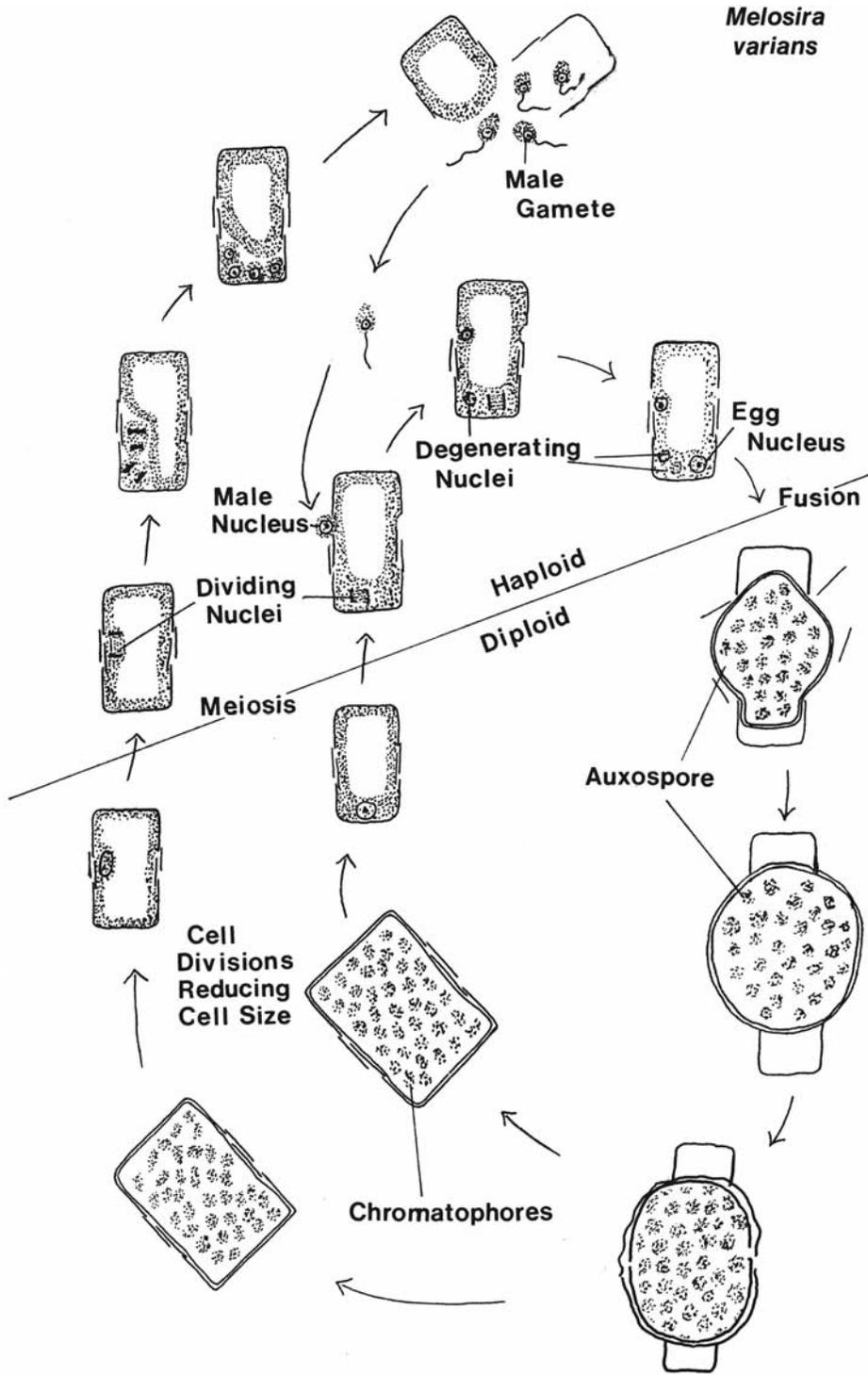
### Biddulphiales

The Biddulphiales or Centrales is primarily a marine planktonic group having cells with gonoid or centric ornamentation (Fig. 17.2). *Melosira*, a common golden-brown diatom found in marine and freshwater environments, consists of cylindrical cells with a greater length than breadth (Figs. 17.35, 17.40, 17.41, 17.42). There are a number of girdle bands which form incomplete rings around the cell without their ends meeting. Both the valve and the girdle bands are ornamented with small spines. The diatom is usually filamentous, with the cells joined end to end by their valves so that to the inexperienced eye it resembles a filamentous chrysophycean alga with the cells always in girdle view. Gametogenesis is initiated after the cells have undergone a number of mitotic cell divisions that have reduced the size of the frustule below a certain critical limit. Von Stosch (1951) has elucidated the following events in the life cycle of *Melosira varians* (Fig. 17.42). In the male cells, gametogenesis starts with the nucleus undergoing two meiotic divisions to produce four

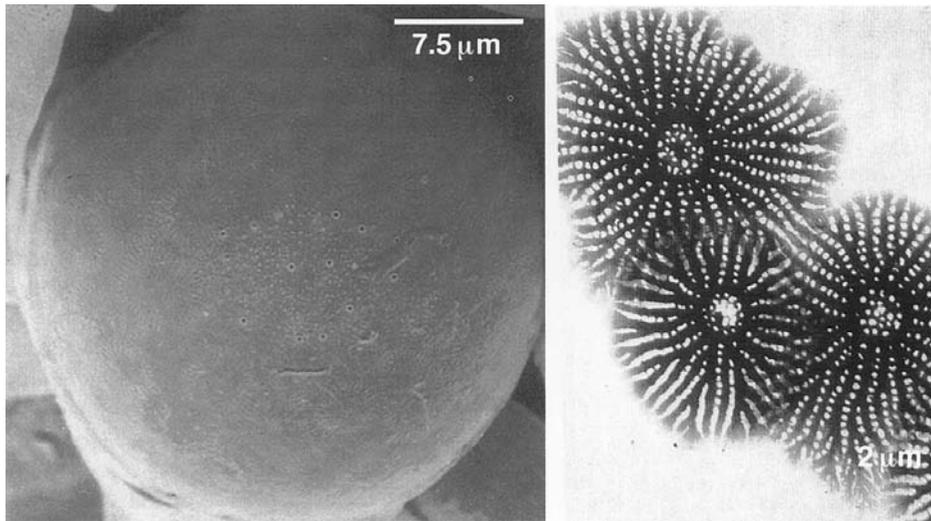
haploid nuclei. A plasma membrane is formed around each nucleus and a small amount of parent cell protoplasm. The remaining protoplasm of the mother cell now lacks a nucleus and begins to degenerate. A single flagellum is produced by each male gamete, the epitheca separates from the hypotheca, and the male gametes are released into the medium. In the female cells, while the nucleus is undergoing the first meiotic division, one of the girdle bands separates from the edge of the valve, leaving an area of protoplasm open to the medium. The male gamete swims to the area of the female cell where the girdle band has separated from the valve edge. During telophase of the first meiotic division of the female cell, the cytoplasm of the male gamete fuses with that of the female cell. One of the nuclei of the first meiotic division in the female cell degenerates while the other nucleus undergoes a second meiotic division. From the second meiotic division, one of the female nuclei again degenerates, and the other becomes the egg nucleus. The male nucleus fuses with the egg nucleus to form the zygote or auxospore nucleus. The resulting auxospore (Fig. 17.43) then swells, pushing apart the hypotheca and epitheca until the auxospore is attached to both or one theca by a small protuberance only. The auxospore has an outer organic wall and an inner wall made of siliceous scales (Crawford, 1974). A new cell wall of maximum dimensions is now produced within



**Fig. 17.41** Semidiagrammatic representation of a cell of *Melosira varians* composed of two valves, V and V', two girdle band series: 1, 2, 3, and, underlapping these, the younger series 1', 2', 3'. (After Crawford, 1971.)



**Fig. 17.42** The life cycle of *Melosira varians*. (Adapted from von Stosch, 1951.)



**Fig. 17.43** Electron micrographs of an auxospore of *Melosira nummuloides* (left) showing the scales covering the auxospore (right). (From Medlin et al., 1993.)

the auxospore. This cell divides to give rise to a new filament.

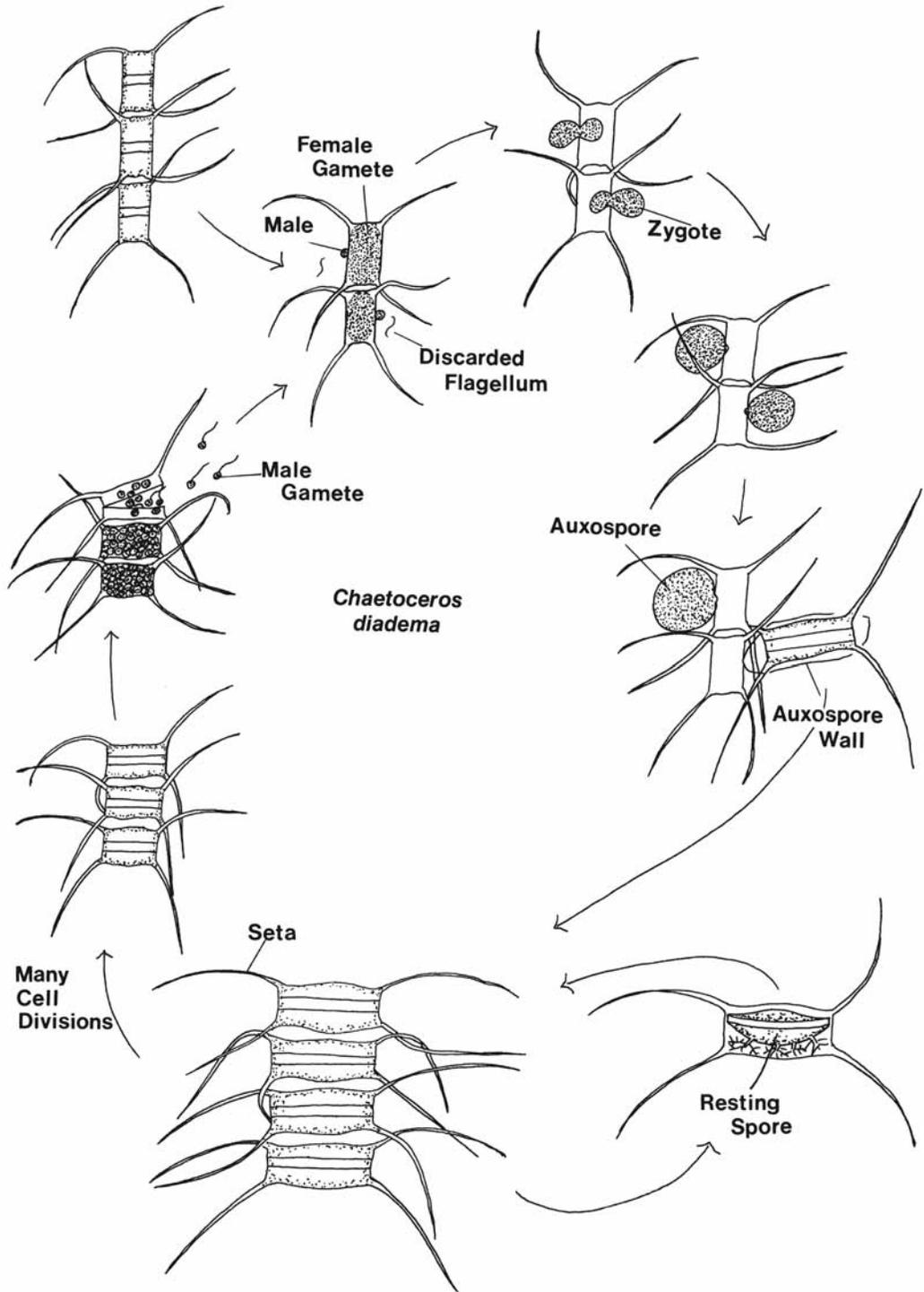
*Melosira* is capable of producing dormant cells in freshwater environments. The dormant cells have the protoplasm condensed to a dark-brown protoplasmic mass in one part of the frustule (Sicko-Goad et al., 1986). The dormant cells settle into the sediment, where they are capable of surviving for up to 20 years. Normally, however, the dormant cells are swept up by normal recirculation of lake waters during lake overturns (Lund, 1959). Within 24 hours of being swept out of the sediments, the dormant cells of *Melosira* differentiate into vegetative cells.

*Chaetoceros* (Figs. 17.6(a), 17.32, 17.44, 17.45) has more than 160 species, the largest number of any planktonic diatom. The genus is widespread in warm and cold waters. The life cycle of *Chaetoceros diadema* has been elucidated by Hargraves (1972) (Fig. 17.44). *Chaetoceros diadema* is a colonial marine diatom, with cells united by their long setae to form filaments. The different phases of the life cycle are characterized by different sizes and shapes of cells. Gametogenesis is initiated at temperatures between 2 and 5 °C (French and Hargraves, 1985). The cells that produce the male gametes are about 1.5 to 2 times as wide as high.

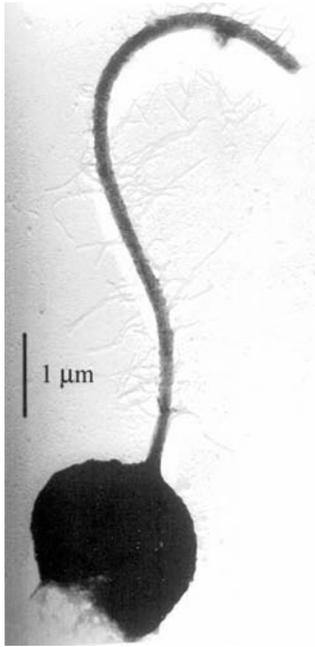
These cells divide to produce 32 male gametes, presumably by meiosis, which are released as uniflagellate swimmers. The cells that produce the female gametes are about twice as high as wide. The exact nuclear events that lead to the production of the egg are not known, but the male gamete (Fig. 17.45) swims to the girdle area of the female cell, casts off its flagellum, and fuses with the female gamete inside the frustule. The cell contents of the auxospore-forming cell are then extruded through the girdle region into a thin hyaline envelope. This cell then produces the auxospore, which, in turn, produces new cells with frustules of maximum size. These large daughter cells remain attached to the auxospore parent wall through two divisions of the daughter cells. The hyaline envelope of the auxospore is sloughed off after the first division. Any of the vegetative cells have the ability to produce resting spores after nitrogen depletion of the culture medium at temperatures that allow vegetative growth (French and Hargraves, 1985). The resting spores germinate to produce cells larger than the original cells. The resting spores occur within the parent cell as heavily siliceous bodies, having usually convex, often dissimilar, upper and lower surfaces bearing spines.

### Bacillariales

The Bacillariales or Pennales order contains cells that occur both in freshwater and in marine

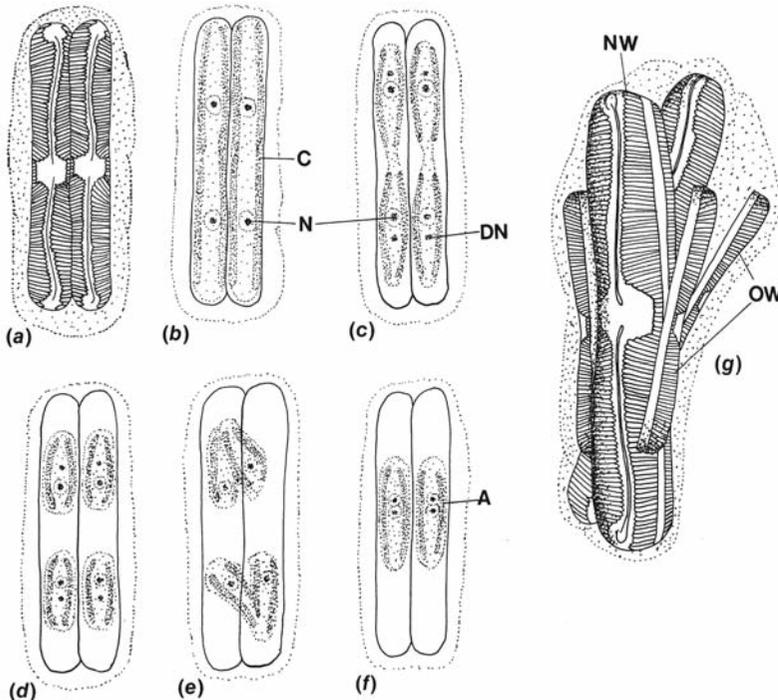


**Fig. 17.44** The life cycle of *Chaetoceros diadema*. (Adapted from Hargraves, 1972.)



**Fig. 17.45** *Chaetoceros lacinosus*. A transmission electron micrograph of a shadowed preparation of a whole mount of a spermatozoid showing the single tinsel flagellum. (From Jensen et al., 2003.)

environments. These cells have either pennate or trellisoid ornamentation (Fig. 17.2). The life cycle of these organisms involves fusion of two gametes by conjugation and common genera such as *Nitzschia* (Figs. 17.26(b), 17.35), *Pseudo-nitzschia* (Figs. 17.27, 17.29), *Navicula* (Figs. 17.2(d), 17.16, 17.25, 17.26(a), 17.33(b), 17.35), *Amphora* (Figs. 17.22(c), (d), 17.35), *Cymbella*, and *Pinnularia* (Figs. 17.3(a), 17.12, 17.18, 17.46) have essentially the same life cycle. In *Pinnularia*, the cells have rounded poles with more or less parallel sides (Fig. 17.46). Auxospore formation is a sexual process initiated after cell divisions have reduced the cell to a certain critical size. Two cells come together and invest themselves in a mucous envelope (Hendey, 1964). The nucleus in each cell undergoes two meiotic divisions followed by cytokinesis into two cells. Two gametes are thus formed per mother cell, each protoplast receiving one functional and one degenerating nucleus. One gamete from each mother cell moves to the contiguous mother cell to fuse with the passive gamete. In this manner, one zygote (auxospore) is formed within each mother cell. These auxospores become greatly enlarged, and eventually new siliceous frustules



**Fig. 17.46** Auxospore formation in *Pinnularia*. (a) Two cells lie next to each other. (b)–(d) Meiosis occurs in each mother cell, resulting in two gametes per mother cell, each with one functional nucleus (N) and one degenerate nucleus (DN). (e) One gamete from each mother cell passes to the other mother cell. (f) There is one auxospore (A) per mother cell frustule. (g) Each auxospore has produced a new large daughter cell (NW), with the old cell walls (OW) of the mother cells still embedded in the mucilage. (C) Chloroplast. (After Hendey, 1964.)

of maximum size are produced within each auxospore. The four discarded valves of the mother cells are often seen attached to the newly formed frustules.

The life cycle of *Pseudo-nitzschia* (Fig. 17.29) is similar to *Pinnularia*.

## REFERENCES

- Alcoverro, T., Conte, E., and Mazzella, L. (2000). Production of mucilage by the Adriatic epipelagic diatom *Cylindrotheca closterium* (Bacillariophyceae) under nutrient limitation. *J. Phycol.* 36:1087–95.
- Andersen, L. W. J., and Sweeney, B. M. (1977). Diel changes in sedimentary characteristics of *Ditylum brightwelli*: Changes in cellular lipid and effects of respiratory inhibitors and ion transport modifiers. *Limnol. Oceanogr.* 22:539–52.
- Andersen, R. A., Medlin, L. K., and Crawford, R. M. (1986). An investigation of the cell wall components of *Actinocyclus subtilis* (Bacillariophyceae). *J. Phycol.* 22:466–79.
- Anderson, O. R. (1975). The ultrastructure and cytochemistry of resting cell formation in *Amphora coffaeformis* (Bacillariophyceae). *J. Phycol.* 11:272–81.
- Anderson, O. R. (1976). Respiration and photosynthesis during resting cell formation in *Amphora coffaeformis* (Ag.) Kütz. *Limnol. Oceanogr.* 21:452–6.
- Bates, S. S. (2000). Domoic-acid-producing diatoms: another genus added. *J. Phycol.* 36:978–85.
- Beattie, A., Hirst, E. L., and Percival, E. (1961). Studies on the metabolism of the Chrysophyceae. Comparative structural investigations on leucosin (chrysolaminarin) separated from diatoms and laminarin from brown algae. *Biochem. J.* 79:531–6.
- Bidle, K. D., and Azam, F. (1999). Accelerated dissolution of diatom silica by marine bacterial assemblages. *Nature* 397:508–12.
- Branton, D., Cohen, C. M., and Tyler, T. (1981). Interaction of cytoskeletal proteins on the human erythrocyte membrane. *Cell* 24:24–32.
- Brzezinski, M. A., and Conley, D. J. (1994). Silicon deposition during the cell cycle of *Thalassiosira weissflogii* (Bacillariophyceae) determined using dual rhodamine 123 and propidium iodide staining. *J. Phycol.* 30:45–55.
- Caldwell, G. S., Bentley, M. G., and Olive, P. J. W. (2004). First evidence of sperm motility inhibition by the diatom aldehyde 2E,4E-decadienal. *Mar. Ecol. Progr. Ser.* 273:97–108.
- Casotti, R., Mazza, S., Brunet, C., Vantrepotte, V., Ianora, A., and Miralto, A. (2005). Growth inhibition and toxicity of the diatom aldehyde 2-trans,4-trans-decadienal on *Thalassiosira weissflogii*. *J. Phycol.* 41:7–20.
- Chepurnov, V. A., Mann, D. G., Sabbe, K., and Vyverman, W. (2004). Experimental studies on sexual reproduction in diatoms. *Int. Rev. Cytol.* 237:91–154.
- Chiovitti, A., Bacic, A., Burke, J., and Wetherbee, R. (2003). Heterogenous xylose-rich glycans are associated with extracellular glycoproteins in the biofouling diatom *Craspedostauros australis* (Bacillariophyceae). *Eur. J. Phycol.* 38:351–60.
- Chiovitti, A., Molino, P., Crawford, S. A., Teng, R., Spurck, T., and Wetherbee, R. (2004). The glucans extracted with warm water from diatoms are mainly derived from intracellular chrysolaminarin and not extracellular polysaccharides. *Eur. J. Phycol.* 39:117–28.
- Clavero, E., Hernandez-Marine, M., Grimalt, J. O., and Garcia-Pichel, F. (2000). Salinity tolerance of diatoms from thalassic hypersaline environments. *J. Phycol.* 36:1021–34.
- Cohn, S. A., and Weitzell, R. E. (1996). Ecological consideration of diatom cell motility. I. Characterization of motility and adhesion in four diatom species. *J. Phycol.* 32:918–39.
- Cohn, S. A., Spurck, T. P., and Pickett-Heaps, J. D. (1989). Perizonium and initial valve formation in the diatom *Navicula cuspidata* (Bacillariophyceae). *J. Phycol.* 25:15–26.
- Cooksey, K. E., and Cooksey, B. (1974). Calcium deficiency can induce the transition from oval to fusiform cells in cultures of *Phaeodactylum tricoratum* Bohlin. *J. Phycol.* 10:89–90.
- Coombs, J., and Volcani, B. E. (1968). Studies on the biochemistry and fine structure of silica shell formation in diatoms. Chemical changes in the wall of *Navicula pelliculosa* during its formation. *Planta* 82:280–92.
- Crawford, R. M. (1971). The fine structure of the frustule of *Melosira varians* C. A. Agardh. *Br. Phycol. J.* 6:175–86.
- Crawford, R. M. (1974). The auxospore wall of the marine diatom *Melosira nummuloides* (Dillw.) C. Ag. and related species. *Br. Phycol. J.* 9:9–20.
- Crawford, S. A., Higgins, M. J., Mulvaney, P., and Wetherbee, R. (2001). Nanostructure of the diatom frustule as revealed by atomic force and scanning electron microscopy. *J. Phycol.* 37:543–54.
- Damste, J. S. S., Muyzer, G., Abbas, B., et al. (2004). The rise of rhizosolenid diatoms. *Science* 304:584–7.
- Davidovich, N. A. and Bates, S. S. (1998). Sexual reproduction in the pennate diatoms *Pseudo-nitzschia multi-*

- series and *P. pseudodelicatissima* (Bacillariophyceae). *J. Phycol.* 34:126–37.
- Davis, C. O., Hollibaugh, J. T., Seibert, D. L. R., Thomas, W. H., and Harrison, P. J. (1980). Formation of resting spores by *Leptocylindrus danicus* (Bacillariophyceae) in a controlled experimental ecosystem. *J. Phycol.* 16:296–302.
- Dawson, P. A. (1973). Observations on the structure of some forms of *Gomphonema parvulum* Kütz. III. Frustule formation. *J. Phycol.* 9:353–64.
- de Brouwer, J. F. C., and Stal, L. J. (2002). Daily fluctuation of exopolymers in cultures of the benthic diatoms *Cylindrotheca closterium* and *Nitzschia* sp. (Bacillariophyceae). *J. Phycol.* 38:464–72.
- Del Amo, Y., and Brzezinski, M. A. (1999). The chemical form of dissolved Si taken up by marine diatoms. *J. Phycol.* 35:1162–70.
- Drum, R. W., and Hopkins, J. T. (1966). Diatom locomotion: An explanation. *Protoplasma* 62:1–33.
- Edgar, L. A. (1979). Diatom locomotion: Computer assisted analysis of cine film. *Br. Phycol. J.* 14:83–101.
- Edlund, M. B., and Stoermer, E. F. (1997). Ecological, evolutionary, and systematic significance of diatom life histories. *J. Phycol.* 33:897–918.
- Erickson, S. J. (1972). Toxicity of copper to *Thalassiosira pseudonana* in unenriched inshore seawater. *J. Phycol.* 8:318–23.
- Fairchild, E., and Sheriden, R. P. (1974). A physiological investigation of the hot spring diatom *Achnanthes exigua*. Grün. *J. Phycol.* 10:1–4.
- Fauré-Fremiet, E. (1951). The tidal rhythm of the diatom *Hantzschia amphioxys*. *Biol. Bull.* 100:173–7.
- Fisher, A. E., and Harrison, P. J. (1996). Does carbohydrate content effect the sinking rate of marine diatoms. *J. Phycol.* 32:360–5.
- French, F. W., and Hargraves, P. E. (1985). Spore formation in the life cycles of the diatoms *Chaetoceros diadema* and *Leptocylindrus danicus*. *J. Phycol.* 21:477–83.
- Granum, E., and Myklesstad, S. M. (2001). Mobilization of  $\beta$ -1,3-glucan and biosynthesis of amino acids induced by  $\text{NH}_4^+$  addition to N-limited cells of the marine diatom *Skeletonema costatum* (Bacillariophyceae). *J. Phycol.* 37:772–82.
- Gross, F. (1940). The development of isolated resting spores into auxospores in *Ditylum brightwelli* (West). *J. Mar. Biol. Assoc. UK* 24:375–80.
- Guillou, L., Chretiennot-Dinet, M.-J., Medlin, L. K., Claustre, H., Loiseaux-de Goer, S., and Vaultot, D. (1999). *Bolidomonas*: a new genus with two species belonging to a new algal class, the Bolidophyceae (Heterokonta). *J. Phycol.* 35:368–81.
- Hargraves, P. E. (1972). Studies on marine planktonic diatoms. I. *Chaetoceros diadema* (Ehr.) Gran.: Life cycle, structural morphology, and regional distribution. *Phycologia* 11:247–57.
- Hasle, G. R. (1995). *Pseudo-nitzschia pungens* and *P. multi-series* (Bacillariophyceae): nomenclature history, morphology and distribution. *J. Phycol.* 31:428–35.
- Hasle, G. R., and Lange, C. B. (1989). Freshwater and brackish water *Thalassiosira* (Bacillariophyceae): taxa with tangentially undulated valves. *Phycologia* 28:120–35.
- Hecky, R. F., Mopper, K., Kilham, P., and Degens, E. T. (1973). The amino acid and sugar composition of diatom cell walls. *Mar. Biol.* 19:323–31.
- Hellebust, J. A. (1971). Glucose uptake by *Cyclotella cryptica*: Dark induction and light inactivation of transport system. *J. Phycol.* 7:345–49.
- Hendey, N. I. (1964). An introductory account of the smaller algae of British coastal waters. Part V. Bacillariophyceae (diatoms). *Fish. Invest. Ser. IV*. H.M.S.O., London.
- Hendey, N. I. (1971). Electron microscope studies and the classification of diatoms. In *The Micropaleontology of Oceans*, ed. B. M. Funnell, and W. R. Riedel, pp. 625–31. Cambridge: Cambridge University Press.
- Higgins, M. J., Molino, P., Mulvaney, P., and Wetherbee, R. (2003). The structure and nanomechanical properties of the adhesive mucilage that mediates diatom-substratum adhesion and motility. *J. Phycol.* 39:1181–93.
- Hildebrand, M. (2004). Silicic acid transport and its control during cell wall silicification in diatoms. In *Biomineralization: Progress in Biology, Molecular Biology and Application*, ed. E. Baeuerlein, pp. 158–76. Weinheim: Wiley-VCH.
- Hoagland, K. D., Rosowski, J. R., Gretz, M. R., and Romer, S. C. (1993). Diatom extracellular polymeric substances: Function, fine structure, chemistry, and physiology. *J. Phycol.* 29:537–66.
- Iler, R. K. (1979). *The Chemistry of Silica*. New York: John Wiley.
- Jeffrey, S. W. (1976). A report of green algal pigments in the central north Pacific Ocean. *Mar. Biol.* 37:33–7.
- Jeffrey, S. W., and Vesik, M. (1977). Effect of blue-green light on photosynthetic pigments and chloroplast structure in the marine diatom *Stephanopyxis turris*. *J. Phycol.* 13:271–9.
- Jensen, K. G., Moestrup, Ø., and Schmid, A.-M. (2003). Ultrastructure of the male gametes from two centric diatoms, *Chaetoceros lacinosus* and *Coscinodiscus wailesii* (Bacillariophyceae). *Phycologia* 42:98–105.

- Johnson, L. M., Hoagland, K. D., and Gretz, M. R. (1995). Effects of bromide and iodide on stalk secretion in the biofouling diatom *Achnanthes longipes* (Bacillariophyceae). *J. Phycol.* 31:401–12.
- Knudson, B. M. (1957). Ecology of the epiphytic diatom *Tabellaria flocculosa* (Roth) Kütz. var. *flocculosa* in three English lakes. *J. Ecol.* 45:93–112.
- Kroger, N. (2001). The sweetness of diatom molecular engineering. *J. Phycol.* 37:657–8.
- Kroger, N., and Sumper, M. (2004). The molecular basis of diatom biosilica formation. In *Biomineralization: Progress in Biology, Molecular Biology and Application*, ed. E. Baeuerlein, pp. 137–58. Weinheim: Wiley-VCH.
- Kroger, N., Deutzmann, R. and Sumper, M. (1999). Polycationic peptides from diatom biosilica that direct silica nanosphere formation. *Science* 286:1129–32.
- Kroger, N., Deutzmann, R. and Sumper, M. (2000). Species-specific polyamines from diatoms control silica morphology. *Proc. Natl. Acad. Sci., USA* 97:14133–8.
- Lauritis, J. A., Coombs, J., and Volcani, B. E. (1968). Studies on the biochemistry and fine structure of silica shell formation in diatoms. IV. Fine structure of the apochlorotic diatom *Nitzschia alba* Lewin and Lewin. *Arch. Mikrobiol.* 62:1–16.
- Lewin, J. C. (1953). Heterotrophy in diatoms. *J. Gen. Microbiol.* 9:305–13.
- Lewin, J. C. (1962). Silicification. In *Physiology and Biochemistry of the Algae*, ed. R. A. Lewin, pp. 445–55. New York: Academic Press.
- Lewin, J. C. (1966). Silicon metabolism in diatoms. V. Germanium dioxide, a specific inhibitor of diatom growth. *Phycologia* 6:1–12.
- Lewin, J. C., and Lewin, R. A. (1960). Auxotrophy and heterotrophy in marine littoral diatoms. *Can. J. Microbiol.* 6:127–34.
- Lewin, J. C., and Lewin, R. A. (1967). Culture and nutrition of some apochlorotic diatoms of the genus *Nitzschia*. *J. Gen. Microbiol.* 46:361–7.
- Li, C-W., Chu, S., and Lee, M. (1989). Characterizing the silica deposition vesicle of diatoms. *Protoplasma* 151:156–63.
- Lund, J. W. G. (1949). Studies on *Asterionella*. I. The origin and nature of the cells producing seasonal maxima. *J. Ecol.* 37:389–419.
- Lund, J. W. G. (1950). Studies on *Asterionella formosa* Hass. II. Nutrient depletion and the spring maximum. *J. Ecol.* 38:1–14, 15–35.
- Lund, J. W. G. (1959). Buoyancy in relation to the ecology of freshwater phytoplankton. *Br. Phycol. Bull.* 7:17.
- Lundholm, N., and Moestrup, O. (2000). Morphology of the marine diatom *Nitzschia navis-varingica*, sp. nov. (Bacillariophyceae), another producer of the neurotoxin domoic acid. *J. Phycol.* 36:1162–74.
- McQuoid, M. R., and Hobson, L. A. (1996). Diatom resting stages. *J. Phycol.* 32:889–902.
- Mann, J. E., and Myers, J. (1968). On pigments, growth and photosynthesis of *Phaeodactylum tricorutum*. *J. Phycol.* 4:349–55.
- Mansour, M. P., Volkman, J. K., Jackson, A. E., and Blackburn, S.I. (1999). The fatty acid and sterol composition of five marine dinoflagellates. *J. Phycol.* 35:710–29.
- Martinez, L. A., Silver, M. W., King, J. M., and Alldredge, A. L. (1983). Nitrogen fixation by floating diatom mats: A source of new nitrogen to oligotrophic ocean waters. *Science* 221:152–4.
- Martin-Jezequel, V., Hildebrand, M., and Brzezinski, M. A. (2000). Silicon metabolism in diatoms: implications for growth. *J. Phycol.* 36:821–40.
- Medlin, L. K., and Kaczmarka, I. (2004). Evolution of the diatoms: V. Morphological and cytological support for the major clades and a taxonomic revision. *Phycologia* 43:245–70.
- Medlin, L. K., Crawford, R. M., and Andersen, R. A. (1986). Histochemical and ultrastructural evidence for the function of the labiate process in the movement of centric diatoms. *Br. Phycol. J.* 21:297–301.
- Medlin, L. K., Williams, D. M., and Sims, P. A. (1993). The evolution of the diatoms (Bacillariophyta). I. Origin of the group and assessment of the monophyly of its major divisions. *Eur. J. Phycol.* 28:261–75.
- Medlin, L. K., Kooistra, W. H. C. F., Gersonde, R., Sims, P. A., and Wellbrock, U. (1997). Is the origin of diatoms related to the end-Permian mass extinction? *Nova Hedwigia* 65:1–11.
- Mehta, S. C., Venkataraman, G. S., and Das, S. C. (1961). The fine structure and the cell wall nature of *Diatoma hiemale* var. *mesodan* (Her.) Grun. *Rev. Algol., N.S.* 6:49–52.
- Miralto, A., Barone, G., Romano, G. et al. (1999). The insidious effect of diatoms on copepod reproduction. *Nature* 402:173–6.
- Moore, J. K., and Villareal, T. A. (1996). Size-ascent rate relationships in positively buoyant marine diatoms. *Limnol. Oceanog.* 41:1514–20.
- Morel, N. M. L., Rueter, J. G., and Morel, F. M. M. (1978). Copper toxicity to *Skeletonema costatum*. *J. Phycol.* 14:43–8.
- Nagai, S., Hori, Y., Manabe, T., and Imai, I. (1995). Restoration of cell size by vegetative cell enlargement

- in *Coscinodiscus wailestii* (Bacillariophyceae). *Phycologia* 34:533–55.
- Nakajima, T., and Volcani, B. E. (1969). 3,4-Dihydroxyproline: a new amino acid from diatom cell walls. *Science* 164:1400–1.
- Navarro, J. N. (1993). Three-dimensional imaging of diatom ultrastructure with high resolution low-voltage SEM. *Phycologia* 32:151–6.
- Nultsch, W. (1956). Studien über die Phototaxis der Diatomeen. *Arch. Protistenk.* 101:1–68.
- Pan, Y., Subba Rao, D. V., and Mann, K. H. (1996). Changes in domoic acid production and cellular chemical composition of the toxigenic diatom *Pseudo-nitzschia multiseries* under phosphate limitation. *J. Phycol.* 32:371–81.
- Perry, C. C., and Keeling-Tucker, T. (2000). Biosilicification: the role of the organic matrix in structure control. *J. Biol. Inorg. Chem.* 5:537–50.
- Pickett-Heaps, J. D., Tippit, D. H., and Andreozzi, J. A. (1979a). Cell division in the pennate diatom *Pinnularia*. III. The valve and associated organelles. *Biol. Cellulaire* 35:195–8.
- Pickett-Heaps, J. D., Tippit, D. H., and Andreozzi, J. A. (1979b). Cell division in the pennate diatom *Pinnularia*. IV. Valve morphogenesis. *Biol. Cellulaire* 35:199–206.
- Pickett-Heaps, J. D., Hill, D. R. A., and Wetherbee, R. (1986). Cellular movement in the centric diatom *Odontella sinensis*. *J. Phycol.* 22:334–9.
- Pickett-Heaps, J. D., Carpenter, J., and Koutoulis, A. (1994). Valve and seta (spine) morphogenesis in the centric diatom *Chaetoceros peruvianus* Brightwell. *Protoplasma* 181:269–82.
- Pohnert, G. (2002). Phospholipase A<sub>2</sub> activity triggers the wound-activated chemical defense in the diatom *Thalassiosira rotula*. *Plant Physiol.* 129:103–11.
- Pohnert, G., and Boland, W. (2002). The oxylipin chemistry of attraction and defense in brown algae and diatoms. *Nat. Prod. Rep.* 19:108–22.
- Pohnert, G., Lumineau, O., Cueff, A., et al. (2002). Are volatile unsaturated aldehydes from diatoms the main line of chemical defence against copepods? *Mar. Ecol. Progr. Ser.* 245:33–45.
- Potapova, M., and Snoeijs, P. (1997). The natural life cycle in the wild populations of *Diatoma moniliformis* (Bacillariophyceae) and its disruption in an aberrant environment. *J. Phycol.* 33:924–37.
- Poulin, M., Masse, G., Belt, S. T., Delavault, P., Rousseau, J.-M., and Rowland, S. J. (2004). Morphological, biochemical and molecular evidence for the transfer of *Gyrosigma nipkowii* Meister to the genus *Halsea* (Bacillariophyta). *Eur. J. Phycol.* 39:181–95.
- Prasad, A. K. S. K., Nienow, J. A., and Livingstone, R. J. (1990). The genus *Cyclotella* (Bacillariophyta) in Choctawhatchee Bay Florida, with special reference to *C. striata* and *C. choctawhatcheana* sp. nov. *Phycologia* 29:418–36.
- Prasad, A. K. S. K., Riddle, K. A., and Nienow, J. A. (2000). Marine diatom genus *Climaconeis* (Berkeleyaceae, Bacillariophyta): two new species, *Climaconeis koenigii*, and *C. colemaniae*, from Florida Bay, U.S.A. *Phycologia* 39:199–211.
- Reavic, E. D., Smol, J.-P., Carignan, R., and Lorrain, S. (1998). Diatom paleolimnology of two fluvial lakes in the St. Lawrence River. A reconstruction of environmental changes during the last century. *J. Phycol.* 34:446–56.
- Reimann, B. E. F., Lewin, J. C., and Volcani, B. E. (1965). Studies on the biochemistry and fine structure of silica shell formation in diatoms. I. The structure of the cell wall of *Cylindrotheca fusiformis* Reimann and Lewin. *J. Cell Biol.* 24:39–55.
- Reisen, W. K., and Spencer, D. J. (1970). Succession and current demand relationships of diatoms on artificial substrates in Prater's Creek, South Carolina. *J. Phycol.* 6:117–21.
- Richardson, J. L., Mody, N. S., and Stacey, M. E. (1996). Diatoms and water quality in Lancaster County (PA) streams: a 45-year perspective. *Pennsylvania Acad. Sci.* 70:30–9.
- Rogall, E. (1939). Über den Feinbau der Lieselmembran der Diatomeen. *Planta* 29:279–91.
- Ross, R., and Sims, P. A. (1972). The fine structure of the frustule in centric diatoms: A suggested terminology. *Br. Phycol. J.* 7:139–63.
- Round, F. E. (1957). The late-glacial and post-glacial diatom succession in the Kentmere valley deposit. I. Introduction, methods and flora. *New Phytol.* 56:98–126.
- Round, F. E. (1961). The diatoms of a core from Esthwaite water. *New Phytol.* 60:43–59.
- Round, F. E., Crawford, R. M., and Mann, D. G. (1990). *The Diatoms*. Cambridge: Cambridge University Press. 747 pp.
- Rueter, J. G. (1983). Effect of copper on growth, silicic acid uptake and soluble pools of silicic acid in the marine diatom, *Thalassiosira weissflogii* (Bacillariophyceae). *J. Phycol.* 19:101–4.
- Rueter, J. G., Chisholm, S. W., and Morel, F. M. M. (1981). Effects of copper toxicity on silicic acid uptake and growth in *Thalassiosira pseudonana*. *J. Phycol.* 17:270–8.
- Sato, S., Nagumo, T., and Tanaka, J. (2004). Auxospore formation and the morphology of the initial cell of

- marine araphid diatom *Gephyria media* (Bacillariophyceae). *J. Phycol.* 40:684–91.
- Schmaljohann, R., and Röttger, R. (1978). The ultrastructure and taxonomic identity of the symbiotic algae of *Heterostegina depressa* (Foraminifera: Nummulitidae). *J. Mar. Biol. Assoc. UK* 58:227–37.
- Schmid, A.-M. M., and Crawford, R. M. (2001). *Ellerbeckia arenaria* (Bacillariophyceae): formation of auxospores and initial cells. *Eur. J. Phycol.* 36:307–20.
- Schmid, A.-M. M., and Volcani, B. E. (1983). Wall morphogenesis in *Coscinodiscus wailesii* Gran and Angst. I. Valve morphology and development of its architecture. *J. Phycol.* 19:387–402.
- Shipe, R. F., Brzezinski, M. A., Pilskaln, C., and Villareal, T. A. (1999). *Rhizosolenia* mats: an overlooked source of silica production in the open sea. *Limnol. Oceanogr.* 44:1282–92.
- Sicko-Goad, L., Stoermer, E. F., and Fahnensteil, G. (1986). Rejuvenation of *Melosira granulata* (Bacillariophyceae) resting cells from anoxic sediments of Douglas Lake, Michigan. I. Light microscopy and  $^{14}\text{C}$  uptake. *J. Phycol.* 22:22–8.
- Smayda, T. J. (1970). The suspension and sinking of phytoplankton in the sea. *Oceanogr. Mar. Biol. Annu. Rev.* 8:353–414.
- Staats, N., de Winder, B., Stal, L. J., and Mur, L. R. (1999). Isolation and characterization of extracellular polysaccharides from the epipelagic diatoms *Cylindrotheca closterium* and *Navicula salinarum*. *Eur. J. Phycol.* 34:161–9.
- Stoermer, E. F., Pankratz, H. S., and Drum, R. W. (1965). The fine structure of *Mastogloia grevillei* Wm. Smith. *Protoplasma* 59:1–13.
- Subba Rao, D. V., Quilliam, M. A., and Pocklington, R. (1988). Domoic acid – a neurotoxic amino acid produced by the marine diatom *Nitzschia pungens* in culture. *Can. J. Fish. Aquat. Sci.* 45:2076–9.
- Sullivan, C. W. (1977). Diatom mineralization of silicic acid. II. Regulation of  $\text{Si}(\text{OH})_4$  transport rates during the cell cycle of *Navicula pelliculosa*. *J. Phycol.* 13:86–91.
- Thomas, W. H., Hollibaugh, J. T., and Seibert, D. L. R. (1980). Effects of heavy metals on the morphology of some marine phytoplankton. *Phycologia* 19:202–9.
- Tilman, D., Kilham, S. S., and Kilham, P. (1976). Morphometric changes in *Asterionella formosa* colonies: phosphate and silicate limitation. *Limnol. Oceanogr.* 21:883–6.
- Venrich, E. L., McGowan, J. A., and Mantyla, A. W. (1973). Deep maxima of photosynthetic chlorophyll in the Pacific Ocean. *Fish. Bull.* 71:41–52.
- Villareal, T. A. (1989). Division cycles in the nitrogen-fixing *Rhizosolenia* (Bacillariophyceae)–*Richelia* (Nostocaceae) symbiosis. *Br. Phycol. J.* 24:357–65.
- Villareal, T. A., and Carpenter, E. J. (1994). Chemical composition and photosynthetic characteristics of *Ethmodiscus rex* (Bacillariophyceae): evidence for vertical migration. *J. Phycol.* 30:1–8.
- von Denffer, D. (1949). Die planktonische Massenkultur pennatur Grunddiatomeen. *Arch. Mikrobiol.* 14:159–202.
- von Stosch, H. A. (1951). Entwicklungsgeschichtliche Untersuchungen an zentrischen Diatomeen. I. Die Auxosporenbildung von *Melosira varians*. *Arch. Mikrobiol.* 16:101–35.
- von Stosch, H. A. (1975). An amended terminology of the diatom girdle. *Nova Hedwigia, Beih.* 53:1–28.
- von Stosch, H. A. (1982). On auxospore envelopes in diatoms. *Bacillaria* 5:127–48.
- von Stosch, H. A., and Fecher, K. (1979). “Internal thecae” of *Eumotia soleirolii* (Bacillariophyceae): Development, structure and function as resting spores. *J. Phycol.* 15:233–43.
- Vrieling, E. G., Gieskes, W. W. C., and Beelen, T. P. M. (1999). Silicon deposition in diatoms: control by the pH inside the silicon deposition vesicle. *J. Phycol.* 35:548–59.
- Wagner, J. (1934). Beiträge zur Kenntnis der *Nitzschia putrida* Benecke, insbesondere ihrer Bewegung. *Arch. Protistenk.* 82:86–113.
- Wang, Y., Chen, Y., Lain, C., and Gretz, M. R. (2000). Extracellular matrix assembly in diatoms (Bacillariophyceae). IV. Ultrastructure of *Achnanthes longipes* and *Cymbella cistula* as revealed by high-pressure freezing/freezing substitution and cryo-field emission scanning electron microscopy. *J. Phycol.* 36:367–78.
- Wetherbee, R., Crawford, S., and Mulvaney, P. (2004). The nanostructure and development of diatom biosilica. In *Biom mineralization Progress Biology, Molecular Biology and Application*, ed. E. Baeuerlein, pp. 177–93. Weinheim: Wiley-VCH.
- White, A. W. (1974). Growth of two facultatively heterotrophic marine centric diatoms. *J. Phycol.* 10:292–300.
- Wilson, D. P. (1946). The triradiate and other forms of *Nitzschia closterium* (Ehrenberg) Wm. Smith, forma *minutissima* of Allen and Nelson. *J. Mar. Biol. Assn., U.K.* 26:235–70.

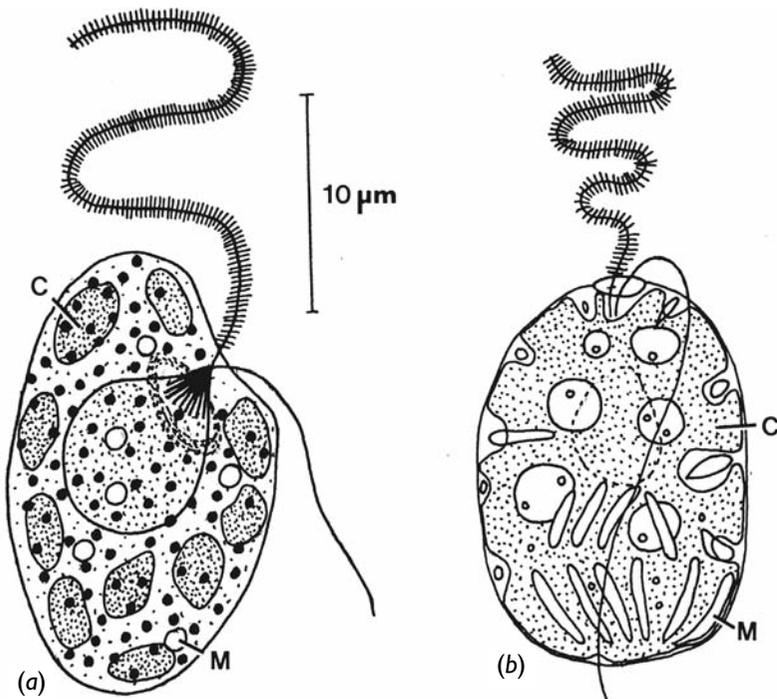
# Heterokontophyta

## RAPHIDOPHYCEAE

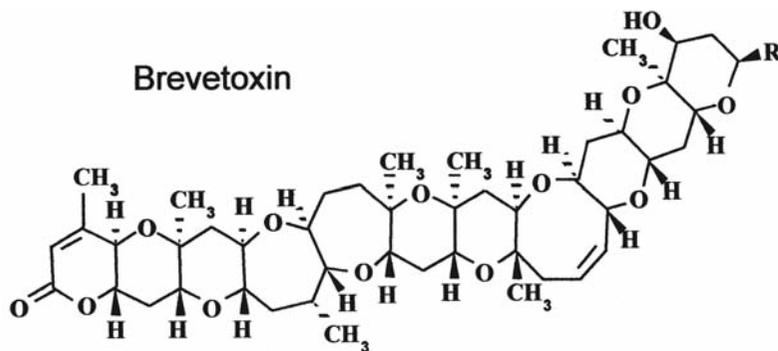
The Raphidophyceae, or chloromonads, have chlorophylls *a* and *c*, and two membranes of chloroplast endoplasmic reticulum. The anterior flagellum is commonly tinsel, whereas the posterior flagellum is naked (Figs. 18.1, 18.2, 18.3). The freshwater species of the Raphidophyceae are green, whereas the marine forms are yellowish and contain the carotenoid fucoxanthin (Vesk and

Moestrup, 1987). The closest relatives of the Raphidophyceae are the Eustigmatophyceae and the Chrysophyceae (Cavalier-Smith and Chao, 1996).

Marine species are euryhaline and eurythermic (tolerant of a wide salinity and temperature range) and occur in temperate and subtropical waters worldwide. Marine genera are *Chattonella* (Fig. 18.3), *Fibrocapsa* (Fig. 18.1(b)), and *Heterosigma* (Fig. 18.1(a)). Many of the marine species produce neurotoxic compounds that are similar to brevetoxin (Fig.18.2).



**Fig. 18.1** (a) *Heterosigma carterae*. (b) *Fibrocapsa japonica*. (C) Chloroplast; (M) mucocyst. ((a) after Leadbeater, 1969; (b) after Hara and Chihara, 1985.)



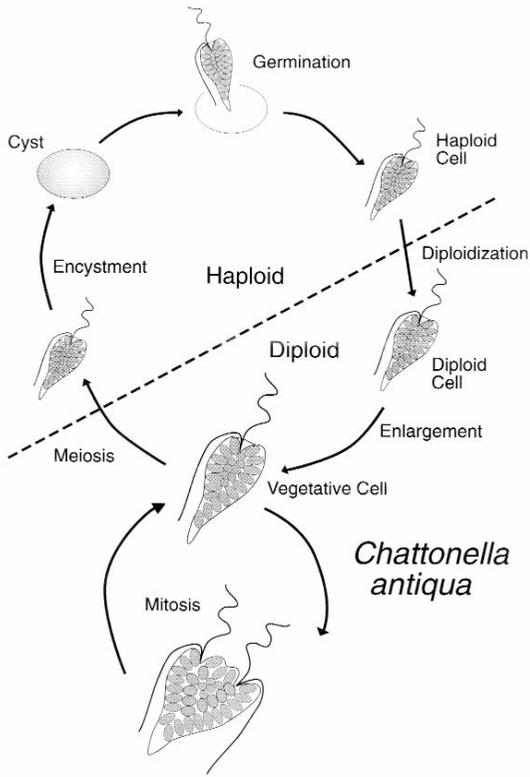
**Fig. 18.2** The chemical structure of brevetoxin.

Uptake of the toxin by fish results in depolarization of nerves supplying the heart. This reduces the heart rate, thereby lowering blood pressure, which in turn affects the transfer of oxygen to the gill lamellae, creating hypoxic conditions that lead to fish mortality (Tyrrell et al., 2001).

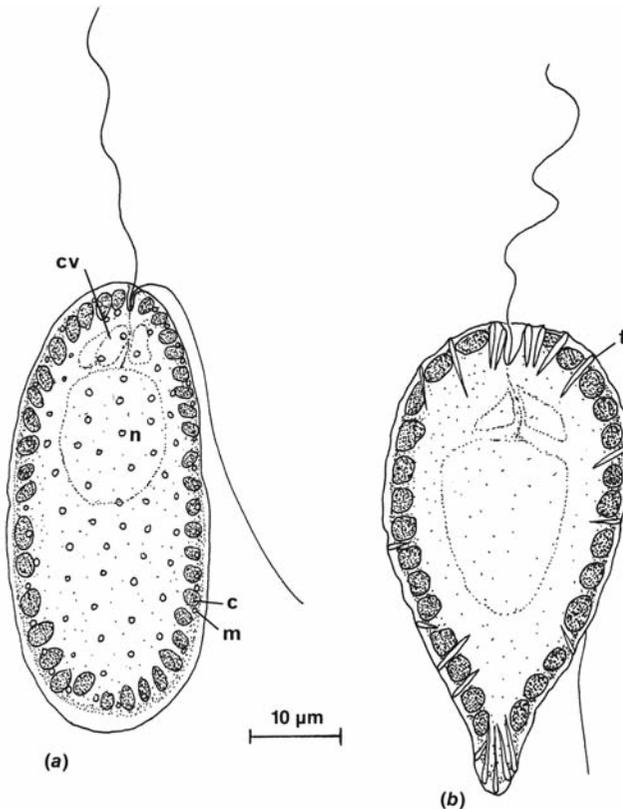
Toxic red-tide blooms of the marine *Chattonella antiqua* and *Heterosigma carterae* (Taylor, 1992) have occurred in the Seto Inland Sea in Japan (Watanabe et al., 1988). These red tides occurred in the summer when a salinity and temperature stratification occurred at a depth of 5–10 meters. There was little mixing of waters above and below the stratified layer resulting in the upper layer being deficient in nutrients while the bottom layer was anaerobic. *Heterosigma carterae* flourishes under these conditions because it has a daily vertical migration of 10 to 15 meters and this allows the alga to move between the stratified layers. This migration enables *H. carterae* to use the nutrients in the lower layers, and light and oxygen in the upper layer, resulting in the red-tide blooms of the organism. The migration is correlated with the production and degradation of cytoplasmic fat particles (Wada et al., 1987). *Heterosigma carterae* has a wide salinity tolerance in culture (3 to 50%) and loses its motility at temperatures below 10 °C. At 5 to 10 °C, the alga forms non-motile masses capable of surviving in continuous darkness for up to 15 weeks (Han et al., 2002). This is a far longer dark period than the motile cells can tolerate and still live. In Narragansett Bay, Rhode Island, blooms of *H. carterae* occur during a period of low nitrogen concentration, but at a time when phosphate levels are near a yearly maximum (Tomas, 1979).

*Chattonella antiqua* is a marine raphidophyte that produces blooms in coastal Japan. In 1972, a bloom killed 500 million dollars worth of caged yellow-tail fish in the Seto Inland Sea (Okaichi, 1989). *C. antiqua* has the highest toxicity during the early to mid-logarithmic phase. After reaching the stationary phase, the toxicity decreases markedly (Khan et al., 1996). The life cycle of *Chattonella antiqua* involves a vegetative propagation phase and a non-motile dormant phase (Fig. 18.3) (Yamaguchi and Imai, 1994). The vegetative diploid cells grow by binary fission under normal growth conditions. Small haploid cells are produced when the nutrients are depleted in the medium. These haploid cells change into cysts under low-light conditions and spend several months dormant in bottom sediments. The period of dormancy usually lasts from the end of summer to the following spring and is enforced by low temperatures (Imai and Itoh, 1987). Swimmers germinate from the cysts and somehow become diploid, although how diploidization occurs is not known. The resulting diploid vegetative cells complete the life history.

In freshwater, Raphidophyceae are associated with mud bottoms or ponds with abundant macrophytes. *Vacuolaria* contains chlorophylls *a* and *c* as well as the carotenoid  $\beta$ -carotene and the xanthophylls lutein epoxide and antheraxanthin (Chapman and Haxo, 1966; Guillard and Lorenzen, 1972). The numerous chloroplasts have three thylakoids per band, and there are two membranes of chloroplast E.R. around the chloroplasts (Koch and Schnepf, 1967; Mignot, 1967). *Vacuolaria* (Fig. 18.4(a)) can be free-swimming or in a palmelloid state. In the palmelloid condition,



**Fig. 18.3** The life cycle of *Chattonella antiqua*. (Adapted from Yamaguchi and Imai, 1994.)



**Fig. 18.4** (a) *Vacuolaria virescens*, showing chloroplasts (c), mucilaginous bodies (m), flagella, contractile vacuoles (cv), and the nucleus (n); (b) *Gonyostomum semen* with chloroplasts, trichocysts (t), contractile vacuoles, and the nucleus. (After Mignot, 1967.)

the cells are more or less spherical, 35 to 50  $\mu\text{m}$  in diameter, yellow-green with each cell surrounded by a thick gelatinous matrix. In the free-swimming state, the organisms vary considerably in shape from broadly ovate to elongate. The flagella emerge from a simple notch slightly to one side of the anterior ventral region. The anteriorly directed flagellum has microtubular hairs, whereas the posteriorly directed flagellum is whiplash. There is no stigma, but the organism is positively phototactic. *Vacuolaria* divides only in the palmelloid state. Mitosis is typically eukaryotic, with the nuclear membrane remaining intact (Heywood and Godward, 1972). No sexual reproduction has been reported in *Vacuolaria*. Occasionally the cells form cysts with the resistant sheaths surrounding the cells (Spencer, 1971).

*Vacuolaria* has muciferous bodies (mucocysts) in the peripheral cytoplasm (Fig. 18.4(a)), which are similar in structure to those in the Prymnesiophyceae and Chrysophyceae. The genus *Gonyostomum* (Fig. 18.4(b)) has trichocysts similar to those in the Dinophyceae (Mignot, 1967).

## REFERENCES

- Cavalier-Smith, T., and Chao, E. E. (1996). 18S rRNA sequence of *Heterosigma carterae* (Raphidophyceae), and the phylogeny of heterokont algae (Ochrophyta). *Phycologia* 35:500–10.
- Chapman, D. J., and Haxo, F. T. (1966). Chloroplast pigments of the Chloromonadophyceae. *J. Phycol.* 2:89–91.
- Guillard, R. R. L., and Lorenzen, C. J. (1972). Yellow-green algae with chlorophyllide *c*. *J. Phycol.* 8:10–14.
- Han, M.-S., Kim, Y.-P. and Cattolico, R. A. (2002). *Heterosigma akashiwo* (Raphidophyceae) resting cell formation in batch culture: strain identity versus physiological response. *J. Phycol.* 38:304–17.
- Hara, Y., and Chihara, M. (1985). Ultrastructure and taxonomy of *Fibrocapsa japonica* (Class Raphidophyceae). *Arch. Protistenk.* 130:133–41.
- Heywood, P., and Godward, M. B. E. (1972). Centromeric organization in the chloromonadophycean alga *Vacuolaria virescens*. *Chromosoma* 39:333–9.
- Imai, I., and Itoh, K. (1987). Annual life cycle of *Chattonella* spp., causative flagellates of noxious red tides in the Inland Sea of Japan. *Marine Biology* 94:287–92.
- Khan, S., Arakawa, O., and Onoue, Y. (1996). A toxicological study of the marine phytoflagellate, *Chattonella antiqua* (Raphidophyceae). *Phycologia* 35:239–44.
- Koch, W., and Schnepf, E. (1967). Einige elektronenmikroskopische Beobachtungen an *Vacuolaria virescens* Ciénk. *Arch. Mikrobiol.* 57:196–8.
- Leadbeater, B. S. C. (1969). A fine structural study of *Olisthodiscus luteus* Carter. *Br. Phycol. J.* 4:3–17.
- Mignot, J.-P. (1967). Structure et ultrastructure de quelques Chloromonadines. *Protistologia* 3:5–24.
- Okaichi, T. (1989). Red tide problems in the Seto Inland Sea, Japan. In *Red Tides: Biology, Environmental Science and Toxicology*, ed. T. Okaichi, D. M. Anderson, and T. Nemoto, pp. 137–42. New York: Elsevier Science Publishing.
- Spencer, L. B. (1971). A study of *Vacuolaria virescens* Cienkowski. *J. Phycol.* 7:274–9.
- Taylor, F. J. R. (1992). The taxonomy of harmful marine phytoplankton. *G. Bot. Ital.* 126:209–19.
- Tomas, C. R. (1979). *Olisthodiscus luteus* (Chrysophyceae). III. Uptake and utilization of nitrogen and phosphorus. *J. Phycol.* 15:5–12.
- Tyrrell, J. V., Bergquist, P. R., Bergquist, P. L., and Scholin, C. A. (2001). Detection and enumeration of *Heterosigma akashiwo* and *Fibrocapsa japonica* (Raphidophyceae) using rRNA-targeted oligonucleotide probes. *Phycologia* 40:457–67.
- Vesk, M., and Moestrup, Ø. (1987). The flagellar root system in *Heterosigma akashiwo* (Raphidophyceae). *Protoplasma* 137:15–28.
- Wada, M., Hara, Y., Kato, M., Yamada, M., and Fujii, T. (1987). Diurnal appearance, fine structure and chemical composition of fatty particles in *Heterosigma akashiwo* (Raphidophyceae). *Protoplasma* 137:134–9.
- Watanabe, M., Kohata, K., and Kunugi, M. (1988). Phosphate accumulation and metabolism by *Heterosigma akashiwo* (Raphidophyceae) during diel vertical migration in a stratified microsm. *J. Phycol.* 24:22–8.
- Yamaguchi, M., and Imai, I. (1994). A microfluorometric analysis of nuclear DNA at different stages in the life history of *Chattonella antiqua* and *Chattonella marina* (Raphidophyceae). *Phycologia* 33:163–70.

## Heterokontophyta

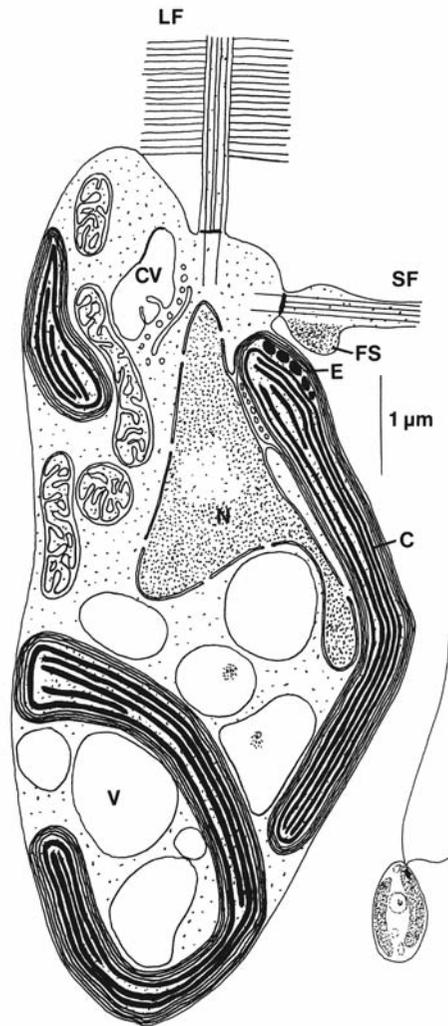
### XANTHOPHYCEAE

The Xanthophyceae contain primarily freshwater and terrestrial algae with a few marine representatives. The class is characterized by motile cells with a forwardly directed tinsel flagellum and a posteriorly directed whiplash flagellum (Figs. 19.1, 19.5(c)). The chloroplasts contain chlorophylls *a* and *c* (Sullivan et al., 1990), lack fucoxanthin, and are colored yellowish-green. The eyespot in motile cells is always in the chloroplast (Figs. 19.1, 19.5(c)), and the chloroplasts are surrounded by two membranes of chloroplast endoplasmic reticulum. The outer membrane of the chloroplast E.R. is usually continuous with the outer membrane of the nucleus. In most non-motile cells the wall is composed of two overlapping halves (Figs. 19.2 (d), (e), (f), 19.3, 19.4). Molecular data have shown the Xanthophyceae is most closely related to the Phaeophyceae (Ariztia et al., 1991; Potter et al., 1997). Although the class is commonly called the Xanthophyceae, the proper name is the Tribophyceae since there is no genus in the class that can lend its name to Xanthophyceae (Hibberd, 1981).

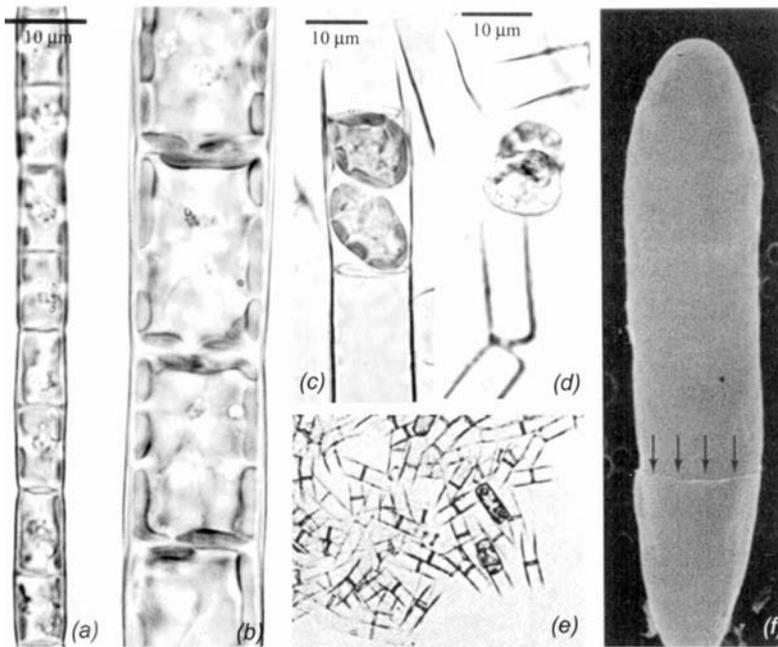
### Cell structure

#### Cell wall

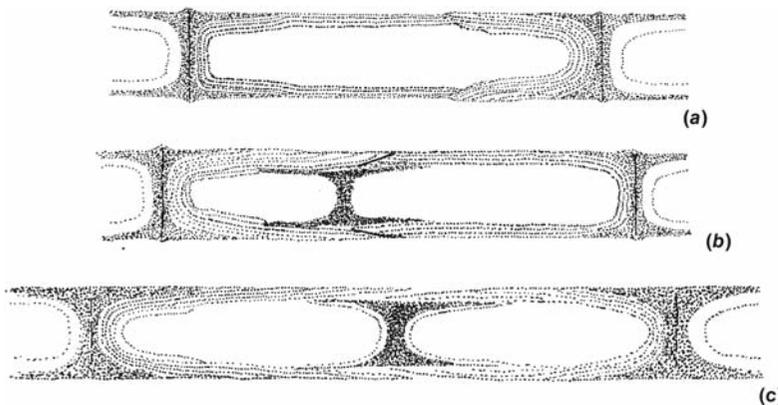
The cell walls of two Xanthophyceae, *Tribonema* (Figs. 19.2, 19.3) (Cleare and Percival, 1973) and *Vaucheria* (Figs. 19.7, 19.8), are composed of cellulose (Parker et al., 1963). In *Vaucheria* cellulose comprises 90% of the wall, with the remaining



**Fig. 19.1** Light and electron microscopical drawing of a zoospore of a typical member of the Xanthophyceae, *Mischooccus sphaerocephalus*. (C) Chloroplast; (CV) contractile vacuole; (E) eyespot; (FS) flagellar swelling; (LF) long flagellum with hairs; (N) nucleus; (SF) short flagellum; (V) vacuole. (Adapted from Hibberd and Leedale, 1971.)



**Fig. 19.2** Light microscopy of filaments of *Tribonema regulare* (a) and *Tribonema utriculosum* (b). (c) *Tribonema viride*, zoosporangium containing two zoospores. (d) Liberated zoospores of *Tribonema regulare*. (e) H-shaped cell walls of *Tribonema regulare* remaining after liberation of zoospores. (f) Scanning electron micrograph of a two-celled filament of *Tribonema viridae* showing the margin of an overlapping H-piece (arrows). (From Lokhorst and Star, 2003.)

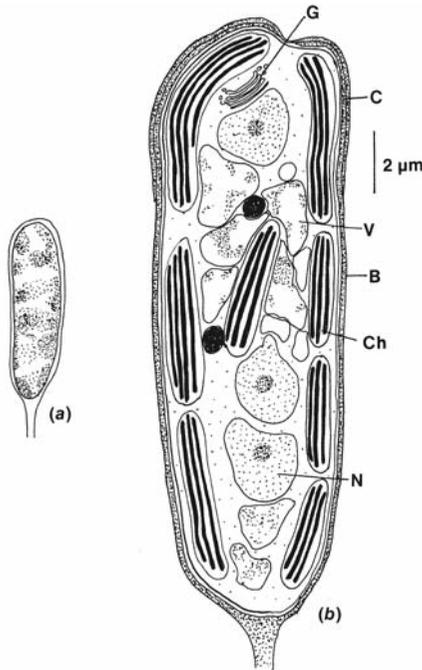


**Fig. 19.3** Wall structure of *Tribonema bombycinum*, after treatment with potassium hydroxide. (a) Two H-pieces articulated to enclose a single protoplast. (b), (c) Recently divided cell showing the intercalation of a new H-piece. (After Smith, 1938.)

portion being amorphous polysaccharides composed primarily of glucose and uronic acids.

Many of the algae in the class have walls composed of two overlapping halves that fit together as do the two parts of the bacteriologist's Petri dish (Figs. 19.2(d), (e), (f), 19.3, 19.4). The two-part nature of the wall cannot be delineated with the light microscope unless the cells have been treated with certain reagents such as concentrated potassium hydroxide. A typical example is the wall of *Ophiocytium majus* (one study places *Ophiocytium* in the closely related Eustigmatophyceae based on the sequence of

mitochondrial cytochrome oxidase (Ehara et al., 1997)), which is tubular in shape (Fig. 19.4). The wall is composed of two parts, a cap of constant size fitted over a tubular basal portion. As the cell grows and increases in length, the tubular basal portion elongates, but the cap remains the same size. The rims of both the cap and the tube are finely tapered and overlap for some distance so that the bipartite condition is not normally apparent in living cells. A layer of intercalary material, presumably functioning as a cementing substance, separates the cap from the tubular part of the wall. Filamentous genera, such as



**Fig. 19.4** *Ophiocytium majus*. (a) Vegetative cell. (b) Semidiagrammatic drawing of the fine structure of a vegetative cell. (B) Basal tubular portion of wall; (C) cap of wall; (Ch) chloroplast; (G) Golgi; (N) nucleus; (V) vesicle. (Adapted from Hibberd and Leedale, 1971.)

*Tribonema* (Figs. 19.2, 19.3), have a wall composed of H-shaped pieces. These alternately overlap each other so that each protoplast is enclosed by halves of two successive H-pieces (Lokhorst and Star, 1988).

### Chloroplasts and food reserves

Two membranes of chloroplast E.R. surround the chloroplasts, the outer membrane of chloroplast E.R. being continuous with the outer membrane of the nuclear envelope (Fig. 19.1) (Hibberd and Leedale, 1971). The thylakoids are grouped into bands of three and in many genera there is a pyrenoid in the chloroplast (Fig. 19.5) (Marchant, 1972). The eyespot consists of globules beneath the chloroplast envelope at the anterior end of the chloroplast (Figs. 19.1, 19.5). Where the short flagellum passes over the eyespot, the flagellar sheath is dilated into the flagellar swelling, which is closely applied to the plasmalemma in the area of the eyespot.

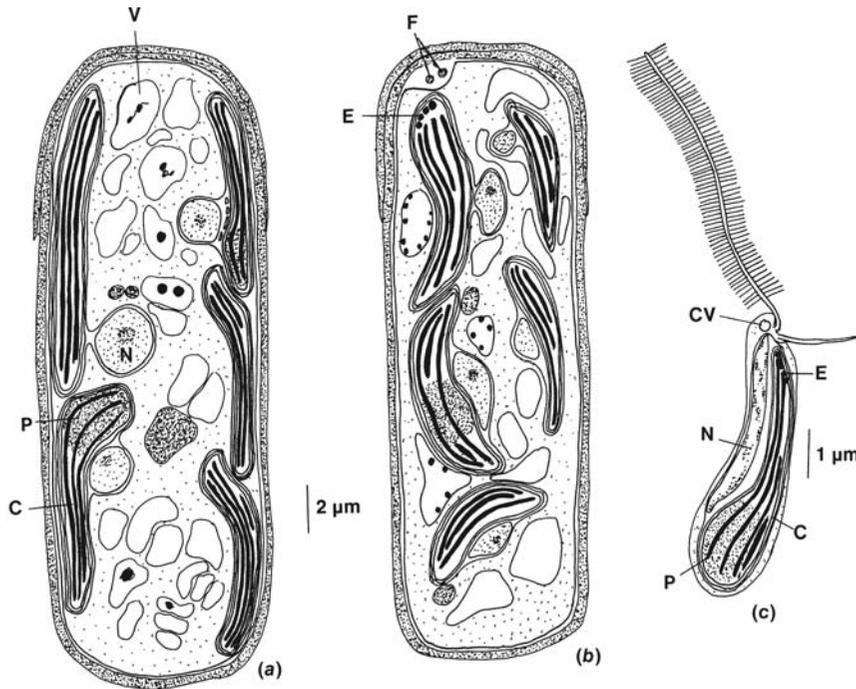
Chlorophylls *a* and *c* are present in the chloroplasts (Sullivan et al., 1990), with the major carotenoids being diadinoxanthin, heteroxanthin and vaucheriaxanthin ester.

Mannitol and glucose accumulate during photosynthesis in the plastids (Cleare and Percival, 1973). The principal storage product is probably a  $\beta$ -1,3 linked glucan similar to paramylon, although lipids have been suggested as also being important.

### Asexual reproduction

Xanthophycean organisms multiply asexually by fragmentation, zoospores, and aplanospores. In addition, they have the ability to form specialized resting spores. Fragmentation is limited to the tetrasporine and filamentous colonies, and is due to the breaking of the colony into parts.

Zoospores are formed by a majority of the genera. The zoospores are biflagellate, with the forward tinsel flagellum usually being four to six times longer than the shorter whiplash flagellum (Figs. 19.1, 19.5). The zoospores are naked and usually pyriform (pear-shaped). Zoospore production has been studied at the fine-structural level in *Pseudobumilleriopsis pyrenoidosa* by Deason (1971) (Fig. 19.5). This alga has rod-shaped cells with several nuclei and laminate chloroplasts. Vegetative cells prior to zoosporogenesis have the nuclei and vacuoles in the center of the cell, whereas the chloroplasts are flattened against the plasmalemma. The first indication of cleavage in zoosporogenesis is the appearance of vacuoles between the ends of adjacent chloroplasts. The chloroplasts move away from the plasmalemma, and each becomes associated with a nucleus. The vacuoles then coalesce and separate the nucleus-chloroplast pairs, each of which becomes a zoospore. Basal bodies are present near the nuclei of the vegetative cells; the basal bodies migrate to one end of the chloroplast as cleavage begins and produce flagella early in zoosporogenesis. One to 16 zoospores are produced, which are released by dissolution and/or separation of the sporangial walls where they overlap. In the zoospore, the chloroplast is massive and has a pyrenoid. The nucleus is elongate, and there are two or more contractile vacuoles present.



**Fig. 19.5** *Pseudobumilleriopsis pyrenoidosa*. (a) Vegetative cell. (b) Cell undergoing zoosporogenesis. (c) Zoospore. (C) Chloroplast; (CV) contractile vacuole; (E) eyespot; (F) flagella; (N) nucleus; (P) pyrenoid; (V) vacuole. (Adapted from Deason, 1971.)

Instead of producing zoospores, the entire protoplast may produce a single aplanospore or divide into a number of parts, each of which becomes an aplanospore. In some cases, environmental conditions determine whether the alga reproduces by zoospores or aplanospores. Submerged thalli of *Botrydium* produce zoospores; those living on damp soil produce aplanospores (Rostafiński and Woronin, 1877) (Fig. 19.6). An aplanospore liberated from a parent cell can grow directly into a new plant, or it may give rise to zoospores, which develop into new plants.

Some flagellates and rhizopodial cells produce cysts or statospores endogenously, similar to those in the Chrysophyceae. In their formation, there is an internal delimitation of a spherical protoplast that is separated from the peripheral portion of the mother cell's protoplast by a membrane. The endogenously differentiated protoplast then secretes a wall with two overlap-

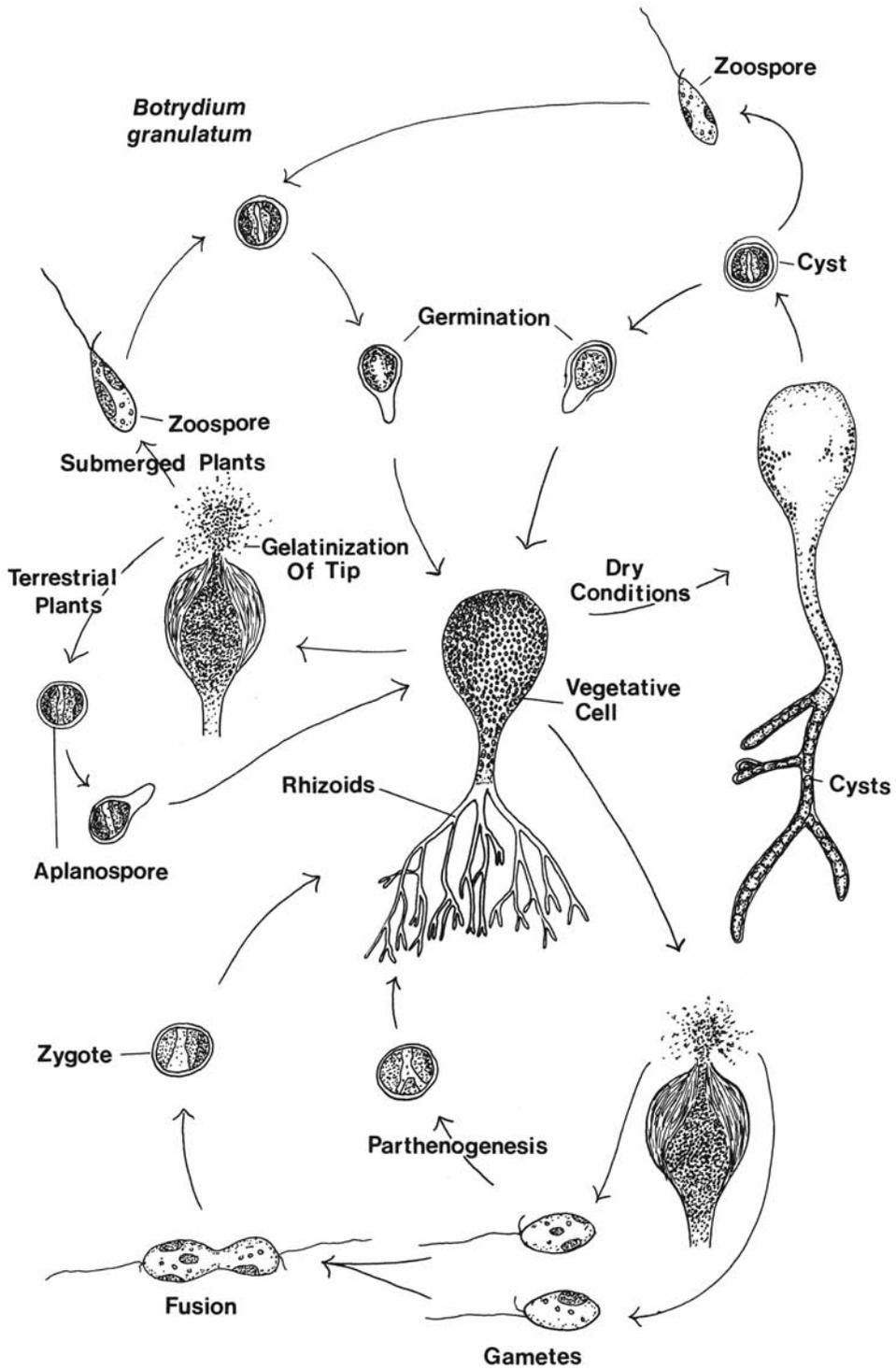
ping halves. Vegetative cells can also change directly into spore-like resting stages, with much thicker walls and more abundant food reserves than the vegetative cells. These spore-like cells, in which the spore wall is not distinct from the parent wall, are called akinetes and are usually found in filamentous genera.

## Sexual reproduction

There are few reliable reports of sexual reproduction in the Xanthophyceae. Sexual reproduction has only been established in three genera: *Botrydium* (Fig. 19.6), *Tribonema* (Fig. 19.2), and *Vaucheria* (Fig. 19.8). In the first two genera, both gametes are flagellated, whereas in *Vaucheria* reproduction is oogamous.

Four orders will be considered here:

- Order 1 Mischococcales: small coccoid cells.
- Order 2 Tribonematales: filamentous organisms, not coenocytic.
- Order 3 Botrydiales: globose multinucleate thallus with colorless rhizoids.
- Order 4 Vaucheriales: filamentous coenocyte.



**Fig. 19.6** The life cycle of *Botrydium granulatum*.  
 (Adapted from Rostafiński and Woronin, 1877; Kolkwitz, 1926; Rosenberg, 1930.)

### Mischococcales

This order is characterized by small coccoid cells and contains about two-thirds xanthophyte algae (Bailey and Andersen, 1998). *Mischococcus* (Fig. 19.1) and *Pseudobumilleriopsis* (Fig. 19.5) are examples of algae in this order.

### Tribonematales

The algae in this order have cylindrical cells uniserially united end to end in branched or unbranched filaments. *Tribonema* is composed of barrel-shaped cells that are two to five times longer than they are wide (Figs. 19.2, 19.3). The wall is composed of two H-shaped pieces overlapping in the middle of the cell. The protoplast is uninucleate and contains a number of discoid chloroplasts. Asexual reproduction is by fragmentation of the filaments, by zoospores, or by aplanospores. Aplanospores are produced more frequently than zoospores and are released by the pulling apart of the two portions of the cell wall. Akinetes can also be formed by the filaments (Smith, 1950). Sexual reproduction is isogamous, one of a uniting pair of gametes coming to rest and withdrawing its flagella just before the other swims up to it and unites with it (Scherffel, 1901).

### Botrydiales

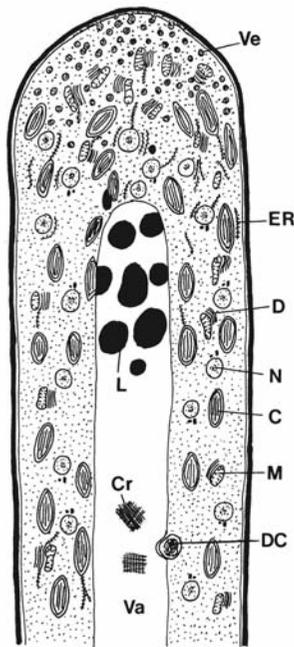
*Botrydium* is a unicellular multinucleate alga consisting of a usually globose aerial portion with chloroplasts and a colorless rhizoidal portion that penetrates the soil (Fig. 19.6). The shape of the aerial part is influenced by environmental conditions. It is usually elongate when growing in shaded habitats and spherical when growing in brightly illuminated places (Moore and Carter, 1926). The aerial portion has a relatively tough wall within which is a delicate layer of cytoplasm containing many nuclei and chloroplasts. The branched rhizoidal system has no chloroplasts but does have many nuclei. The cells are incapable of vegetative division, and the only method by which new plants may be formed is by production of zoospores or aplanospores. According to Rakován and Fridvalsky (1970), the formation of either aplanospores or zoospores begins at night, and the cells must be illuminated 8 to 9 hours after the beginning of the process for flagella to develop. If there is no illumination, aplanospores

develop. In the formation of these spores, three to five chloroplasts become associated with a nucleus in the mother cell, and cleavage occurs with each zoospore containing the above organelles. If the spore is an aplanospore, then a wall is secreted; if it is a zoospore, no wall is formed. Motile gametes are apparently formed in a similar manner (Iyengar, 1925; Miller, 1927), and sexual reproduction can be isogamous or anisogamous, with the cells being either homothallic or heterothallic. The gametes become apposed by their anterior ends when uniting in pairs to form a zygote. Gametes that have not fused develop parthenogenetically into thalli. A germinating zygote develops directly into a new vegetative thallus.

*Botrydium* also produces cysts or resting spores during periods of dry conditions (Miller, 1927). In *B. granulatum*, the protoplast migrates into the rhizoids where division occurs to produce a large number of thick-walled cysts. These cysts can either germinate directly to form a new thallus or give rise to zoospores.

### Vaucheriales

There is only one genus, *Vaucheria* (Figs. 19.7, 19.8), in this order. *Vaucheria* diverged early in evolution from other members of the order (Bailey and Andersen, 1998). *Vaucheria* has a relatively thin cell wall within which the cytoplasm is restricted to the periphery of the coenocyte, with the center being occupied by a large central vacuole (Fig. 19.7). In the cytoplasm, the numerous elliptical chloroplasts with pyrenoids are to the outside, whereas the nuclei are toward the center. Growth of the filaments is restricted to the apex which has a large number of vesicles, mitochondria, and dictyosomes. Chloroplasts, nuclei, and the large central vacuole are not found at the growing tip (Ott and Brown, 1974a). The large central vacuole contains lipids, degenerated chloroplasts, and crystals and extends the entire length of the filament except for the area immediately behind the growing tip. Cytoplasmic streaming takes place in the area of the large central vacuole and directly involves the nuclei, mitochondria, and their associated dictyosomes. The cytoplasmic streaming involves two separate systems, the first based on microtubules that move the nuclei, and the



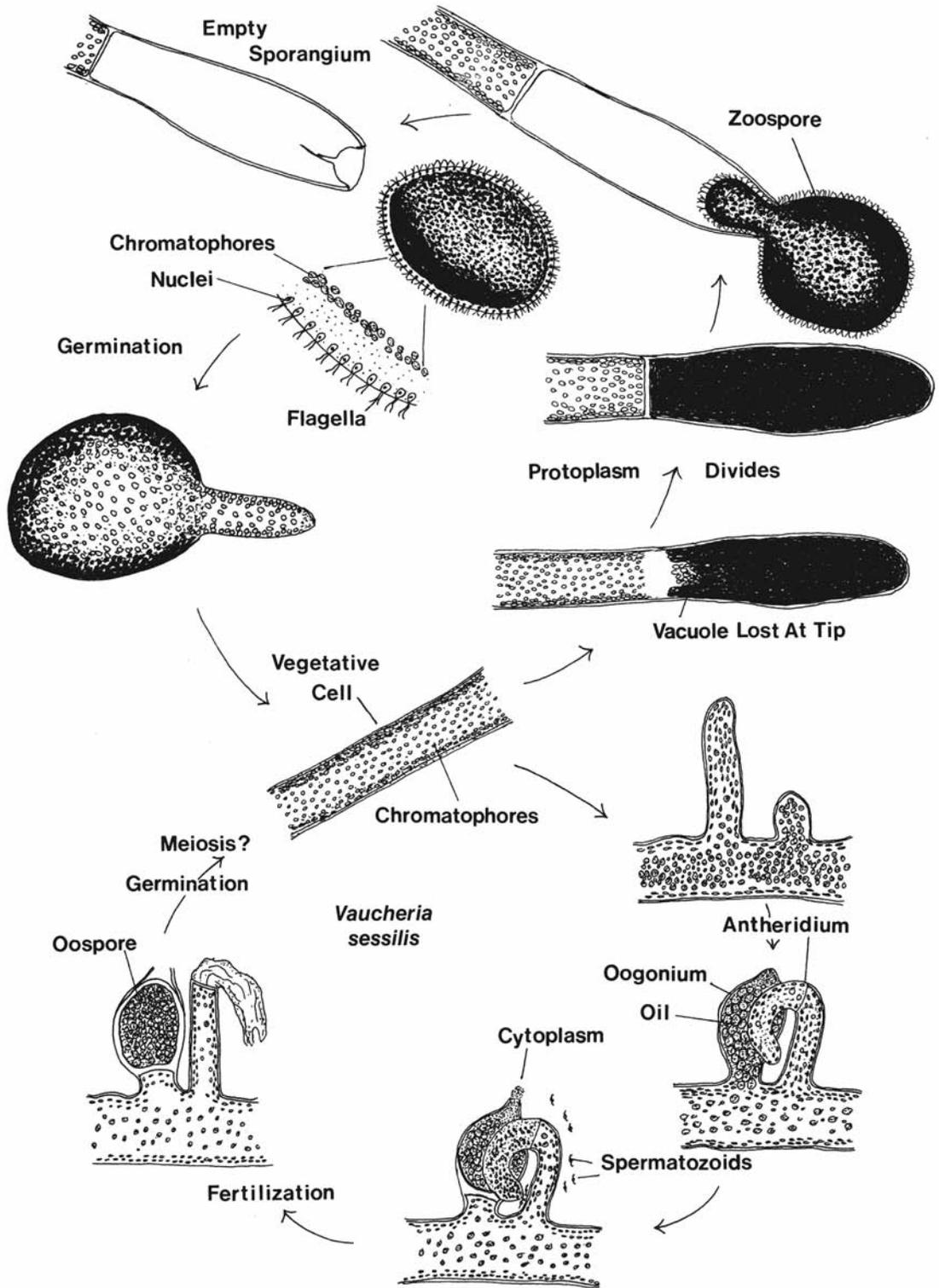
**Fig. 19.7** Schematic representation of the tip of a vegetative filament of *Vaucheria dillwynii*. (C) Chloroplast; (Cr) crystal; (D) dictyosome; (DC) degenerate chloroplast; (ER) endoplasmic reticulum; (L) lipid body; (M) mitochondrion; (N) nucleus; (Va) vacuole; (Ve) vesicle. (After Ott and Brown, 1974a.)

second based on microfilaments that move the mitochondria and their associated dictyosomes. The chloroplasts do not migrate in patterns of definite streaming but have a more or less random movement, not associated with either microtubules or microfilaments.

Although *Vaucheria* can develop transverse septa that block off injured portions of the coenocyte, there is little reproduction by accidental breaking of filaments. Asexual reproduction of aquatic individuals is usually by means of multi-flagellate, multinucleate zoospores (Birckner, 1912) (Fig. 19.8), which are produced singly in club-shaped sporangia at the swollen ends of filaments. In their production large numbers of chloroplasts and nuclei stream into the tip of the filament, the central vacuole decreases in size, and the tips appear dark green. A band of colorless protoplasm now appears at the base of the developing sporangium, which breaks in the middle to form two protoplasts. The two protoplasts

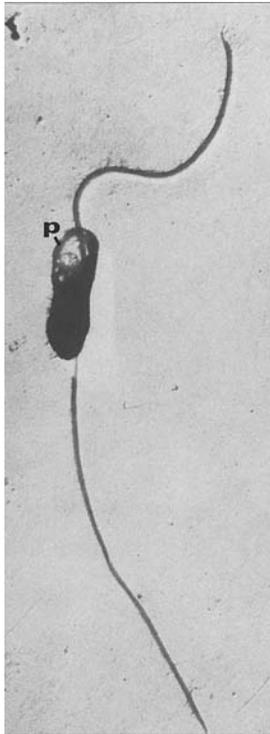
approach each other, and a septum is formed. Within the sporangium, vesicles are produced (Ott and Brown, 1974b), around which nuclei become oriented with a pair of basal bodies between each nucleus and the vesicle membrane. Flagella are produced through the vesicle membrane, and the vesicles migrate to the plasmalemma. The nuclei with their flagella pairs thus come to lie in the peripheral area of the cell. The wall at the apex of the sporangium gelatinizes, forming a narrow aperture; the zoospore pushes its way through the aperture and swims in the medium. The nuclei in the sporangium are separated from each other by a number of vacuoles, and one flagellum of each pair is slightly longer than the other (Greenwood et al., 1957). There is no eyespot or pyrenoid in the zoospore (Greenwood, 1959). The zoospores are sluggish in their movements, swimming for only about 15 minutes. On coming to rest, the flagella are withdrawn, and a thin wall is secreted. Germination follows almost immediately by the protrusion of one or two tubular outgrowths, one of which attaches itself to the substratum by means of a colorless lobed holdfast. Instead of producing zoospores, terrestrial individuals may have the entire contents of the sporangium develop into an aplanospore. Zoospores can be obtained if vegetative filaments kept moist for some days are soaked in water, or transferred from a nutritive solution into distilled water, or removed from running water to still water (Klebs, 1896; Starr, 1964).

Sexual reproduction is oogamous and usually homothallic with meiosis occurring before the production of gametes (Al-Kubaisi and Schwantes, 1981). The life cycle is therefore diplontic with the diploid phase predominant. Sex organs are common on filaments growing in damp soil or in quiet water, but are infrequent if they are growing in flowing water. The antheridia and oogonia are borne adjacent to each other and on a common lateral branch or on adjacent lateral branches. The sex organs are cut off by a septum. The oogonium has a single egg and is filled with oil and chloroplasts. The mature oogonium produces a beak, the tip of which gelatinizes, forming an aperture. A portion of colorless cytoplasm of the egg projects through the aperture, and the egg contracts.



**Fig. 19.8** The life cycle of *Vaucheria sessilis*.

The antheridia usually develop as strongly curved cylindrical tubes that become cut off by a septum, usually fairly high up in the tube. The mature antheridium has the spermatozooids produced in a specific area between the central and peripheral cytoplasm (Moestrup, 1970). The central and peripheral cytoplasm contain those parts of the cytoplasm that are not included in the spermatozooids: the chloroplasts, vacuoles, and many mitochondria (Ott and Brown, 1978). An aperture appears in the antheridium, and the spermatozooids are released. The spermatozooids are cylindrical posteriorly but have a flattened proboscis in the anterior portion (Fig. 19.9). There is a forward-projecting tinsel flagellum with two lateral rows of hairs, and a slightly longer trailing smooth flagellum. The nucleus is elongated and worm-like, as are the three or four mitochondria. There is neither a chloroplast nor an eyespot, but there is a Golgi body near the basal bodies of the flagella. The proboscis consists of eight or nine

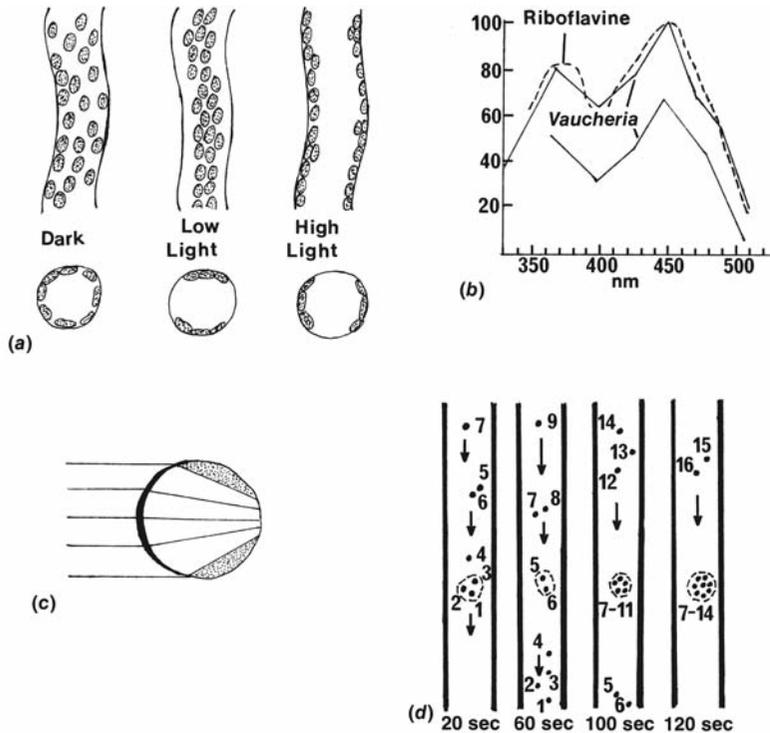


**Fig. 19.9** Shadowcast whole mount of a sperm of *Vaucheria synandra*. A proboscis (p) is present on the anterior part of the cell. (From Moestrup, 1970.)

microtubules running beneath the plasmalemma with vesicles in between the microtubules.

Fertilization is accomplished by the spermatozooids fusing with the egg protoplasm through the aperture in the oogonium. The zygote secretes a wall, and the oil droplets fuse to form a small number of central droplets. The oospore is colored by the oil and the degeneration products of chlorophyll. It remains in the oogonium until it is liberated by the decay of the oogonial wall. The oospore then remains dormant for a few months before germinating, probably by meiosis, into a new filament.

*Vaucheria* is one of the algae that exhibit chloroplast orientation movements in the light. In the dark, the chloroplasts are uniformly distributed in the peripheral cytoplasm (Fig. 19.10); in low-intensity light, they are oriented to the top and bottom of the coenocyte to trap the maximum light; in high light intensities, they are along the sides of the filament, thereby receiving less light (Fischer-Arnold, 1963; for a review, see Haupt and Schönbohm, 1970). The action spectrum for chloroplast movement in *Vaucheria* is similar to that of higher plants and closely resembles that of the action spectrum of flavins, from which it has been concluded that flavins located in the plasma membrane act as the photoreceptive pigments (Blatt, 1983). That the photoreceptor is not the chloroplast can be demonstrated with a microbeam of light. Irradiation with a microbeam results in chloroplast movement, whether or not the chloroplasts receive light. In addition to being able to perceive light, the cell also has the ability to determine the direction of the light. The theoretical basis for this perception is derived from a lens effect by the cell on the light that it receives (Fig. 19.10(c)). The cell acts as a collecting lens, focusing light to the rear of the cell. This results in the light bypassing some parts of the cell along the flanks, thus establishing a front-to-rear gradient. The movement of the chloroplasts relies on energy from two metabolic processes, respiration and photosynthesis. Inhibitors of respiration and photosynthesis abolish the chloroplast movement, whereas inhibitors of photosynthesis just slow down the chloroplast movement. If ATP is added along with photosynthetic inhibitors, chloroplast movement is normal.



**Fig. 19.10** (a) Position of chloroplasts of *Vaucheria* in the dark, and in low and high light intensities. (b) Action spectrum of chloroplast orientation movement in *Vaucheria* under low light (lower curve) and high light (upper curve) intensities. Vertical axis is relative quantum efficiency. (c) Diagrammatic representation of the lens effect of light passing through a *Vaucheria* coenocyte. (d) Movements of single chloroplasts in *Vaucheria* under low light intensity. A beam of light (dashed area) causes accumulation of chloroplasts in the illuminated area. (After Haupt and Schönbohm, 1970.)

There are two possible mechanisms to explain chloroplast movement. In the first or “active movement” the chloroplast moves relative to the rest of the protoplasm, whereas in the second or “passive movement” the protoplasm moves, carrying with it the chloroplast and other organelles. In *Vaucheria*, passive movement occurs. If chloroplast movement is followed at high or low intensity, it can be seen that not only chloroplasts, but also other organelles and inclusions are rearranged by light. Furthermore, if only a small area of the filament is irradiated with a spot of light, a strong accumulation of cytoplasm plus inclusions can be observed at this place (Fig. 19.10(d)). The organelles are actually trapped in a portion of the cytoplasm as they stream through the cell. Illumination of an area of *Vaucheria* results in the formation of an actin fiber network that acts as a trapping mechanism (Blatt, 1983).

## REFERENCES

- Al-Kubaisi, K. H., and Schwantes, H. O. (1981). Cytophotometrische Untersuchungen zum Generationswechsel autotropher und heterotropher siphonaler Organismen (*Vaucheria sessilis* und *Saprolegnia ferax*). *Nova Hedwigia* 34:301–16.
- Ariztia, E. V., Andersen, R. A., and Sogin, M. L. (1991). A new phylogeny for chromophyte algae using 16S-like rRNA sequences from *Mallomonas papillosa* (Synurophyceae) and *Tribonema aequale* (Xanthophyceae). *J. Phycol.* 27:428–36.
- Bailey, J. C., and Andersen, R. A. (1998). Phylogenetic relationships among nine species of the Xanthophyceae inferred from *rbcl* and 18S rRNA gene sequences. *Phycologia* 37:458–66.
- Birckner, V. (1912). Die Beobachtung von Zoosporenbildung bei *Vaucheria aversa* Hass. *Flora* 104:167–71.
- Blatt, M. R. (1983). The action spectrum for chloroplast movements and evidence for blue-light-photoreceptor cycling in the *Vaucheria*. *Planta* 159:267–76.
- Cleare, M., and Percival, E. (1973). Carbohydrates of the freshwater alga *Tribonema aequale*. II. Preliminary photosynthetic studies with  $^{14}\text{C}$ . *Br. Phycol. J.* 8:181–4.
- Deason, T. R. (1971). The fine structure of sporogenesis in the Xanthophycean alga *Pseudobumilleriopsis pyrenoidosa*. *J. Phycol.* 7:101–7.

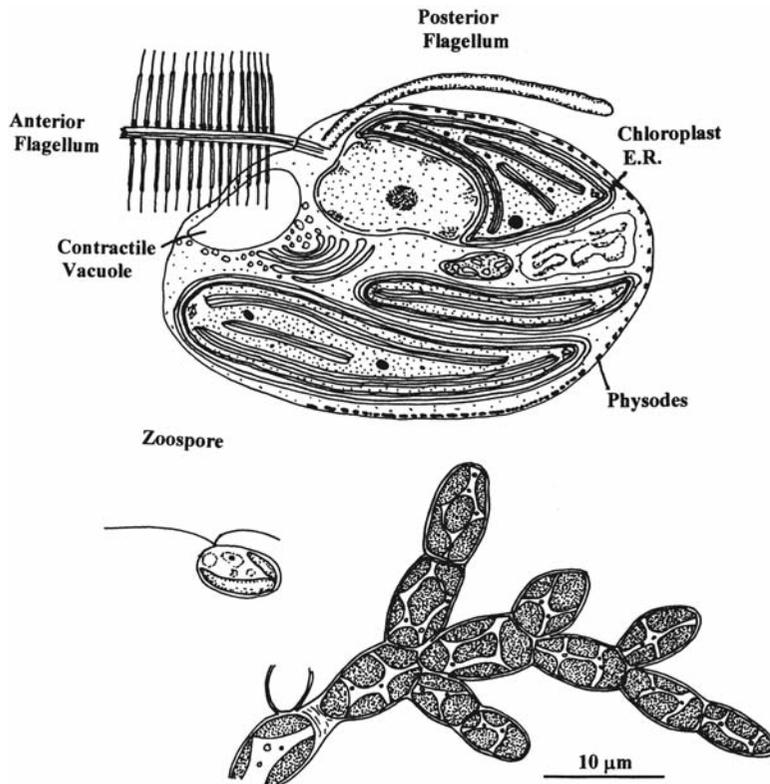
- Ehara, M., Hayashi-Ishimura, Y., Inagaki, Y., and Ohama, T. (1997). Use of a deviant mitochondrial genetic code in yellow-green algae as a landmark for segregating members within the phylum. *J. Mol. Evol.* 45:119–24.
- Fischer-Arnold, G. (1963). Untersuchungen über die Chloroplastenbewegung bei *Vaucheria sessilis*. *Protoplasma* 56:495–506.
- Greenwood, A. D. (1959). Observations on the structure of the zoospores of *Vaucheria*. II. *J. Exp. Bot.* 10:55–68.
- Greenwood, A. D., Manton, I., and Clarke, B. (1957). Observations on the structure of the zoospores of *Vaucheria*. *J. Exp. Bot.* 8:71–86.
- Haupt, W., and Schönbohm, E. (1970). Light-oriented chloroplast movements. In *Photobiology of Microorganisms*; ed. P. Halldal, pp. 283–307. London: Wiley-Interscience.
- Hibberd, D. J. (1981). Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synonym Xanthophyceae). *Bot. J. Linn. Soc.* 82:93–119.
- Hibberd, D. J., and Leedale, G. F. (1971). Cytology and ultrastructure of the Xanthophyceae. II. The zoospore and vegetative cell of coccoid forms, with special reference to *Ophiocytium majus* Naegeli. *Br. Phycol. J.* 6:1–23.
- Iyengar, M. O. P. (1925). Note on two species of *Botrydium* from India. *J. Indian Bot. Soc.* 4:193–201.
- Klebs, G. (1896). *Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen*. Jena.
- Kolkwitz, R. (1926). Zur Ökologie und Systematik von *Botrydium granulatum* (L) Grev. *Ber. Dtsch. Bot. Ges.* 44:533–40.
- Lokhorst, G. M., and Star, W. (1988). Mitosis and cytokinesis in *Tribonema regulare* (Tribophyceae, Chrysophyta). *Protoplasma* 145:7–15.
- Lokhorst, G. M., and Star, W. (2003). The flagellar apparatus in *Tribonema* (Xanthophyceae) reinvestigated. *Phycologia* 42:31–43.
- Marchant, H. J. (1972). Pyrenoids of *Vaucheria woroniniana*. Heering. *Br. Phycol. J.* 7:81–4.
- Miller, V. (1927). Untersuchungen über die Gattung *Botrydium* Wallroth. II. Spezieller Teil. *Ber. Dtsch. Bot. Ges.* 45:161–70.
- Moestrup, Ø. (1970). On the fine structure of the spermatozooids of *Vaucheria sescuplicaria* and on later stages in spermatogenesis. *J. Mar. Biol. Assoc. UK* 50:513–23.
- Moore, G. T., and Carter, N. (1926). Further studies on the subterranean algal flora of the Missouri Botanical Garden. *Ann. Mo. Bot. Gard.* 13:101–40.
- Ott, D. W., and Brown, R. M., Jr. (1974a). Developmental cytology of the genus *Vaucheria*. I. Organization of the vegetative filament. *Br. Phycol. J.* 9:111–26.
- Ott, D. W., and Brown, R. M., Jr. (1974b). Developmental cytology of the genus *Vaucheria*. II. Sporogenesis in *V. fontinalis* (L) Christensen. *Br. Phycol. J.* 9:333–51.
- Ott, D. W., and Brown, R. M. (1978). Developmental cytology of the genus *Vaucheria*. IV. Spermatogenesis. *Br. Phycol. J.* 13:69–85.
- Parker, B. C., Preston, R. D., and Fogg, G. E. (1963). Studies of the structure and chemical composition of the cell walls of Vaucheriaceae and Saprolegniaceae. *Proc. R. Soc. Lond. [B]* 158:435–45.
- Potter, D., Saunders, G. W., and Andersen, R. A. (1997). Phylogenetic relationships of the Raphidophyceae and Xanthophyceae as inferred from nucleotide sequences of the 18S ribosomal RNA gene. *Am. J. Bot.* 84:966–72.
- Rakován, J. N., and Fridvalsky, L. (1970). Electron microscope studies on the gonidiogenesis of *Botrydium granulatum* (L) Grev. (Xanthophyceae). *Ann. Univ. Sci. Budap. Sect. Biol.* 12:209–12.
- Rosenberg, M. (1930). Die geschlechtliche Fortpflanzung von *Botrydium granulatum* Grev. *Oesterr. Bot. Z.* 79:289–96.
- Rostafiński, J., and Woronin, M. (1877). Ueber *Botrydium granulatum*. *Bot. Ztg.* 35:649–71.
- Scherffel, A. (1901). Kleiner Beitrag zur Phylogenie einiger Gruppen neiderer Organismen. *Bot. Ztg.* 59:143–58.
- Smith, G. M. (1938). *Cryptogamic Botany*, Vol. 1. *Algae and Fungi*. New York and London: McGraw-Hill.
- Smith, G. M. (1950). *The Fresh-Water Algae of the United States*, 2nd edn. New York and London: McGraw Hill.
- Starr, R. C. (1964). The culture collection of algae at Indiana University. *Am. J. Bot.* 51:1013–44.
- Sullivan, C. M., Entwistle, T. J., and Rowan, K. S. (1990). The identification of chlorophyll *c* in the Tribophyceae (= Xanthophyceae) using spectrophotofluorometry. *Phycologia* 29:285–91.

# Heterokontophyta

## PHAEOTHAMNIOPHYCEAE

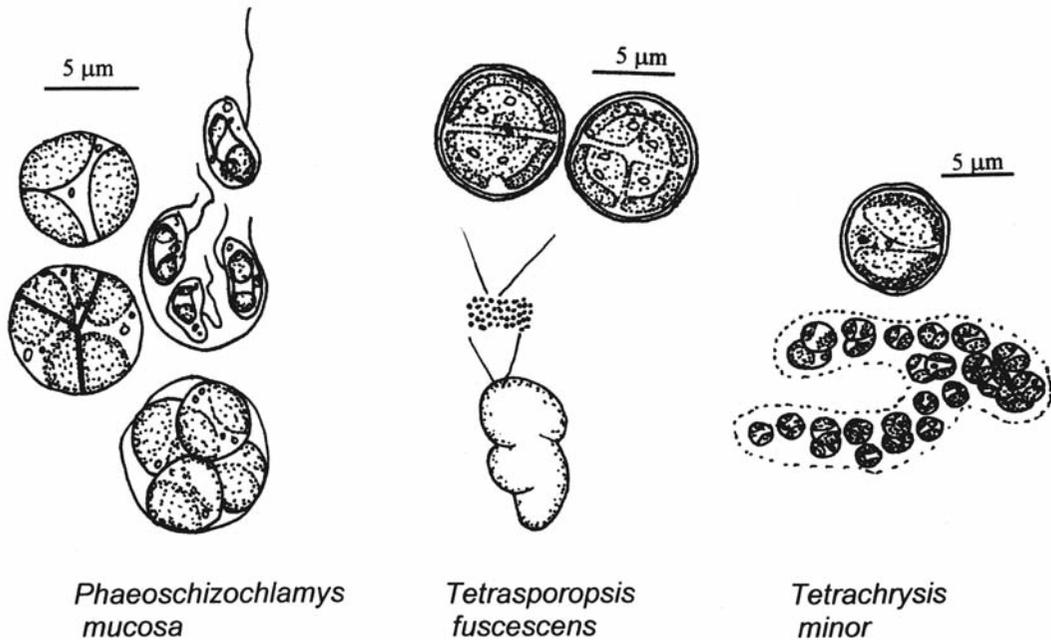
Recent nucleotide sequencing has uncovered an evolutionary line of golden-brown algae not related to other golden-brown algae (Bailey et al., 1998). These algae have been placed in the class Phaeothamniophyceae, a class that is most closely

related to the Xanthophyceae and Phaeophyceae. The cytology of these three classes is similar (Fig. 20.1). The cells have two membranes of chloroplast endoplasmic reticulum with the outer membrane of chloroplast E.R. continuous with the outer membrane of the nuclear envelope. The chloroplasts have a ring-shaped genophore and girdle lamellae. The flagella are inserted



**Fig. 20.1** A filament and zoospore of *Phaeothamnion polychrysis*. Also included is the fine structure of a zoospore.

*Phaeothamnion polychrysis*



**Fig. 20.2** Some algae classified in the Phaeothamniophyceae.

laterally into the motile cells. The anterior tinsel flagellum has tripartite hairs that lack lateral filaments. The posterior flagellum lacks hairs. New daughter cells are formed by **eleutheroschisis** (parent cell wall is completely cast off and new daughter cell walls are formed). Vesicles under the plasma membrane appear similar to the **physodes** that occur in the Phaeophyceae. The Phaeothamniophyceae is the only class of algae where fucoxanthin and heteroxanthin occur together. Endogenous siliceous cysts (statospores) are not produced by these algae.

*Phaeothamnion* is a filamentous brown alga that produces zoospores that settle to produce new filaments (Fig. 20.1) (Andersen et al., 1998). *Tetrachrysis* occurs in environments such as peat ponds and has cells embedded in a common mucilage (Dop et al., 1980) (Fig. 20.2). *Tetrasporopsis* is a colonial freshwater alga that consists of a brown, gelatinous, bladdery sac (Entwistle and Andersen, 1990) (Fig. 20.2). *Phaeoschizochlamys* occurs among detritus or suspended between other freshwater algae. The cells occur in mucilage of different shapes up to 0.5 mm in

diameter (Fig. 20.2). The cells divide to form four autospores containing two chloroplasts.

## REFERENCES

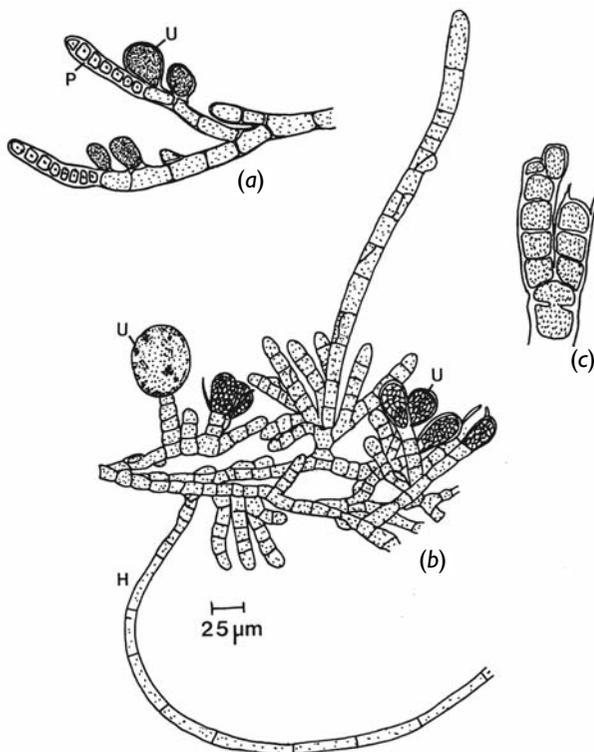
- Andersen, R. A., Potter, D., Bidigare, R. R., Latasa, M., Rowar, K., and O'Kelly, C. J. (1998). Characterization and phylogenetic position of the enigmatic golden alga *Phaeothamnion confervicola*: ultrastructure, pigment composition and partial SSU rDNA sequence. *J. Phycol.* 34:286–98.
- Bailey, J. C., Bidigare, R. R., Christensen, S. J., and Andersen, R. A. (1998). Phaeothamniophyceae classis nova: a new lineage of chromophytes based upon photosynthetic pigments, *rbcl* sequence analysis and ultrastructure. *Protist* 149:245–63.
- Dop, A. J., Kosterman, Y., and van Oers, F. (1980). Coccoid and palmelloid benthic Chrysophyceae from the Netherlands. *Acta Bot. Neerl.* 29:87–102.
- Entwistle, T. J., and Andersen, R. A. (1990). A re-examination of *Tetrasporopsis* (Chrysophyceae) and a description of *Dermatochrysis* gen. nov. (Chrysophyceae): a monostromatic algae lacking cell walls. *Phycologia* 29:263–74.

# Heterokontophyta

## PHAEOPHYCEAE

The Phaeophyceae, or brown algae, derive their characteristic color from the large amounts of the carotenoid fucoxanthin in their chloroplasts as well as from any phaeophycean tannins that might be present. The chloroplasts also have chlorophylls  $a$ ,  $c_1$ , and  $c_2$ . There are two membranes of chloroplast E.R., which are usually continuous with the

outer membrane of the nuclear envelope. The storage product is laminarin. There are no unicellular or colonial organisms in the order, and the algae are basically filamentous, pseudoparenchymatous, or parenchymatous. They are found almost exclusively in the marine habitat, there being only four genera containing freshwater species, that is, *Heribaudiella*, *Pleurocladia*, *Bodanella*, and *Sphacelaria* (Fig. 21.1) (Schloesser and Blum, 1980). A number of marine forms penetrate into brackish water, where



**Fig. 21.1** Some freshwater brown algae. (a) *Pleurocladia lacustris*. (b) *Sphacelaria lacustris*. (c) *Heribaudiella fluviatilis*. (H) Hair; (P) pleurilocular sporangia; (U) unilocular sporangia. ((b) after Schloesser and Blum, 1980.)

they often form an important part of the salt marsh flora. These brackish water plants have almost totally lost the ability to reproduce sexually, and propagate by vegetative means only. Most of the Phaeophyceae grow in the intertidal belt and the upper littoral region. They dominate these regions in colder waters, particularly in the Northern Hemisphere, where the number of phaeophycean species is less than that of the Rhodophyceae, but the number of phaeophycean plants is much greater. In the tropics, the only place where large numbers of Phaeophyceae are found is the Sargasso Sea of the Atlantic.

The Phaeophyceae probably evolved from an organism in the Phaeothamniophyceae, which have motile cells similar to those in the Phaeophyceae, but lack the characteristic unilocular and plurilocular sporangia of the Phaeophyceae (Bailey et al., 1998).

## Cell structure

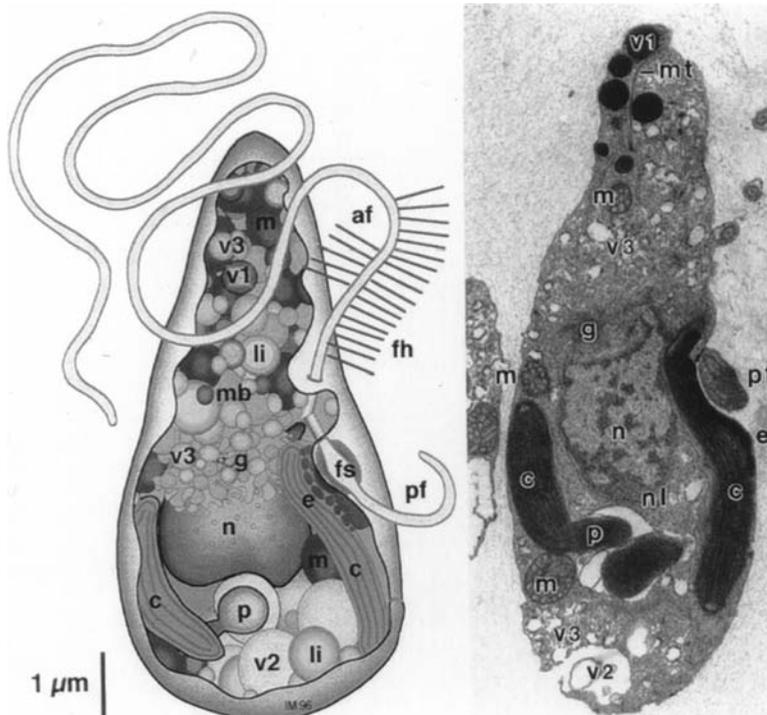
The cell structure (Figs. 21.2, 21.3) is in many ways similar to that of the Chrysophyceae,

Prymnesiophyceae, Bacillariophyceae, and Xanthophyceae, which are closely related to the Phaeophyceae. The main difference lies in the large amounts of extracellular polysaccharides surrounding the protoplast.

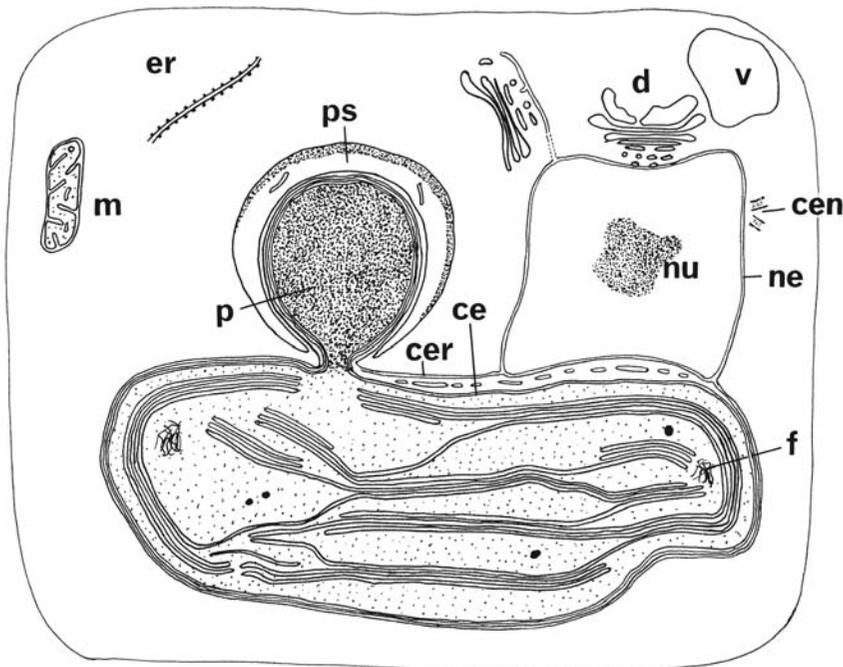
## Cell walls

Phaeophycean cell walls are generally composed of at least two layers, with **cellulose** making up the main structural skeleton (Kloareg and Quatrano, 1988). The amorphous component of the cell wall is made up of **alginic acid** and **fucoidin**, whereas the mucilage and cuticle are composed primarily of alginic acid (Evans and Holligan, 1972a; Vreeland, 1972). Alginic acid is basically made up of  $\beta$ -1,4 linked mannuronic acid units that have a variable amount of guluronic acid units attached through C-1 and C-4 linkages (Fig. 1.7). Fucoidin is composed primarily of  $\alpha$ -1,2 linked sulfated-fucose units, with a lesser amount of  $\alpha$ -1,4 linked sulfated-fucose units (Fig. 1.7). The relative quantities of alginic acid and fucoidin vary between species, different parts of the plant, and different environments.

Calcification of the wall occurs only in some



**Fig. 21.2** Left: Diagrammatic representation of a male gamete of *Ectocarpus siliculosus* showing the distribution of cellular organelles. Right: Transmission electron micrograph of a thin section of a male gamete of *E. siliculosus*. (af) Anterior flagellum; (c) chloroplast; (e) eyespot; (fh) flagellar hairs (present along entire length, for clarity only shown on part of the flagellum); (fs) proximal swelling of the posterior flagellum; (g) Golgi apparatus; (li) lipid body; (m) mitochondrion; (mb) microbody; (mt) microtubules; (n) nucleus; (p) pyrenoid; (pf) posterior flagellum; (v1) physode; (v2) storage granule; (v3) vesicles with cell wall or adhesive material. (From Maier, 1997a.)



**Fig. 21.3** Diagram of a hypothetical brown algal cell.

(ce) Chloroplast envelope; (cen) centrioles; (cer) chloroplast endoplasmic reticulum; (d) dictyosome; (er) endoplasmic reticulum; (f) DNA fibrils; (m) mitochondrion; (ne) nuclear envelope; (nu) nucleolus; (p) pyrenoid; (ps) pyrenoid sac; (v) vacuole. (From Bouck, 1965.)

species of *Padina* where calcium carbonate is deposited as needle-shaped crystals of aragonite in concentric bands on the surface of the fan-like thallus (Borowitzka et al., 1974).

The parenchymatous Phaeophyceae have plasmodesmata or pores between most of the cells. These pores are bounded by the plasmalemma, and protoplasm is continuous from one cell to the next through them. In the Laminariales, Fucales, and Dictyotales, the pores are grouped in primary pit areas, whereas in the more primitive parenchymatous Phaeophyceae the plasmodesmata are scattered in the cell wall (Bisalputra, 1966; Bourne and Cole, 1968; Cole, 1970).

### Flagella and eyespot

Generally the motile cells of the Phaeophyceae (always zoospores or gametes, as there are no motile vegetative cells) have a long anterior tinsel flagellum with tripartite hairs and a shorter pos-

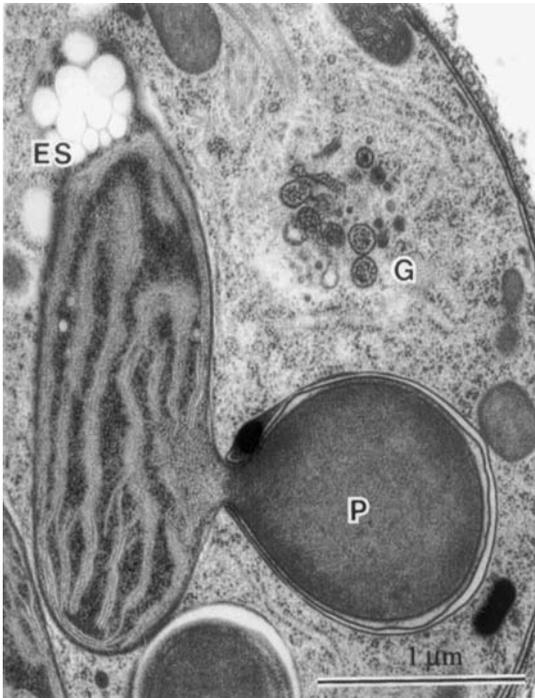
teriorly directed whiplash flagellum (Fig. 21.2) (Bouck, 1969; Loiseaux and West, 1970). The Fucales (Fig. 21.44) are an exception to this, with the posterior flagellum of the spermatozoid being longer than the anterior flagellum. The posterior flagellum usually has a swelling near the base, and this swelling fits into a depression of the cell immediately above the eyespot. The eyespot consists of 40 to 80 lipid globules arranged in a single layer between the outermost band of the thylakoids and the chloroplast envelope. The **eyespot (stigma)** acts as a concave mirror focusing light onto the flagellar swelling, which is the photoreceptor site for phototaxis in brown-algal flagellate cells (Kawai et al., 1996). Light at 420 and 460 nm is most effective in phototaxis in the brown algae, and is probably detected by a flavin-like substance in the flagellar swelling of the posterior flagellum (Kawai et al., 1991).

### Chloroplasts and photosynthesis

The chloroplasts of the Phaeophyceae have three thylakoids per band and are surrounded by the chloroplast envelope and two membranes of chloroplast E.R. (Figs. 21.3, 21.4). The outer membrane of the chloroplast E.R. is generally continu-

ous with the outer membrane of the nuclear envelope in the Ectocarpales but appears to be discontinuous in the Dictyotales, Laminariales, and Fucales. Membrane-bounded tubules are common in the area between the chloroplast E.R. and chloroplast envelope where the latter two are not closely appressed (Bouck, 1965; Evans, 1968). Microfibrils of DNA occur in the plastids, and in *Sphacelaria* sp. there is a ring-shaped genophore inside the outermost band of thylakoids (Bisalputra and Burton, 1969). The DNA microfibrils are both linear and circular and are attached to the thylakoid membranes. The plastids contain chlorophylls *a*, *c*<sub>1</sub>, and *c*<sub>2</sub>, with the major carotenoid being fucoxanthin.

All the phaeophyceean orders have representatives with pyrenoids (Figs. 21.3, 21.4) (Chi, 1971), but their presence, even in one species, can vary according to the stage of the plant. If the species is one that has pyrenoids only in some stages, then a pyrenoid is usually present in the eggs and/or sporelings but absent in the macroscopic phase,



**Fig. 21.4** A transmission electron micrograph of a portion of a cell of *Scytosiphon lomentaria*. (ES) Eyespot; (g) Golgi; (P) pyrenoid. (From Nagasato and Motomura, 2002.)

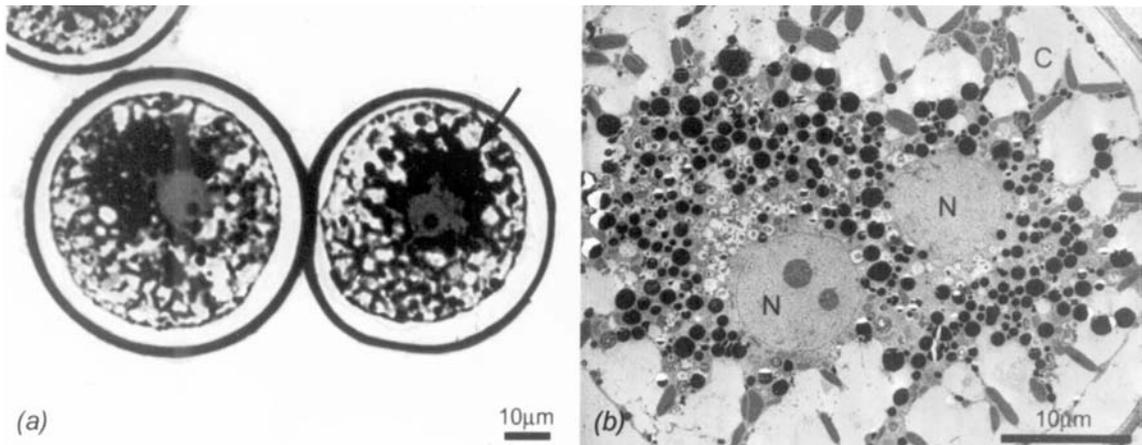
spermatozoids, and/or zoospores (Bourne and Cole, 1968; Evans, 1968; Bisalputra et al., 1971). A pyrenoid in the Phaeophyceae is usually a stalk-like structure set off from the main body of the chloroplast and containing a granular substance not traversed by thylakoids. Surrounding the pyrenoid but outside the chloroplast E.R. is a membrane-bounded sac that presumably contains the reserve product. The long-term storage product is **laminarin** (Fig. 1.7), a  $\beta$ -1,3 linked glucan. The sugar alcohol, **D-mannitol** (Fig. 1.7) is, however, the accumulation product (up to 25% of the dry weight of some *Laminaria* species in the autumn) of photosynthesis.

In a number of brown algae, the mannitol concentration in the cell increases or decreases as the salinity of the surrounding medium increases or decreases (Reed et al., 1985). This osmoregulatory mechanism prevents the cells from bursting in hypotonic media or shrinking in hypertonic media. The increase in mannitol concentration occurs in the dark as well as in the light, showing that photosynthesis is not involved in the process.

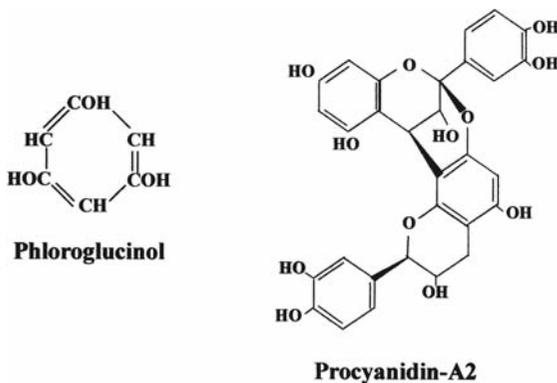
The brown algae are unique among the algae in having uptake of inorganic carbon, and therefore photosynthetic carbon fixation, stimulated by blue light (Forster and Dring, 1994). Most of the Phaeophyceae live in the littoral zone where they receive a generous amount of light. Photosynthesis is usually limited by the supply of inorganic carbon in this environment. The Phaeophyceae have evolved a mechanism to increase the amount of inorganic carbon uptake, but only when the cells are illuminated by blue light, thereby conserving the energy required for the process when the cells are in the dark. The only brown algae that do not have this mechanism are the furoids, which appear to have evolved a separated carbon-concentrating mechanism.

### Phlorotannins and physodes

**Phlorotannins** (phaeophyceean tannins) are stored in physodes (Fig. 21.5) in the cytoplasm of many brown algae. Phlorotannins are formed by polymerization of phloroglucinol (1,3,5-tri-hydroxybenzene) (Fig. 21.6) through the acetate-malonate pathway (Schoenwaelder and Clayton, 2000; Pavia



**Fig. 21.5** Physodes in zygotes of *Hormosira banksii*.  
 (a) Bright-field microscopy of sectioned zygotes showing accumulation of physodes around the nucleus (arrow).  
 (b) Transmission electron micrograph showing physodes around the nucleus. (From Schoenwaelder and Clayton, 2000.)



**Fig. 21.6** Chemical structure of phloroglucinol, the basic building block of polyphenols and the chemical structure of the polyphenol procyanidin.

et al., 2003). The phlorotannins in the physodes appear as a colorless, highly refractive acidic fluid that stains red with vanillin and hydrochloric acid. The tannins are non-glycosidic (do not contain sugars), bind proteins, have strong reducing action, and are astringent to the taste. They are readily oxidized in air, resulting in the formation of a black pigment, **phycophaein**, giving dried brown algae their characteristic black color.

The phlorotannin content of brown algae varies from 1% to 15% of dry mass. Phlorotannins occur

at high levels in brown algae of the temperate and tropical Atlantic, whereas levels are low (less than 2% of dry mass) in the tropical Pacific and Indo-Pacific (Targett and Arnold, 1998). In temperate areas, the fucoids (Fucales) have high concentrations of phlorotannins whereas kelps (Laminariales) have low concentrations of phlorotannins. The same species of brown alga will have a higher phlorotannin content when deprived of nitrogen (Van Alstyne and Pelletreau, 2000).

Phlorotannins have been postulated to function in (1) deterring grazing by herbivores, (2) absorbing ultraviolet radiation, and (3) serving as a component of cell walls (Henry and Van Alstyne, 2004). The effect of phlorotannins as a chemical defense against herbivores has been best studied. Phlorotannins deter feeding by inhibiting the glycosidases of gastropods (Shibata et al., 2002). Phlorotannins of high molecular weight inhibit herbivory more than phlorotannins of low molecular weight (Targett and Arnold, 1998; Kubanek et al., 2004). There is variation among herbivores in the effectiveness of phlorotannins against grazing. Herbivores with digestive systems containing surfactants (wetting agents) or high pH are able to tolerate phlorotannins better. Phlorotannins are not normally secreted outside the cell (Shibata et al., 2002). It is necessary for the cells to be damaged before the phlorotannins are released.

Phlorotannins are always present in brown algae and thus are a “constitutive” chemical defense. Stimulation of phlorotannin synthesis by

grazing would be “inducible” chemical defense, which does not occur in most brown algae (Luder and Clayton, 2004). Synthesis of phlorotannins may be induced by the growth regulator jasmonic acid as it is in higher plants (Arnold et al., 2001).

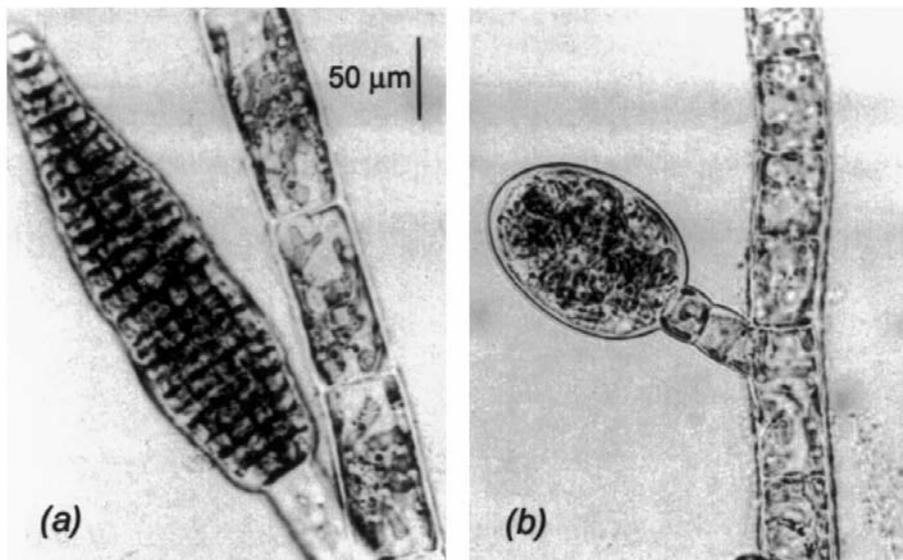
Eggs of the Phaeophyceae contain phenolic vesicles just under the plasma membrane that are discharged outside the cells by exocytosis after fertilization. It has been postulated that the discharge of these phenolic vesicles has a toxic effect on spermatozooids and acts as a polyspermy block before the primary wall is secreted (Clayton and Ashburner, 1994). These peripheral phenolic vesicles are distinguishable from physodes, which also contain phenolic compounds, but which are significantly larger and tend to be localized around the egg nucleus.

## Life history

The unilocular sporangium (Fig. 21.7(b)) is generally considered to be the site of meiosis, the haploid zoospores that are released forming the gametophyte generation. The gametophyte then produces the gametes, which fuse to form the

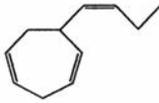
zygote (Bell, 1997). Although meiotic divisions have been considered the rule in the unilocular sporangium, a disturbingly large number of investigations have not found this to be the case. In these investigations there is a “direct” type of life history, with no meiosis or fusion occurring. More research is needed in this area to clarify the situation. In the phaeophycean life cycle, a **plethysmothallus** is a filamentous stage (or one composed of compacted filaments) that can multiply itself by spores (usually zoospores from plurilocular sporangia) (Papenfuss, 1951).

The thallus of many Phaeophyceae is relatively large and complex with a number of different types of growth that include: (1) **diffuse**, with most of the cells of the plant capable of cell division (*Ectocarpus*, Fig. 21.17 and *Petalonia*, Fig. 21.20); (2) **apical**, with a single cell at the apex giving rise to the cells beneath (*Dictyota*, Fig. 21.11 and *Sphacelaria*, Fig. 21.13); (3) **trichothallic**, where a cell divides to form a hair above and a thallus below (*Cutleria*, Fig. 21.14 and *Dermarestia*, Fig. 21.15); (4) **promeristem**, with a non-dividing apical cell controlling a large number of smaller meristematic, dividing promeristematic cells beneath it (*Fucus*, Fig. 21.41); (5) **intercalary**, with



**Fig. 21.7** *Ectocarpus fasciculatus*, plurilocular (a) and unilocular (b) sporangia. (From Dixon et al., 2000.)

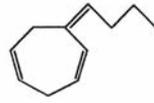
### Monosubstituted Cycloheptadienes



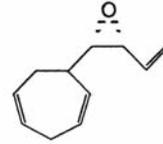
Ectocarpene  
*Ectocarpus*  
*Sphacelaria*



Desmarestene  
*Desmarestia*

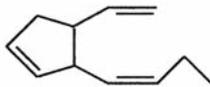


Dictyotene  
*Dictyota*



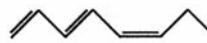
Lamoxirene  
*Laminaria*, *Macrocystis*

### 3,4- Disubstituted Cyclopentene



Multifidene  
*Cutleria*, *Chorda*

### Linear Saturated Hydrocarbon



Fucoserratene  
*Fucus*

**Fig. 21.8** The chemical structure of some brown algal pheromones with the names of the algae that secrete the pheromones. (Modified from Pohnert and Boland, 2002.)

a zone of meristematic cells forming tissue above and below the meristem (*Laminaria*, Figs. 21.24, 21.23); (6) **meristoderm**, with a layer of usually peripheral cells dividing periclinally (parallel to the surface of the thallus) to form a tissue below the meristoderm (usually cortex) and occasionally anticlinally (perpendicular to the surface of the thallus) to add more cells to the meristoderm (*Fucus*, Fig. 21.41).

Investigations on the sexual hormones of the brown algae in the 1970s and 1980s by Müller (Fig. 21.9) and his associates represent a major advancement in the knowledge of the brown algae (Müller, 1982; Maier and Müller, 1986). A **sexual hormone (sirenine, pheromone)** is a diffusible substance that coordinates cellular activities during sexual reproduction. Two types of biological effects mediated by sexual hormones occur in the brown algae: (1) the explosive discharge of spermatozooids from antheridia, and (2) the attraction of male gametes by female gametes or eggs. All sexual hormones in the brown algae are unsaturated hydrocarbons (have at least one double or triple bond) (Fig. 21.8) (Müller et al., 1982). With the exception of the *Fucus* sperm attractant, all of the sexual hormones in the brown algae are C<sub>8</sub> to C<sub>11</sub> olefins (unsaturated

open-chain hydrocarbons containing at least one double bond), most of them incorporating a five- or seven-membered ring structure. All of the attractions in the brown algae identified so far are highly volatile and hydrophobic. Very small amounts of the pheromones are released, only 0.6 fmol per cell per hour from *Ectocarpus siliculosus* gametes. However, the non-polar nature of the pheromones contrasts strongly with the highly polar nature of water, making the pheromones easily recognizable to the responding cells. Perception of the pheromone starts with the simple partition from water of the non-polar pheromone into the lipid plasma membrane of the gamete that contains the macromolecular receptors (Pohnert and Boland, 2002). The substances have a very strong tendency to leave the aqueous solution and escape into the air. This characteristic helps to avoid the buildup of chronic concentrations which would decrease the efficiency of the gradients around the female cells. The attractants probably do not function more than 0.5 mm away from the female cells, and thus attraction is clearly a short-distance phenomenon (Müller, 1982). The sexual hormones include **ectocarpene** from *Ectocarpus* (Müller et al., 1971), **desmarestene** from *Desmarestia aculeata* (Müller et al., 1982), **lamoxirene** from *Laminaria* (Müller et al., 1985a,b), **multifidene** from *Cutleria multifida* (Jaenicke et al., 1974), **dictyopterene C'** from *Dictyota dichotoma* (Müller et al., 1981), and

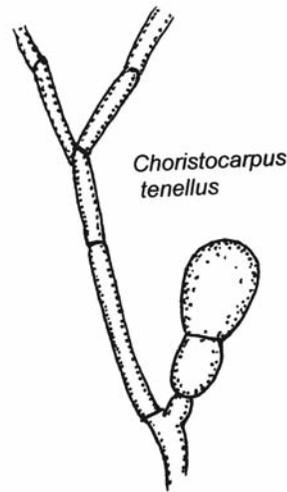


**Fig. 21.9** **Dieter G. Müller** Born January 24, 1935, in Stuttgart, Germany. From 1956 to 1961, Dr. Müller studied at the Universität Tübingen; from 1961 to 1963, he was a Postdoctoral Fellow at the University of Pennsylvania; from 1964 to 1973, he was Wissenschaftlicher Mitarbeiter at the Max-Planck-Institut für Züchtungsforschung at Köln-Vogelsang. In 1973, he received his Habilitation at the University of Köln; and since 1973, he has been professor in the Fakultät für Biologie at the Universität Konstanz. In 1964, Dr. Müller received strong support from Professor J. Straub, director of the Max-Planck-Institut, Köln, to work out in detail the life cycle of *Ectocarpus siliculosus*. After evidence for a sexual hormone was discovered, a cooperation scheme was established with the Institut für Biochemie at the University of Köln. These two events led to the characterization of the sexual hormones of the brown algae.

**fucoserratene** from *Fucus serratus* and *F. vesiculosus* (Müller and Jaenicke, 1973) (Fig. 21.9).

## Classification

The Phaeophyceae are an ancient lineage, originating between 150 (Medlin et al., 1997) and 200 million years ago (Lim et al., 1986). The Xanthophyceae and Phaeothamniophyceae are the closest known sister taxa to the Phaeophyceae. The first true brown alga was probably similar to the extant *Choristocarpus tenellus* (Fig. 21.10) with creeping filaments, apical growth, and an isomorphic life history (de Reviere and Rousseau, 1999; Draisma et al., 2001).



**Fig. 21.10** *Choristocarpus tenellus* showing the uniseriate filament and a propagule with an apical cell.

Within the Phaeophyceae, the Fucales are a sister group to the remainder of the algae in the class. The early divergence of the Fucales is consistent with the presence of a proboscis in the spermatozooids of *Vaucheria* and *Fucus* (de Reviere and Rousseau, 1999). Fossils similar to *Cutleria* occur in 25 million-year-old Miocene deposits while fossils similar to algae in the Laminariales and Fucales occur in 16–20 million year old Miocene deposits.

The orders considered here are presented in an evolutionary sequence with the Dictyotales and Sphacelariales being the most ancient and the Ectocarpales and the Laminariales the most recent (de Reviere and Rousseau, 1999; Draisma et al., 2001).

- Order 1 **Dictyotales**: growth by an apical cell; meiosis occurring in the production of four to eight non-motile spores; oogamous sexual reproduction.
- Order 2 **Sphacelariales**: growth by an apical cell; daughter cells divided longitudinally to give a polysiphonous structure; isogamous sexual reproduction.
- Order 3 **Cutleriales**: trichothallic growth forming a fan-like thallus in at least one generation; anisogamous sexual reproduction.

- Order 4 **Desmarestiales**: trichothallic growth forming axial cells; oogamous sexual reproduction.
- Order 5 **Ectocarpales**: thallus consisting of filaments or filaments compacted together; reproduction isogamous or anisogamous.
- Order 6 **Laminariales**: diploid thallus parenchymatous resulting from an intercalary meristem between the stipe and blade; reproduction oogamous.
- Order 7 **Fucales**: growth primarily by a promeristem; gametophyte reduced to egg and sperm; oogamous sexual reproduction.

### Dictyotales

This order has organisms that grow by means of an apical cell or by a marginal row of apical cells. There is an isomorphic alternation of erect, flattened, parenchymatous thalli. A distinctive character of this order is the modification of the unilocular sporangia to produce four to eight large aplanospores. Sexual reproduction is oogamous. The Dictyotales are common in warmer waters throughout the world.

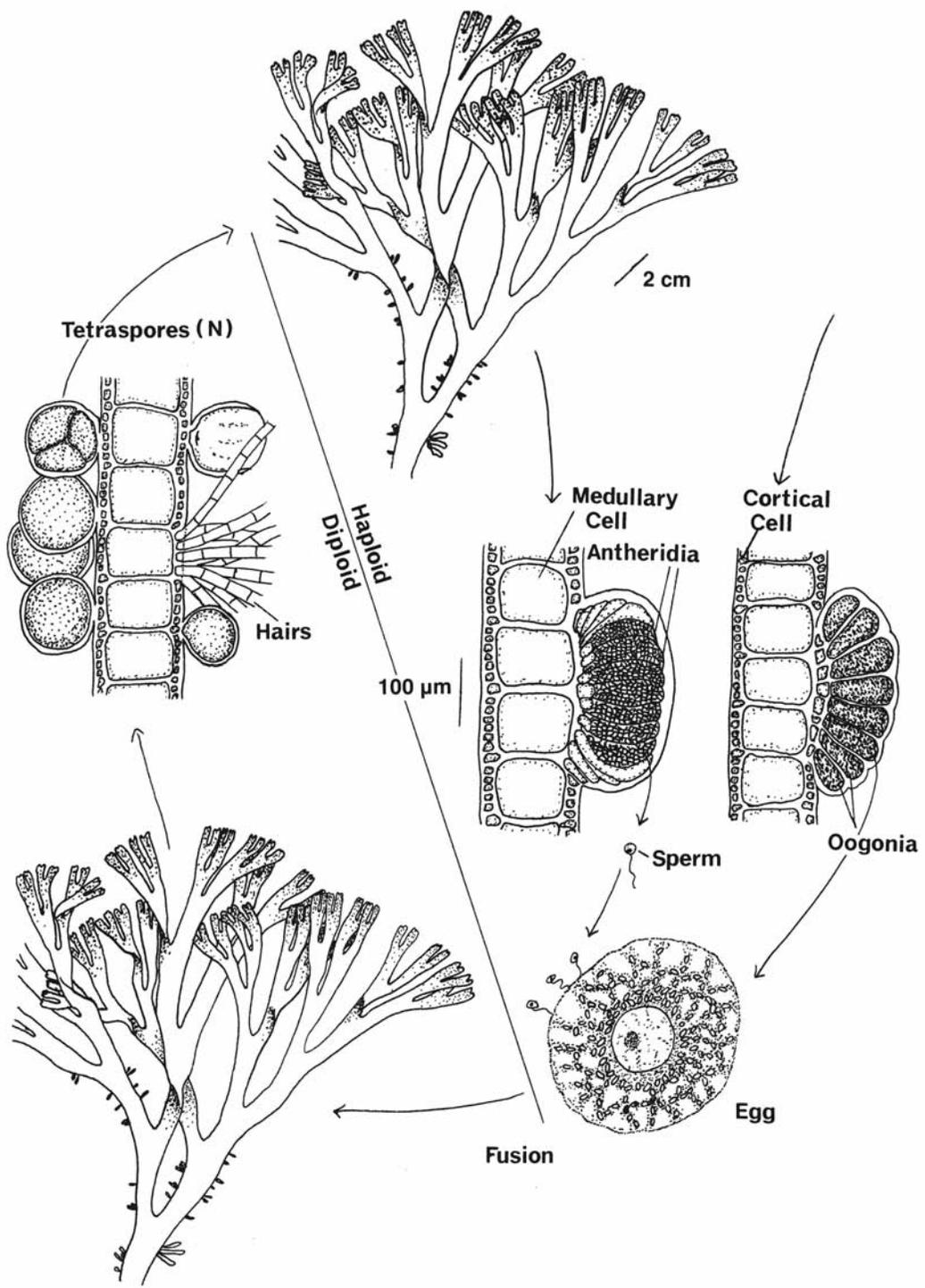
*Dictyota dichotoma* has a single apical cell that forms the flattened annual thallus (Fig. 21.11). The mature thallus consists of three layers: a middle layer composed of large cells with few or no chloroplasts, surrounded on both sides by a layer of small cells densely packed with chloroplasts. Gametophytes form sex organs in projecting sori. Gametogenesis can be artificially induced by exposure of the gametophytes to blue light (Kumke, 1973). An oogonium develops from a surface cell that divides into a stalk cell and the oogonium proper. Each oogonium produces a single egg, which is liberated through the gelatinized apex of the wall. There are usually 25 to 50 oogonia in a sorus with sterile oogonia at the margin. The deep-brown color of the female sori contrasts with the white glistening spots that comprise the male sori. The male sori can be recognized early in their development by the disintegration of the chloroplasts in the cells. Like the oogonia, the antheridia develop from surface cells. These cells enlarge and divide horizontally into a stalk cell and a primary spermatogenous cell. This cell

divides and redivides in vertical and horizontal planes into between 650 and 1500 compartments (Williams, 1904). The content of each locule becomes a pear-shaped sperm with a single, laterally inserted, tinsel flagellum and an anterior eyespot (Phillips and Clayton, 1993). Although there is only one emergent flagellum, a second basal body is present (Manton, 1959), indicating a derivation from a biflagellate ancestor. The mature sperms are set free by dissolution of the walls of the antheridium. The male sorus is surrounded by elongated sterile cells that are regarded as undeveloped antheridia. The egg secretes the pheromone **dictyotene** (Fig. 21.8) (Pohnert and Boland, 2002) that attracts the sperm. The sperm fertilizes the egg to produce the zygote that germinates into the sporophyte; unfertilized eggs can germinate parthenogenetically but seldom develop normally and soon abort. The sporophytes produce haploid aplanospores (tetraspores) on the surface of the thallus. The tetrasporangia occur singly or in small groups. The naked tetraspores are released by gelatinization of the apex of the sporangium, and soon after liberation the large motionless spores secrete a cellulose wall and develop into the gametophytes.

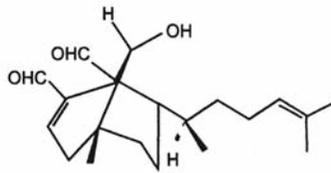
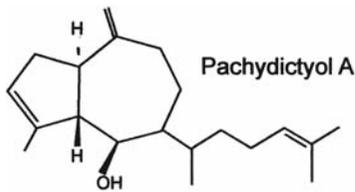
In *D. dichotoma*, the gametes are released at regular intervals. This was first noticed by Williams (1905) in Great Britain, where the gametes are released fortnightly. Müller (1962) showed that moonlight is the synchronizing factor for the release. When he grew the alga in natural light, gametes were released every 14 to 15 days. If the alga was grown under artificial conditions with a 14 hours light:10 hours dark cycle, then few gametes were released, and there was no synchrony. If the artificially lighted cultures had the lights left on all night, then 10 days later a burst of gametes was released. The lights being left on all night simulated moonlight.

Species of *Dictyota* produce terpenoids, such as pachydictyol and (6R)-6-hydroxydichotoma-3,14-diene-1,17,dial (Fig. 21.12), that inhibit grazing of *Dictyota* by herbivorous fish, amphipods, and sea urchins (Schmitt et al., 1998; Pereira et al., 2000).

The only calcified genus in the Phaeophyceae, *Padina*, is in the Dictyotales.



**Fig. 21.11** The life cycle of *Dictyota dichotoma*. (Adapted from Thuret and Bornet, 1878; Williams, 1898; Taylor, 1960.)



(6R)-6-hydroxydichotoma-3,14-diene-1,17-dial

**Fig. 21.12** The chemical structure of two secondary metabolites secreted by *Dictyota* spp. that act as antifoulants.

## Sphacelariales

This order is characterized by an apical meristematic cell that divides transversely to produce the daughter cells. The algae produce distinctive vegetative propagules. Another less precise characteristic is blackening of the cell walls when treated with bleaching liquid (Draisma et al., 2002).

*Sphacelaria* (Fig. 21.13) grows attached to rocks or other algae and has one or more freely branched shoots arising from a discoid holdfast. The apical cell undergoes transverse divisions, with subsequent longitudinal septation of the daughter cells to produce a polysiphonous structure. Although the maturing axes and branches undergo septation into smaller and smaller cells, they do not enlarge; thus the diameter of the filament is essentially the same from the base to the apex. Older axes, though, may become corticated by downward-growing filaments. The erect axes are usually abundantly branched, usually in a regular, distichous manner.

Asexual reproduction is by means of **propagula** (Fig. 21.13), which are specialized branchlets of distinctive form that are produced throughout the vegetative parts of the plants. They are formed much more frequently than sporangia or gametangia. Each propagule has an apical cell and usually two to three protuberances. After falling from the parent plant and contacting a suitable substrate, the propagule develops into a new plant. Propagula are formed only at temperatures above 12 °C and under daylight conditions longer than 12 hours (Colijn and van den Hoek, 1971).

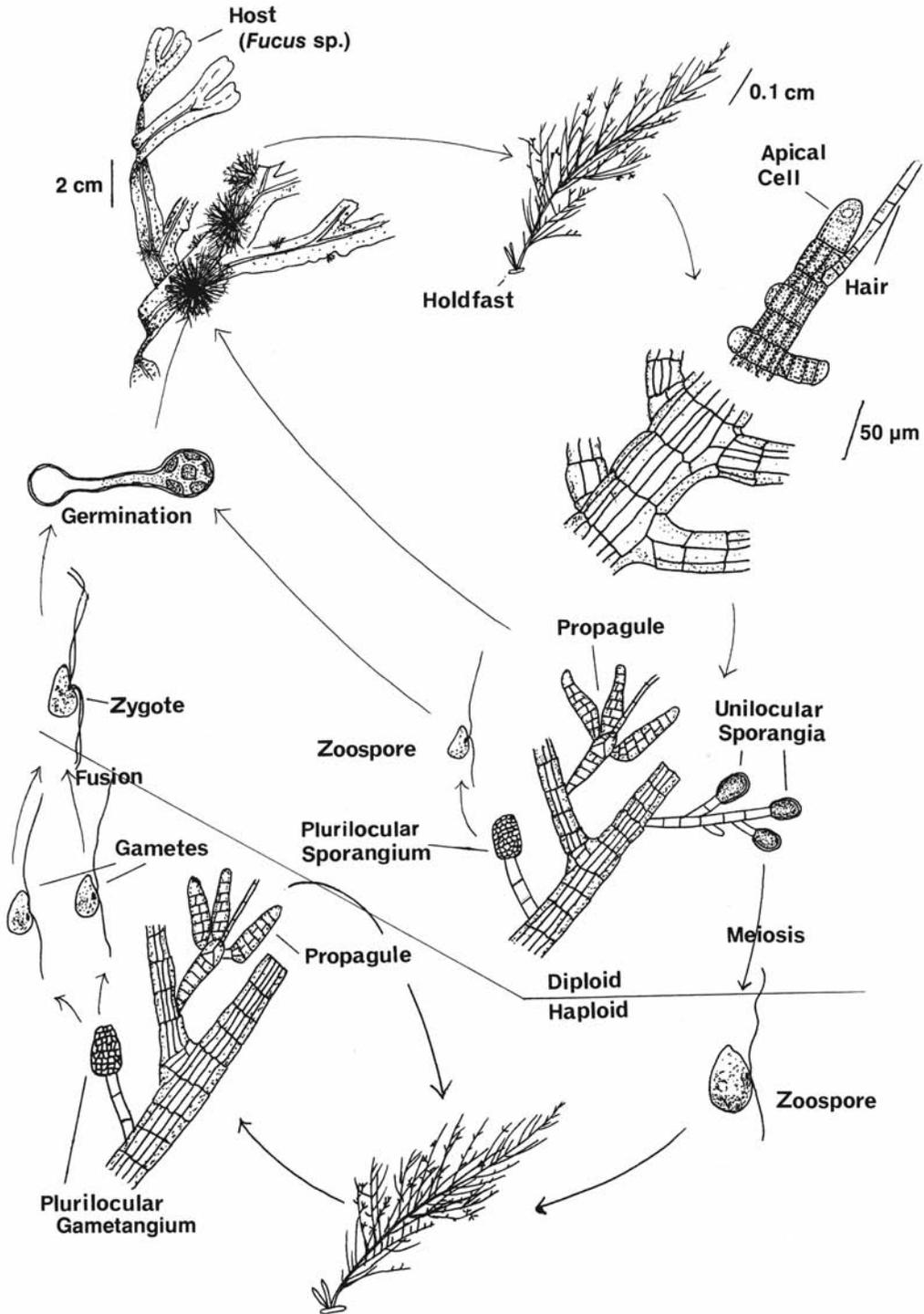
In *S. bipinnata*, the sporophyte forms both unilocular and plurilocular sporangia terminally on branches (Fig. 21.13). The plurilocular sporangia produce zoospores that re-form the parent

sporophyte. Meiosis occurs in the production of zoospores in the unilocular sporangia (Clint, 1927). Over 200 zoospores are released through an apical pore in the unilocular sporangium (Papenfuss, 1934). The zoospores germinate to presumably form gametophytes that are similar to the sporophytes. The gametophytes produce plurilocular gametangia of one type, which release isogamous gametes. **Ectocarpene** (Fig. 21.8) is used as a pheromone in gamete attraction (Pohnert and Boland, 2002). The fusion of gametes takes place while they are motile and produces a quadriflagellate zygote that may continue moving for several hours. The life cycle of another species, *S. furcigera*, involves anisogamy and unisexual gametophytes, which are somewhat smaller than the sporophytes (van den Hoek and Flinterman, 1968). The life cycle is controlled by temperature and photoperiod.

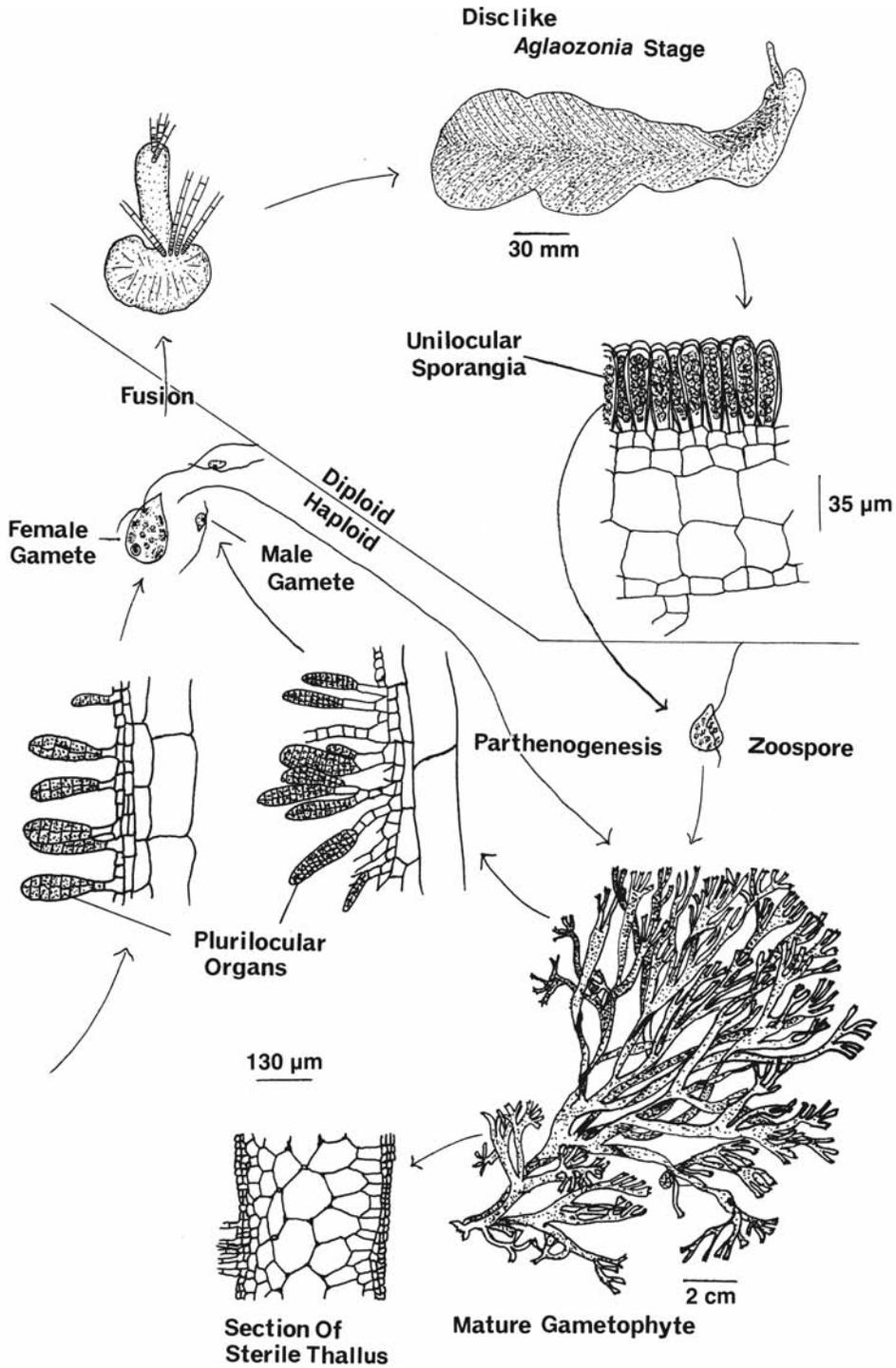
## Cutleriales

This order contains only two genera, *Cutleria* and *Zanardinia*. The genera show an alternation of generations that is heteromorphic in *Cutleria* and isomorphic in *Zanardinia*. The thallus is flattened, blade-like, or disc-like, with entirely or partially trichothallic growth. The sporophytes produce only unilocular sporangia, whereas the gametophytes are heterothallic and markedly anisogamous.

*Cutleria* is a warm-water plant of the Northern Hemisphere that may be closely related to *Saccorhiza* in the Laminariales (Rousseau et al., 1997). The gametophyte is an erect, flattened blade with numerous dichotomies (Fig. 21.14). Growth is trichothallic at the base of many erect uniseriate hairs at the upper margin of the blade. The cells that are cut off below the hairs contribute to the thallus. The innermost of these cells gradually enlarge to form the medulla, whereas



**Fig. 21.13** The life cycle of *Sphacelaria* (*S. cirrhosa* and *S. bipinnata*). (Adapted from Savaugau, 1900–14; Papenfuss, 1934; Taylor, 1957.)



**Fig. 21.14** The life cycle of *Cutleria multifida*. (Adapted from Kuckuck, 1899; Savaugeau, 1899.)

the outer ones undergo divisions to form the cortex. The gametophytes are heterothallic, with the sex organs developing in clusters on the surface of the thallus. A superficial epidermal cell may develop directly into a male gametangium, or it may develop into a branched hair that bears several gametangia. The male gametangium consists of a stalk cell on which there are 20 or more tiers of cells, each tier composed of eight cells. The protoplast of each cell forms a biflagellate male gamete, which escapes through a pore in the gametangial wall to the outside. Female gametangia develop similarly to the male, but with a smaller number of larger cells. Female gametangia are four to seven tiers high, with only four cells in each tier. Free-swimming male gametes are pyriform, with a single reddish chloroplast at the place of flagella insertion. Free-swimming female gametes are also pyriform, but are much larger and have a dozen or so chloroplasts. The female gametes release a highly volatile, low-molecular-weight compound, **multifidene** (Fig. 21.8) (Pohnert and Boland, 2002) that attracts the male gametes (Müller, 1974). When the gametes fuse, the male gametes are actively swimming while the female are sluggish or immobile. Fusion of the two nuclei follows within a few hours, and the zygote begins to develop into the sporophyte within a day. Unfertilized female gametes develop parthenogenetically into gametophytes.

The zygote germinates to produce the sporophyte, which was first described as a separate genus, *Aglaozonia*. At first, growth is trichothallic and vertically upward into a columnar structure. Upward growth ceases when the plant is about 10 days old, and all further growth is laterally outward from the base of the column. Repeated cell division at the base of the column forms a flat, disc-like tissue that expands laterally as a result of division and redivision of the marginal cells. The sporophyte is homologous to a minute erect thallus subtended by an enlarged fertile holdfast. The disc-like portion of the thallus is several cells thick, and the outer cells are differentiated into an epidermis-like layer. The holdfast is attached to the substratum by numerous multicellular rhizoids growing from the ventral epidermal cells. The unilocular sporangia are formed in sori on the dorsal surface of the sporophyte. A single epi-

dermal cell divides into one to six stalk cells and a single terminal unilocular sporangium. In the unilocular sporangium 8, 16, or 32 large, pyriform, haploid zoospores are formed, each with several chloroplasts. The zoospores escape through a large apical pore in the sporangial wall, swarm for 10 to 90 minutes, then settle down, round up, and secrete a wall. The zoospores then divide to form the gametophyte.

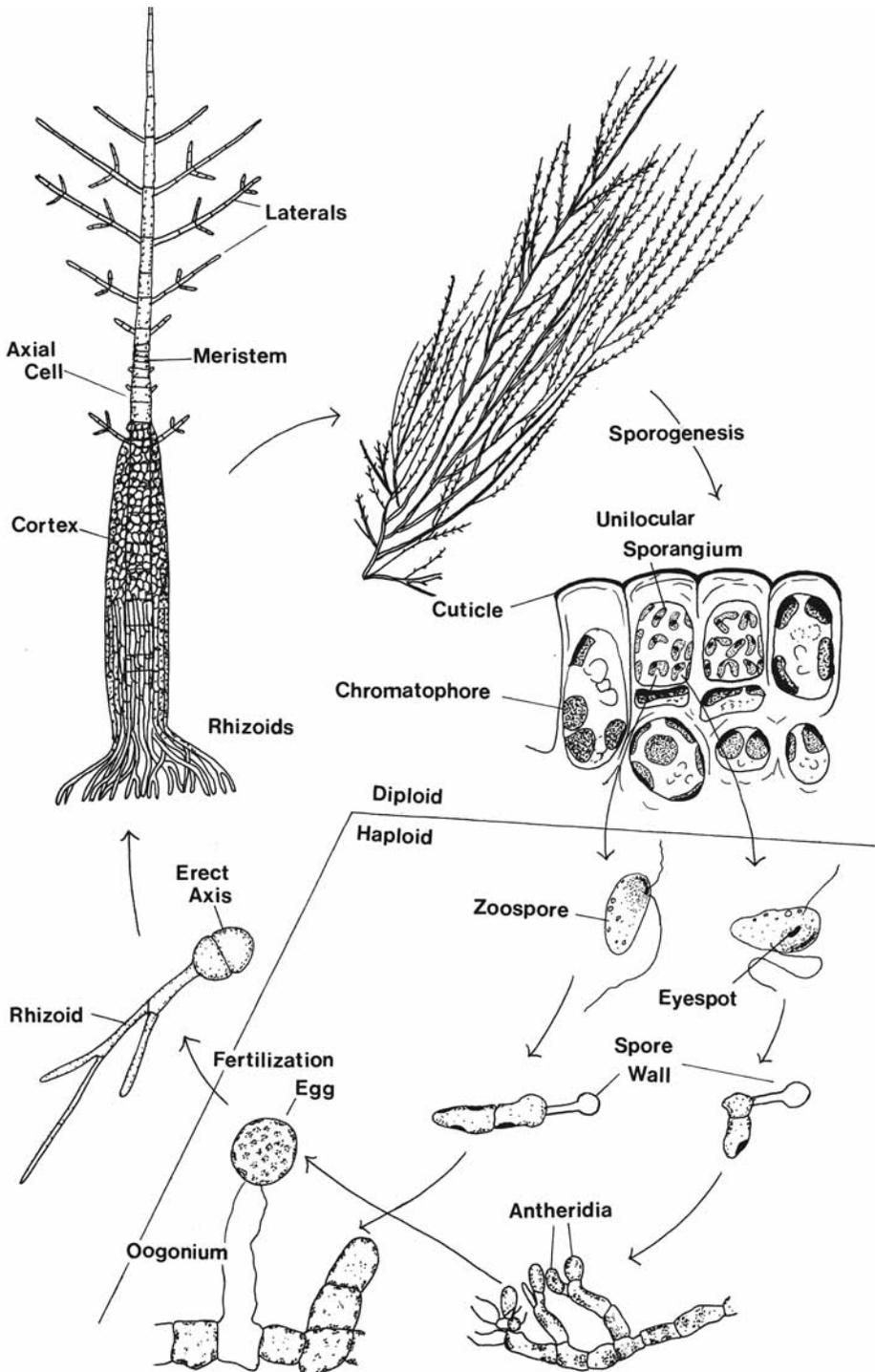
Although germlings from zygotes and from zoospores of the *Aglaozonia* sporophytes have not been grown to maturity in culture, they have been grown to a sufficiently advanced stage to show that the two stages are alternate generations of each other. In Europe, the sporophyte is perennial and fruits in winter or spring, whereas the gametophyte is a spring annual that disappears during the summer.

Fossils of a plant similar in structure to *Cutleria*, called *Limnophycus paradoxa*, have been described from the Miocene (25 million years old) deposits in Germany.

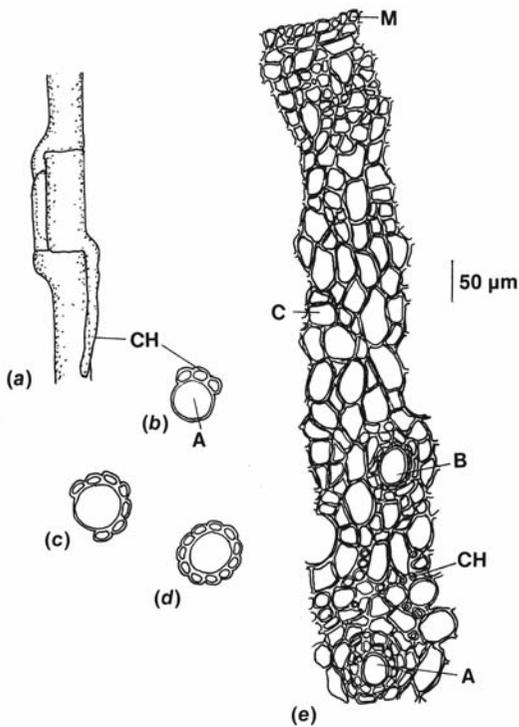
### Desmarestiales

In this order there is an alternation of a large macroscopic sporophyte with a small filamentous gametophyte (Fig. 21.15). The gametophyte forms oogonia and sperm, with the result that reproduction is oogamous. Growth of the sporophyte is trichothallic, and the main axis is corticated by downward-growing cells. In this treatment, the organisms sometimes considered in the Sporochnales are placed in the Desmarestiales, as suggested by Russell and Fletcher (1975).

Sporophytes of *Desmarestia* may reach a length of 2 to 3 m and occur primarily in the sublittoral region in colder waters of both the Northern and the Southern Hemisphere. The plants (Figs. 21.15, 21.16) have trichothallic growth from an intercalary meristem composed of flattened cells that cut off cells to the terminal hair above, and cells to the thallus below. The cells of the terminal hair continually wear away, and each of these cells usually bears one or two unbranched laterals. The cells produced below the meristem cut off two opposite laterals in one plane, which lengthen by means of a basal meristem. Cortication of the thallus begins by the basal cells of the lateral cutting off a number of cells that gradually form a



**Fig. 21.15** The life cycle of *Desmarestia*. (Adapted from Schreiber, 1932; Chapman and Burrows, 1971.)



**Fig. 21.16** *Desmarestia*. (a)–(d) Cortication of an axial cell (A) by corticating hyphae (CH). (e) Partial section of a mature thallus. (B,C) Laterals; (M) meristoderm.

complete one-layered envelope around the elongating axial cell that produced the lateral. The cells of this one-layered envelope soon undergo periclinal division, with the outer layer that is produced behaving as a meristem. This meristem then produces the cells of the cortex to the inside, with the result that the axial cells and laterals are progressively buried. In addition to this primary growth, the cells of the inner cortex are capable of secondary growth, enlarging to form hyphae that push their way downward between the cells of the cortex. Trumpet hyphal cells occur in the medulla. These cells have perforate end walls with callose and probably function in conduction of nutrients as do the sieve filaments in the Laminariales (Moe and Silva, 1981).

*D. aculeata* (Fig. 21.15) produces unilocular sporangia on the sporophytes in the winter by the tangential division of a surface cell of the cortex. Meiosis apparently occurs in the production of a few biflagellate zoospores formed in each small

unilocular sporangium. The zoospores have an eyespot and a single chloroplast. Released zoospores settle, lose their flagella, and round up within 24 hours. The cell contents move out into a germ tube immediately after settling. The sporelings produce female and male gametophytes in roughly similar numbers, the male gametophytes having small cells and being less densely pigmented than the female gametophytes. Gametophytes grow vegetatively only in red light, with differentiation of oogonia and antheridia occurring under blue or white light (Müller and Lüthe, 1981). About 11 days to 3 weeks after germination of spores, conical antheridia and lateral oogonia appear on the gametophytes. The tubular oogonia dehisce apically to liberate the egg, which usually adheres to the gelatinized aperture. A single spermatozoid is released from each antheridium through a narrow apical aperture. The freshly released eggs secrete three volatile chemicals that cause the antheridia to burst and attract free spermatozoids to the eggs. The three sexual hormones are **desmarestene**, **ectocarpene**, and **viridene** (Fig. 21.8). Desmarestene is the most potent of the three sexual hormones (Müller et al., 1982). Sporophyte development begins with the production of a tube from one side of the zygote and a lightly pigmented rhizoid from the opposite pole. The initial tube goes on to produce an oppositely branched uniseriate filament, which forms a trichothallic meristem below most of the lateral branches. Cortication of the thallus begins, as has already been described, to produce a mature sporophyte and complete the life cycle. Gametophytes of *Desmarestia* (Andersen, 1982) and the Laminariales have several features in common, including clusters of unicellular antheridia, vertically elongated intercalary oogonia, attachment of the extruded egg to the oogonial apex, and growth of the young sporophyte upon the oogonial apex.

Some of the species of *Desmarestia* accumulate large amounts of malic acid, lowering the pH of the vacuolar sap to as low as 2. In collecting seaweeds, the species of *Desmarestia* should be kept separate because some of their cells will rupture, releasing acid and killing the other seaweeds.

In the Antarctic waters, members of the Desmarestiales provide the bulk of the biomass of benthic seaweeds. They are perennial, covering

large areas of water to depths of about 40 m. The largest and most abundant species (*D. anceps* and *D. menziesii*) form thickets, but not the protective canopy characteristic of many kelps. The Antarctic possesses the only cold-water flora without Laminariales, although in sub-Antarctic waters there are vast stands of kelps (*Macrocystis* and *Lessonia*) (Moe and Silva, 1977).

The Desmarestiales and the Laminariales have a number of similar development characteristics (Tan and Druehl, 1996) that include (1) vegetative development of the gametophyte in red light; (2) requirement of white or blue light for development of antheridia and oogonia; (3) existence of spermatozoid-releasing and -attracting factors secreted by eggs; (4) unusually long and flexible hind flagella; (5) lack of eyespots in spermatozooids; and (6) formation of sexual organs by the gametophyte, representing an exhaustive and almost lethal effort for the gametophytes.

### Ectocarpales

These algae consist of filaments or of filaments compacted together. In the order it is possible to see the gradual morphological evolution from a filamentous structure to **pseudoparenchymatous (haplostichous)** complex structures of compacted filaments (from the Ectocarpaceae to the Ralfsiaceae, and Splachnidiaceae). Along another line, the filamentous thallus has evolved by the division of the filament into true **parenchymatous (polystichous)** thalli (from the Ectocarpaceae to the Scytosiphonaceae). Most of the algae in the order are **heterotrichous**, with the thallus consisting of two different parts: (1) the prostrate creeping disc that functions as a holdfast, and (2) the erect filamentous, bulbous, or foliose stage. In some of the algae, both systems are evident (*Scytosiphon*, Fig. 21.19), whereas in others the erect stage is reduced to filaments of a few cells and the thallus is crustose (*Ralfsia*, Fig. 21.18), and in yet others the erect stage is predominant with the prostrate system reduced to a small holdfast (*Petalonia*, Fig. 21.20). Even within the same alga, there can be a stage that consists of only a thin crust, whereas the alternate stage has a well-developed erect stage.

Nucleic-acid sequencing studies have shown a strong relationship between the Ectocarpales,

Desmarestiales and Laminariales (Draisma et al., 2001).

Four of the families in the Ectocarpales will be considered here.

Family 1 Ectocarpaceae: plants with free-filamentous construction with no adherence of filaments to each other.

Family 2 Ralfsiaceae: algae with a basal layer supporting erect filaments that are compacted together to form a tissue.

Family 3 Scytosiphonaceae: parenchymatous thalli with mostly diffuse growth.

Family 4 Splachnidiaceae: plants with trichothallic growth and unilocular sporangia formed in conceptacles.

### Ectocarpaceae

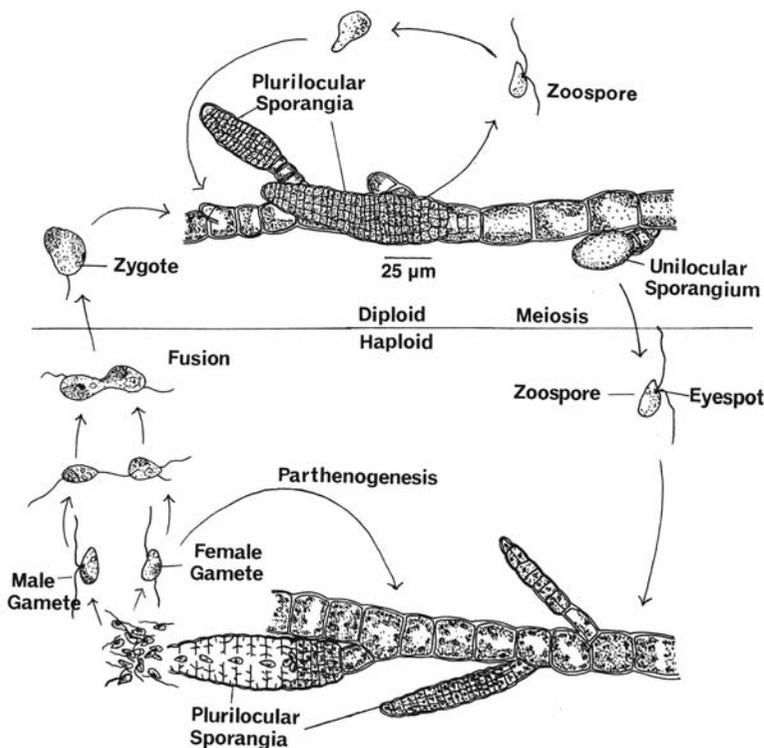
These organisms have free-filamentous construction with no adherence of the filaments to each other. *Ectocarpus* is the prevalent genus, and is composed of freely branched, uniseriate filaments differentiated into prostrate and erect systems. The prostrate parts are rhizoid-like and often penetrate the substrate. Growth can be diffuse or more or less clearly trichothallic, with intercalary cell divisions confined to certain areas of the filaments. Some workers divide up the family into different genera on the basis of cytology and morphology, whereas others consider that the family contains the single genus *Ectocarpus* (Russell and Garbary, 1978).

The life cycle of *E. siliculosus* (Fig. 21.17) can be taken as representative of the family (Papenfuss, 1935). The haploid and diploid phases are both filamentous, but the diploid filaments have longer cells than the haploid filaments. The diploid plants produce unilocular and plurilocular sporangia either on the same plant or on separate plants. These sporangia discharge their zoospores between 0600 and 1200 hours. The mother cell of a unilocular sporangium can be distinguished from a branch initial by the spherical shape and large nucleus of the mother cell. The cell is initially vacuolate, but the physodes and vacuoles are soon extruded from the cell and become lodged in the wall (Loiseaux, 1973). The chloroplasts and nuclei of the unilocular sporangium divide in regular sequence, with the

chloroplasts next to the wall and the nuclei in the center of the cell (Knight, 1929). The nuclei divide meiotically. A chloroplast then becomes associated with a nucleus, and a zoospore is delimited around it. A small perforation occurs at the apex of the unilocular sporangium, and up to 32 haploid zoospores ooze out of the sporangium in a gelatinous matrix. The perforation is small, and zoospores are relatively large, being twice the size of gametes and zoospores from plurilocular sporangia. The zoospores initially swim in a straight pattern, then display circling movements as they explore appropriate surfaces for settling (Iken et al., 2001). The zoospores prefer to settle on a hydrophobic surface, preferably one with a microbial film. The zoospores germinate within 2 to 3 hours to produce haploid filaments.

The plurilocular organs (Fig. 21.17) are modified lateral branches that are divided into as many as 660 cubical cells, each containing a motile cell. The plurilocular sporangia on the diploid filaments produce zoospores that remain motile for 3 to 5 hours, settle, and within 2 to 5 hours germinate to produce diploid filaments like

the parent. The germ tube of the sporeling arises from the narrow, anterior flagellated end of the zoospore, which is always oriented toward the light. The plurilocular organs on the haploid filaments are smaller than those on the diploid filaments, and produce either zoospores or gametes. The motile gametes are all of the same size but differ physiologically. The female gametes settle down about 5 minutes after liberation and secrete a sexual hormone called **ectocarpene** [all-cis-1-(cycloheptadien-2',5'-yl)-1-butene] (Fig. 21.8) (Müller et al., 1971). Male gametes (Fig. 21.17) (Maier, 1997a,b) move very rapidly (269  $\mu\text{m}$  per second) in a straight line in open seawater when no female gametes are around (Müller, 1978). The motile male gametes (which can remain motile for up to 8 hours) swim in circular paths on encountering ectocarpene, the diameter of the circular path decreasing in response to increasing ectocarpene concentration (Müller, 1982). As soon as the female gamete is reached, a firm contact is established between the apical part of the front flagellum of the male gamete and the plasma membrane of the female gamete. The posterior



**Fig. 21.17** The life cycle of *Ectocarpus siliculosus*.

ends of the two gametes fuse to form the zygote. The process of fusion takes about 20 seconds, and after fusion the zygote loses its attraction for male gametes as indicated by the dispersion of the male gametes near the zygote. The zygotes take 2 to 3 days to germinate, and the sporelings develop more slowly than those from diploid zoospores. Some of the unfused gametes have the ability to germinate parthenogenetically to give rise to haploid filaments again. This germination is slow, requiring 36 to 48 hours. Clonal populations of *E. siliculosus* from different parts of the world are interfertile, indicating that there has been little genetic isolation of the species (Müller, 1979). Ectocarpene isolated from *E. siliculosus* attracts male gametes of two other species of *Ectocarpus*, indicating that ectocarpene can be artificially synthesized and is effective in attracting male gametes.

*Ectocarpus* has a fairly wide tolerance to changes in temperatures and salinity. Several studies (Boalch, 1961; Edwards, 1969) have shown that *E. siliculosus* will grow and produce sporangia at temperatures between 10 and 29 °C. Müller (1962) found that at 13 °C this species produces unilocular sporangia, at 19 °C plurilocular sporangia, and at 16 °C both types of sporangia. It has the ability to grow in salinities from 0.5 to 1.5 times that of seawater at 20 °C, and 0.25 to 1.75 times that of seawater at 15 °C. The alga is an obligate photoautotroph and will not grow on any supplied carbon source in the dark. Although it will not grow in the dark, it has the ability to survive up to 150 days of darkness and still remain viable.

### Ralfsiaceae

These algae have a basal layer of branched, radiating, laterally coalesced filaments attached to the substratum by the cell wall or by rhizoids. From this basal layer, chloroplast-bearing filaments arise that are compacted together to give a firm tissue. *Ralfsia* (Fig. 21.18) probably has an isomorphic alternation of generations. The brown crust-like diploid plants produce unilocular sporangia at the base of loosely associated multicellular paraphyses. Zoospores are most likely produced by meiosis in the unilocular sporangium, and the zoospores give rise to haploid crusts. These gametophytes form plurilocular sporangia that are ter-

restrial on erect filaments and probably produce motile cells that can act as gametes or zoospores (Kylin, 1934; Edelman et al., 1968; Hollenberg, 1969).

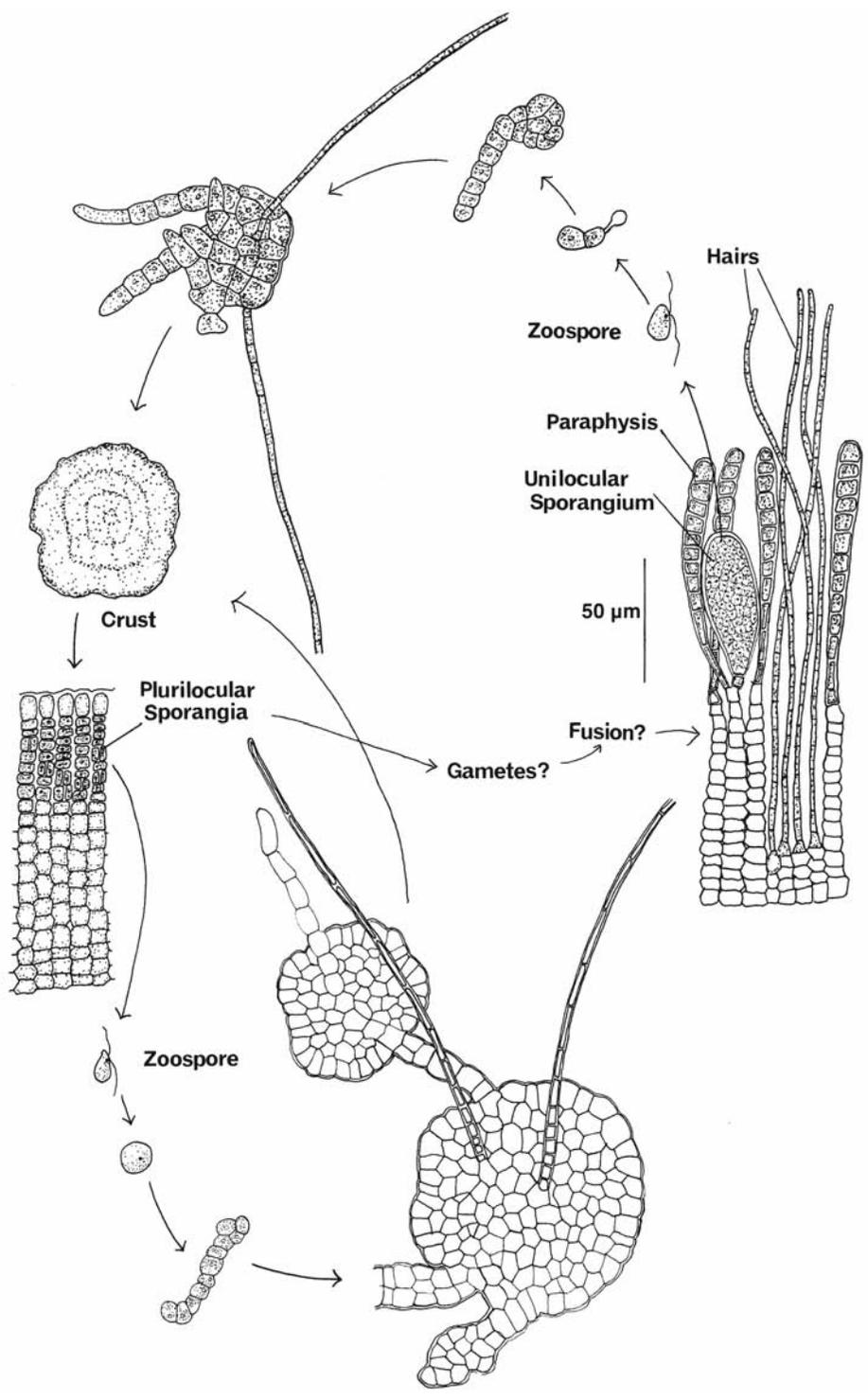
Some of the algae that have been placed in this family are the alternate phase of the life cycle of other higher algae and as such have been removed from the family.

### Scytosiphonaceae

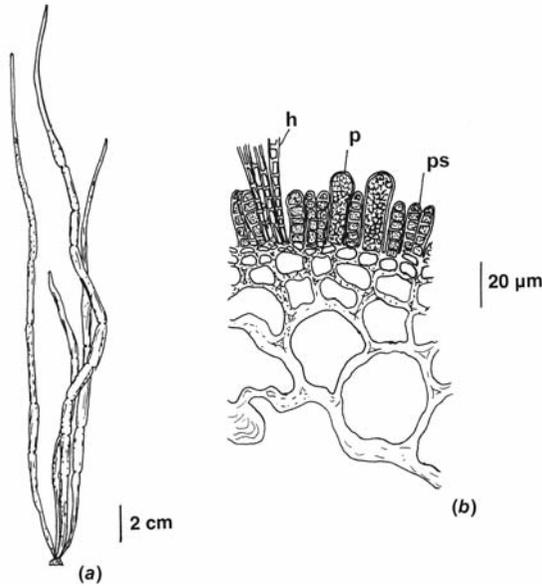
The Scytosiphonaceae, Dictyosiphonaceae, and Punctariaceae are sometimes grouped together in a separate order, the Dictyosiphonales. The members of these three families all have parenchymatous thalli resulting from diffuse or apical growth, and both gametes are motile. These algae, though, have many structural similarities to the more complex members of the Ectocarpales such as the Splachnidiaceae, and will be considered as members of the Ectocarpales, as suggested by Russell and Fletcher (1975).

In the Scytosiphonaceae, growth is diffuse although in some older plants growth can be suprabasal. The macroscopic phase of the plant produces plurilocular sporangia, but not unilocular sporangia. *Scytosiphon lomentaria* (Fig. 21.19) is a common intertidal rock-pool alga. It has a narrow, cylindrical thallus up to 50 cm long, gradually tapering from apex to base. The thallus has occasional constrictions, and the plants grow in tufts with smaller individuals showing no or few constrictions. *Petalonia* (Fig. 21.20) has flat leafy fronds consisting of larger medullary cells covered with smaller cortical cells. In the North Atlantic, *Petalonia fascia* and *Scytosiphon lomentaria* occur in the same area. This area is limited on the north by the 0 °C summer isotherm and on the south by the 17 °C winter isotherm (Fig. 17.9) (van den Hoek, 1982).

The life cycle of *Petalonia* (Fig. 21.20) and *Scytosiphon* (Fig. 21.19) has precipitated some controversy. Workers are more or less divided into the North American School (Wynne, 1969; Edelman et al., 1970; Loiseaux, 1970; Kapraun and Boone, 1987), who claim that meiosis and fusion of gametes do not occur, and the Japanese and Australian school (Nakamura, 1965, 1972; Tatewaki, 1966; Clayton, 1980), who believe that meiosis occurs in the unilocular sporangia and



**Fig. 21.18** The life cycle of *Ralfsia confusa*. (Adapted from Hollenberg, 1969.)



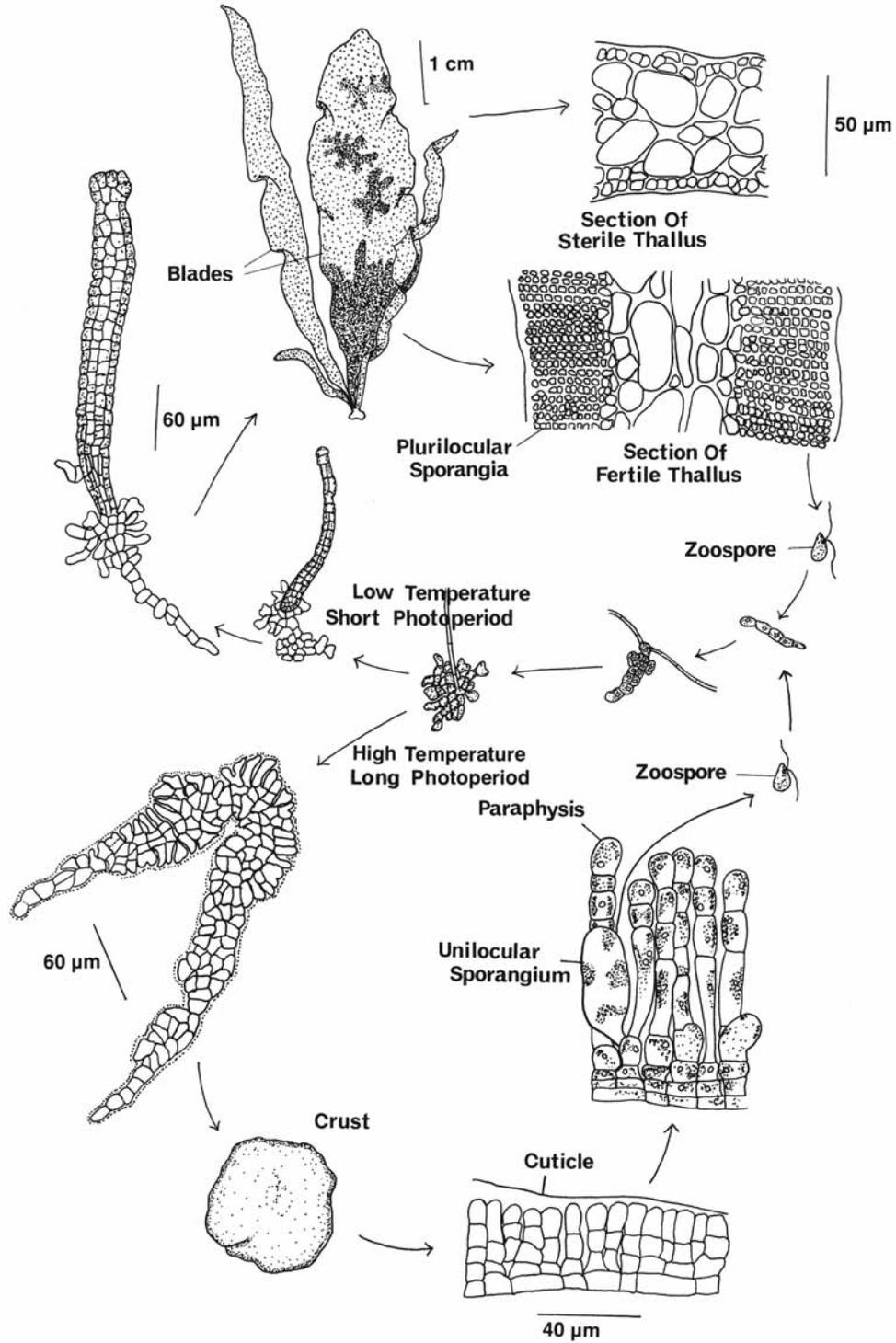
**Fig. 21.19** *Scytosiphon lomentaria*. (a) Whole plant. (b) Portion of a section of the hollow plant showing hairs (h), paraphyses (p), and plurilocular sporangia (ps). (After Taylor, 1957.)

that the motile cells from the plurilocular sporangia function as gametes. The difference in results may be due to different local strains of the same alga. Both groups agree, however, that there are two different morphological phases, a macroscopic phase that produces plurilocular organs and a crustose phase that forms unilocular sporangia.

According to Wynne (1969), *P. fascia* (Fig. 21.20) has the following life cycle. The foliose thalli are annual and usually have erect lanceolate blades on a small discoid holdfast. The blades produce plurilocular sporangia that release negatively phototactic zoospores, with a period of motility lasting up to 24 hours. These zoospores settle and form filamentous germlings with the protoplast not being evacuated from the spore cell. The filaments branch, with the branches spreading laterally to form discs, with each cell having a single parietal plastid and a large pyrenoid. These discs can develop along two different lines, depending on the environmental conditions. Under short days and low temperature, the discs produce erect, uniseriate processes, the blade initials; these then

become parenchymatous by subsequent longitudinal divisions, yielding the upright flattened blade. The surface cell of the blade undergoes numerous anti- and periclinal divisions to form dense lateral files that are the plurilocular organs. The sori cover most of the thallus except for the holdfast and the margin of the blades. The zoospores of the plurilocular organs settle and germinate to form new discs like the original ones. Under long days and high temperature, the discs develop into polystromatic crusts that resemble species of *Ralfsia*. After 4 weeks in culture these crusts reach maturity and are composed of a basal layer of cuboidal cells seldom exceeding six to ten cells in thickness and a layer of paraphyses supported by the basal layer. The paraphyses consist of four to six cells with a conspicuous cuticle covering the paraphyses. This cuticle is evidently secreted by the terminal cells of the paraphyses. A unilocular sporangium is formed by the basal cell of a paraphysis undergoing an unequal division to produce a cell that protrudes from one side. This cell enlarges laterally and upward, a basal cell is laid down, and a unilocular sporangium is formed. The sporangium cleaves up into 128, 256, or more zoospores, which are released by dissolution of the apex of the sporangium. The zoospores free themselves from the enveloping mucilage, swim away, exhibiting negative phototaxis, and, after several hours, settle down. The germlings give rise to the original discs again. Therefore, in the above life cycle the motile cells produced by both the unilocular and plurilocular organs behave similarly, germinating to form discs.

From the above discussion it can be seen that the morphology of the plant is dependent on environmental conditions. In addition, Lüning and Dring (1973) have shown that the type of light will affect the morphology of *Petalonia* and *Scytosiphon*. Among other effects, in red light the prostrate system consists of sparsely branched, uniseriate filaments, whereas under blue or white light it consists of profusely branched filaments. Hsiao (1969, 1970) showed that there are certain minimal concentrations of iodine in the water necessary for the different forms of *P. fascia*. In order to have the formation of the crust-like *Ralfsia* stage,  $4.0 \times 10^{-5}$  M KI was necessary, and, for the formation of blades,  $4.0 \times 10^{-6}$  M KI. Filamentous stages



**Fig. 21.20** The life cycle of *Petalonia fasciata*. (After Smith, 1969; Wynne, 1969.)

of the alga will grow in an iodine-free medium. He also found that the crusts and blades grow at moderate temperatures, whereas the filamentous stages grow at the more extreme temperatures.

### Splachnidiaceae

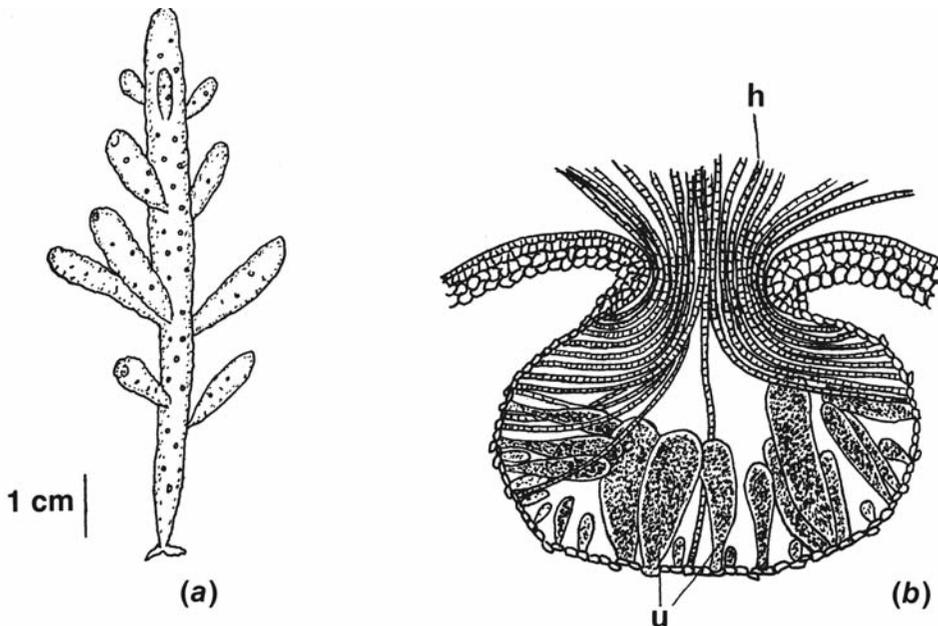
This family contains one genus, *Splachnidium*, a perennial alga of the Southern Hemisphere. The plant consists of a gelatinous, monopodially branched, hollow thallus attached by a basal disc (Fig. 21.21). The alga is interesting, because along with *Notheia* (Nizamuddin and Womersely, 1960; Gibson and Clayton, 1987) and *Acroseira*, it has a number of morphological and reproductive similarities with the Fucales. Nucleic-acid sequencing, however, has shown that these algae are not closely related to the Fucales and represent convergent evolution (de Reviere and Rousseau, 1999). Like members of the Fucales, *Splachnidium* forms its reproductive bodies in conceptacles, the thallus is divided into a medulla with hyphae and a cortex, and there is a type of trichothallic growth (found in young fucalian sporophytes). The conceptacles originate in the immediate origin of the apex by localized cell division, leading to

overarching of certain parts of the surface. Hairs are produced from the inner surface of the conceptacles and project through the ostiole, a situation similar to that in the Fucales. The large unilocular sporangia containing the zoospores arise successively from cells of the inner lining. The zoospores germinate to form filamentous thalli that produce plurilocular sporangia containing gametes. After fusion, the large macroscopic phase is formed again (Price and Ducker, 1966).

### Laminariales

The members of this order are parenchymatous with growth from an intercalary meristem between the stipe and blade. The plants have an alternation of a large sporophyte with a microscopic gametophyte. Sexual reproduction is oogamous. With the exception of *Chorda* (Fig. 21.35) and *Saccorhiza* (Fig. 21.29), the Laminariales lack an eyespot and an associated flagellar swelling in the motile cells (Henry and Cole, 1982; Henry, 1987).

The Laminariales are very large plants that are usually distributed in the colder waters of the world. Many of the genera have sporophytes that



**Fig. 21.21** *Splachnidium*. (a) Whole plant. (b) Section of a conceptacle with hairs (h) and unilocular sporangia (u).

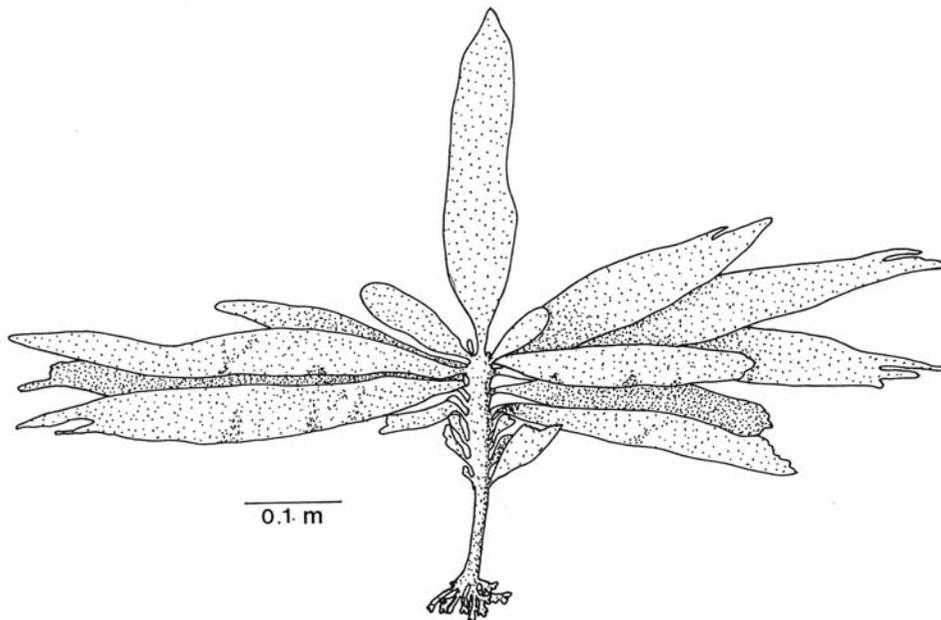
are able to grow vegetatively in warmer waters, but their microscopic gametophytes fail to produce gametes above 10 to 15 °C, thereby preventing the distribution of plants in waters warmer than this (Sjötun and Schoschina, 2002). The first fossils of Laminariales are dated 16 million years ago and it appears that the order originated about this time in the North Pacific during a strong polar cooling trend (Estes and Steinberg, 1988; Saunders and Dreuhl, 1992).

### Morphology and anatomy

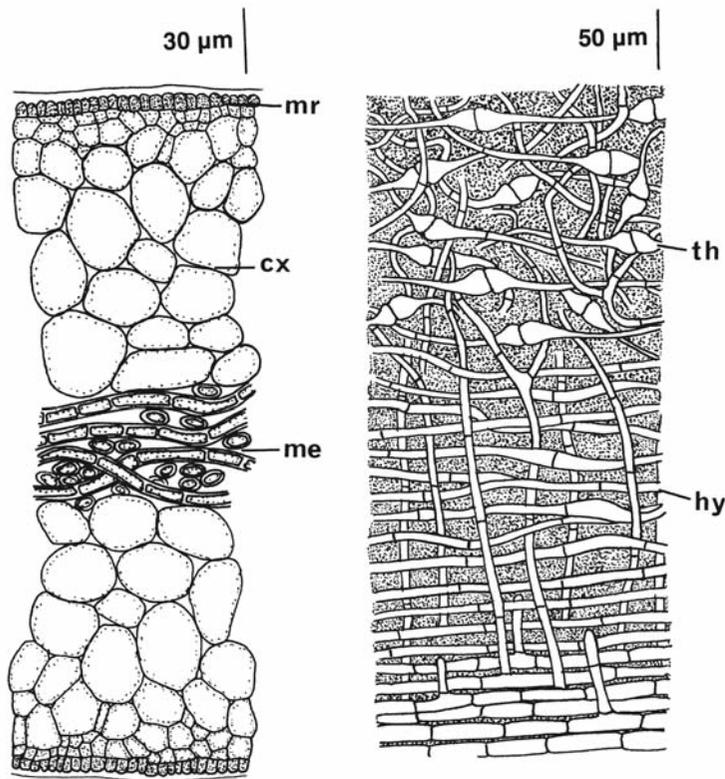
With the exception of the genus *Chorda* (Fig. 21.35), the sporophytes are differentiated into a holdfast, stipe, and blade (Figs. 21.27, 21.29). An intercalary meristem between the stipe and blade adds tissue to both. The blade length often remains about the same because the increase in length at the base often equals the loss by abrasion at the apex. The blades of most genera last for one year, but in many cases the stipe and the base of the blade are perennial. Examples of recorded life spans for kelps are 13 years for *Pterygophora californica* (Fig. 21.22), 11 to 18 years for *Laminaria*

*hyperborea* (Fig. 21.30(d)), and 4 to 8 years for *Macrocystis pyrifera* (Fig. 21.32) (Lobban, 1978). The most frequent cause of death appears to be the tearing of the alga from the rocks by storms. The blades usually stop growing in late summer and begin to disintegrate in the autumn after the plant has discharged its zoospores. The sporophytes are very durable and will withstand a great deal of stress without breaking. When members of the order are washed up on the beach, the plant is usually intact, with the haptera firmly attached to a stone that has come loose from the bottom. The haptera accomplish their firm attachment to the substratum by the growth of rhizoidal cells from the outer meristoderm cells (Tovey and Moss, 1978). These rhizoidal cells fill every microscopic crevice of the substratum until an exact profile of the substratum is built up. Mucilage is also secreted by the cells. The haptera grow downward, not in response to gravity but because they are negatively phototropic, growing away from light (Buggeln, 1974).

There are three different tissues in the sporophyte: the central medulla, the cortex, and the



**Fig. 21.22** A small plant of *Pterygophora californica*. (After Smith, 1969.)

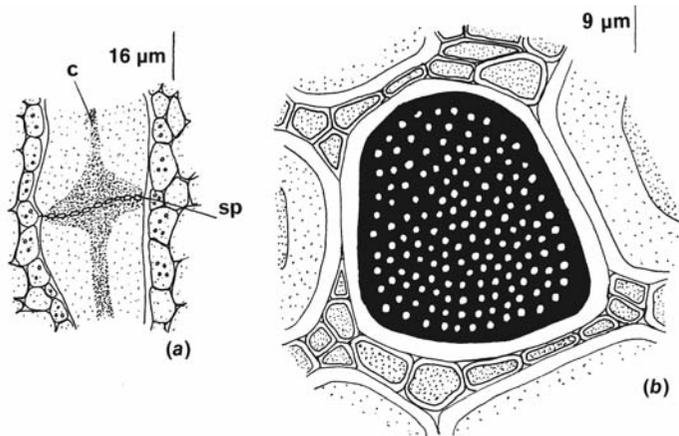


**Fig. 21.23** Sections of lamina (left) and the central portion of a stipe (right) of a member of the Laminariales. (cx) Cortex; (hy) hyphae; (me) medulla; (mr) meristoderm; (th) trumpet hyphae.

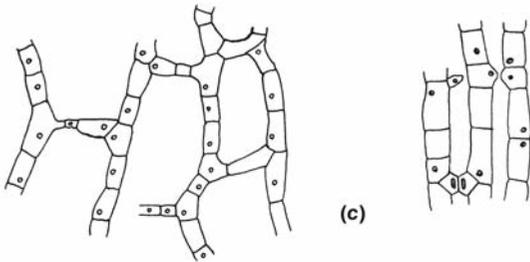
epidermis (Figs. 21.23, 21.25). The haptera lacks a medulla, but all three tissues are present in the stipe and blade. The stipe and blade have the same anatomy, the only difference being the cylindrical to elliptical shape of the stipe and the flattened shape of the blade. At the thallus surface are the photosynthetic, meristematic cells of the meristoderm, which add to the girth of the organ (Fig. 21.23). The meristoderm is composed of small cells that cut off daughter cells to the inside, which in turn form the cells of the outer cortex. The meristoderm is usually covered with a layer of mucilage. The meristoderm in the blade is active throughout the life of the blade, dividing primarily periclinally. In the stipe of some genera (e.g., *Laminaria*), the meristematic activity is transferred to a cortical layer at a depth of four to eight cells beneath the surface, with the result that the tissue to the outside is shed as the stipe increases in width.

Inside the meristoderm are the larger cells of the inner and outer cortex followed by the tan-

gled elongate cells that make up the medulla. The outer cortex differentiates cells to the inner cortex, whereas the inner cortex differentiates cells to the medulla. The cells of the inner cortex and medulla often form cross connections between adjacent cells (Fig. 21.24(c)). In their formation, two adjacent parent cells cut off small cells that elongate toward each other. When they meet, the end walls dissolve, and the cells are continuous. Another type of cells are the **hyphae**, which originate as outgrowths of cells of the cortex and develop into slender, often branched cells of considerable length that grow into the mucilage of the medulla. The medullary cells are in longitudinal rows; and, because they do not have the ability to divide after they are formed, they are drawn out into long cells by the elongation of the thallus from expansion of cells and the meristematic activity of the meristoderm. These medullary elements are often called **trumpet hyphae** because as they are drawn out, the centers become constricted whereas the septal



**Fig. 21.24** (a), (b) Sections of a sieve cell from the medulla of an organism in the Laminariales. The sieve plate (sp) has pores with associated callose (c). (c) Cells of the inner cortex and medulla with cross connections. ((a), (b) after Scagel, 1971.)

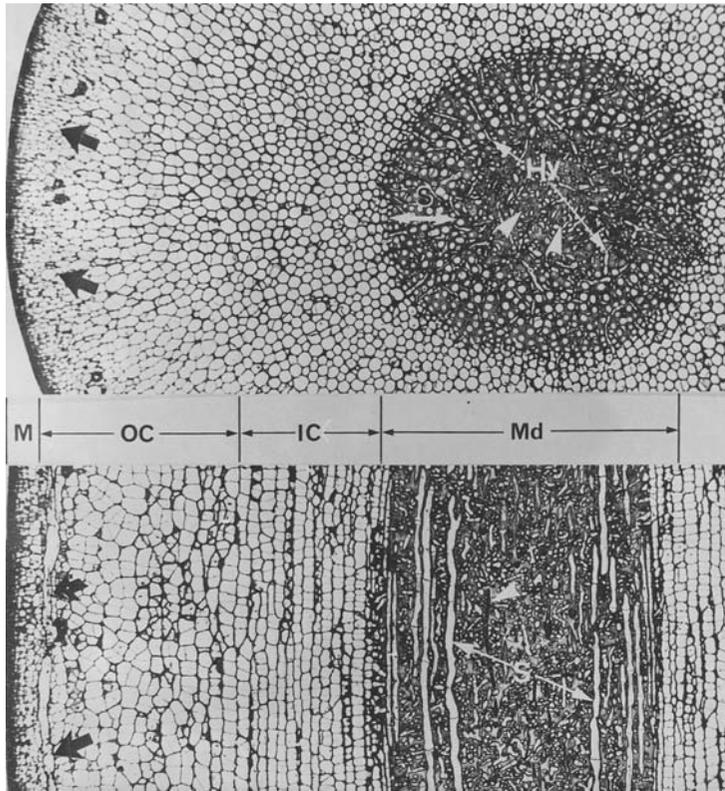


areas maintain their original diameter (Figs. 21.23, 21.24(a), (b), 21.25). Another name for trumpet hyphae is **sieve cells** because there are sieve plates with pores separating the cells. The pores of the sieve plates appear to have evolved from plasmodesmata, which are common in other Phaeophyceae.

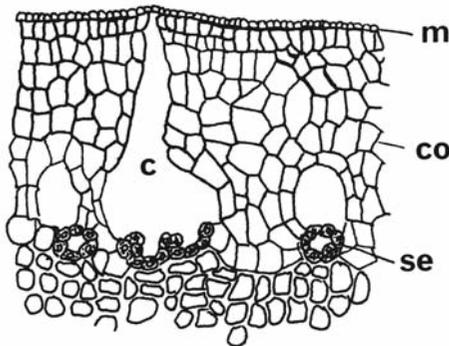
Within the Laminariales there is an evolutionary development of sieve cells (Sideman and Scheirer, 1977). In *Laminaria*, the sieve plates have pores ranging from 0.06 to 0.09  $\mu\text{m}$  in diameter; no **callose** associated with the pores; and nuclei, vacuoles, and mitochondria in the sieve cells. As evolution proceeded through *Alaria* (Fig. 21.39) and *Nereocystis* (Fig. 21.38) to *Macrocystis* (Fig. 21.32), the pores in the sieve plates became larger, callose became associated with the pores, and the cells lost organelles. *Macrocystis* has sieve cells with 2.4- to 6.0- $\mu\text{m}$  pores, callose associated with the pores, and only mitochondria in the cells. The sieve cells of *Macrocystis* differ from sieve elements of the angiosperms in that there are no companion cells present, and there is no large central vacuole. The presence of large

numbers of mitochondria in sieve cells of *Macrocystis* probably reflects the lack of a companion cell, with the mitochondria producing the energy necessary for cell processes. In the Laminariales, there is active transport of the products of photosynthesis through the sieve cells, mostly as mannitol (Parker, 1965). The rate at which the mannitol moves depends on the type of sieve cell present (Lüning et al., 1972). In the well-developed sieve cells of *Macrocystis*, the organic products move as fast as 65 to 78  $\text{cm hour}^{-1}$ , both toward the base for storage in the basal region and toward the apex to provide the rapidly growing apex with substrates (Sargent and Lantrip, 1952; Parker, 1963, 1965). In *Laminaria* and *Saccorhiza*, with their less developed sieve walls, the rate of transport is about five times slower than in *Macrocystis* (Cabello-Pasini and Alberte, 2001).

In the stipes and blades of some of the Laminariales, there is an interconnected system of mucilage canals in the cortex (Grenville et al., 1982) (Figs. 21.25, 21.26). In *Laminaria saccharina* and *L. hyperborea*, these mucilage canals are lined



**Fig. 21.25** Cross (*upper*) and nearly radial longitudinal (*lower*) sections through a stipe of *Macrocyctis integrifolia*, showing the anatomy of a member of the Laminariales. The mucilage ducts (black arrows) are arranged in a ring at the fringe of the outer cortex (OC). Note the sieve elements in radial rows (S) in the outer region of the medulla and the obliterated sieve elements (white arrowheads) in the center of the medulla. Hyphal cells (Hy) anastomose through the medulla. (IC) Inner cortex; (M) meristoderm; (Md) medulla; (OC) outer cortex. (From Shih et al., 1983.)



**Fig. 21.26** *Laminaria cloustoni*, transverse section of a stipe showing mucilage canals (c) and secretory cells (se). (m) Meristoderm; (co) cortex. (After Guignard, 1892.)

by secretory cells which are linked by plasmodesmata (Evans et al., 1973). The secretory cells produce and secrete fucoidin into the canal, from which it goes to the outside of the thallus. These are the only cells that secrete fucoidin in the thallus, and, like secretory cells in other organisms,

they have numerous large Golgi bodies surrounding the nucleus.

### Life cycle

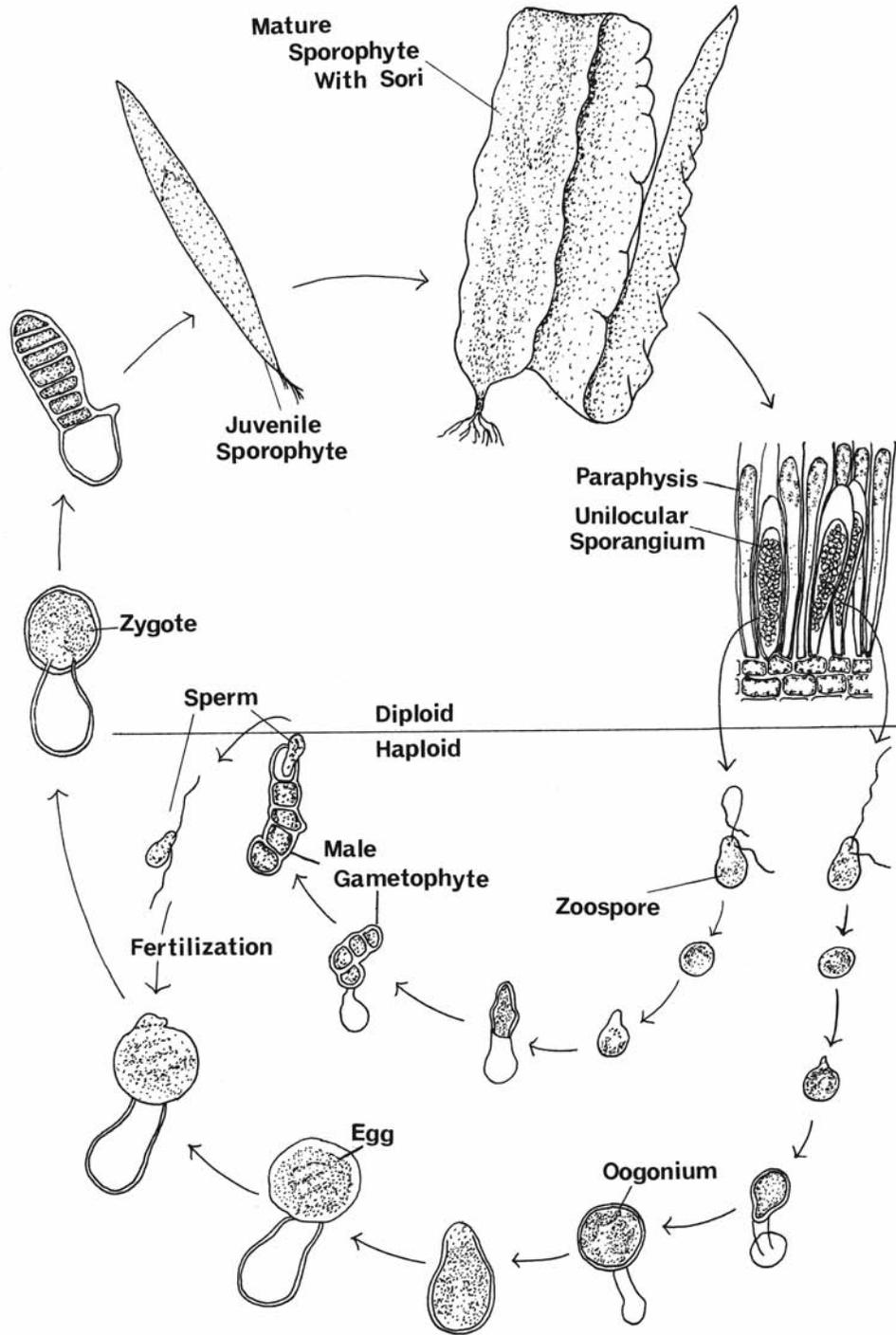
The life cycle of a member of the Laminariales involves the alternation of a large sporophyte with a microscopic gametophyte (Figs. 21.27, 21.29).

The intercalary meristem between the stipe and the blade secretes chemicals that travel distally to inhibit the formation of reproductive sori on the sporophyte (Lüning et al., 2000). The production of the inhibitory chemicals is greatest during the rapid growth in the first part of the year. Activity of the intercalary meristem and growth slows as the season progresses resulting in decreased production of the chemical inhibitors. The level of inhibitory substances decreases to a point where the formation of sori occurs on the sporophyte in the second part of the growing season. The sori contain unilocular sporangia intermingled with paraphyses. First, a superficial cell widens to form a basal cell and a paraphysis. The basal cell widens, and paraphysis elongates. The upper end of the

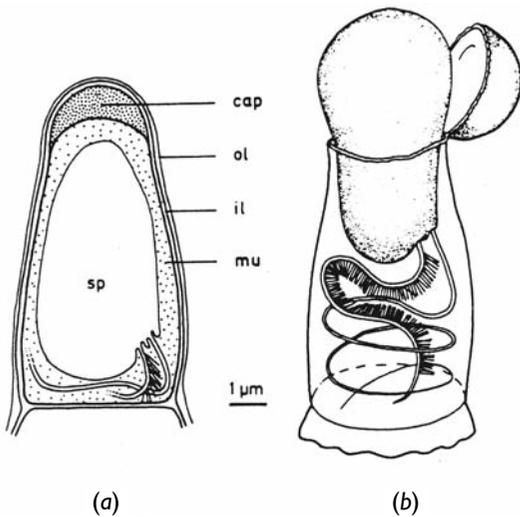
paraphysis becomes swollen and mucilaginous, forming a covering over the basal cells. The basal cell now produces a unilocular sporangium next to the paraphysis. Thirty-two (*Laminaria*) to 128 (*Saccorhiza*) haploid zoospores are formed in the unilocular sporangium (Motomura et al., 1997), and the zoospores are released through the thickened apex of the sporangium. The zoospores have a single chloroplast (in *Chorda*, they have a number of chloroplasts) and may or may not have an eyespot (Evans, 1966). The zoospores are positively chemotactic towards nutrients (Amsler and Neushel, 1989) and can be transported for several kilometers (Reed et al., 1988) during the 48 hours that they swim about. After settling, the zoospores produce the gametophytes. The gametophytes in most of the Laminariales are dioecious, with separate male and female plants. However, *Chorda* (Fig. 21.35) is monoecious, and there are conflicting reports as to whether the gametophytes of *Saccorhiza* (Fig. 21.29) are monoecious or dioecious (Norton, 1972; Henry, 1987). In *Laminaria* (Fig. 21.27), Evans (1965) showed that there is probably an X/Y sex-determining mechanism, with segregation taking place at the meiotic division in the unilocular sporangium. The zoospores contain glycoproteins in small vesicles in the peripheral cytoplasm that are released when the zoospores settle (Oliveira et al., 1980). These glycoproteins adhere the cells to the substratum. The settled zoospore secretes a thin wall around itself, with a slender germ tube emerging; the protoplasm of the zoospore moves out of the original spore and into a swelling at the tip of the germ tube; a wall is formed between the swelling and the original spore; the cell in the swelling now divides to form the gametophyte. It is only at this stage that the female gametophyte looks different from the male. The male gametophyte has smaller cells and is more branched than the female. The male gametophytes produce small colorless antheridia (Fig. 21.28). In the female gametophyte, elongated oogonia are formed that produce a single egg. Under long-day conditions (16 hours light:8 hours dark), eggs are released during the dark cycle, mostly during the first 30 minutes of darkness (Lüning, 1981). The release is apparently controlled by a circadian rhythm. After the female cell has emerged, the thick plastic edges of the wall contract and

form a platform on which the egg remains for some time. The sexual hormone **lamoxirene** is secreted by the eggs as they are released in at least 21 species in the families Laminariaceae, Alariaceae, and Lessoniaceae (Lüning and Müller, 1978; Müller et al., 1979, 1985). Spermatozoids are ejected from the antheridia within a few seconds of exposure to lamoxirene. The spermatozoids are attracted to the eggs where fertilization takes place (Motomura, 1991). The chemical formula of lamoxirene is 1-(1',2'-cis-epoxibut-3'-enyl)-cyclohepta-2,5-diene; it has a molecular weight of 162 and the empirical formula  $C_{11}H_{14}O$  (Fig. 21.8) (Marner et al., 1984). The sexual hormones ectocarpene and desmarestene (Fig. 21.8) are also present, but they do not have hormonal activity in *Laminaria* or *Macrocystis*. In the Laminariales, the zygote germinates to form a flat proembryo that subsequently develops into the mature sporophyte.

Environmental conditions, particularly light and temperature, usually control the life cycle in the Laminariales. Sporophytes will normally not grow at temperatures above 18 to 20 °C (Cheng, 1969; Nakahara and Nakamura, 1973), and sori will not be produced at these temperatures. If a mature sorus of *Laminaria* is placed in water at 20 °C, the sporangia cease to discharge zoospores and disintegrate. The gametophytes are also subject to environmental control. Gametophytes will grow for varying periods of time before forming gametangia. In some cases oogonia are produced by the settled zoospore, whereas at other times the gametophyte grows indefinitely without forming gametes. Lüning and Dring (1972, 1975) showed that if gametophytes are grown in red light at 15 °C, they will grow indefinitely without ever becoming fertile. If these gametophytes are subjected to 6 to 12 hours of blue light, they will produce gametes. Also the gametophytes will not produce gametes if the temperature is above 10 to 12 °C (Sundene, 1963; Vadas, 1972; Nakahara and Nakamura, 1973); or if the water has less than 5  $\mu\text{g liter}^{-1}$  of  $\text{NO}_3\text{-N}$  (Hsiao and Druehl, 1973). The gametophytes have the ability to withstand long periods of darkness and then resume growth when light again becomes available (Kain, 1966). Thus in nature the size of the gametophyte and the time of gametogenesis are probably controlled by environmental conditions.



**Fig. 21.27** The life cycle of *Laminaria japonica*. (After Cheng, 1969.)



**Fig. 21.28** The antheridium of *Laminaria digitata*.

(a) Mature antheridium. (il) Inner layer of cell wall; (ol) outer layer of cell wall; (mu) mucilage; (sp) spermatozoid. The cap is pushed away and the spermatozoid is forced out of the antheridium. (From Maier, 1982.)

## Ecology

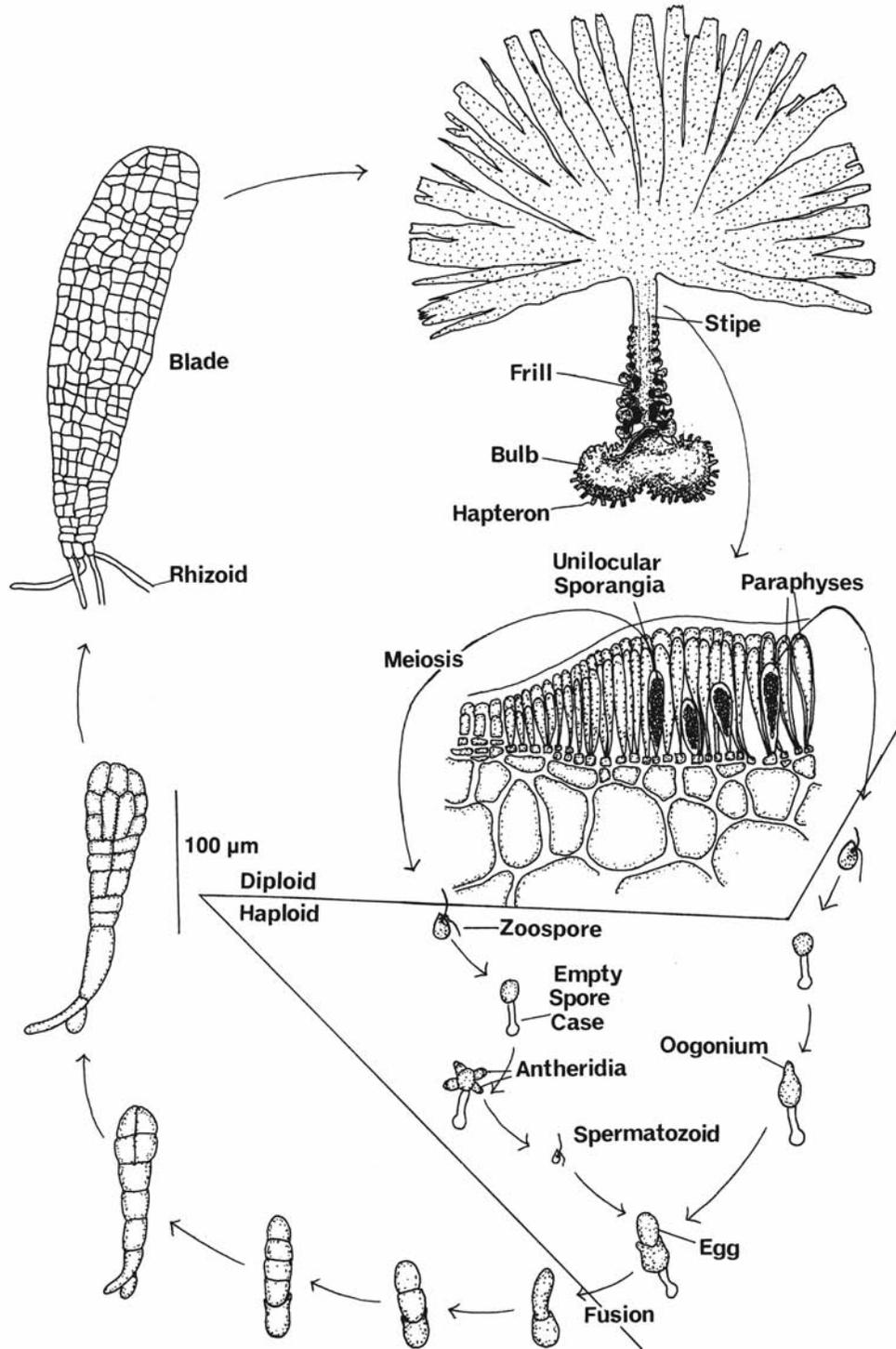
*Laminaria* plants from northern waters are generally much larger with longer stipes and have a greater blade area than those from more southerly waters, a phenomenon that is probably due to their being older plants rather than their having greater growth rates (Larkum, 1972).

*Laminaria hyperborea* (Fig. 21.30(d)) and *L. digitata* (Fig. 21.30(c)) form dense underwater forests in the eastern cold temperate northern Atlantic. Similarly, *Laminaria solidungula* produces dense growths in the Alaskan Beaufort Sea. These kelps exhibit a seasonal growth pattern with a phase of fast growth prevailing from early winter to early summer, and a summer and autumn phase with a reduced or complete cessation of growth. These growth patterns are an ecological strategy, whereby storage of photosynthate during the well-lit summer and autumn enables the algae to start growth by remobilization and translocation of stored carbohydrates early in the dark winter when nutrient supply is optimal due to plankton remineralization. This **circannual** (approximately annual) **rhythm** is under endogenous photoperiodic control (Henley and Dunton, 1997; Schaffelke and Lüning, 1994).

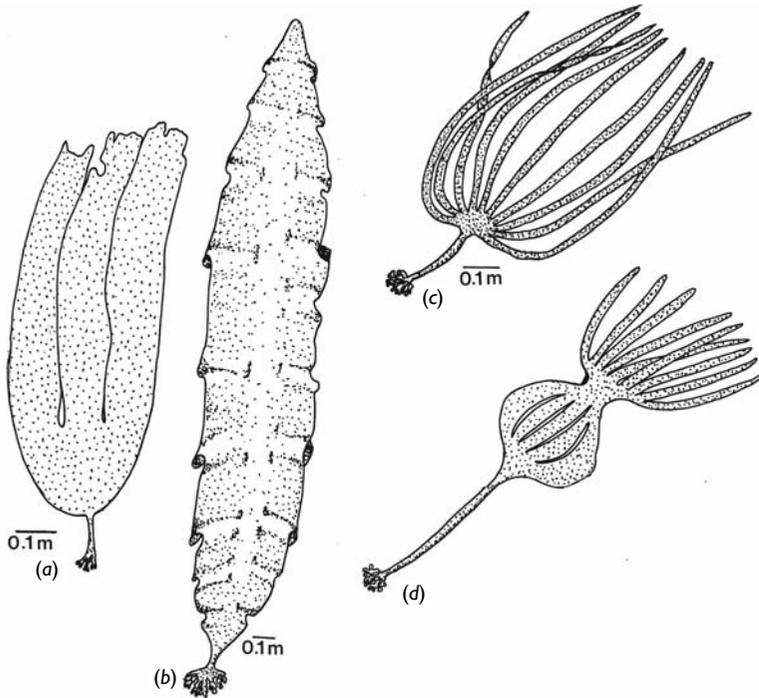
Another factor affecting the morphology of the sporophyte is the environment in which the plant is growing. *Saccorhiza polyschides* sporophytes growing in weak current produce curved blades that are heart-shaped (cordate) at their base (Fig. 21.31). These blades lack subdivisions (digits) and are so fragile that they tear under their own weight when removed from the water. In contrast, plants growing in strong current develop very long, flat, tough blades, narrowly triangular (cuneate) at the base and divided into as many as 30 digits. In habitats without current but exposed to wave action, the sporophytes produce short, flat, extremely tough blades with only three to ten digits. Anatomically, the greater toughness of blades is the result of a larger number of cortical cells increasing the thickness of the thallus (Norton and Burrows, 1969). Somewhat similar results with *Laminaria digitata* (Fig. 12.30(c)) (Sundene, 1962(a)) and *Alaria esculenta* (Fig. 21.39) (Sundene, 1962(b)) have been reported.

Many species of *Laminaria*, such as *L. hyperborea* (Fig. 12.30(d)), produce very thick stands or forests of sporophytes beneath the low-tide mark. As the depth increases, the plants become sparser, forming an open “park” (Larkum, 1972). *Macrocystis* and *Laminaria* have primary production rates that rank among the highest in the world, reaching an annual net production in the range of 1000 to 2000 g m<sup>-1</sup> of carbon (Mann and Chapman, 1975). The forests of the giant kelp *Macrocystis pyrifera* (Fig. 21.32) form continuous beds up to 8 km long and 1 km wide along the Pacific Coast of North and South America (Gaines and Roughgarden, 1987). The extent of these forests and the density of plants vary greatly in space and time because of storms (Dayton et al., 1984), herbivores, and predators (Duggins, 1980), and also major current features such as El Niño. The decline in canopy area due to large winter storms of the 1982–83 El Niño was particularly dramatic (Dayton and Tegner, 1984).

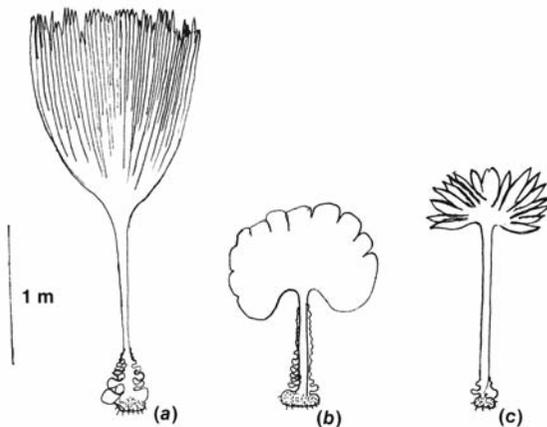
These stands of *Macrocystis* and *Laminaria* are often depopulated by storms which dislodge the plants and cast them on the beach. A small mollusc, *Patina pellucida*, frequently browses the holdfast system, thereby weakening the attachment of the plants to the substratum and making



**Fig. 21.29** The life cycle of *Saccorhiza* sp. (Adapted from Norton and Burrows, 1969.)



**Fig. 21.30** Morphology of some species of *Laminaria*. (a) *L. groenlandica*. (b) *L. saccharina*. (c) *L. digitata*. (d) *L. hyperborea*. ((a) after Scagel, 1971.)



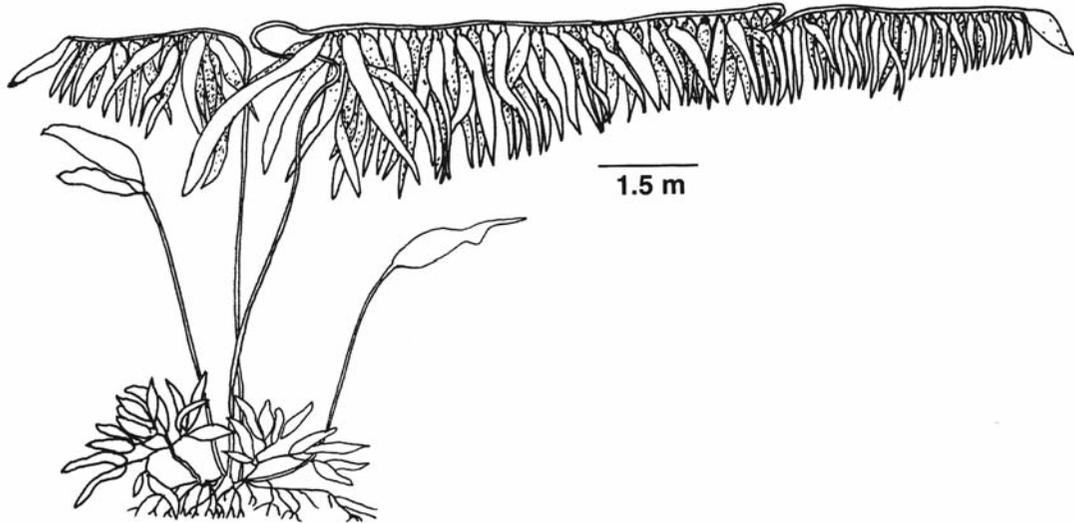
**Fig. 21.31** *Saccorhiza polyschides* sporophytes from (a) strong current, (b) weak current, and (c) area with wave action. (After Norton and Burrows, 1969.)

them more susceptible to storm damage. Sea urchins, such as *Paracentrotus lividus* will also eat members of the Laminariales, and one study (Norton, 1978) showed that in their appetites they exhibit a preference for *Saccorhiza polyschides* (Fig. 21.31) to *Laminaria saccharina* (Fig. 21.30(b)). In the last two centuries there has been a marked

decrease in the shallow-water kelp forests in the North Pacific, due to the near extinction of sea otter. Prior to this, the sea otters ate large quantities of sea urchins that ate the kelp. With their normal predators gone, the sea urchins were free to devastate the kelp forests (Estes and Steinberg, 1988).

The species of Laminariales present in a particular area is determined by the environment of the area. Around Vancouver Island, British Columbia, Druehl (1967) noticed that there were three prevalent forms of *Laminaria*: *L. saccharina* (Fig. 12.30(b)) and a long- and short-stipe form of *L. groenlandica* (Fig. 12.30(a)). The two forms of *L. groenlandica* occurred in surf: the long-stipe form in heavy surf, and the short-stipe form in moderate surf. *Laminaria saccharina* was found only in areas that had no surf. In the United Kingdom, Boney (1966) found that *Laminaria hyperborea* (Fig. 12.30(b)) dominates in regions of moderate or severe wave action, whereas *L. saccharina* is in sheltered areas.

Even in the long term, populations of *Laminaria* can vary. Walker (1956) reported that a *Laminaria* population off the coast of Scotland varied in density over a 10-year period, and that



**Fig. 21.32** *Macrocyctis pyrifera*.

this variation showed a strong correlation with sunspot activity and weather changes.

Concentric rings of dense tissue at the base of the stipe can be used to indicate the age of perennial laminarian algae such as *Laminaria* and *Ecklonia*. Dark rings are produced by cortical meristem during slow growth in autumn and winter, and pale rings are produced in winter and spring (Novaczek, 1981; Klinger and DeWeede, 1988).

Invasion of *Laminaria* thalli by bacteria (such as *Pseudoalteromonas bacteriolytica*) results in degradation of the extracellular polysaccharides of the alga, producing breakdown products such as oligoguluronates. These oligoguluronates initiate a defense mechanism by the *Laminaria*. First there is an increase in respiration of the *Laminaria* cells, followed by the production of hydrogen peroxide ( $H_2O_2$ ). The concentration of hydrogen peroxide is high enough to kill the invading bacterial cells (Potin et al., 1999).

### Metabolism and composition

The proportion of laminarin and mannitol (Fig. 1.7) as dry matter increases steeply during the active photosynthetic period from April to September. On the other hand, the proportion of alginic acid and cellulose in the dry matter decreases during this period. The opposite occurs

from October to April, with the relative amounts of alginic acid and cellulose increasing. These variations are much greater in the frond, which has a high growth rate, than in the stipe, where the growth is slower.

Black (1954a) showed that in a mature frond of *L. saccharina* (Fig. 21.30(b)), where the part near the tip was 7 months old, there was a marked variation in composition along the length. Near the stipe (i.e., the actively growing region and, therefore, the youngest) there was, on a fresh-weight basis, about 3% mannitol and little or no alginic acid or laminarin. About a third of the way along the frond mannitol was at a maximum of about 6%, with laminarin 2% and alginic acid 2.5%, whereas two-thirds of the way up the frond the mannitol content was only 2%, with laminarin at 6% and alginic acid 4%. Variations in the composition of whole fronds can, therefore, be due largely to changes in proportions of old and new tissues.

According to Percival and McDowell (1967), seasonal variations in the Laminariaceae are consistent with the following observations:

- 1 Mannitol is the first product of photosynthesis to accumulate in appreciable quantities and is the main carbohydrate in tissues that are increasing by active cell division.
- 2 During photosynthesis in tissues that are largely growing by cell enlargement, there is

an increase in the proportion of dry solids, made up of mannitol and salts of alginic acid and laminarin. Protein and cellulose are also being synthesized during this period (Pueschel and Karb, 2001). Formation of mannitol and laminarin continues after the other constituents have built up to a constant level in each unit of tissue, thereby increasing the dry solids content and reducing the proportion of alginate, cellulose, and protein on a dry-weight basis. Thus, in late summer, mannitol and laminarin are at high levels and alginic acid, cellulose, and protein are at a minimum on this basis.

- 3 Laminarin may be formed from mannitol, and during active growth mannitol can be formed faster than its rate of conversion into laminarin so that both substances increase in amount. When growth slows down or stops owing to lack of nutrients, shortage of light, or low temperatures, laminarin increases with loss of mannitol. In late summer, there may be a temporary reduction in mannitol content owing to depletion of phosphate in the water, whereas the laminarin content does not drop until later.
- 4 During spore formation and periods when respiration is greater than photosynthesis, both laminarin and mannitol are used up. As there is little change in the amounts of other constituents, the proportion of alginate, cellulose, and protein, calculated on a dry-weight basis, increases.

#### Economic uses

Over 4 million tons of kelps are harvested annually, mainly from mariculture in Asia (China, Japan, Korea) or from natural populations in Europe and North and South America. Overall, the algae in the Laminariales are the largest marine crop in terms of annual landings (Asensi et al., 2001).

The first use of kelps was for kelp ash (Kupper et al., 1998). The brown seaweeds were collected and dried on the shore, and the dried seaweeds were burned in a kiln with the product after burning being a hard cake. The cake constituted the kelp ash. The first use of kelp ash began sometime in the seventeenth century when French peasants

used it for glazing pottery and making low-quality glass. This use lasted for about 200 years until the discovery of Barilla soda, made from certain coastal salt-rich plants. This substance produced a better-quality glass, and kelp ash ceased to be used for glass. In 1811, it was discovered that kelp ash contained large amounts of iodine. Using the best seaweeds properly burned, the kelp contained 1.4% to 1.8% iodine, or about 15 kg per ton. At that time iodine was in demand as a cure for goiter, an enlargement of the thyroid gland caused by lack of iodine (even today much of the table salt consumed is iodized, although not from kelp ash). In 1846, there were 20 manufacturers of iodine in Glasgow alone. Subsequently the discovery of mineral deposits of iodine, particularly in Chile, caused a decline in the kelp ash industry.

The current industrial use of kelps is for the alginate that they contain, which has a variety of uses. Algin was first discovered by Stanford in the early 1880s, although it was not obtained in a purified state until 1896 by Krefting. Algin comprises about 10% of the dry weight of the kelps (Smith, 1955), and is mostly the salt of alginic acid. The main area of algin production is the West Coast of the United States, where large stands of *Macrocystis* grow (Fig. 21.32), with the upper portion of the thallus growing on or near the surface of the water. Because of the large numbers of *Macrocystis* and the way they grow, it is possible to harvest the alga by a motor-driven barge equipped with scythe-like blades 3 feet below the surface of the water. The five-person crew of a barge can harvest 300 tons of kelp a day. The development of algin industries in other parts of the world has been hindered by the high cost of harvesting kelps by hand. One of the uses of algin is in the making of ice cream; practically all manufacturers of ice cream add algin before freezing their product. This prevents the water in the ice cream from forming coarse ice crystals, and thus results in a smoother product (Smith, 1955). About half of the consumption of algin is in the making of ice cream and other dairy products. Also, the water-retaining properties of algin are utilized in a variety of ways in the baking industry, including the addition of algin to frostings to prevent undue drying. The colloidal nature of algin makes it useful as a suspending and emulsifying agent. In the rubber

industry, it is used as a creaming and stabilizing agent in the processing of natural and synthetic rubber latex. When added to paints, alginates keep the pigments in suspension and make a product that can be brushed on a surface without showing brush marks. Alginates are also used as suspending agents in a wide variety of pharmaceutical products.

The first medicinal use of brown algae in the literature occurred in Li Shih-Chen's sixteenth-century herbal *Pen Ts'ao Kang Mu* ("Outlines of Chinese Materia Medica"), where brown algae were listed as a cure for goiter by virtue of the iodine they contained. In Europe, goiter was treated by giving kelp pills or kelp ash, which medicinally was known as *Aethiops vegetabilis*. Short pieces of *Laminaria* stipe were used in surgery for opening fistulae (natural openings), because of the swelling properties of the stipe. Brown algae have been recommended as cures for a number of medicinal purposes without any real benefit being derived. For example, the Indians of Sitka, Alaska, used stipes of the bull kelp (*Nereocystis*) as a cure for headaches. The afflicted individual would place the thin end of the stipe in one ear and the bulb or pneumatocyst on a hot stone. Steam would then be generated in the bulb and pass through the hollow stipe and subsequently into the ear of the individual, supposedly clearing up the headache.

Brown seaweeds have also been used as green manure for the soil and as food for animals and humans. The large brown seaweeds are fed to cattle, horses, and sheep in many northern countries, either as a whole food source during the winter or more commonly as a supplement to other foods. Seaweeds are used as a food source by humans, especially in Japan and China. "Kombu" is a food made from members of the Laminariales in Japan. The algae are collected from July to October, are spread out on beaches to dry, and the stipe and hapteron are cut off. The blade is left until a gloss appears on it; then it is sent to the factory. At the factory a number of different forms of kombu are made, one of the most common being green (*ao*) kombu. This is prepared by boiling the alga, dyeing it green, pressing it, and then shredding it. The shavings are laid out on mats, dried, and packaged. This green kombu can be

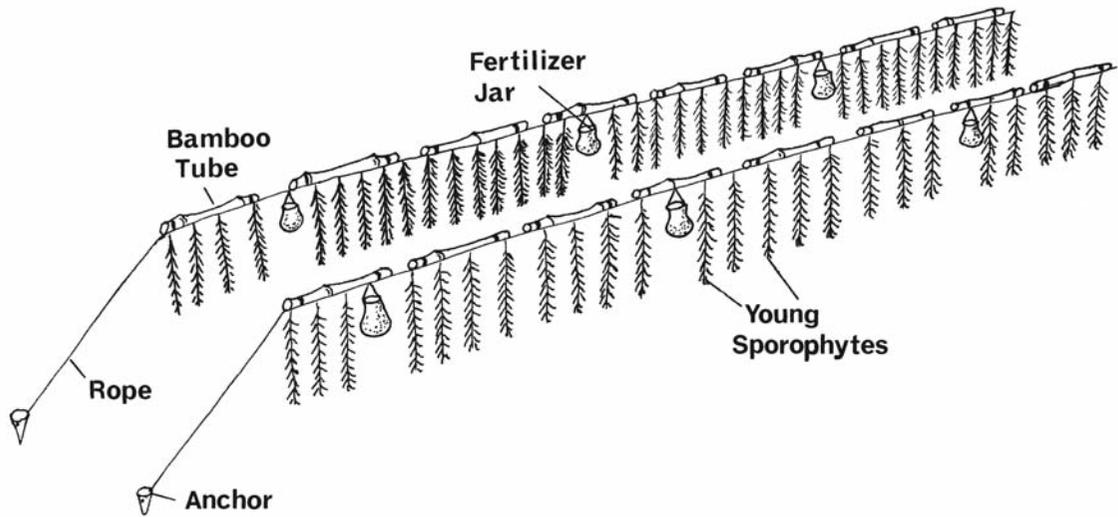
boiled with meat, fish, and soups, or it can be used as a vegetable. In mainland China, *Laminaria*, known as "haidai" (sea belt), has been widely used as a food and medicine for over 1500 years. When properly prepared, especially if cooked with pork and soybean sauce, it is an inexpensive dish. It is especially popular during the season when green vegetables are not readily available. Every kilogram of dried kelp can produce 262 kcal of energy plus a number of important vitamins and minerals (Cheng, 1969; Tseng, 1981).

Naturally occurring populations of Laminariales usually serve as a source of algae. The only exception to this is in mainland China where in 1946, following the evacuation of the Japanese, the culture of kelps on rafts was initiated (Fig. 21.33). The first step in the growing of kelps is the placing of short bamboo splint ladders in the sea, hanging from rafts in late autumn. Zoospores settle on the splints and form gametophytes and gametes, with the zygotes germinating to form young sporophytes. By January, the young sporophytes are removed from the splints and transferred to ropes that make up the rafts. The most popular type of raft is the single-line bamboo tube raft (Fig. 21.33). A typical raft is constructed by tying bamboo end to end in a single line to a rope 60 m long. This floating raft is anchored at each end by a rope, which is driven into the sea bottom. On each raft are hung earthenware jars containing ammonium nitrate, which seeps through the jars, thereby fertilizing the surrounding water. Young sporophytes averaging 10 cm in length are attached to the ropes by inserting the basal end of the stipe through the strands of the rope. The lines are then hung in the water attached to the bamboo tubes. With proper care, sporophytes may reach 3 m or more in length within 4 to 5 months, and are then harvested (Cheng, 1969) (Fig. 21.34).

### Classification

The Laminariales are divided into four families:

- Family 1 Chordaceae: sporophyte hollow, whip-like, and not differentiated into a stipe and blade.
- Family 2 Laminariaceae: transition zone with intercalary meristem not subdivided



**Fig. 21.33** Single-line bamboo rafts used for kelp cultivation in mainland China. The porous earthenware jars are filled with fertilizer that seeps out into the ocean. (After Cheng, 1969.)



**Fig. 21.34** Harvesting *Laminaria* grown on rafts in mainland China. (From Cheng, 1969.)

so that there is a simple primary stipe; sori not on special organs.

Family 3 Lessoniaceae: transition zone with intercalary meristem subdivided so that there are a number of secondary stipes in addition to the primary stipe.

Family 4 Alariaceae: sori borne on special sporophylls.

#### CHORDACEAE

This family has a single genus, *Chorda* (Fig. 21.35) which is not closely related to the rest of the algae in the order (Draisma et al., 2001). The plant is an annual, hollow, whip-like alga that grows in the sublittoral regions of the Northern Hemisphere. The cylindrical sporophyte is long (up to 2.6 m) but seldom wider than 1 cm. The genus differs from other Laminariales in two characteristics: the sporophyte has a meristematic zone beneath the apex, and the young sporophyte has one or more apical hairs. In both of these characteristics, the genus resembles some members of the Dictyosiphonaceae of the Ectocarpales, such as *Dictyosiphon*. *Chorda* has characteristics that

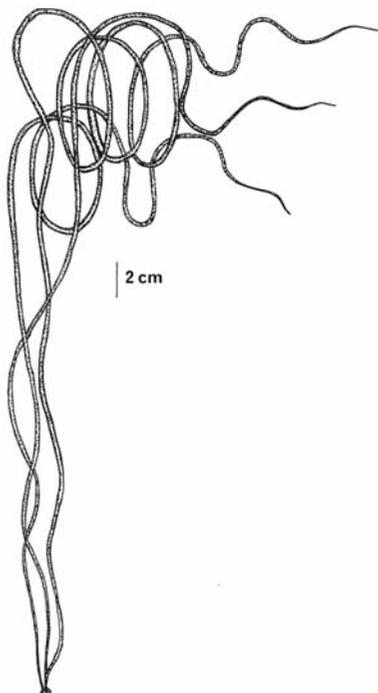


Fig. 21.35 *Chorda filum*.

might be considered intermediate between the Ectocarpales and the Laminariales.

*Chorda* has a life cycle similar to *Laminaria* (Fig. 21.27) except that the gametophyte is monoecious (oogonia and antheridia on the same thallus) (Maier, 1984). Freshly released eggs give off a sexual hormone that causes explosive discharge of spermatozoids from the antheridia and subsequent chemotaxis toward the egg (Maier et al., 1984; Müller et al., 1985).

#### LAMINARIACEAE

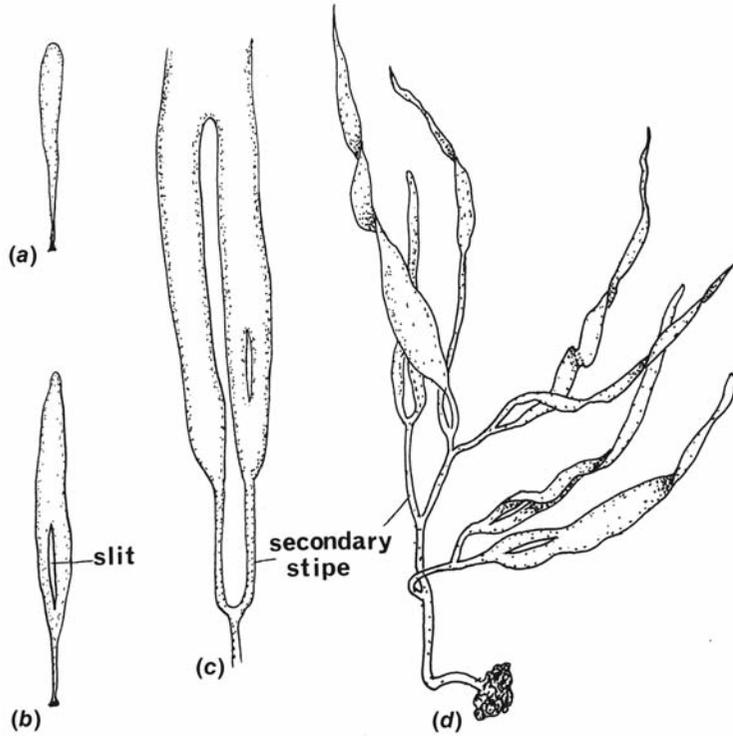
In this family the sporophyte is divided into a holdfast, stipe, and blade. The blade is produced whole by the intercalary meristem even though it may later fragment into a number of digits. *Laminaria* (Fig. 21.27) has already been discussed. *Saccorhiza bulbosa* (Fig. 21.29) is an annual found on the Atlantic shores of Europe and North Africa. The mature sporophyte has a divided (digitate) blade, sometimes exceeding 2 m in length, borne at the end of a flattened stipe. The stipe is spirally twisted in its lower portion, and below the twist there is a large inverted bell-shaped outgrowth that covers the holdfast and the basal part of the stipe. The bell develops in the young plant from the intercalary basal meristem, and the stipe subsequently develops undulating wings. The blades have cryptostomata with hairs.

#### LESSONIACEAE

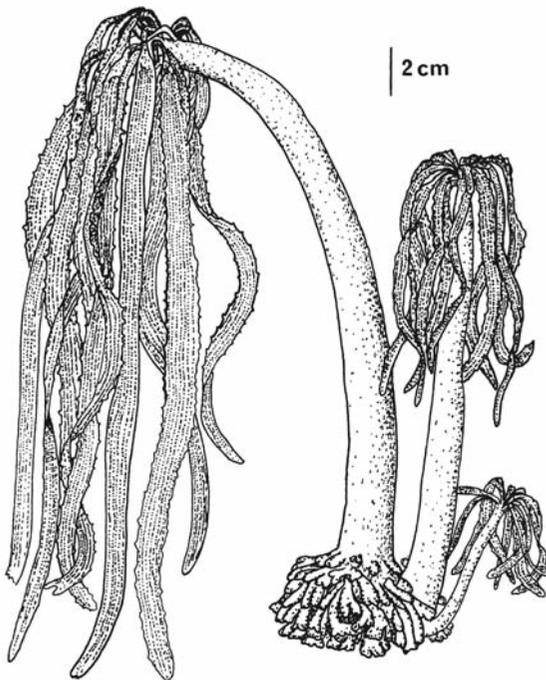
Although any splitting of the blade in the Laminariaceae does not extend down into the basal intercalary meristem, in the Lessoniaceae it does. This means that the secondary blades produced have their own secondary stipes.

*Lessonia nigrescens*, a species from the Southern Hemisphere, has entire young blades that soon develop a median split, resulting in a blade with two segments, each with its own secondary stipe (Fig. 21.36). The blades continue to split until the mature, much-divided sporophyte is formed. *Postelsia palmaeformis* (sea palm) has a short thick primary stipe that supports the secondary blades and stipes (Fig. 21.37). The plants occur on the Pacific Coast of North America in habitats exposed to the full violence of the waves.

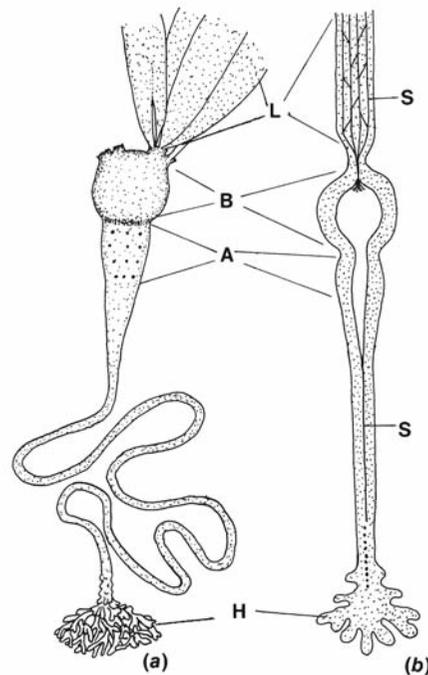
The largest of the algae in the family are *Nereocystis* (Fig. 21.38), *Pelagophycus*, and *Macrocystis*



**Fig. 21.36** *Lessonia nigrescens*. (a)–(c) Successive stages in the splitting of the blade to produce secondary stipes and secondary blades. (d) Mature plant.



**Fig. 21.37** *Postelsia palmaeformis*.



**Fig. 21.38** Sporophyte of *Nereocystis*. (a) Whole plant. (b) Semidiagrammatic longitudinal section. (A) Apophysis of stipe; (B) gas-filled bulb of stipe; (H) holdfast; (L) lamina; (S) sieve filaments. (After Nicholson, 1970.)

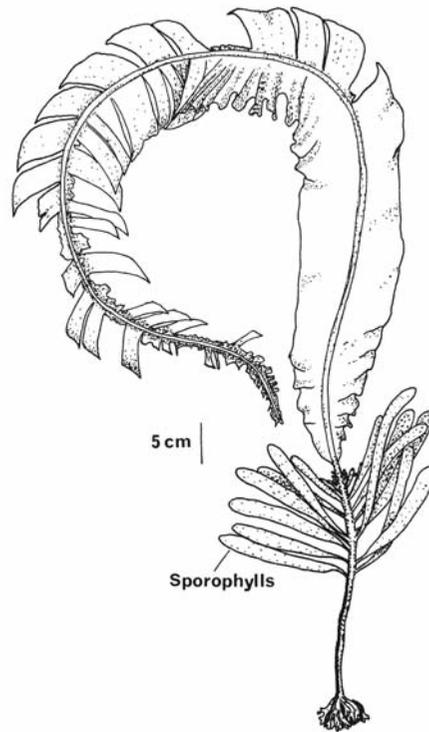
(Fig. 21.32). *Nereocystis luetkeana* (bull kelp) has a tough whip-like stipe up to 25 m long that terminates in a large air bladder (Fig. 21.38). This air bladder supports secondary stipes and blades that hang down from the surface of the sea. The remarkable aspect of this large seaweed is that it is an annual plant that can grow as much as 6 cm a day. *Julescraneia grandicornis* is a fossil alga from Miocene diatomite of California that resembles *Nereocystis* (Parker and Dawson, 1966). *Macrocystis* (Fig. 21.32) may grow up to 50 m in length and has a life of 5 years, although the individual secondary blades have a life of only 6 months. As in *Lessonia* there is successive splitting of the primary blade, but in *Macrocystis* the growth of one of the two segments is arrested. This leads to a long curtain type of thallus. Each of the segments has an air bladder at the base of the secondary stipe, allowing the secondary blade to hang down from the surface of the water.

#### ALARIACEAE

The sporophytes in this family have the sori formed on special sporophylls. *Alaria* (Fig. 21.39) has a lamina with a wavy margin and a midrib. The short stipe produces thick tongue-shaped sporophylls in the summer, which, after maturation of the sori, are shed during autumn and winter, leaving scars on the stipe. During the winter the blade wears down to the basal meristematic zone, and a new blade is produced the following season.

#### Fucales

The organisms in this order are parenchymatous with growth from an apical cell. The haploid generation is reduced to the egg and sperm, with the remainder of the life cycle being diploid. The gametes are borne in special cavities, the **conceptacles**, and gametic union is always oogamous. Conceptacles may be scattered over the surface of the thallus, but more frequently they are limited to the inflated tips of special branches, the **receptacles**. The Fucales are worldwide in distribution, but those of the Arctic and north temperate seas differ considerably from those of the Antarctic and south temperate waters, *Fucus* (Fig. 21.40) is a common genus in northern waters, whereas in tropical and subtropical waters *Sargassum* (Fig.



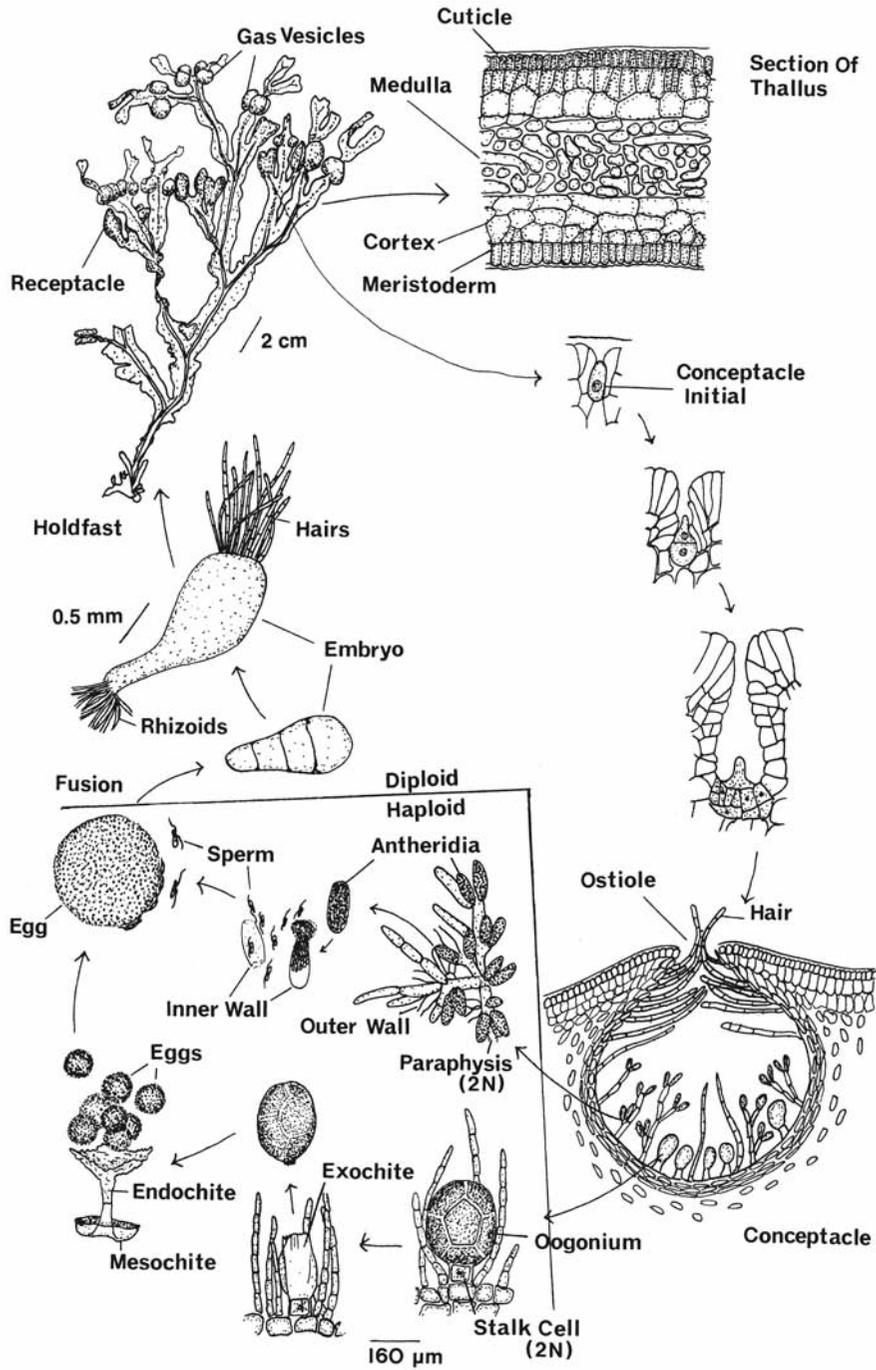
**Fig. 21.39** *Alaria esculenta*. The old distal part of the blade is partly eroded away, and the sporophylls are fully developed. (After Taylor, 1957.)

21.51) is present. In Australian waters, *Cystophora* is a predominant member of the flora, with the large *Durvillea* being common in sub-Antarctic waters.

Gene sequencing has revealed that the Fucales diverged early from the remainder of the Phaeophyceae (Draisma et al., 2001).

#### Morphology and anatomy

The genus *Fucus* will be used as the representative genus in this order (Fig. 21.40). The thallus is much branched and is supported by a short narrow stalk that is attached to a discoid holdfast. The branching is dichotomous, with each flattened segment having a prominent central midrib surrounded on both sides by a narrower wing. The wings usually bear scattered **cryptoblasts**, which are basically sterile conceptacles with large numbers of hairs, that facilitate the uptake of nutrients from the seawater (Hurd et al., 1993). At certain times of the year, the tips of the branches



**Fig. 21.40** The life cycle of *Fucus* sp. (*F. vesiculosus* and *F. serratus*). (Adapted from Thuret, 1854; Oltmanns, 1889; Nienburg, 1931; Taylor, 1957.)

are swollen into receptacles that contain the fertile conceptacles. The inflation of the receptacles is due to the production of a large amount of mucilage.

Each branch has an apical cell at its apex (Fig. 21.41). The apical cell divides several times a year, resulting in the formation of a dichotomy or fork, with one arm of the fork being longer than the other. The apical cell in mature *Fucaceae* is a four-sided pyramid with a flattened base. In the other families and in young *Fucaceae*, the apical cell is a three-sided pyramid. According to Moss (1967), the apical cell itself does not divide (except in the formation of forks), and instead stimulates the cells around it to divide. Thus a meristematic zone extends beneath and around the sides of the apical cell and is called the **promeristem**. The cells of the **promeristem**, similar to meristematic cells in other organisms, are very small. At the sides of the promeristem the cells enlarge and divide only transversely, yielding the flattened wings. Mucilage is deposited between the derivatives of the promeristem, causing the files of cells to separate, remaining in contact only where there are pits. The apical cell shows apical dominance, inhibiting the development of laterals beneath it (Moss, 1965, 1970). If the apical cell is destroyed, then the underlying lateral will develop into a new apical cell.

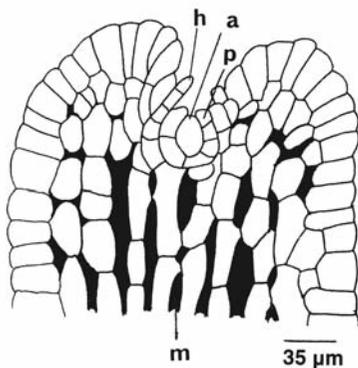
The second meristem in the apical area of the thallus is the **meristoderm** or outer row of cells

derived from the promeristem. This is a closely packed layer of brick-shaped cells that divides anticlinally at first and then periclinally to produce new tissues to the inside.

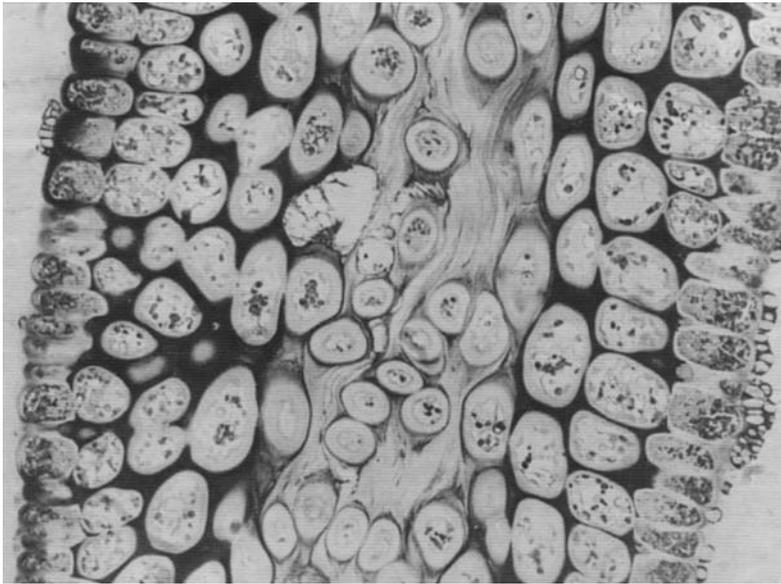
The anatomy of the *Fucales* is similar to that of the *Laminariales*, with a mucilaginous cuticle covering the epidermal layer of cells (Figs. 21.41, 21.42). Inside this is the cortex with the medulla in the center. Hyphae are produced by the inner cortical cells, but there are no trumpet hyphae present. There is an orientation of organelles within the epidermal cells. They have an outer layer of alginic acid vesicles with a basal nucleus and chloroplasts (Fulcher and McCully, 1969; Rawlence, 1973). The cap of alginic acid vesicles may shield the chloroplasts and nucleus from intense illumination, especially at low tide when the plants are usually exposed. The organelles of the cortical cells are arranged just the reverse of the epidermal cells, having an outer layer of chloroplasts. The chloroplasts of the medullary and hyphal cells are much reduced.

Like the *Laminariales*, the *Fucales* are able to translocate organic materials (Floc'h and Penot, 1972). Mannitol (Fig. 1.7) is the form of photosynthate translocated. The growing apex acts as a sink, with the mannitol translocated to the growing apex from the blades of the alga (Diouris, 1989). Structurally, the *Fucales* have a system of conducting elements very similar to the *Laminariales*. In the *Fucales*, the sieve elements run from the apical meristem to the base of the plant. The medullary elements have sieve plates ( $1\ \mu\text{m}$  thick) at their ends. The pores in the sieve plates enable a continuous system of cytoplasm for the translocation of materials both longitudinally and transversely through the cross connections. The pores in the sieve plates are  $0.1\ \mu\text{m}^2$  or less, which makes them smaller than the pores reported in the *Laminariales* (*Alaria* has pores ranging in size from  $0.1$  to  $0.3\ \mu\text{m}^2$ ) (Moss, 1983).

**Gas vesicles (air bladders)** (Fig. 21.40) originate not far from the apex as a result of growth of the surface layers of cells accompanied by an increase in the thickness of the cortex. This leads to the rupture of the medulla, the remnants of which are commonly around the edge of the hollow. The air bladders are apparently filled with gases similar to those in the atmosphere.



**Fig. 21.41** Upper portion of a mature juvenile thallus of *Fucus*, showing the apical depression, apical cell (a), remnants of terminal hairs (h), the promeristem (p), and the medulla (m). (After Oltmanns, 1889.)



**Fig. 21.42** *Fucus virsoides*.

Transverse section of a mature thallus. A mucilaginous cuticle covers the outer epidermal layer in the cortex with the medulla in the center. (From Mariani et al., 1985.)

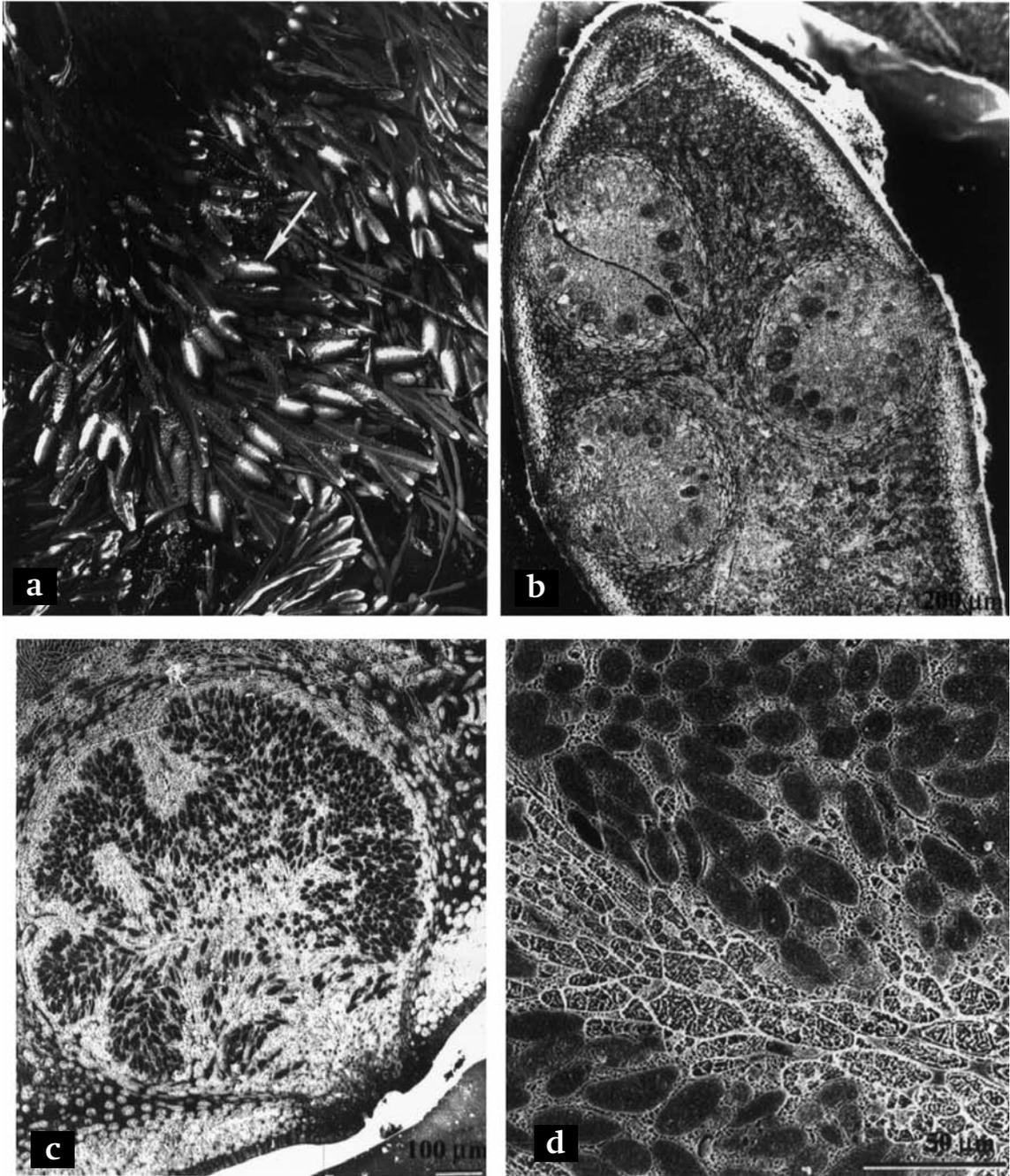
### Life cycle

*Fucus* will again be used as an example of a typical member of the Fucales (Fig. 21.40). The gametes are borne in conceptacles (Fig. 21.43) that are similar to the cryptoblasts except that the colorless hairs are restricted to a small area near the aperture. The wall of the conceptacle is lined with flat cells that bear branched paraphyses with few chloroplasts. The conceptacles originate from an initial that is a superficial cell of the thallus. The initial divides into an outer tongue cell and an inner basal cell. The tongue cell either degenerates or does not contribute to the development of the conceptacle. The basal cell then divides to form the floor of the conceptacle. At the same time, the cells surrounding the original initial grow and divide so that the derivatives of the initial cell become open to the outside. The mature conceptacle consequently has cells lining the floor derived from the conceptular initial and cells lining the walls derived from the cells surrounding the original initial.

The gametes are released into the seawater during daytime and at times when there is little water motion, reducing the amount of gamete dilution, and ensuring high rates of fertilization (usually about 95%) (Pearson and Brawley, 1998; Ladah et al., 2003). Tide-pool populations of fucoids release their gametes during low tide

when the tide pools are isolated and calm. Intertidal populations release their gametes at slack high tide when the water is calmest.

The plants are either monoecious or dioecious, and in the monoecious forms the antheridia and oogonia can be in the same or different conceptacles. The antheridia are usually formed on paraphyses. Antheridial parent cells are distinguished by dense cytoplasm with few vacuoles, a large central vacuole, and chloroplasts with only a few thylakoids (Berkaloff and Rousseau, 1979). Following meiosis, which occurs during the first two divisions of the primary nucleus, the nuclei undergo four mitoses. Mature antheridia (Fig. 21.43(c), (d)) thus contain 64 nuclei, each of which becomes incorporated into a spermatozoid. Cells in the receptacle release potassium and chloride ions into the mucilage of the conceptacle. This results in swelling of the mucilage that carries the antheridia (or oogonia in the female conceptacle) out of the conceptacle into the seawater (Speransky et al., 2001). The wall of the antheridium is composed of two layers. At liberation, the outer wall ruptures, releasing the inner wall containing the spermatozooids and mucilage. This packet passes out of the conceptacle and into the sea, where the inner wall gelatinizes at one or both ends, releasing the spermatozooids.



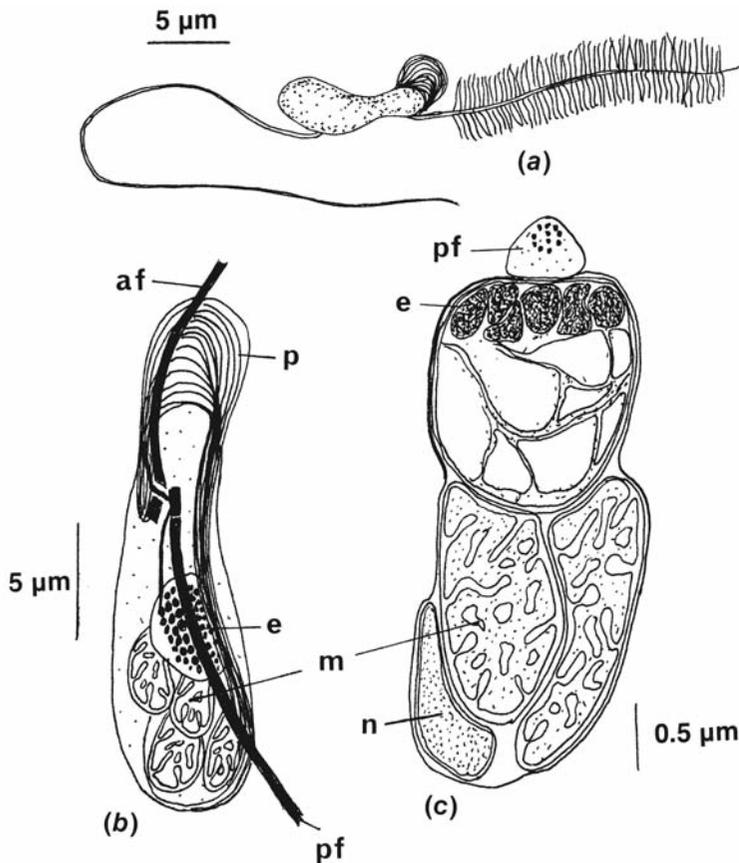
**Fig. 21.43** *Fucus vesiculosus*. (a) Whole plant showing receptacle (arrow). (b) Cross section of a female receptacle showing three conceptacles. (c) Cross section of a male conceptacle. (d) Branched antheridial filaments bearing antheridia. (b), (c), (d) are scanning electron micrographs of frozen tissue. (From Speransky et al., 1999.)

The spermatozooids are spherical at first and then uncoil to give the elongated biflagellate form (Fig. 21.44). There is an eyespot consisting of a single layer of pigment globules inside a reduced chloroplast. The basal portion of the posterior flagellum is closely applied to the plasmalemma in the area of the eyespot. The anterior portion of the cell contains 13 microtubules that make up the **proboscis**, a structure that may function in the detection of the female sex attractant. The proboscis microtubules pass from the area of the basal bodies, extending themselves in one plane in front of the spermatozoid, and then pass beneath the plasmalemma to the posterior portion of the cell (Manton and Clarke, 1950, 1951, 1956).

The oogonia are usually borne on a stalk cell that is embedded in the wall of the conceptacle (Figs. 21.40, 21.43 (b)). The oogonial cell undergoes three nuclear divisions, yielding eight haploid

nuclei. The cytoplasm then cleaves into eight eggs. The wall of the oogonium is composed of three layers, the thin outer layer or **exochite**, the thick middle layer or **mesochite**, and the thin inner layer or **endochite**. When the oogonium is mature, the exochite ruptures, releasing the packet of eggs, still surrounded by the other two wall layers, into the sea. In the sea, the mesochite ruptures apically, slips backward, and exposes the eggs within the endochite. The endochite rapidly dissolves, releasing the eggs.

The sperm are attracted to the eggs by a species-non-specific pheromone, **fucoserraten** (Fig. 21.8), released by the eggs (Müller and Jaenicke, 1973). The species-specific recognition between eggs and sperm is based on specific oligosaccharides on the eggs and sperm. The oligosaccharide side chains of the egg-surface glycoproteins contain fucosyl, mannosyl, and/or glucosyl residues (Wright et al., 1995a). The surface of



**Fig. 21.44** Spermatozoid of *Fucus*. (a) Whole spermatozoid. (b) Semidiagrammatic drawing showing the anterior flagellum (af), eyespot (e), mitochondria (m), proboscis (p), and the posterior flagellum (pf). (c) Section through a spermatozoid illustrating the close appression of the posterior flagellum (pf) to the eyespot area (e) of the reduced chloroplast. (n) Nucleus. (After Manton and Clarke, 1956.)

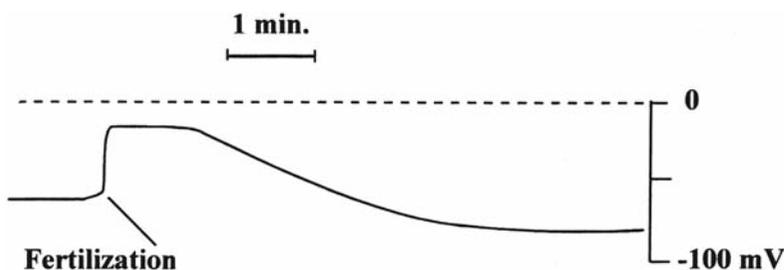
the egg is not homogeneous, but instead is organized into different domains, each containing different glycoproteins (Stafford et al., 1992). Likewise, the sperm contain glycoproteins organized into domains on the anterior flagellum plasma membrane, the mastigonemes of the anterior flagellum, and the sperm body (Jones et al., 1988). On reaching the egg surface, sperm exhibit a characteristic behavior involving movement over the plasma membrane of the egg and a probing or “searching” of the egg membrane with the anterior flagellum (Brawley, 1991). The glycoproteins on the sperm eventually bind to the complementary glycoproteins on the egg. This results in two “blocks” to further sperm penetration (Wright et al., 1995b):

- 1 A “fast block” within seconds (Fig. 21.45) caused by depolarization of the plasma membrane due to  $\text{Na}^+$  and  $\text{Ca}^{2+}$  influx. Excess sperm detach from the egg following depolarization.
- 2 A “slow block” results from the formation of a cell wall around the zygote (Fig. 21.46) by the release of cellulose, phenolics, sulfated fucans, vanadate peroxidase, and alginates from cortical vesicles to form the adhesive **glycocalyx** (Vreeland et al., 1998; Schoenwaelder and Clayton, 1999).

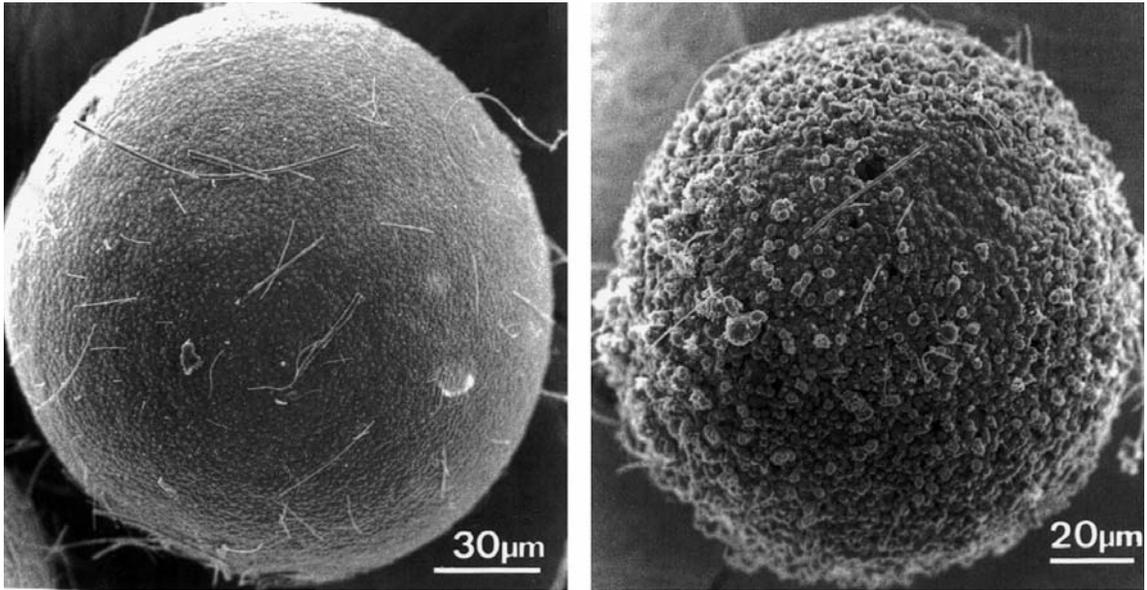
The male nucleus migrates to the female nucleus along associated microtubules. As it migrates, the nuclear envelope of the male nucleus breaks up. The egg nucleus becomes convoluted along the surface nearest the advancing male nucleus. Immediately prior to nuclear fusion, many egg mitochondria accumulate in the vicinity of the male nucleus (Brawley et al., 1976).

The spherical zygote germinates by forming a primary rhizoid from one side, while the rest of the zygote gives rise to the embryo. There are a number of stages in the determination of which part of the zygote will develop into the rhizoid and which will develop into the embryo (Fig. 21.47) (Belanger and Quatrano, 2000; Bisgrove and Kropf, 2004):

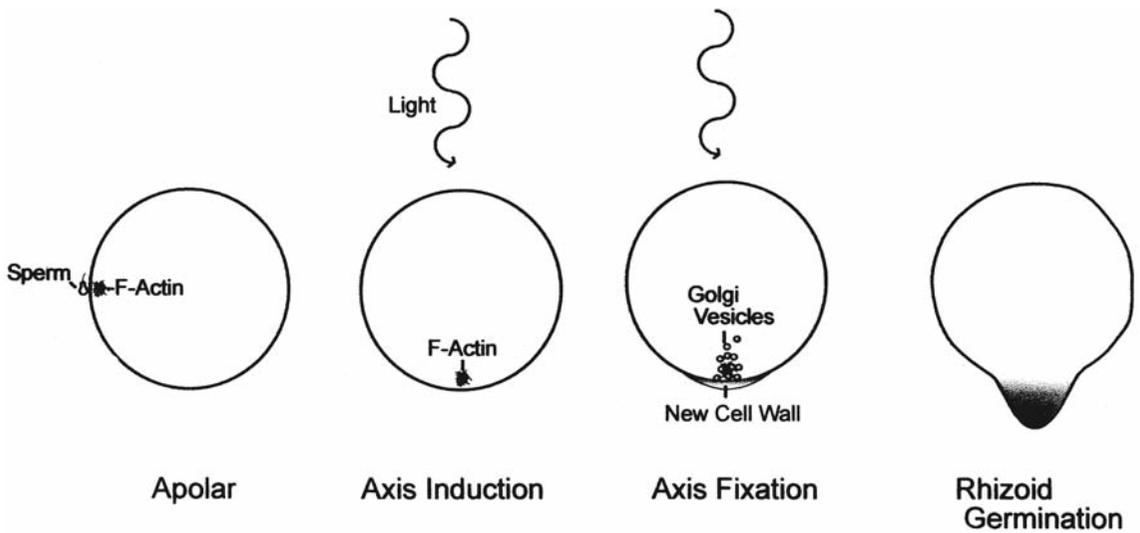
- 1 **Apolar** – In the early stages of development, the furoid zygotes are apolar and appear to have no inherent order. However, staining of the cells shows the accumulation of F-actin under the plasma membrane in the area of the zygote where the sperm entered.
- 2 **Axis induction** – A potential polar axis is generated within the zygote. The axis is determined by a number of environmental stimuli, including the direction of incident light, the position of neighboring zygotes, water currents, chemical or ionic gradients, and electrical fields. If none of these stimuli occurs, the axis will be where the sperm originally entered the zygote. During axis induction, the polarity is labile and can be re-oriented by the subsequent exposure to a stimulus in a different direction. F-actin microfilaments are produced *de novo* in the area where the rhizoid will develop (e.g., the shaded area of the zygote in light is responsible for axis induction).
- 3 **Axis fixation** – The F-actin microfilaments guide Golgi vesicles to the plasma membrane where the vesicles release components of a cell wall that is different in composition from the glycocalyx. At this point the axis is fixed and is no longer susceptible to re-orientation by the environment.



**Fig. 21.45** The fast block after fertilization of a *Fucus* egg by a sperm. The potential of the plasma membrane of the egg drops as  $\text{Na}^+$  flows into the zygote after fertilization.



**Fig. 21.46** Scanning electron micrographs of the egg (left) and zygote (right) of the furoid *Acrocarpia paniculata*. After fertilization, the zygote extrudes droplets of material that produce the glycocalyx. (From Schoenwaelder and Clayton, 1998.)



**Fig. 21.47** Diagram showing the key events in establishing a polar axis and site of rhizoid germination in a *Fucus* embryo.

4 **Rhizoid germination** – The spherical symmetry of the zygote is broken by the emergence of a polar bulge representing the emergence of the rhizoid. The direction of the rhizoid germination defines the direction of the polar axis. Unequal cytokinesis follows, perpendicular to the direction of rhizoid germination, yielding an embryo consisting of two freshly differentiated cells, a large rounded thallus cell, which is the precursor of the frond and receives most of the chloroplasts, and a smaller rhizoid cell, which generates the stipe and holdfast.

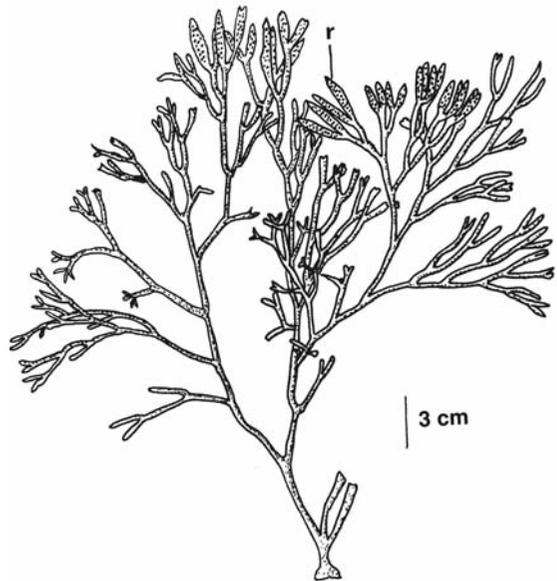
The embryo divides to form a minute cylindrical plant with at least one apical cell with trichothallic growth, producing a hair above (which may function in the uptake of nutrients (Steen, 2003)) and the thallus below. Eventually the apical cell ceases production of the hair and becomes the three-sided apical cell, which later becomes four-sided. It is only after the initiation of the apical cell that the thallus begins to assume its mature flattened shape.

The Fucales show a periodicity in the formation of receptacles and conceptacles. In *Fucus* and *Ascophyllum* (Fig. 21.49) in the North Atlantic, receptacle initiation is a short-day phenomenon, with receptacles being initiated in a 8:16 and 12:12 light-dark photoperiod and inhibited under a 16:8 and continuous light photoperiod (Bird and McLachlan, 1976; Terry and Moss, 1980). White light in the dark period inhibits the short-day response. In the field, conceptacle development commences during September and October, and continues during the following spring until gametes are mature and are liberated during April and May. Following gamete discharge, the conceptacle-bearing receptacles and the rest of the lateral shoot are shed. *Halidrys* (Fig. 21.50) (Moss and Shearer, 1973) has a different periodicity, with vegetative growth in the spring and summer followed by initiation of the conceptacles. The gametes are then shed during the winter. Even though this is the darkest period of the year, the gametes are able to germinate and secure themselves to a substrate with their rhizoids. These germlings are then able to sustain themselves during the periods of little

light and subsequently grow normally as soon as there is sufficient daylight. Many of the Fucales show a temperature tolerance similar to that of the Laminariales, with 20 °C being the highest temperature at which eggs of *Halidrys* will germinate.

### Ecology

Both the geographical distribution and the location on the shore of a member of the Fucales depend on the ability of the fertilized egg to settle and germinate under the environmental conditions present (Chapman, 1995). Embryos of *Pelvetia fastigata* (Fig. 21.48) will almost all survive if they settle under adult *Pelvetia* thalli. Those that settle on exposed rock will almost all die. Within red-algal tufts, most of the younger embryos survive, with survival declining with the increasing age of the settling embryos (Brawley and Johnson, 1991). Sedimentation on top of the embryos reduces embryo survival, particularly if hydrogen sulfide is present (Chapman and Fletcher, 2002; Bergstrom et al., 2003). In addition, the newly attached zygotes also fall prey to browsing molluscs – in particular, the limpets and *Littorina* species or periwinkles. The mollusc pressure on fucoid development is one



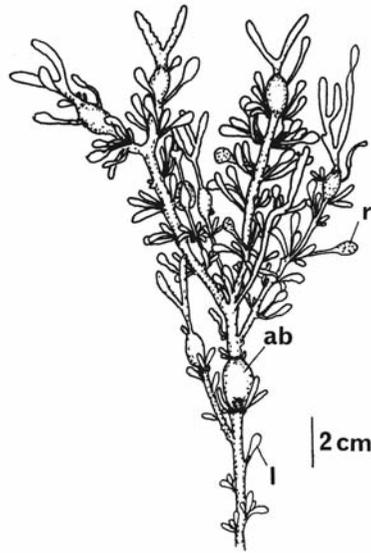
**Fig. 21.48** *Pelvetia fastigata*. (r) Receptacle. (After Smith, 1969.)

of the key factors in determining the number of plants present.

Lodge (1948) carried out an experiment on the Isle of Man to determine the rate of recolonization of a shore with primarily fucoid algae. A strip of shore 5 m wide was cleared of all macroscopic biological life. In the first spring after the clearance, green algae (*Enteromorpha*, *Urospora*, and *Chaetomorpha*) covered the shore, along with diatoms. *Fucus* germlings then developed beneath the green algae, starting first at the high-tide mark and then establishing themselves toward the low-water mark. The main *Fucus* species during the first year was *F. vesiculosus*, whereas during the second year *F. serratus* became prominent. After the fucoids became established, the green algae gradually disappeared, and a sparse undergrowth of red algae appeared (*Dumontia*, *Laurencia*). After two years the *Fucus* plants dominated the shore, covering a more diverse undergrowth. By this time the limpets had started to recolonize the shore, slowing down colonization by algae. Seven years after the beginning of the experiment, the shore had returned to its original condition.

The littoral Fucales are adapted to the difficult conditions in which they live, being able to withstand freezing temperatures and summer temperatures up to 34 to 36 °C (Malm and Kautsky, 2003). The zonation of the different fucoid species is due partly to their ability to photosynthesize better when exposed to air (Madsen and Maberly, 1990) and partly to withstand desiccation during both germination and growth. The plants that live higher up in the littoral zone have thicker walls, more fucoidin, and a higher water content, and reach their dry weight on evaporation later than those lower in the littoral zone. The proportion of polysaccharides also reflects the fucoid position in the littoral zone. *Fucus spiralis* and *Pelvetia canaliculata*, which grow highest in the littoral zone, contain the highest amount of fucoidin, 18% to 24% of the dry weight. *Fucus serratus*, which grows near the low-tide mark, has much less fucoidin, about 13% on a dry-weight basis (Black, 1954b).

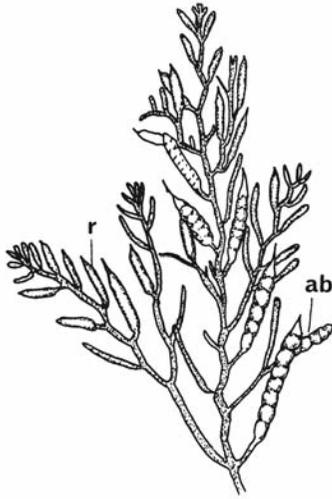
Generally the life-span of most shore fucoids is about 2 to 3 years (Boney, 1966). The only exception to this being *Ascophyllum* (Fig. 21.49), which



**Fig. 21.49** *Ascophyllum nodosum*. Small portion of the distal end of a plant in late summer condition. (ab) Air bladder; (l) lateral; (r) receptacle. (After Taylor, 1957.)

has an average age from 12 to 15 years. The average age of the *Ascophyllum* plants will vary according to their position in the littoral zone. On the Welsh coast, plants from the top of the littoral zone were found to be 4 to 5 years old, whereas those from the bottom of the zone were 5 to 15 years old (David, 1943). Although being long-lived, *Ascophyllum* produces a paucity of sporelings and it takes a couple of decades for recolonization of a denuded area. This has resulted in severely depleted populations in areas where the alga is commercially exploited (Bacon and Vadas, 1991).

Morphology of fucoid plants will vary with environment. Moss and Sheader (1973) showed that in *Halidrys siliquosa* (Fig. 21.50) the germlings at 10 °C in total darkness produced long rhizoids and a short thallus. If the same plants were grown at a high light intensity (5936 lux), the thalli were long and unbranched, whereas the rhizoids remained short but were pigmented. If the thalli were grown at 20 °C under high light intensity, branched thalli were obtained. Vesiculation will also vary with the environment. *Fucus vesiculosus* growing in areas subjected to very severe wave action will lack vesicles, whereas those growing in calmer areas have gas



**Fig. 21.50** *Halidrys siliquosa*. (ab) Air bladder; (r) receptacle.

vesicles. A minimum branch length seems necessary before vesicle formation can begin, and vesiculation is postponed to the following year if growth has not attained this minimum length (Boney, 1966).

Although the members of the order are normally lithophytes, they are also widely represented by unattached growth-forms lacking holdfasts and propagating mostly vegetatively. These free-living forms arise by vegetative growth of detached branches of the normal attached form that have been transported to a sheltered habitat, or by the development of zygotes in a quiet environment into unattached plants. These unattached plants are referred to as **ecads**, a term for a plant whose morphology has been altered by growth in an unusual environment. Most of these ecads are found in sheltered habitats such as bays and salt marshes.

Fucoids are basically salt-water algae. Attempts to colonize habitats of lower salinity fail, probably because the “fast block” after fertilization (see section on reproduction of *Fucus*) relies on depolarization of the egg membrane by  $\text{Na}^+$  (e.g.,  $\text{NaCl}$ ) flowing in from seawater (Serrão et al., 1999). Low salinity causes loss of the fast block and more than one sperm fertilizing the egg. This commonly results in lost embryos because polyspermy is almost always lethal.

In discussing salt marsh ecads, Boney (1966) states that the fucoids show the following characteristics: (1) vegetative propagation as the main means of propagation; (2) absence of a holdfast; (3) dwarf habit; (4) spiral twisting of the thallus; and (5) profuse branching. Many of the salt marsh forms grow embedded (but not attached by holdfasts) in a muddy substratum, and some are entwined around the stem bases of the dominant angiosperms. The protection afforded by the canopy of angiosperms enables the fucoids to survive at high levels in the marshes.

*Ascophyllum nodosum* ecad *mackaii* is an unattached form common in Scotland (Gibb, 1957; Moss, 1971). Normally if a plant of *Ascophyllum* becomes detached from rocks in the intertidal zone, it is cast up by the waves and soon disintegrates. In the unusually calm waters at the head of some Scottish lochs, the detached thalli are gently covered and uncovered as the tide advances and recedes, but they never dry out and disintegrate. The external forms of the attached plant and of the ecad are in complete contrast. The attached plant is flattened in one plane and generally dichotomizes once a year in the spring after differentiation of an air bladder. During the summer a series of lateral nodes are developed, from which receptacles are produced as laterals the following year. This yearly cycle of differentiation is completely lacking in the marsh form of the ecad. Here there are no air bladders and no regular dichotomy to mark off one year’s growth from another. Instead, apical branching is frequent and in all planes, giving rise to the characteristic cushion form of the ecad. Also there are no lateral nodes and thus no lateral meristems to give rise to lateral branches. Instead, receptacles are sometimes differentiated behind the apices of any branch, apparently at random.

The form of the ecad is caused by the destruction of the apical meristem and the lateral nodes of the ecad. The branches are eventually regenerated from wound-healing tissue to give rise to the reduced ecad form. Growth of the ecad is slow, and large tufts on the shore may have taken several years to grow. The ecad often still has a prominent apical cell, but there is little meristematic activity when compared to the

attached plant. This results in a thallus with little cortex.

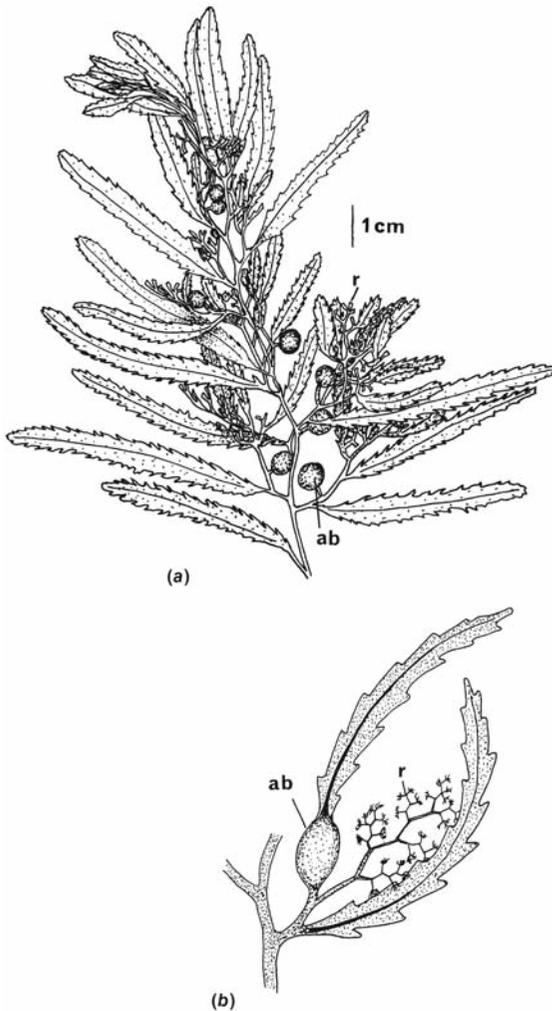
The receptacles of the ecad are small when compared to those of the attached plant. The eggs are also small and of variable size. The number of germlings produced from the ecad eggs is low. The time of gamete discharge in the ecad approximates that of nearby attached plants.

The Sargasso Sea is in a gyre in the Atlantic Ocean between 20° and 35° north latitude and is made up of huge masses of floating *Sargassum*

(gulfweed) (Fig. 21.51). The immense tracts of *Sargassum* are formed by continuous vegetative reproduction of the plants in the area. Holdfasts are never found. Occasionally, conceptacles can be seen, but they are always functionless, and the maintenance of the population is entirely by vegetative means.

## REFERENCES

- Amsler, C. D., and Neushel, M. (1989). Chemotactic effects of nutrients on spores of the kelps *Macrocystis pyrifera* and *Pterygophora californica*. *Mar. Biol.* (Berlin) 102:557–64.
- Andersen, R. J. (1982). The life history of *Desmarestia firma* (C. Ag.) Skottsb. (Phaeophyceae, Desmarestiales). *Phycologia* 21:316–22.
- Arnold, T. M., Targett, N. M., Tanner, C. E., Hatch, W. L., and Ferrari, K. E. (2001). Evidence for methyl jasmonate-induced phlorotannin production in *Fucus vesiculosus* (Phaeophyceae). *J. Phycol.* 37:1026–9.
- Asensi, A., Gall, E. A., Marie, D., Billot, C., Dion, P., and Kloareg, B. (2001). Clonal propagation of *Laminaria digitata* (Phaeophyceae) sporophytes through a diploid cell-filament suspension. *J. Phycol.* 37:411–17.
- Bacon, L. E., and Vadas, R. L. (1991). A model for gamete release in *Ascophyllum nodosum* (Phaeophyta). *J. Phycol.* 27:166–73.
- Bailey, J. C., Bidigare, R. R., Christensen, S. J., and Andersen, R. A. (1998). Phaeothamniophyceae classis nova: a new lineage of chromophytes based upon photosynthetic pigments, *rbcl* sequence analysis and ultrastructure. *Protist* 149:245–63.
- Belanger, K. D., and Quatrano, R. S. (2000). Polarity: the role of localized secretion. *Curr. Opin. Plant Biol.* 3:67–72.
- Bell, G. (1997). The evolution of the life cycle of brown seaweeds. *Biol. J. Linnean Soc.* 60:21–38.
- Bergstrom, L., Berger, R., and Kautsky, L. (2003). Negative effects of nutrient enrichment on the establishment of *Fucus vesiculosus* in the Baltic Sea. *Eur. J. Phycol.* 38:41–6.
- Berkaloff, C., and Rousseau, B. (1979). Ultrastructure of male gametogenesis in *Fucus serratus* (Phaeophyceae). *J. Phycol.* 15:163–73.
- Bird, N. L., and McLachlan, J. (1976). Control of formation of receptacles in *Fucus distichus* L., subsp. *distichus* (Phaeophyceae, Fucales). *Phycologia* 15:79–84.



**Fig. 21.51** *Sargassum filipendula*. (a) Part of a main branch. (b) *S. longifolium*. (ab) Air bladder; (r) receptacle. ((a) after Taylor, 1960.)

- Bisalputra, T. (1966). Electron microscopic study of protoplasmic continuity in certain brown algae. *Can. J. Bot.* 44:89–93.
- Bisalputra, T., and Burton, H. (1969). The ultrastructure of the chloroplast of a brown alga *Sphacelaria* sp. II. Association between the chloroplast DNA and the photosynthetic lamellae. *J. Ultrastruct. Res.* 29:224–35.
- Bisalputra, T., Shields, M., and Markam, J. W. (1971). *In situ* observations of the fine structure of *Laminaria* gametophytes and embryos in culture. I. Methods and the ultrastructure of the zygote. *J. Microscopie* 10:83–98.
- Bisgrove, S. R., and Kropf, D. L. (2004). Cytokinesis in brown algae: studies of asymmetric division in fucoid zygotes. *Protoplasma* 223:163–73.
- Black, W. A. P. (1954a). Concentration gradients and their significance in *Laminaria saccharina* (L.) Lamour. *J. Mar. Biol. Assoc. UK* 33:49–60.
- Black, W. A. P. (1954b). The seasonal variation in the combined L-fucose content of the common British Laminariaceae and Fuaceae. *J. Sci. Food Agric.* 5:445–54.
- Boalch, G. T. (1961). Studies on *Ectocarpus* in culture. *J. Mar. Biol. Assoc. UK* 41:287–304.
- Boney, A. D. (1966). *A Biology of Marine Algae*. London: Hutchinson.
- Borowitzka, M. A., Larkum, A. W. D., and Nockolds, C. E. (1974). A scanning electron microscope study of the structure and organisation of the calcium carbonate deposits of algae. *Phycologia* 13:195–203.
- Bouck, G. B. (1965). Fine structure and organelle associations in brown algae. *J. Cell Biol.* 26:523–37.
- Bouck, G. B. (1969). Extracellular microtubules. The origin, structure and attachment of flagellar hairs in *Fucus* and *Ascophyllum* antherozoids. *J. Cell Biol.* 40:446–60.
- Bourne, V. L., and Cole, K. (1968). Some observations on the fine structure of the marine brown alga *Phaeostrophion irregulare*. *Can. J. Bot.* 46:1369–75.
- Brawley, S. H. (1991). The fast block against polyspermy in fucoid algae is an electrical block. *Dev. Biol.* 144:94–106.
- Brawley, S. H., and Johnson, L. E. (1991). Survival of fucoid embryos in the intertidal zone depends upon developmental stage and microhabitat. *J. Phycol.* 27:179–86.
- Brawley, S. H., Wetherbee, R., and Quatrano, R. S. (1976). Fine structural studies of the gametes and embryo of *Fucus vesiculosus* L. (Phaeophyta). *J. Cell Sci.* 20:233–54.
- Buggeln, R. G. (1974). Negative phototropism of the haptera of *Alaria esculenta* (Laminariales). *J. Phycol.* 10:80–2.
- Cabello-Pasini, A., and Alberte, R. S. (2001). Expression of carboxylating enzymes in *Laminaria setchellii* (Phaeophyceae). *Phycologia* 40:351–8.
- Chapman, A. R. O. (1995). Functional ecology of fucoid algae: twenty-three years of progress. *Phycologia* 34:1–32.
- Chapman, A. R. O., and Burrows, E. M. (1971). Field and culture studies of *Desmarestia aculeata* (L.) Lamour. *Phycologia* 10:63–76.
- Chapman, A. S., and Fletcher, R. L. (2002). Differential effects of sediments on survival and growth of *Fucus serratus* embryos (Fucales, Phaeophyceae). *J. Phycol.* 38:894–903.
- Cheng, T-H. (1969). Production of kelp. A major source of China's exploitation of the sea. *Econ. Bot.* 23:215–36.
- Chi, E. Y. (1971). Brown algal pyrenoids. *Protoplasma* 72:101–4.
- Clayton, M. N. (1980). Sexual reproduction – A rare occurrence in the life history of the complanate form of *Scytosiphon* (Scytosiphonaceae, Phaeophyta) from southern Australia. *Br. J. Phycol.* 15:105–18.
- Clayton, M. N., and Ashburner, C. M. (1994). Secretion of phenolic bodies following fertilization in *Durvillaea potatorum* (Durvillaeales, Phaeophyta). *Eur. J. Phycol.* 29:1–9.
- Clint, H. B. (1927). The life history and cytology of *Sphacelaria bipinnata* Sauv. *Publ. Hartley Bot. Lab. Liverpool* 3:1–19.
- Cole, K. (1970). Ultrastructural characteristics in some species in the order Scytosiphonales. *Phycologia* 9:275–83.
- Colijn, F., and van den Hoek, C. (1971). The life history of *Sphacelaria furcigera* Kütz. (Phaeophyceae). II. The influence of daylength and temperature on sexual and vegetative reproduction. *Nova Hedwigia* 21:899–922.
- David, H. M. (1943). Studies in the autecology of *Ascophyllum nodosum* Le Jol. *J. Ecol.* 31:178–98.
- Dayton, P. K., and Tegner, M. J. (1984). Catastrophic storms, El Niño, and patch stability in a southern California kelp community. *Science* 224:283–5.
- Dayton, P. K., Currie, V., Gerrodette, T., Keller, B. D., Rosenthal, G., and Ven Tresca, D. (1984). Patch dynamics and stability of some California kelp communities. *Ecol. Monogr.* 54:253–89.
- de Reviere, B., and Rousseau, F. (1999). Towards a new classification of the brown algae. In *Progress*

- in *Phycological Research*, ed. F. E. Round and D. J. Chapman, Vol. 13, pp. 107–201. Bristol: Biopress Ltd.
- Diouris, M. (1989). Long-distance transport of  $^{14}\text{C}$ -labelled assimilate in the Fucales: nature of translocated substances in *Fucus serratus*. *Phycologia* 28:504–11.
- Dixon, N. M., Leadbeater, B. S. C., and Wood, K. R. (2000). Frequency of viral infection in a field population of *Ectocarpus fasciculatus* (Ectocarpales, Phaeophyceae). *Phycologia* 39:528–63.
- Draisma, S. G. A., Prud'homme van Reine, W. F., Stam, W. T., and Olsen, J. L. (2001). A reassessment of phylogenetic relationships within the Phaeophyceae based on Rubisco large subunit and ribosomal DNA sequences. *J. Phycol.* 37:586–603.
- Draisma, S. G. A., Olsen, J. L., Stam, W. T., and Prud'homme van Reine, W. F. (2002). Phylogenetic relationships within the Sphacelariales (Phaeophyceae): *rbcl*, RUBISCO spacer and morphology. *Eur. J. Phycol.* 37:385–401.
- Druehl, L. D. (1967). Distribution of two species of *Laminaria* as related to some environmental factors. *J. Phycol.* 3:103–8.
- Duggins, D. O. (1980). Kelp beds and sea otters: An experimental approach. *Ecology* 61:447–53.
- Edelstein, T., Chen, L., and McLachlan, J. (1968). Sporangia of *Ralfsia fungiformis* (Gunn.) Setchell and Gardner. *J. Phycol.* 4:157–60.
- Edelstein, T., Chen, L. C.-M., and McLachlan, J. (1970). The life cycle of *Ralfsia clavata* and *R. borneti*. *Can. J. Bot.* 48:527–31.
- Edwards, P. (1969). Field and cultural studies on the seasonal periodicity of growth and reproduction of selected Texas benthic marine algae. *Contrib. Mar. Sci. Univ. Texas* 14:59–114.
- Estes, J. A., and Steinberg, P. D. (1988). Predation, herbivory, and kelp evolution. *Paleobiology* 14:19–36.
- Evans, L. V. (1965). Cytological studies in the Laminariales. *Ann. Bot.* 29:541–62.
- Evans, L. V. (1966). Distribution of pyrenoids from some brown algae. *J. Cell Sci.* 1:449–54.
- Evans, L. V. (1968). Chloroplast morphology and fine structure in British fucoids. *New Phytol.* 67:173–8.
- Evans, L. V., and Holligan, M. S. (1972). Correlated light and electron microscope studies on brown algae. I. Localization of alginic acid and sulphated polysaccharides in *Dictyota*. *New Phytol.* 71:1161–72.
- Evans, L. V., Simpson, M., and Callow, M. E. (1973). Sulphated polysaccharide synthesis in brown algae. *Planta* 110:237–52.
- Floc'h, J. Y., and Penot, M. (1972). Transport du  $^{32}\text{P}$  et du  $^{86}\text{Rb}$  chez quelques algues brunes: Orientation des migrations et voies de conduction. *Physiologie Végétale* 10:677–86.
- Forster, R. M., and Dring, M. J. (1994). Influence of blue light on the photosynthetic capacity of marine plants from different taxonomic, ecological and morphological groups. *Eur. J. Phycol.* 29:21–7.
- Fulcher, R. G., and McCully, M. E. (1969). Histological studies on the genus *Fucus*. IV. Regeneration and adventure embryony. *Can. J. Bot.* 47:1643–9.
- Gaines, S. D., and Roughgarden, J. (1987). Fish in offshore kelp forests affect recruitment to intertidal populations. *Science* 235:479–81.
- Gibb, D. C. (1957). The free-living forms of *Ascophyllum nodosum* (L.) Le Jol. *J. Ecol.* 45:49–83.
- Gibson, G., and Clayton, M. N. (1987). Sexual reproduction, early development and branching in *Notheia anomala* (Phaeophyta) and its classification in the Fucales. *Phycologia* 26:363–73.
- Grenville, D. J., Peterson, R. L., Barrales, H. L., and Gerrath, J. F. (1982). Structure and development of the secretory cells and duct system in *Macrocystis pyrifera* (L.) C. A. Agardh. *J. Phycol.* 18:232–40.
- Guignard, L. (1892). Observations sur l'appareil mucifere des Laminariacées. *Ann. Sci. Nat. Bot.* VII 15:1–46.
- Henley, W. J., and Dunton, K. H. (1997). Effects of nitrogen supply and continuous darkness on growth and photosynthesis of the arctic kelp *Laminaria solidungula*. *Limnol. Oceanog.* 42:209–16.
- Henry, B. E., and Van Alstyne, K. L. (2004). Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. *J. Phycol.* 40:527–33.
- Henry, E. C. (1987). Primitive reproductive characters and a photoperiodic response in *Saccorhiza dermatodea* (Laminariales, Phaeophyceae). *Br. Phycol. J.* 22:23–31.
- Henry, E. C., and Cole, K. M. (1982). Ultrastructure of swarmers in the Laminariales (Phaeophyceae). I. Zoospores. *J. Phycol.* 18:550–69.
- Hollenberg, G. J. (1969). An account of the Ralfsiaceae (Phaeophyta) of California. *J. Phycol.* 5:290–301.
- Hsiao, S. I. C. (1969). Life history and iodine nutrition of the marine brown alga, *Petalonia fascia* (O. F. Müll.) Kuntze. *Can. J. Bot.* 47:1611–16.
- Hsiao, S. I. C. (1970). Light and temperature effects on the growth, morphology, and reproduction of *Petalonia fascia*. *Can. J. Bot.* 48:1359–61.

- Hsiao, S. I. C., and Druehl, L. D. (1973). Environmental control of gametogenesis in *Laminaria saccharina*. IV. In situ development of gametophytes and young sporophytes. *J. Phycol.* 9:160–4.
- Hurd, C. L., Galvin, R. S., Norton, T. A., and Dring, M. J. (1993). Production of hylaine hairs by intertidal species of *Fucus* (Fucales) and their role in phosphate uptake. *J. Phycol.* 29:160–5.
- Iken, K., Amsler, C. D., Greer, S. P., and McClintock, J. B. (2001). Qualitative and quantitative studies of the swimming behaviour of *Hincksia irregularis* (Phaeophyceae) spores: ecological implications and parameters for quantitative swimming assays. *Phycologia* 40:359–66.
- Jaenicke, L., Müller, D. G., and Moore, R. E. (1974). Multifidene and auctantene, C<sub>11</sub> hydrocarbons in the male attracting essential oil from the gynogametes of *Cutleria multifida* (Smith) Grev. (Phaeophyta). *J. Am. Chem. Soc.* 96:3324–5.
- Jones, J. L., Callow, J. A., and Green, J. R. (1988). Monoclonal antibodies to sperm antigens of the brown alga *Fucus serratus* exhibit region-, gamete-, species- and genus-preferential binding. *Planta* 176:298–306.
- Kain, J. M. (1966). The role of light in the ecology of *Laminaria hyperborea*. *Br. Ecol. Soc. Symp.* 6:319–34.
- Kapraun, D. F., and Boone, P. W. (1987). Karyological studies of three species of Scytosiphonaceae (Phaeophyta) from coastal North Carolina. *J. Phycol.* 23:318–22.
- Kawai, H., Kubota, M., Kondo, T., and Watanabe, M. (1991). Action spectra for phototaxis in zoospores of the brown alga *Pseudochorda gracilis*. *Protoplasma* 161:17–22.
- Kawai, H., Nakamura, S., Mimuro, M., Furuya, M., and Watanabe, M. (1996). Microspectrofluorometry of the autofluorescent flagellum in phototactic algal zooids. *Protoplasma* 191:172–7.
- Klinger, T., and DeWeede, R. E. (1988). Stipe rings, age and size in populations of *Laminaria setchelli* Silva (Laminariales, Phaeophyta) in British Columbia. *Phycologia* 27:234–40.
- Kloareg, D., and Quatrano, R. S. (1988). Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharides. *Oceanogr. Mar. Biol. Rev.* 26:234–40.
- Knight, M. (1929). Studies in the Ectocarpaceae. II. The life-history and cytology of *Ectocarpus siliculosus* Dillw. *Trans. R. Soc. Edinburgh* 56:307–32.
- Kubanek, J., Lester, S. E., Fenical, W., and Hay, M. E. (2004). Ambiguous role of phlorotannins as chemical defenses in the brown alga *Fucus vesiculosus*. *Mar. Ecol. Progr. Ser.* 277:79–93.
- Kuckuck, P. (1899). Über den generationwechsel von *Cutleria multifida* (Engl. Bot.) Grev. *Wiss. Meeresunters. Helgoland N.F.* 3:95–116.
- Kumke, J. (1973). Beiträge zur Periodizität der Oogon-Entleerung bei *Dictyota dichotoma* (Phaeophyta). *Z. Pfl. Physiol.* 70:191–210.
- Kupper, F. C., Schweigert, N., Gall, E. A., Legendre, J.-M., Vilter, H., and Kloareg, B. (1998). Iodine uptake in Laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide. *Planta* 207:163–71.
- Kylin, H. (1934). Zur Kenntnis der Entwicklungsgeschichte einiger Phaeophyceen. *Lunds Univ. Årsskr. N.F. Avd.* 30:1–19.
- Ladah, L., Bermudez, R., Pearson, G., and Serrao, E. (2003). Fertilization success and recruitment of dioecious and hermaphroditic fucoid seaweeds with contrasting distributions near their southern limit. *Mar. Ecol. Progr. Ser.* 262:173–83.
- Larkum, A. W. D. (1972). Frond structure and growth in *Laminaria hyperborea*. *J. Mar. Biol. Assoc. UK* 52:405–18.
- Lim, B.-L., Kawai, H., Hori, H., and Osawa, S. (1986). Molecular evolution of 5S ribosomal RNA from red and brown algae. *Japanese J. Genet.* 61:169–76.
- Lobban, C. S. (1978). The growth and death of the *Macrocystis* sporophyte (Phaeophyceae, Laminariales). *Phycologia* 17:196–212.
- Lodge, S. M. (1948). Algal growth in the absence of *Patella* on an experimental strip of seashore. *Proc. Trans. Liverpool Biol. Soc.* 61:78–83.
- Loiseaux, S. (1970). *Streblonema anomalum* S. et G. and *Composonema sporangiiferum* S. et G. stages in the life history of a minute *Scytosiphon*. *Phycologia* 9:185–91.
- Loiseaux, S. (1973). Ultrastructure of zoidogenesis in unilocular zoidocysts of several brown algae. *J. Phycol.* 9:277–89.
- Loiseaux, S., and West, J. A. (1970). Brown algal mastigonemes: Comparative ultrastructure. *Trans. Am. Microsc. Soc.* 89:524–32.
- Luder, U. H., and Clayton, M. N. (2004). Induction of phlorotannins in the brown macroalga *Ecklonia radiata* (Laminariales, Phaeophyta) in response to simulated herbivory – the first microscopic study. *Planta* 218:928–37.
- Lüning, K. (1981). Egg release in gametophytes of *Laminaria saccharina*: Induction by darkness and inhibition by blue light and U.V. *Br. Phycol. J.* 16:379–93.

- Lüning, K., and Dring, M. J. (1972). Reproduction induced by blue light in female gametophytes of *Laminaria saccharina*. *Planta* 104:252–6.
- Lüning, K., and Dring, M. J. (1973). The influence of light quality on the development of the brown algae *Petalonia* and *Scytosiphon*. *Br. Phycol. J.* 8:333–8.
- Lüning, K., and Dring, M. J. (1975). Reproduction, growth and photosynthesis of gametophytes of *Laminaria saccharina* grown in blue and red light. *Mar. Biol.* 29:195–200.
- Lüning, K., and Müller, D. G. (1978). Chemical interaction in sexual reproduction of several Laminariales (Phaeophyceae): Release and attraction of spermatozooids. *Z. Pflanzenphysiol.* 89:333–41.
- Lüning, K., Schmitz, K., and Willenbrink, J. (1972). Translocation of <sup>14</sup>C-labelled assimilates in two *Laminaria* species. *Proc. VII Int. Seaweed Symp.* 420–5.
- Lüning, K., Wagner, A., and Buchholz, C. (2000). Evidence for inhibitors of sporangium formation in *Laminaria digitata* (Phaeophyceae) during the season of rapid growth. *J. Phycol.* 36:1129–34.
- Madsen, T. V., and Maberly, S. C. (1990). A comparison of air and water as environments for photosynthesis by the intertidal alga *Fucus spiralis* (Phaeophyta). *J. Phycol.* 26:24–30.
- Maier, I. (1982). New aspects of pheromone-triggered spermatozoid release in *Laminaria digitata* (Phaeophyta). *Protoplasma* 113:137–43.
- Maier, I. (1984). Culture studies of *Chorda tomentosa* (Phaeophyta, Laminariales). *Br. Phycol. J.* 19:95–106.
- Maier, I. (1997a). The fine structure of the male gamete of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae). I. General structure of the cell. *Eur. J. Phycol.* 32:241–53.
- Maier, I. (1997b). The fine structure of the male gamete of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae). II. The flagellar apparatus. *Eur. J. Phycol.* 32:255–66.
- Maier, I., and Müller, D. G. (1986). Sexual pheromones in algae. *Biol. Bull.* 170:145–75.
- Maier, L., Müller, D. G., Gassmann, G., Boland, W., Marner, F.-J., and Jaenicke, L. (1984). Pheromone-triggered gamete release in *Chorda tomentosa*. *Naturwissenschaften* 71:48–9.
- Malm, T., and Kautsky, L. (2003). Differences in life-history characteristics are consistent with the vertical distribution pattern of *Fucus serratus* and *Fucus vesiculosus* (Fucales, Phaeophyceae) in the central Baltic Sea. *J. Phycol.* 39:880–7.
- Mann, K. H., and Chapman, A. R. O. (1975). Primary production of marine macrophytes. In *Photosynthesis and Productivity in Different Environments*, ed. J. P. Cooper, *Int. Biol. Prog.* 3:307–33. Cambridge: Cambridge University Press.
- Manton, I. (1959). Observations on the internal structure of the spermatozoid of *Dictyota*. *J. Exp. Bot.* 10:448–61.
- Manton, I., and Clarke, B. (1950). Electron microscope observations of the spermatozoid of *Fucus*. *Nature* 166:973–4.
- Manton, I., and Clarke, B. (1951). Electron microscope observations on the zoospores of *Pylaiella* and *Laminaria*. *J. Exp. Bot.* 23:242–6.
- Manton, I., and Clarke, B. (1956). Observations with the electron microscope on the internal structure of the spermatozoid of *Fucus*. *J. Exp. Bot.* 7:416–32.
- Mariani, P., Tolomio, C., and Braghetta, P. (1985). An ultrastructural approach to the adaptive role of the cell wall in the intertidal alga *Fucus viruosoides*. *Protoplasma* 128:208–17.
- Marner, F.-J., Müller, B., and Jaenicke, L. (1984). Lamoxirene, the *Laminaria*-pheromone: Structural proof of the spermatozoid releasing and attracting factor of Laminariales. *Z. Naturforsch.* 39c:689–91.
- Medlin, L. K., Kooistra, W. H. C. F., Potter, D., Saunders, G. W., and Andersen, R. A. (1997). Phylogenetic relationships of the “golden algae” (haptophytes, heterokont chromophytes) and their plastids. In *Origins of Algae and Their Plastids*, ed. D. Bhattacharya, pp. 187–219. Vienna: Springer-Verlag.
- Moe, R. L., and Silva, P. C. (1977). Antarctic marine flora: Uniquely devoid of kelps. *Science* 196:1206–8.
- Moe, R. L., and Silva, P. C. (1981). Morphology and taxonomy of *Himantothallus* (including *Phaeoglossum* and *Phyllogigas*), an Antarctic member of the Desmarestiales (Phaeophyceae). *J. Phycol.* 17:15–29.
- Moss, B. (1965). Apical dominance in *Fucus vesiculosus*. *New Phytol.* 64:387–92.
- Moss, B., (1967). The apical meristem of *Fucus*. *New Phytol.* 66:67–74.
- Moss, B. (1970). Meristems and growth control in *Ascophyllum nodosum* (L.) Le Jol. *New Phytol.* 69:253–60.
- Moss, B. (1971). Meristems and morphogenesis in *Ascophyllum nodosum* ead *mackii* (Cotton). *Br. Phycol. J.* 6:187–93.
- Moss, B. (1983). Sieve elements in the Fucales. *New Phytol.* 93:433–7.
- Moss, B., and Shearer, A. (1973). The effect of light and temperature upon the germination and growth of *Halidrys siliquosa* (L.) Lygnb. (Phaeophyceae, Fucales). *Phycologia* 12:63–8.

- Motomura, T. (1991). Immunofluorescence microscopy of fertilization and parthenogenesis in *Laminaria angusta* (Phaeophyta). *J. Phycol.* 27:248–57.
- Motomura, T., Ichimura, T., and Melkonian, M. (1997). Coordinative nuclear and chloroplast division in unilocular sporangia of *Laminaria angustata* (Laminariales, Phaeophyceae). *J. Phycol.* 33:266–71.
- Müller, D. (1962). Über jahres und lunarperiodische Erscheinungen bei einigen Braunalgen. *Bot. Mar.* 4:140–55.
- Müller, D. (1974). Sexual reproduction and isolation of a sex attractant in *Cutleria multifida* (Smith) Grev. (Phaeophyta). *Biochem Physiol. Pflanz.* 165:212–15.
- Müller, D. (1978). Locomotive responses of male gametes to the species specific sex attractant in *Ectocarpus siliculosus* (Phaeophyta). *Arch. Protistenk.* 120:371–77.
- Müller, D. (1979). Genetic affinity of *Ectocarpus siliculosus* (Dillw.) Lyngb. from the Mediterranean, North Atlantic and Australia. *Phycologia* 18:312–18.
- Müller, D. (1982). Sexuality and sex attraction. In *The Biology of Seaweeds* eds. C. S. Lobban, and M. J. Wynne, pp. 661–74. Los Angeles and Berkeley: Univ. Calif. Press.
- Müller, D., and Jaenicke, L. (1973). Fucoserraten, the female sex attractant of *Fucus serratus* L. (Phaeophyta). *FEBS Lett.* 30:137–9.
- Müller, D., and Lüthe, N. M. (1981). Hormonal interaction in sexual reproduction of *Desmarestia aculeata* (Phaeophyceae). *Br. Phycol. J.* 16:351–6.
- Müller, D., Jaenicke, L., Donike, M., and Akindobi, T. (1971). Sex attractant in a brown alga: Chemical structure. *Science* 171:815–17.
- Müller, D., Gassmann, G., and Lüning, K. (1979). Isolation of a spermatozoid-releasing and -attracting substance from female gametophytes of *Laminaria digitata*. *Nature* 279:430–1.
- Müller, D., Gassmann, G., Boland, W., Marner, F., and Jaenicke, L. (1981). *Dictyota dichotoma* (Phaeophyceae): Identification of sperm attractant. *Science* 212:1040–1.
- Müller, D., Peters, A., Gassmann, G., Boland, W., Marner, F.-J., and Jaenicke, L. (1982). Identification of a sexual hormone and related substances in the marine alga *Desmarestia*. *Naturwissenschaften* 69:290–1.
- Müller, D., Maier, I., and Gassmann, G. (1985). Survey on sexual pheromone specificity in Laminariales (Phaeophyceae). *Phycologia* 24:475–84.
- Nagasato, C., and Motomura, T. (2002). New pyrenoid formation in the brown alga, *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). *J. Phycol.* 38:800–6.
- Nakahara, H., and Nakamura, Y. (1973). Parthenogenesis, apogamy and apospory in *Alaria crassifolia* (Laminariales). *Mar. Biol.* 18:327–32.
- Nakamura, Y. (1965). Development of zoospores in *Ralfsia*-like thallus, with special reference to the life cycle of the Scytosiphonales. *Bot. Mag. Tokyo* 78:109–10.
- Nakamura, Y. (1972). A proposal on the classification of the Phaeophyta. In *Contributions to the Systematics of Benthic Marine Algae of the North Pacific*, ed. I. A. Abbott, and M. Kurogi, pp. 147–56. New York: Academic Press.
- Nicholson, N. L. (1970). Field studies on the giant kelp *Nereocystis*. *J. Phycol.* 6:177–82.
- Nienberg, W. (1931). Die Entwicklung der Keimlinge von *Fucus vesiculosus* und ihre Bedeutung für die Phylogenie der Phaeophyceen. *Wiss. Meeresunters. Abt. Kiel N.F.* 21:49–63.
- Nizamuddin, M., and Womersley, H. B. S. (1960). Structure and systematic position of the Australian brown alga, *Notheia anomala*. *Nature* 187:673–4.
- Norton, T. A. (1972). The development of *Saccorhiza dermatodea* (Phaeophyceae, Laminariales) in culture. *Phycologia* 11:81–6.
- Norton, T. A. (1978). The factors influencing the distribution of *Saccorhiza polyschides* in the region of Lough Ine. *J. Mar. Biol. Assoc. UK* 58:527–36.
- Norton, T. A., and Burrows, E. M. (1969). Studies on marine algae of the British Isles. 7. *Saccorhiza polyschides* (Lightf.) Batt. *Br. Phycol. J.* 4:19–53.
- Novaczek, I. (1981). Stipe growth rings in *Ecklonia radiata* (C. Ag.) J. Ag. (Laminariales). *Br. Phycol. J.* 16:363–71.
- Oliveira, L., Walker, D. C., and Bisalputra, T. (1980). Ultrastructural, cytochemical and enzymatic studies on the adhesive “plaques” of the brown alga, *Laminaria saccharina* (L.) Lamour. and *Nereocystis leutkeana* (Nert.) Post. et Rupr. *Protoplasma* 104:1–15.
- Oltmanns, F. (1889). Beiträge zur Kenntniss der Fucaceen. *Bibl. Bot.* 3(14):1–100.
- Papenfuss, G. F. (1934). Alternation of generations in *Sphacelaria bipinnata* Sauv. *Bot. Not.* 437–44.
- Papenfuss, G. F. (1935). Alternation of generations in *Ectocarpus siliculosus*. *Bot. Gaz.* 96:421–46.
- Papenfuss, G. F. (1951). Phaeophyta. In *Manual of Phycology*, ed. G. M. Smith, pp. 119–38. Waltham, MA: Chronica Botanica.
- Parker, B. C. (1963). Translocation in the giant kelp *Macrocystis*. *Science* 140:891–2.

- Parker, B. C. (1965). Translocation in the giant kelp *Macrocystis*. I. Rates, direction, quantity of  $C^{14}$ -labelled products and fluorescein. *J. Phycol.* 1:41–6.
- Parker, B. C., and Dawson, E. Y. (1966). Non-calcareous marine algae from California Miocene deposits. *Nova Hedwigia* 10:273–95.
- Pavia, H., Toth, G. B., Lindgren, A., and Aberg, P. (2003). Intraspecific variation in the phlorotannin content of the brown alga *Ascophyllum nodosum*. *Phycologia* 42:378–83.
- Pearson, G. A., and Brawley, S. H. (1998). A model for signal transduction during gamete release in the furoid alga *Pelvetia compressa*. *Plant Physiol.* 118:305–13.
- Percival, E., and McDowell, R. H. (1967). *Chemistry and Enzymology of Marine Algal Polysaccharides*. London: Academic Press.
- Pereira, R. C., Cavalcanti, D. N., and Teixeira, V. L. (2000). Effects of secondary metabolites from the tropical Brazilian brown alga *Dictyota menstrualis* on the amphipod *Parhyale hawaiiensis*. *Mar. Ecol. Progr. Ser.* 205:95–100.
- Phillips, J. A., and Clayton, M. N. (1993). Comparative flagellar morphology of spermatozooids of the Dictyotales (Phaeophyceae). *Eur. J. Phycol.* 28:123–7.
- Pohnert, G., and Boland, W. (2002). The oxylipin chemistry of attraction and defense in brown algae and diatoms. *Nat. Prod. Rep.* 19:108–22.
- Potin, P., Bouarab, K., Kupper, F., and Kloareg, B. (1999). Oligosaccharide recognition signals and defense reactions in marine plant-microbe interactions. *Curr. Opin. Microbiol.* 2:276–83.
- Price, I. R., and Ducker, S. C. (1966). The life history of the brown alga *Splachnidium rugosum*. *Phycologia* 5:261–73.
- Pueschel, C. M., and Korb, R. E. (2001). Storage of nitrogen in the form of protein bodies in the kelp *Laminaria solidungula*. *Mar. Ecol. Progr. Ser.* 218:107–14.
- Rawlence, D. J. (1973). Some aspects of the ultrastructure of *Ascophyllum nodosum* (L.) Le Jolis (Phaeophyceae, Fucales) including observations on cell plate formation. *Phycologia* 12:17–28.
- Reed, D. C., Laur, D. R., and Ebeling, A. W. (1988). Variation in algal dispersal and recruitment: the importance of episodic events. *Ecol. Monogr.* 58:321–55.
- Reed, R. H., Davison, I. R., Chudek, J. A., and Foster, R. (1985). The osmotic role of mannitol in the Phaeophyta: An appraisal. *Phycologia* 24:35–47.
- Rousseau, F., Leclerc, M.-C., and de Reviere, B. (1997). Molecular phylogeny of European Fucales (Phaeophyceae) based on partial large-subunit rDNA sequence comparisons. *Phycologia* 36:438–46.
- Russell, G., and Fletcher, R. L. (1975). A numerical taxonomic study of the British Phaeophyta. *J. Mar. Biol. Assoc. UK* 55:763–83.
- Russell, G., and Garbary, D. (1978). Generic circumscription in the family Ectocarpaceae (Phaeophyceae). *J. Mar. Biol. Assoc. UK* 58:517–25.
- Sargent, M. C., and Lantrip, L. W. (1952). Photosynthesis, growth and translocation in giant kelp. *Am. J. Bot.* 39:99–107.
- Saunders, G. W., and Druehl, L. D. (1992). Nucleotide sequences of the small-subunit ribosomal RNA genes from selected Laminariales (Phaeophyta). Implications for kelp evolution. *J. Phycol.* 28:544–9.
- Savaugau, C. (1899). Les Cutlériacées et leur alternance de générations. *Ann. Sci. Nat. Bot. Ser.* 8 10:265–62.
- Savaugau, C. (1900–14). Remarques sur les Sphacélariacées. Bordeaux. 634 pp. in *J. Bot.* 14–18.
- Scagel, R. F. (1971). *Guide to Common Seaweeds of British Columbia*. Victoria: British Columbia Prov. Museum.
- Schaffelke, B., and Lüning, K. (1994). A circannual rhythm controls seasonal growth in the kelps *Laminaria hyperborea* and *L. digitata* from Helgoland (North Sea). *Eur. J. Phycol.* 29:49–56.
- Schloesser, R. E., and Blum, J. L. (1980). *Sphacelaria lacustris* sp. nov., a freshwater brown alga from Lake Michigan. *J. Phycol.* 16:201–7.
- Schmitt, T. M., Lindquist, N., and Hay, M. E. (1998). Seaweed secondary metabolites as antifoulants: effects of *Dictyota* spp. diterpenes on survivorship, settlement, and development of marine invertebrate larvae. *Chemoecology* 8:125–31.
- Schoenwaelder, M. E. A., and Clayton, M. N. (1999). The presence of phenolic compounds in isolated cell walls of brown algae. *Phycologia* 38:161–6.
- Schoenwaelder, M. E. A., and Clayton, M. N. (2000). Physode formation in embryos of *Phyllospora comosa* and *Hormosira banksii* (Phaeophyceae). *Phycologia* 39:1–9.
- Schreiber, E. (1932). Über die Entwicklungsgeschichte und die systematische Stellung der Desmarestiaceen. *Z. Bot.* 25:561–82.
- Serrão, E. A., Pearson, G., Kautsky, M. L., and Brawley, S. H. (1996). Successful external fertilization in turbulent environments. *Proc. Natl. Acad. Sci., USA* 93:5286–90.
- Shibata, T., Tamaguchi, K., Nagayama, K., Kawaguchi, S., and Nakamura, T. (2002). Inhibitory activity of brown algal phlorotannins against glycosidases from

- the viscera of the turban shell *Turbo cornutus*. *Eur. J. Phycol.* 37:493–500.
- Shih, M. L., Floch, J-Y., and Srivastava, L. M. (1983). Localization of <sup>14</sup>C-labeled assimilates in sieve elements of *Macrocystis integrifolia* by histoautoradiography. *Can. J. Bot.* 61:157–63.
- Sideman, E. J., and Scheirer, D. C. (1977). Some fine structural observations on developing and mature sieve elements in the brown alga *Laminaria saccharina*. *Am. J. Bot.* 64:649–57.
- Sjøtun, K., and Schoschina, E. V. (2002). Gametophytic development of *Laminaria* spp. (Laminariales, Phaeophyta) at low temperature. *Phycologia* 41:147–52.
- Smith, G. M. (1955). *Cryptogamic Botany*, Vol. 1, 2nd edn. New York: McGraw-Hill.
- Smith, G. M. (1969). *Marine Algae of the Monterey Peninsula, California*, 2nd edn. Stanford, Calif.: Stanford University Press.
- Speransky, V. V., Speransky, S. S., and Brawley, S. H. (1999). Cryoanalytical studies of freezing damage and recovery in *Fucus vesiculosus* (Phaeophyceae). *J. Phycol.* 35:1264–75.
- Speransky, V. V., Brawley, S. H., and McCully, M. E. (2001). Ion fluxes and modification of the extracellular matrix during gamete release in furoid algae. *J. Phycol.* 37:555–73.
- Stafford, C. J., Green, J. R., and Callow, J. A. (1992). Organization of glycoproteins into plasma membrane domains on *Fucus serratus* eggs. *J. Cell Sci.* 101:437–48.
- Steen, H. (2003). Apical hair formation and growth of *Fucus evanescens* and *F. serratus* (Phaeophyceae) germlings under various nutrient and temperature regimes. *Phycologia* 42:26–30.
- Sundene, O. (1962a). Growth in the sea of *Laminaria digitata* sporophytes from cultures. *Nytt Mag. Bot.* 9:5–24.
- Sundene, O. (1962b). The implications of transplant and culture experiments on the growth and distribution of *Alaria esculenta*. *Nytt Mag. Bot.* 9:155–74.
- Sundene, O. (1963). Reproduction and ecology of *Chorda tomentosa*. *Nytt Mag. Bot.* 10:159–71.
- Tan, I. H., and Druehl, L. D. (1996). A ribosomal DNA phylogeny supports the close evolutionary relationships among the Sporochneales, Desmarestiales, and Laminariales (Phaeophyceae). *J. Phycol.* 32:112–18.
- Targett, N. M., and Arnold, T. M. (1998). Predicting the effects of brown algal phlorotannins on marine herbivores in tropical and temperate oceans. *J. Phycol.* 34:195–205.
- Tatewaki, M. (1966). Formation of a crustacean sporophyte with unilocular sporangia in *Scytosiphon lomentaria*. *Phycologia* 6:62–6.
- Taylor, W. R. (1957). *Marine Algae of the Northeastern Coast of North America*. Ann Arbor: University of Michigan Press.
- Taylor, W. R. (1960). *Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas*. Ann Arbor: University of Michigan Press.
- Terry, L. A., and Moss, B. L. (1980). The effect of photoperiod on receptacle initiation in *Ascophyllum nodosum* (Lo) Le Jol. *Br. Phycol. J.* 15:291–301.
- Terry, L. A., and Moss, B. L. (1981). The effect of irradiance and temperature on the germination of four species of Fucales. *Br. Phycol. J.* 16:143–51.
- Thuret, G. (1854). Recherches sur la fécondation des Fucacées. *Ann. Sci. Nat. Bot.* IV 2:197–214.
- Thuret, G., and Bornet, E. (1878). *Études Phycologiques*. Paris.
- Tovey, D. J., and Moss, B. L. (1978). Attachment of the haptera of *Laminaria digitata* (Huds.) Lamour. *Phycologia* 17:17–22.
- Tseng, C. K. (1981). Commercial cultivation. In *The Biology of Seaweeds*, ed. C. S. Lobban, and M. J. Wynne, pp. 680–725. Berkeley and Los Angeles: Univ. Calif. Press.
- Vadas, R. L. (1972). Ecological implications of culture studies on *Nereocystis luetkeana*. *J. Phycol.* 8:196–203.
- Van Alstyne, K. L., and Pelletreau, K. N. (2000). Effect of nutrient enrichment on growth and phlorotannin production in *Fucus gardneri* embryos. *Mar. Ecol. Progr. Ser.* 206:33–43.
- van den Hoek, C. (1982). The distribution of benthic marine algae in relation to the temperature regulation of their life histories. *Biol. J. Linn. Soc.* 18:81–144.
- van den Hoek, C., and Flinterman, A. (1968). The life history of *Sphacelaria furcigera* Kütz. (Phaeophyceae). *Blumea* 16:193–242.
- Vreeland, V. (1972). Immunocytochemical localization of the extracellular polysaccharide alginic acid in the brown seaweed *Fucus distichus*. *J. Histochem. Cytochem.* 20:358–67.
- Vreeland, V., Waite, J. H., and Epstein, M. (1998). Polyphenols and oxidases in substratum adhesion by marine algae and mussels. *J. Phycol.* 34:1–8.
- Walker, F. T. (1956). Periodicity of the Laminariaceae around Scotland. *Nature* 177:1246.
- Williams, J. L. (1898). Reproduction in *Dictyota dichotoma*. *Ann. Bot.* 12:559–60.
- Williams, J. L. (1904). Studies in the Dictyotaceae. I. The cytology of the gametophyte generation. *Ann. Bot.* 18:183–204.

- Williams, J. L. (1905). Studies in the Dictyotaceae. II. The periodicity of sexual cells in *Dictyota dichotoma*. *Ann. Bot.* 19:531–60.
- Wright, P. J., Callow, J. A., and Green, J. R. (1995a). The *Fucus* (Phaeophyceae) sperm receptor for eggs. II. Isolation of a binding protein which partially activates eggs. *J. Phycol.* 31:592–600.
- Wright, P. J., Green, J. A., and Callow, J. A. (1995b). The *Fucus* (Phaeophyceae) sperm receptor for eggs. I. Development and characteristics of a binding assay. *J. Phycol.* 31:584–91.
- Wynne, M. J. (1969). Life history and systematic studies of some Pacific North American Phaeophyceae (brown algae). *Univ. Calif. Publ. Bot.* 50:1–88.

# Prymnesiophyta

## PRYMNESIOPHYCEAE

The Prymnesiophyta are a group of uninucleate flagellates characterized by the presence of a haptonema between two smooth flagella. The Prymnesiophyta have two membranes of chloroplast endoplasmic reticulum, as do the Cryptophyta and the Heterokontophyta, but differ in having flagella without mastigonemes. Molecular data also show that the Prymnesiophyta are distinct from the Cryptophyta and Heterokontophyta (Bhattacharya and Ehlting, 1995; Medlin et al., 1994). Until 1962, the organisms were considered part of the Chrysophyceae, at which time Christensen split them off into a separate class, the Haptophyceae (named after the presence of the haptonema). The name Haptophyceae was a descriptive name and not based on a genus in the class; thus the name was later changed to Prymnesiophyceae, based on the genus *Prymnesium* (Fig. 22.7) (Hibberd, 1976). The fossil record of the Prymnesiophyceae is known from the Carboniferous (approximately 300 000 000 years ago) (Faber and Preisig, 1994; Jordan and Chamberlain, 1997).

The cells are commonly covered with scales. In many cases, the scales are calcified, thereby producing coccoliths. The chloroplasts lack girdle lamellae and most contain chlorophylls *a* and *c*<sub>1</sub>/*c*<sub>2</sub>,  $\beta$ -carotene, diadinoxanthin, and diatoxanthin (Zapata et al., 2004). The storage product is chrysolaminarin (leucosin) in vesicles in the posterior end of the cell (Janse et al., 1996). The anterior end of the cell has a large Golgi apparatus and sometimes a contractile vacuole.

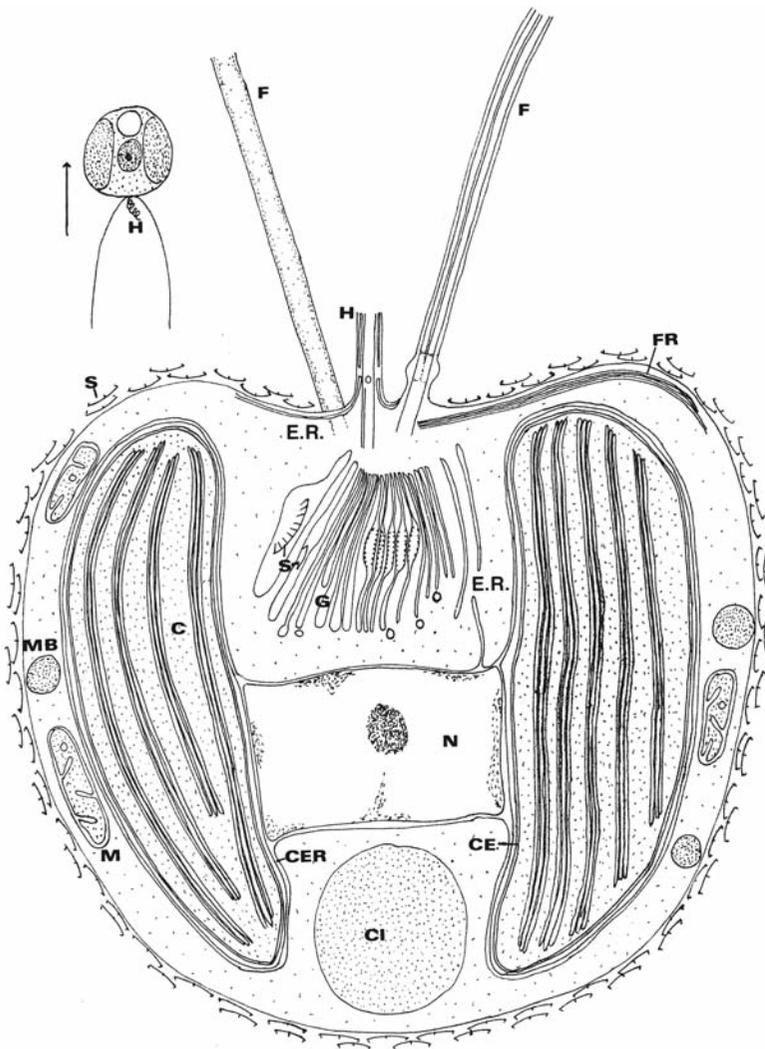
The Prymnesiophyceae are primarily marine organisms, although there are some freshwater representatives. They make up a major part of the marine nanoplankton and constitute about 45% of the total phytoplankton cells in the middle latitudes of the South Atlantic. They decrease in frequency toward the poles although some still occur in polar waters (Manton et al., 1977).

## Cell structure

### Flagella

Most of the Prymnesiophyceae have two smooth flagella of approximately the same length (Figs. 22.1, 22.3(a)). The Pavlovales is the exception, where one flagellum is longer than the other and is usually covered by small cylindrical to club-shaped hollow scales 70 nm long and 20 nm wide (Fig. 22.3(b)) (van der Veer, 1969; Green and Manton, 1970). Because the class is characterized by two more or less equal, smooth flagella, a number of genera, such as *Diacronema* (Fig. 22.4), *Isochrysis* (Fig. 22.18(b)), and *Dicrateria*, which have rudimentary or no haptonema, are grouped in the Prymnesiophyceae (Green and Pienaar, 1977). There is usually no flagellar swelling associated with an eyespot as occurs in many other golden-brown flagellates with two membranes of chloroplast E.R. (Hibberd, 1976), although there are exceptions (Green and Hibberd, 1977).

During swimming, the flagellar end of the cell can be forward with the flagella sweeping outward and backward down the sides of the body (Fig. 22.5(a)), or the flagellar end may be directed



**Fig. 22.1** A light and electron microscopical drawing of a cell of a typical member of the Prymnesiophyceae, *Chrysochromulina* sp. A rapidly swimming individual is shown, with the arrow indicating the direction of movement. (C) Chloroplast; (CE) chloroplast envelope; (CER) chloroplast endoplasmic reticulum; (CI) chrysolaminarin vesicle; (E.R.) endoplasmic reticulum; (F) flagellum; (FR) flagellar root; (G) Golgi body; (H) haptonema; (M) mitochondrion; (MB) muciferous body; (N) nucleus; (S) scale. (Adapted from Hibberd, 1976.)

backward (Fig. 22.5(b)). Movement is usually rapid, the cells swimming only for a short distance in one direction, after which they rapidly change the position of the flagella and move off in the opposite direction. In *Pavlova* (Fig. 22.3(b)), the flagellar action is a little different, with the longer flagellum directed forward and the shorter flagellum trailing or directed outward.

### Haptonema

A **haptonema** is a filamentous appendage arising near the flagella but thinner and with different properties and structure. The haptonema ranges from a few profiles of E.R. that represent a reduced haptonema in *Imatonia rotunda* (Fig. 22.18(c), (d))

(Green and Pienaar, 1977) to a short bulbous structure in *Hymenomonas roseola* (Fig. 22.6(a)) to the 80- $\mu\text{m}$ -long whip-like structure in *Chrysochromulina parva* (Fig. 22.6(b)) (Manton, 1967a). The haptonema of *Prymnesium parvum* has an internal structure similar to the haptonema of other Prymnesiophyceae and will be used as an example of haptonemal structure (Fig. 22.7) (Manton, 1964). In transverse section the haptonema is composed of three concentric membranes surrounding a core containing seven microtubules. The core is covered by the innermost of the three membranes so that there is no contact between the core microtubules and the outer portion of the haptonema. The space



**Fig. 22.2** Irene Manton 1904–1988. Dr. Manton was a graduate of Cambridge University. She was a lecturer in botany at the University of Manchester from 1929 to 1945 and Professor of Botany at the University of Leeds from 1945 to 1969. Dr. Manton was one of the foremost cytologists during the 1950s and 1960s when the new science of electron microscopy was adding vast amounts of new information to the understanding of algae. The work of Dr. Manton and her colleagues led to the distinct features of the haptophytes and the recognition of these organisms as a distinct group of algae.

between the innermost and middle membranes is a vesicle continuous over the tip of the core. The haptonema is commonly covered with small body scales.

The microtubules in the haptonema slide, relative to one another, probably through the calcium-binding protein centrin (Lehtreck, 2004), to produce two basic movements, coiling and bending (Greyson et al., 1993):

- 1 Coiling** is a sensory response to obstacles (Kawachi and Inouye, 1994). The haptonema coils instantly when forward-swimming cells

encounter obstacles. The flagella are thrown backward and generate propulsive forces, resulting in backward swimming.

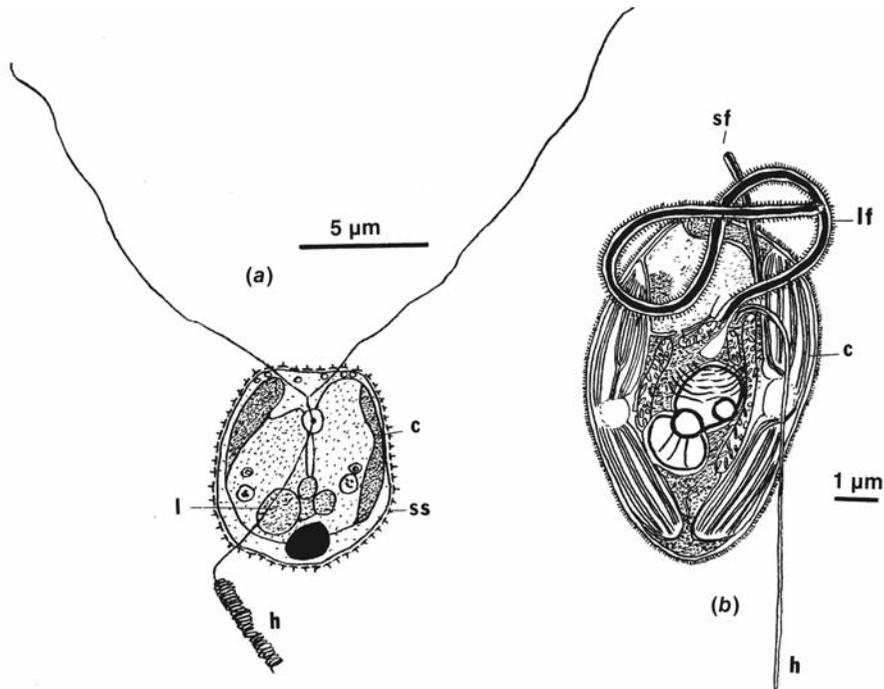
- 2 Bending** by the haptonema occurs during food capture by the cells (Inouye and Kawachi, 1994; Kawachi et al., 1991). Prey particles adhere to the haptonema as the cells swim with the haptonema projecting ahead of the cell, and the flagella beating alongside. The adhering particles are transported down to a particular point on the haptonema, about 2  $\mu\text{m}$  distal from the base, called the **particle-aggregating center** (Fig. 22.8). The particles accumulate at the particle-aggregating center, resulting in the production of a massive aggregate. In the aggregate, individual particles tightly adhere to one another, suggesting that some sort of cementing material is secreted. After reaching a certain size, the aggregate moves to the tip of the haptonema. The haptonema bends into a sigmoid shape and eventually delivers the aggregate to the posterior end of the cell, where the aggregate is injected into a food vacuole.

## Chloroplasts

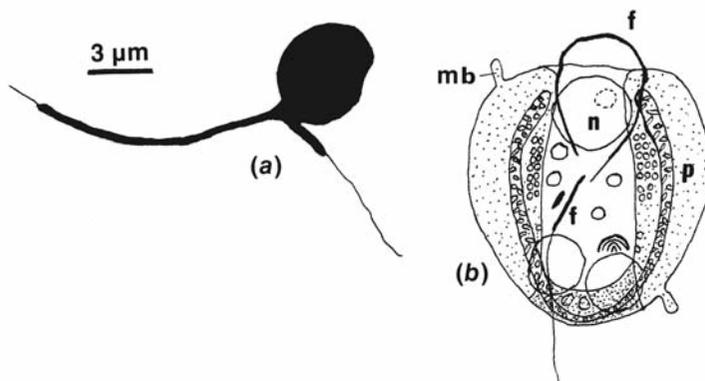
Usually there are two elongate discoid chloroplasts in each cell (Fig. 22.1). Each chloroplast is surrounded by four membranes: the two membranes of the chloroplast envelope, and outside of them the two membranes of the chloroplast E.R. The thylakoids are aggregated into bands of three. A girdle band of thylakoids is usually absent (Hibberd, 1976). A pyrenoid is commonly present in the center of the chloroplast or as a bulge to one side. Eyespots are not common in the Prymnesiophyceae although *Pavlova* has an eyespot, which consists of a group of lipid droplets inside the anterior end of a chloroplast (Green, 1973) (Fig. 22.21).

## Other cytoplasmic structures

Two types of membrane-bounded vesicles are in the cytoplasm, the first containing lipids and the second the storage product. The storage product is usually stated as being chrysolaminarin (leucosin) (Janse et al., 1996), although in a study of *Pavlova mesolychnon* (Fig. 22.3(b)) it was found that one of



**Fig. 22.3** (a) Ventral view of a saddle-shaped cell of *Chrysochromulina ephippium* with the haptonema loosely coiled. (b) *Pavlova mesolychnon*. (c) Chloroplast; (h) haptonema; (l) leucosin vesicle; (lf) long flagellum; (ss) spined scale; (sf) short flagellum. ((a) after Parke et al., 1956; (b) after van der Veer, 1969.)

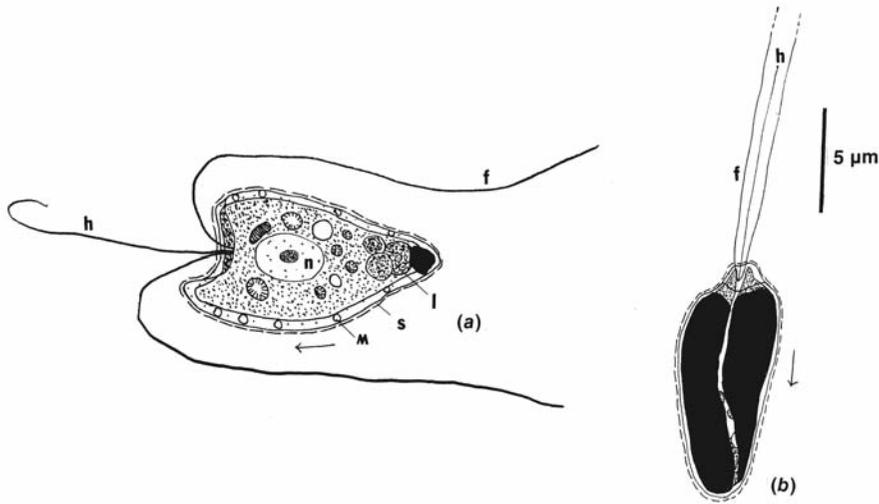


**Fig. 22.4** (a) Drawing of a direct preparation of *Diacronema vlkianum* showing the hair points at the tip of the flagella and the absence of lateral hairs. (b) A resting cell of *D. vlkianum*. (f) Flagellum; (mb) muciferous body (discharged); (n) nucleus; (p) plastid. (After Fournier, 1969.)

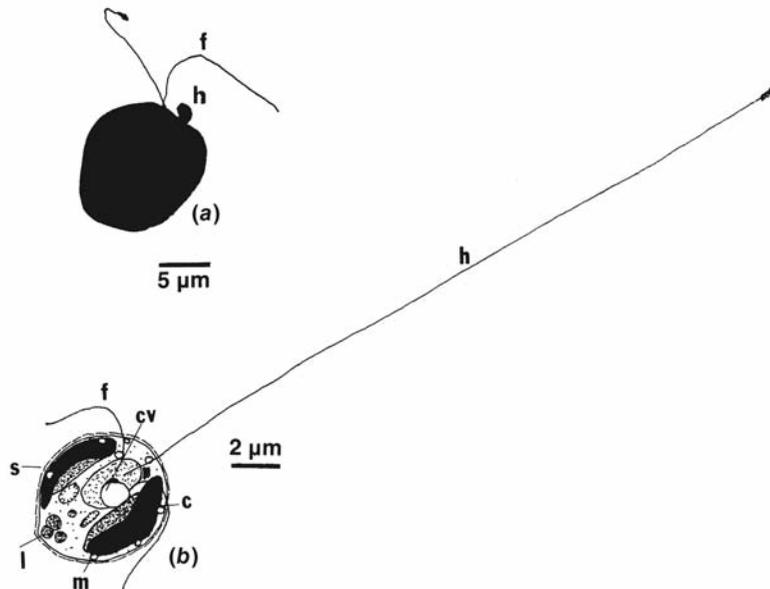
the storage products present in the cells was a  $\beta$ -1,3 linked glucan similar to paramylon in the Euglenophyceae (Kreger and van der Veer, 1970).

In some of the Prymnesiophyceae and particularly the genus *Chrysochromulina*, muciferous bodies are under the plasma membrane (Figs. 22.1, 22.5(a), 22.10(b)). They have the same structure as

the muciferous bodies in the Raphidophyceae, Dinophyceae, and Chrysophyceae, and consist of a single-membrane-bounded vesicle filled with semi-opaque contents. When they are discharged outside the cell, they appear as substantial cylinders of opaque material of uniform diameter. The function of muciferous bodies is not known.

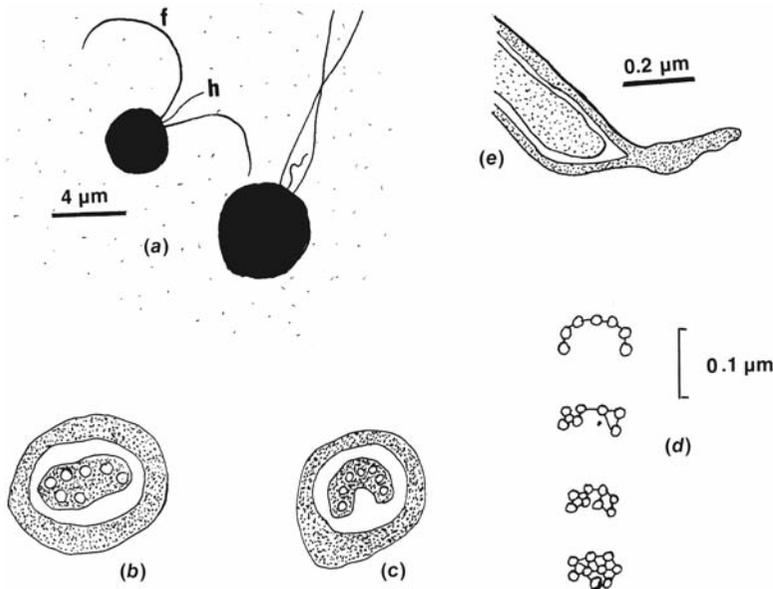


**Fig. 22.5** *Chrysochromulina polylepis*. (a) Cell with flagella in position for swimming with flagellar pole forward. (b) Cell swimming with flagellar pole to the rear. (f) Flagellum; (h) haptonema; (l) leucosin vesicle; (m) muciferous body; (n) nucleus; (s) scale. (After Manton and Parke, 1962.)



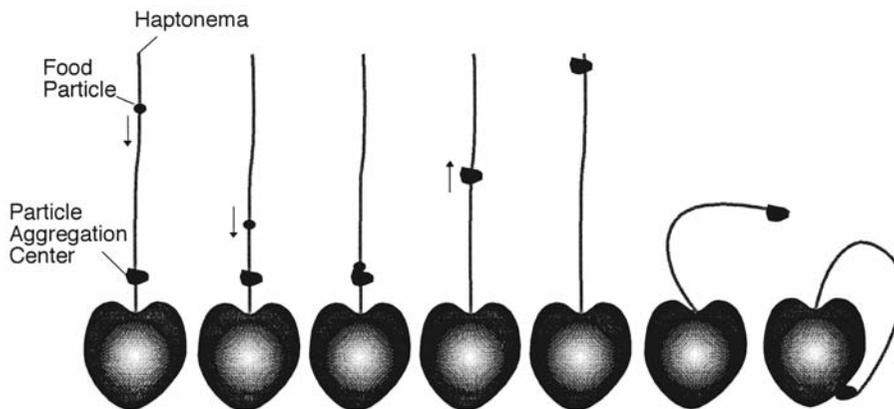
**Fig. 22.6** (a) *Hymenomonas roseola*, a dried cell showing the flagella and the short bulbous haptonema (which actually arises between the two flagella). (b) A slowly gliding cell of *Chrysochromulina parva*. (c) Chloroplast; (cv) contractile vacuole; (f) flagellum; (h) haptonema; (l) leucosin; (m) muciferous body; (s) scale. ((a) after Manton and Peterfi, 1969; (b) after Parke et al., 1962.)

*Phaeocystis globosa* has vesicles that contain tightly-wound filaments of chitin (Chrétiennot-Dinet et al., 1997). Each vesicle contains five chitin filaments that attach near the base of another chitin filament (Fig. 22.9). The chitin filaments produce a five-sided star at their base when they are released from their vesicles.



**Fig. 22.7** *Pymnesium parvum*. (a)

Drawing of two cells dried and shadow-cast showing the two smooth flagella (f) and the short haptonema (h) on each cell. (b) Transverse section of a haptonema showing the structure of the middle region of seven microtubules in the core surrounded by the membrane-bounded cavity and the haptonemal wall bounded by the plasmalemma externally. (c) Transverse section of a haptonema near the point of union with the cell showing the crescentic shape of the core characteristic of this region. (d) Progressive levels of haptonema tubules from immediately below the plasmalemma to the distal points of the microtubules. (e) Longitudinal section of a tip of a haptonema. ((a) after Manton, 1966; (b)–(e) after Manton, 1968.)

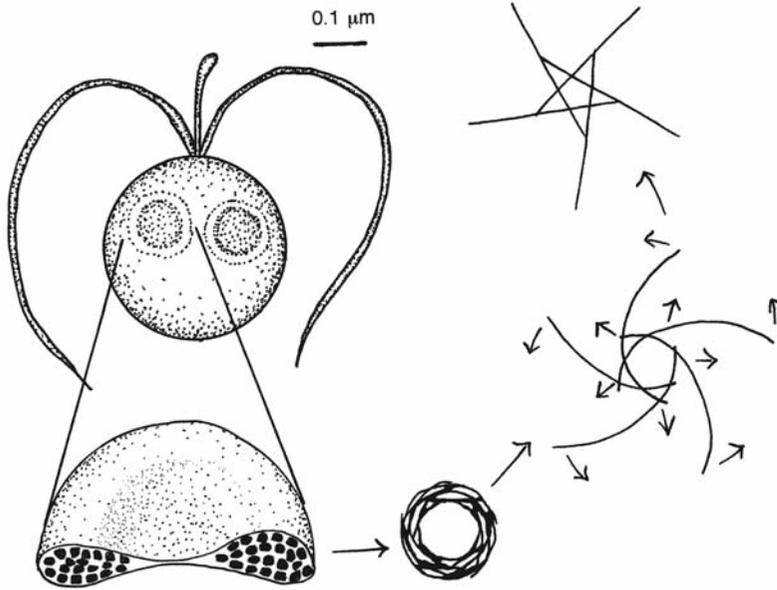


**Fig. 22.8** The sequence of events by which food particles adhere to a haptonema and are moved toward the particle aggregation center. The mass of particles at the particle aggregation center then moves to the tip of the haptonema; the haptonema bends to deposit the mass of particles at the posterior end of the cell where the particles are taken up in food vacuoles. (Adapted from Kawachi et al., 1991.)

Surface protrusions containing cytoplasm are common in the Prymnesiophyceae; they are called **pseudopodia**. Also, cells can extrude slender trailing filaments from their surfaces, which can be straight or branched. These filaments,

called **filopodia**, eventually become segmentally constricted and break up into droplets. Many of the organisms are phagocytic and consequently have food vesicles in the cytoplasm in which they digest bacteria and other small algae. They are not selective in taking up material into the food vesicles, and will take up indigestible detritus as rapidly as bacteria and other algae.

Like many of the other algal groups, the Prymnesiophyceae participate in symbiotic events. Invertebrate radiolaria can harbor prymnesiophyte algal symbionts (Anderson et al., 1983).



**Fig. 22.9** *Phaeocystis globosa* showing a cell with two vesicles containing coiled filaments made of chitin. The coiled filaments unwind when they are discharged outside the cell, forming a 5-ray star structure. (After Chrétiennot-Dinet et al., 1997.)

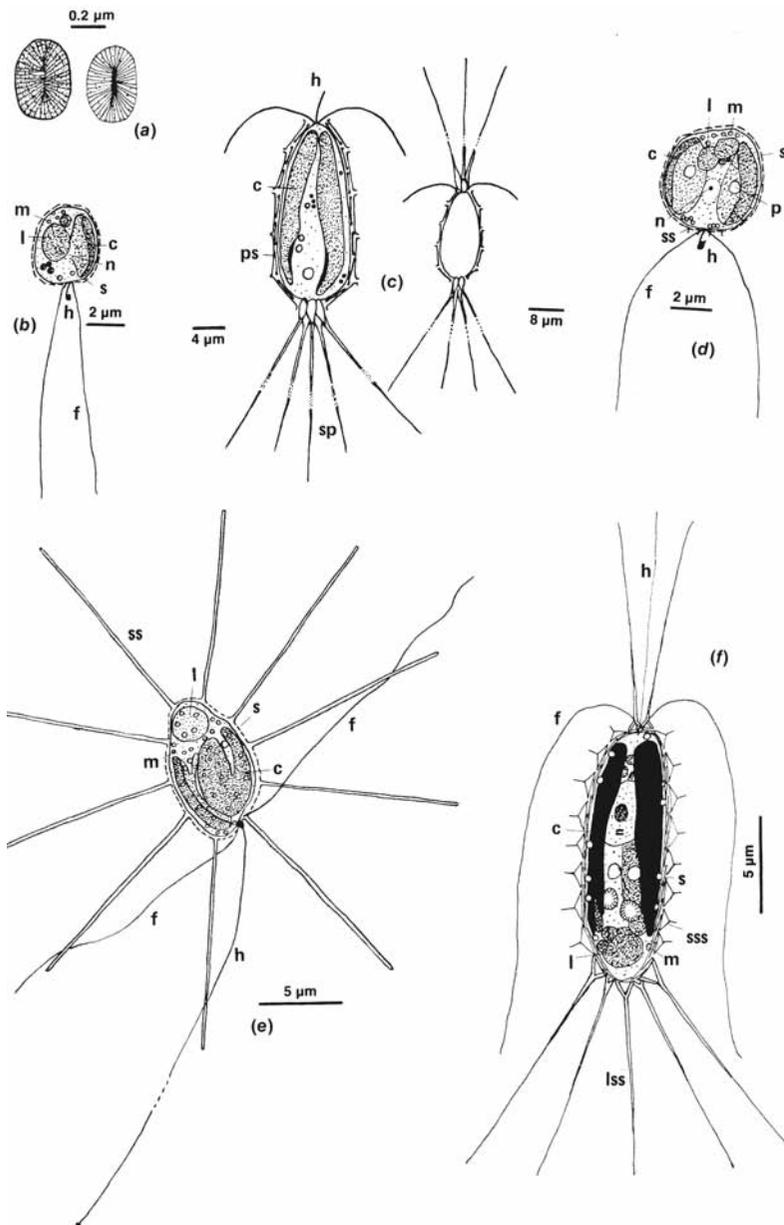
The symbionts are held in the rhizopodial network surrounding the central capsule of the radiolarian. The algal symbionts fix carbon dioxide, and some of the photosynthate passes to the radiolarian.

## Scales and coccoliths

Most of the members of the Prymnesiophyceae have a cell covering consisting of a number of elliptical scales (Figs. 22.1, 22.10). The elliptical scales are embedded in a mucilaginous substance, and in some organisms a layer of calcified coccoliths is outside the scales. The structure of the scales can vary considerably, but all of them appear to have an elliptical organic scale constituting either the whole structure of the scale or just the base plate on which the rest of the structure is based. The elliptical organic scale (plate scale) has radiating ridges extending to the edge (Fig. 22.10(a)) (Parke and Manton, 1962). *Chrysochromulina minor* (Fig. 22.10(b)) (Parke et al., 1955) and *Chrysochromulina parva* (Fig. 22.6(b)) (Parke et al., 1962) have a cell covering of only plate scales of the type just described. In other Prymnesiophyceae the rims of the elliptical base plate have become turned up to form a variety of different types of scales ranging from

very shallow tub-shaped scales with the edges of the scales barely turned up, to very long spined structures with the spine being the very elongated upturned rim of the organic scale (Fig. 22.10(c)) (Manton, 1972). A number of different types of scales can be present on the same organism. *Chrysochromulina kappa* (Fig. 22.10(d)) has plate scales around most of the body with a few short-spined scales near the flagella (Parke et al., 1955). *Chrysochromulina ericina* (Fig. 22.10(e)) has a cell covering consisting of plate scales with about 30 long-spined scales intermixed with the plate scales (Parke et al., 1956). *Chrysochromulina pringsheimii* (Fig. 22.10(f)) has an even more complex cell covering consisting of four types of scales. Long-spined scales are at either end of the cell with small-spined scales covering the rest of the cell. Underneath this spined layer is a layer of plate scales, and, finally, near the base of the flagella are a number of small plate scales (Parke and Manton, 1962).

The organic scales originate in the Golgi apparatus (Figs. 22.1, 22.13) (Hawkins and Lee, 2001). A scale, when first formed in a Golgi vesicle, is closely enveloped by the vesicle membrane no matter how elaborate the shape of the scale is (long-spined, etc.); but immediately before the scale is liberated to the outside of the cell, this close contact is lost (Manton, 1967b).



**Fig. 22.10** (a) Small plate scales of *Chrysochromulina pringsheimii*, the upper face on the left and the lower face on the right.

(b) *Chrysochromulina minor*, individual swimming with the flagella and haptonema behind the body in the position characteristic of the species during rigid swimming.

(c) *Chrysochromulina parkae*, two forms. (d) *Chrysochromulina kappa*, swimming with the flagella and haptonema behind the body, the characteristic position of species during rapid swimming.

(e) *Chrysochromulina ericina*, individual with dividing chloroplasts anchored by a haptonema which is partially extended; the flagella are in the characteristic position when the cells are stationary.

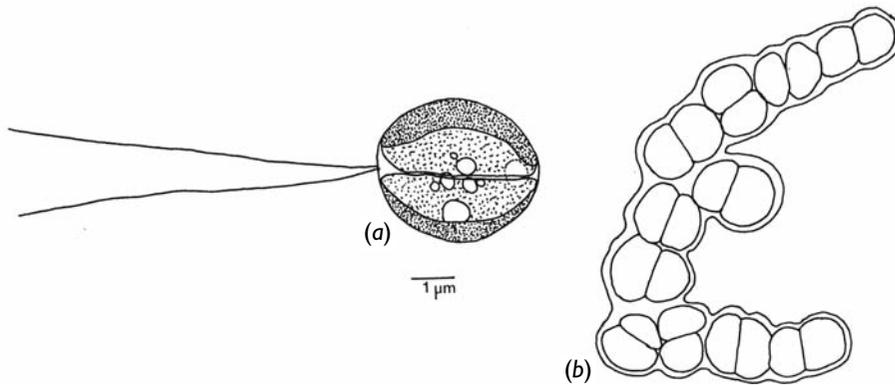
(f) *Chrysochromulina pringsheimii*, individual swimming with the flagella and haptonema in front of the body, and the haptonema fully extended.

(c) Chloroplast; (f) flagellum; (h) haptonema; (l) leucosin vesicle; (lss) long-spined scale; (m) muciferous body; (n) nucleus; (p) pyrenoid; (ps) plate scale; (s) scale; (sp) spine; (ss) spined scale; (sss) small-spined scale. ((a), (f) after Parke and Manton, 1962; (b), (d) after Parke et al., 1955; (c) after Green and Leadbeater, 1972; (e) after Parke et al., 1956.)

There is a diurnal rhythm in the production of scales. Manton and Parke (1962) showed that in *Chrysochromulina polylepis* the greatest production of scales is in the late afternoon with the least in the early morning hours. The time of nuclear division is the reverse, with the most mitotic figures appearing in the early morning.

Even in the non-motile filamentous stages of the Prymnesiophyceae, the cell wall is composed

of scales embedded in a gelatinous matrix. In *Pleurochrysis scherffelii* (Fig. 22.11) the cells of the filamentous stage are covered in cellulosic scales produced by the single Golgi apparatus. By the use of the cinemotography, Brown (1969) showed that during wall formation the whole protoplast revolves so that the scale secretions of the Golgi apparatus are received more or less evenly by all portions of the cell wall.



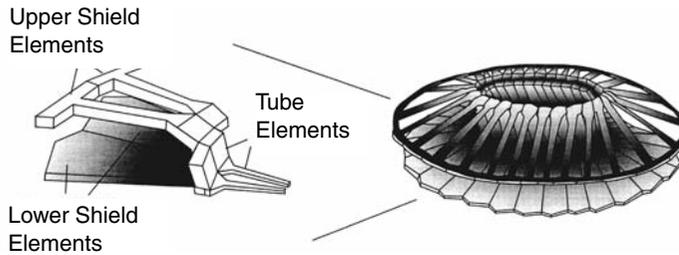
**Fig. 22.11** *Pleurochrysis scherffellii*. (a) Motile cell. (b) Filamentous stage. (After Pringsheim, 1955.)

**Coccoliths** are calcified scales of the Prymnesiophyceae. They were originally described as minute carbonate discs in Cretaceous deposits and thought to be of inorganic origin (Marsh, 2004). Later they were in sea bottom oozes brought up by the first Atlantic cable survey in 1858. Their algal nature was not recognized until 1898. Coccoliths are basically organic scales that have calcium carbonate ( $\text{CaCO}_3$ ) deposited on one surface in a characteristic pattern depending on the species of the alga. Under ordinary conditions anhydrous  $\text{CaCO}_3$  exists in nature in two crystalline forms (Fig. 4.7), **calcite** (rhombohedral) and **aragonite** (orthorhombic), which differ in structure, hardness, specific gravity, and solubility. In coccoliths the form of  $\text{CaCO}_3$  is usually calcite. The calcite is attached to the outer surface of a plate scale, which has a pattern of radiating ridges (Figs. 22.13, 22.24) whereas the inner side of the scale (toward the cell) is virtually patternless. The coccoliths of *Emiliania huxleyi* (Fig. 22.16(b)) are relatively simple and can serve as an example of coccolith structure although it should be realized that there is a multitude of different coccolith forms, some very complex (Fig. 22.16). The coccoliths of *E. huxleyi* (Figs. 22.12, 22.16(b)) are composed of a number of hollow crystals of calcite arranged around the periphery of a plate scale (Young et al., 1992). Each coccolith consists of an upper and lower shield joined by a tube. The tube and the shields are composed of subunits.

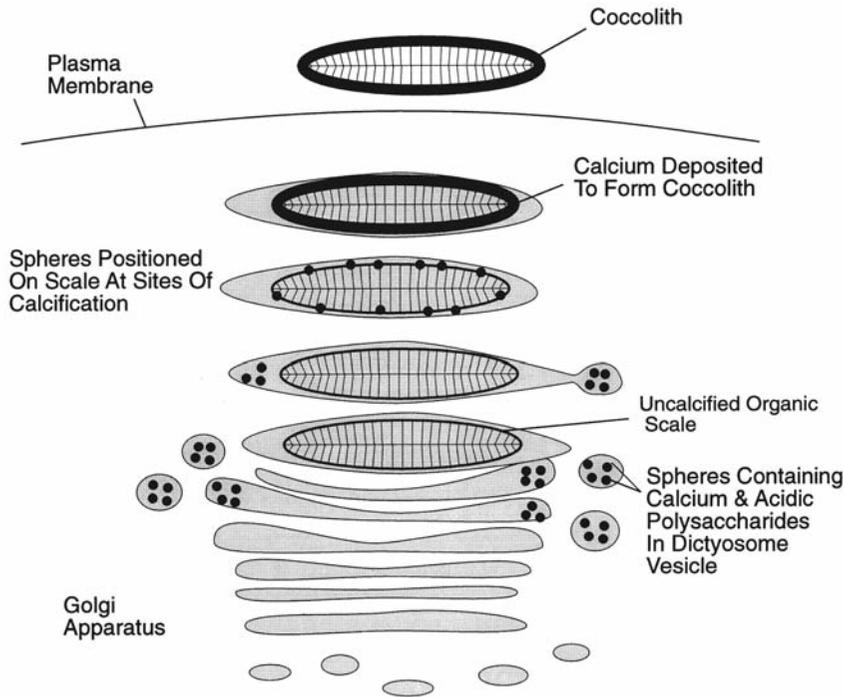
Coccolith formation begins with the production of an uncalcified organic plate-scale in the

center of Golgi cisternae (Figs. 22.13, 22.14) (Marsh, 1996, 2004). Highly acidic polysaccharides are produced as 25 nm diameter spheres in the peripheral part of Golgi cisternae. The highly acidic polysaccharides are extremely negative polyanions that sequester large numbers of calcium ions. The 25 nm diameter spheres are pinched off the Golgi cisternae as vesicles, which eventually fuse with a vesicle containing an uncalcified organic plate-scale. The 25 nm diameter spheres aggregate on the scales in the area of future calcium deposition. Calcium is deposited in the area of the 25 nm diameter spheres to produce calcified coccoliths. The remaining material in the spheres is reorganized into an amorphous coat that surrounds the mature crystals of calcium carbonate that make up the coccoliths. The coccoliths are carried in the vesicles to the plasma membrane where the coccoliths are deposited outside the cell. Two to seven coccoliths are produced per day by *Emiliania huxleyi* (Balch et al., 1993; Linschooten et al., 1991).

Coccoliths are detached from cells in layers at the same time as other coccoliths are produced. During logarithmic growth, about the same number of coccoliths are detached as are produced. In stationary growth, however, the rate of coccolith detachment increases about threefold, while coccolith production drops off (Balch et al., 1993). Coccolithophorids have greater rates of calcification when nitrogen and phosphorus are limiting in the seawater (Corstjens and Gonzales, 2004).



**Fig. 22.12** Construction of a heterococcolith of *Emiliana huxleyi*. The heterococcolith is composed of a tube joining the upper and lower shields, each of which is composed of subunits. (Adapted from Young et al., 1992.)

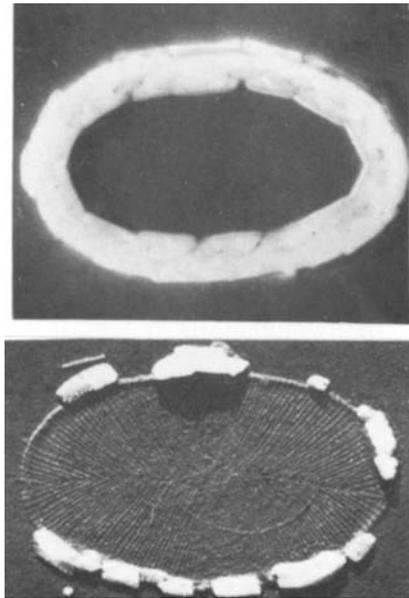


**Fig. 22.13** Coccolith formation in *Pleurochrysis*. Spheres containing calcium bound to acidic polysaccharides are produced in vesicles by the Golgi apparatus. These vesicles fuse with Golgi vesicles containing uncalcified organic scales. The calcium-containing spheres become positioned on the scales at the sites of calcification and the calcium is released. The calcified scales are carried to the plasma membrane, releasing the coccoliths outside of the cell. (Adapted from Marsh, 1994.)

Coccoliths can have an outer covering of heterococcoliths that contain assemblages of morphologically complex and diverse  $\text{CaCO}_3$  elements (Figs. 22.12, 22.14, 22.16), which are composed of similar (often rhombohedral) calcite crystals (Fig. 22.15). The two types of coccoliths can occur in the same organism with heterococcoliths on the

diploid cells and holococcoliths on the haploid cells (Geisen et al., 2002). In *Coccolithus*, holococcoliths occur in the motile *Crystallolithus* stage (Fig. 22.15) whereas heterococcoliths occur in the non-motile stage. The base plates of heterococcoliths are produced in Golgi cisternae followed by calcification in the same cisternae. However, only the base plates of holococcoliths are produced in Golgi cisternae. These base plates are discharged outside the cell where calcification occurs within an outer vestment (Rowson et al., 1986).

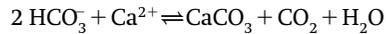
The coccolithophorids (algae with coccoliths) (Fig. 22.16) are common in tropical waters because these warm waters have a low partial pressure of carbon dioxide and are usually saturated or supersaturated with calcium carbonate,



**Fig. 22.14** Top: Intact heterococcolith of *Hymenomonas carterae*. Bottom: The calcium carbonate making up the heterococcolith is partially dissolved away, revealing the scale on which the calcium carbonate is deposited. (From Outka and Williams, 1971.)

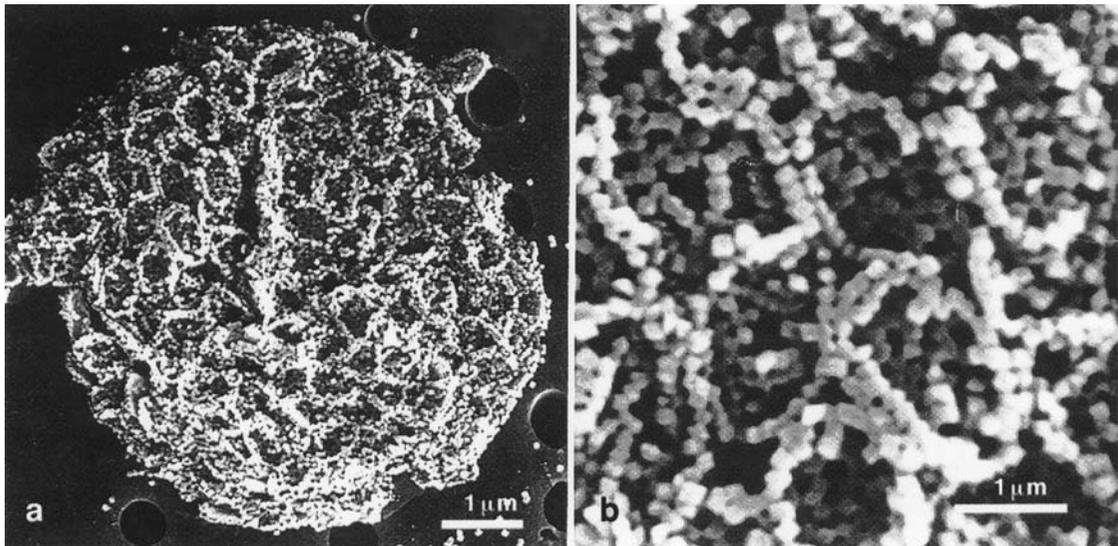
the concentrations being especially high in the upper layers. Supersaturation of calcium carbonate is favorable for the formation of coccoliths, and the distribution of coccolithophorids shows a close correlation with the degree of saturation of seawater by calcium carbonate. In the seas of polar regions, the degree of saturation does not even reach 90%.

The calcification reaction is:

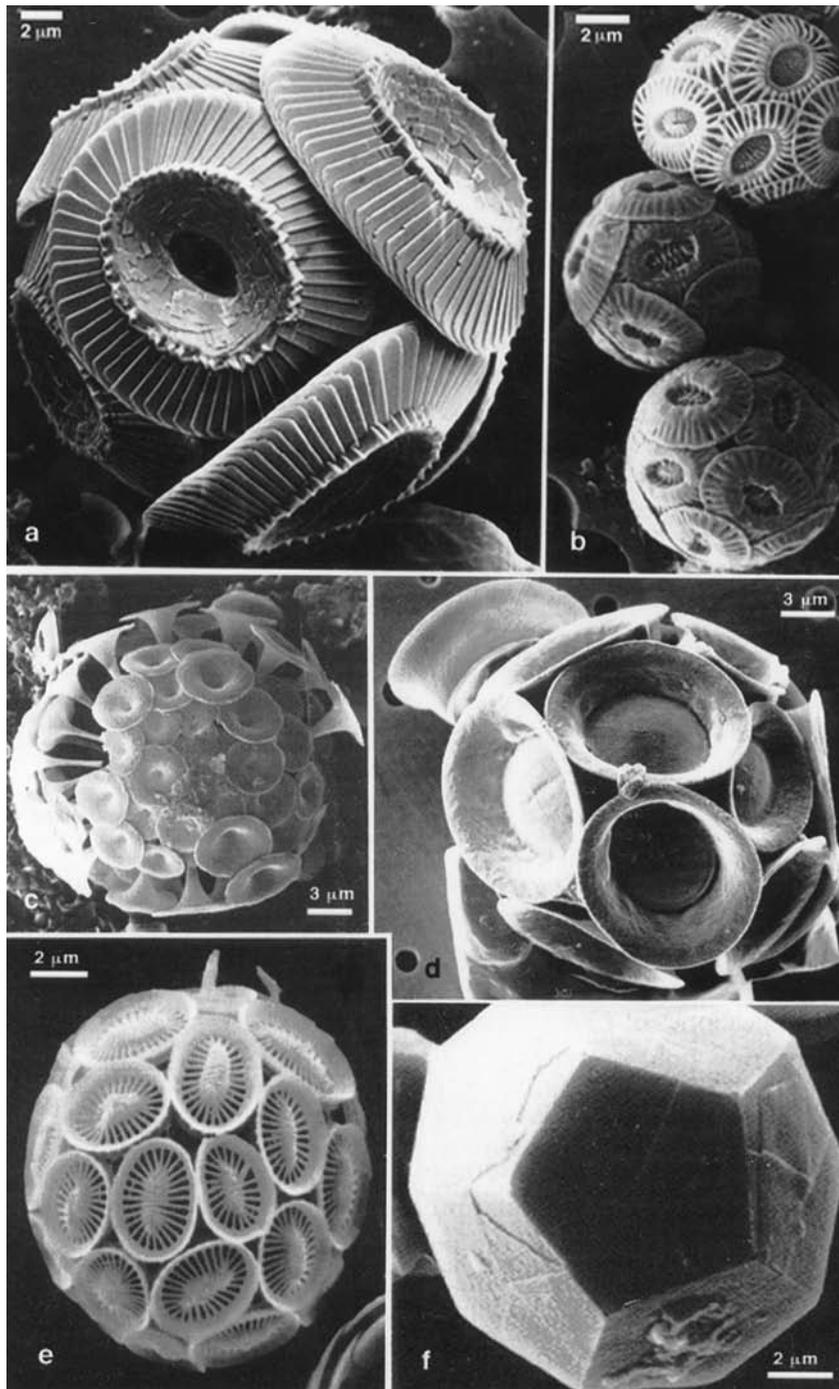


Carbon dioxide is released in calcification, and this may make coccolithophorids more competitive by increasing the amount of  $\text{CO}_2$  available for photosynthesis inside the cell (Nielsen, 1995). This may be particularly important in seawater where the pH of 8.2 results in a very low concentration of dissolved  $\text{CO}_2$  of about  $10 \mu\text{M}$  compared to a total dissolved carbonate (mostly  $\text{HCO}_3^-$ ) of  $2000 \mu\text{M}$ .

The membrane of the coccolith vesicle contains an  $\text{H}^+$ -ATPase that pumps  $\text{H}^+$  out of the coccolith vesicle, removing protons produced during dehydration of bicarbonate and increasing the rate of calcification (Corstjens and Gonzales, 2004; Gonzales, 2004).



**Fig. 22.15** Scanning electron micrograph of holococcoliths covering a cell of *Crystallolithus hyalinus*. (From Faber and Preisig, 1994.)



**Fig. 22.16** Scanning electron micrographs of coccolithophore cells. (a) *Coccolithus pelagicus*. (b) *Emiliania huxleyi*. (c) *Discosphaera tubifera*. (d) *Pontosphaera syracusana*. (e) *Syracosphaera nodosa*. (f) *Braarudosphaera bigelowii*. (From Faber and Preisig, 1994.)

The external coccoliths can be removed by lowering the pH of the culture medium. Cells of *Coccolithus huxleyi* decalcified in this manner may acquire a complete coccolith envelope (about 15 coccoliths) within 15 hours of their being transferred back to a normal medium in the light. Complete recalcification in *Cricosphaera* sp. may require 40 hours. In both instances, cell division is not a prerequisite for the formation of new coccoliths. If the organisms are grown in artificial seawater, the coccoliths dissolve if the product of the concentrations of calcium and carbonate is appreciably smaller than the solubility product of calcite. In *C. huxleyi*, coccoliths are still formed inside cells even when the calcium content of the medium is reduced to levels where external coccoliths dissolve, although coccolith production is retarded at calcium concentrations less than half that of normal seawater. A photochemical process is apparently directly associated with coccolith production because when light is turned off, there is a sharp drop in coccolith production.

*Pleurochrysis carterae* has biflagellate cells surrounded by a coccosphere consisting of a single layer of 100 to 200 coccoliths. The cells incorporate calcium into extracellular coccoliths at a more or less constant rate throughout a 16-hour light:8-hour dark cycle. The cells divide during the dark periods with a concomitant decrease in cell size during the dark period followed by an increase in cell size during the light period. The cells form coccoliths in the light as well as in the dark at a similar rate (van der Wal et al., 1987) (although *Emiliania huxleyi* (Fig. 22.16(b)) produces coccoliths only during the light period (Linschooten et al., 1991).

Although coccolithophorids constitute a minor part of recent calcareous oozes (bottom sediments composed of calcified remains of organisms) in the ocean, in the Cretaceous they dominated the calcareous nanoplankton (Tasch, 1973). This domination paralleled an increase in  $\text{Ca}^{2+}$  in seawater at the time (Brennan et al., 2004). Coccolithophorids provided the major constituent of Mesozoic (Jurassic and Cretaceous) and Tertiary chalks and marls. The abundance of coccolithophorids in these chalks can be demonstrated by taking a piece of ordinary blackboard chalk, pulverizing it, mixing it with distilled

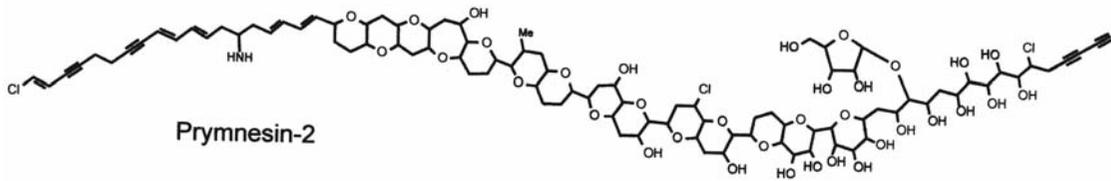
water in a test tube, and letting it stand for 20 minutes. Draw some of the solution into a pipette, dispose of the first four to five drops, and place the next few drops on a slide. Place a cover slip on the slide, and view it at a magnification of 400 to 500 $\times$ . Many coccoliths and other remains will be seen.

Coccoliths in sedimentary rocks can be used as markers in the discovery and mode of deposition of oil deposits. For example, the oil shales of the Kimmeridge Clays in England are sandwiched between limestone bands that are composed mostly of coccoliths of one species, *Ellipsagelosphaera britannica* (Gallois, 1976). Other oil-bearing rocks have similar characteristic coccoliths. Therefore petroleum geologists know that when a drill core shows certain coccoliths that are associated with petroleum, there is a good chance of finding oil in that stratum of rock.

## Toxins

The prymnesiophycean alga *Prymnesium parvum* (Fig. 22.7) secretes the potent exotoxin **prymnesin** (Fig. 22.17). The toxin causes fish mortalities by increasing cell membrane permeability and disturbing cellular ion balance (Fistarol et al., 2003). The toxin is most effective against aquatic gill-breathing animals, such as fish and molluscs. In Amphibia, only the gill-containing tadpole stage is sensitive to immersion in solutions containing the ichthyotoxin. The rapidity of the action of *Prymnesium* toxin on immersed fish suggests that the immediate target must be an exposed organ, probably the gill. Experiments have shown that the toxins affect the permeability of the gill, resulting in the increased sensitivity of the fish. In fish removed promptly from such toxin solutions, the gill damage is repaired within hours. In Israel, Shilo (1967) has found that it is possible to control *P. parvum* in fish breeding ponds by adding small amounts of ammonium salt, which causes the algal cells to lyse.

Secretion of prymnesin by *Prymnesium parvum* immobilizes prey organisms and enables *P. parvum* to more easily seize its prey (Skovgaard and Hansen, 2003). *Prymnesium* produces higher quantities of toxin when phosphorus is limiting



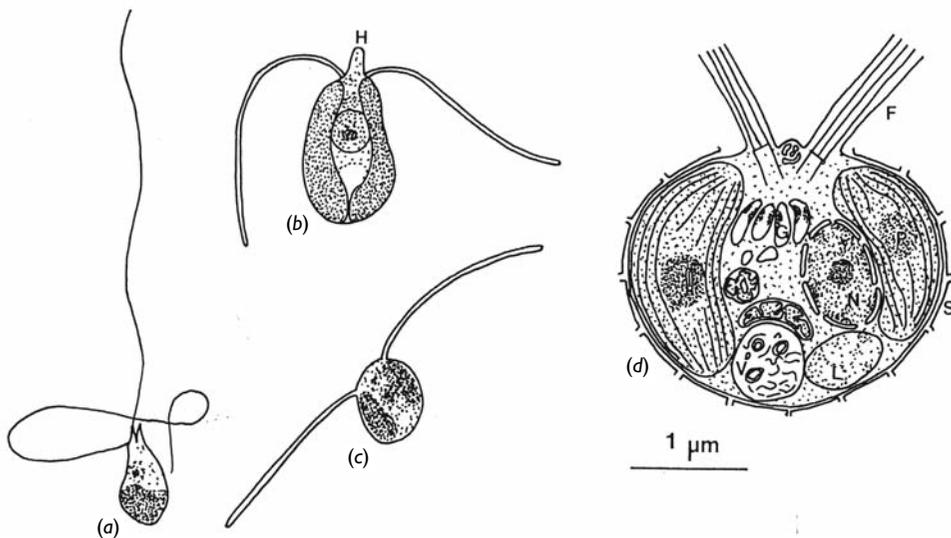
**Fig. 22.17** The chemical structure of prymnesin-2. (After Igarashi et al., 1996.)

(Legrand et al., 2001). Secretion of the toxin results in greater kill of prey organisms which are subsequently ingested by the *Prymnesium* cells. The phosphorus in the prey organisms alleviates the phosphorus deficiency in the *Prymnesium* cells, resulting in decreased production of toxin.

Some species of *Chrysochromulina* produce toxins that kill fish, mussels, and ascidians (Hansen et al., 1995; Moestrup, 1994; Simonsen and Moestrup, 1997). The best documented fish kills have occurred off the coast of Norway and Sweden. The large blooms of *Chrysochromulina* causing the fish kills have been associated with a lack of predation by the normal ciliate grazers of

*Chrysochromulina*. It appears that the long spines on the surface of the *Chrysochromulina* cells make them too large to be taken up by the ciliates (Hansen et al., 1995).

In the North Sea of Europe, and in the seas off Antarctica, blooms of the prymnesiophyte *Phaeocystis* (Figs. 22.4, 22.18(a)) occur as macroscopic lobed colonies or “bladders” in the spring and fall. *Phaeocystis* colonies are hollow, balloon-like structures with individual cells lying beneath a thin mucous skin (Hamm et al., 1999; Solomon et al., 2003). Grazing by invertebrates results in colonies of larger size, the larger size induced by chemicals released into the water by the grazing (Tang, 2003). Colony formation and enlargement is a defense mechanism that results in clogging of the filtration apparatus of the grazers (Haberman et al., 2002).



**Fig. 22.18** (a) Motile cell of *Phaeocystis poucheti*. (b) *Isochrysis galbana*. (c), (d) *Imantonia rotunda*. (F) Flagellum; (H) haptonema; (L) leucosin vesicle; (N) nucleus; (P) pyrenoid; (S) scale. ((d) after Reynolds, 1974.)

In the North Sea, herring avoid the ocean areas where there are *Phaeocystis* blooms because of its unpalatability to the fish (Savage, 1930). Also, for a number of years biologists were puzzled by the near sterility of the intestines of certain Antarctic birds in regard to Protozoa and microorganisms. This condition was shown to be due to the large quantities of *Phaeocystis* in their diet, with *Phaeocystis* producing large amounts of acrylic acid which has a strong bactericidal action. *Phaeocystis* secretes 16% to 64% of the carbon assimilated in photosynthesis as polysaccharides of varying molecular weight. As much as  $7 \mu\text{g liter}^{-1}$  of acrylic acid, and at least  $0.3 \text{ mg liter}^{-1}$  of polysaccharides, can be released in the formation of a dense bloom (Guillard and Hellebust, 1971; van Rijssel et al., 1997).

## Classification

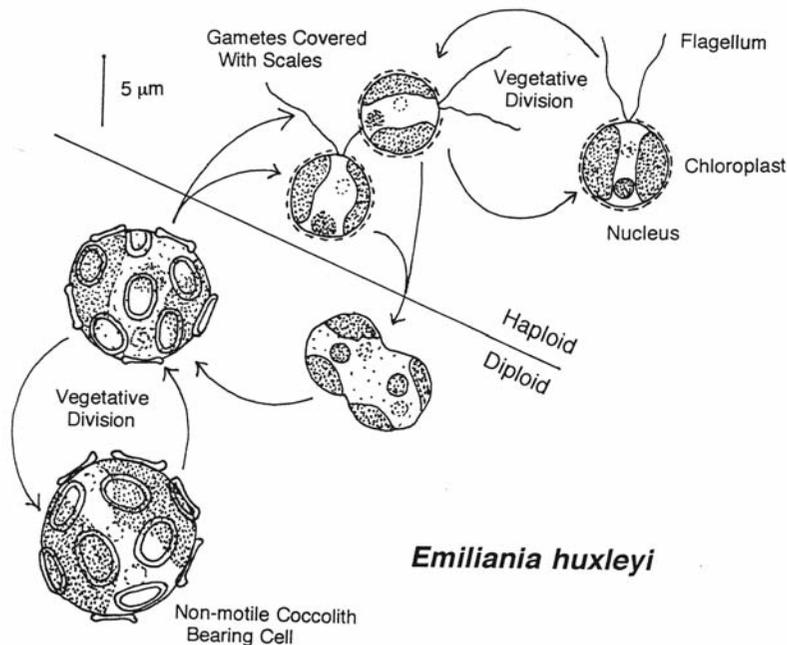
The Prymnesiophyceae can be divided into two orders (Edvardsen et al., 2000; Fujiwara et al., 2001):

- Order 1 Prymnesiales: cells with two equal smooth flagella, no eyespot, scales commonly covering the cell body.
- Order 2 Pavloales: cells with two unequal flagella often covered with hairs and deposits, eyespots may be present.

## Prymnesiales

*Emiliana huxleyi* (Fig. 22.16(b)) is typical of the order with motile cells that have two flagella. The life cycle of *E. huxleyi* (Fig. 22.19) probably involves a diploid, non-motile phase with coccoliths, which alternates with a haploid phase that has scales but no coccoliths (Paasche, 2002).

Every spring from 1978 to 1983, large areas (10 to 100 square miles) of water off the Atlantic Coast of France and southern England gave a strong reflectance of visible light to the Nimbus space satellite. Samples showed that the water contained mostly the coccolithophorid *Emiliana huxleyi* (Fig. 22.16(b)). The large coccoliths of *E. huxleyi* resulted in the high light reflectance which was picked up by the satellite (Holligan et al., 1983).

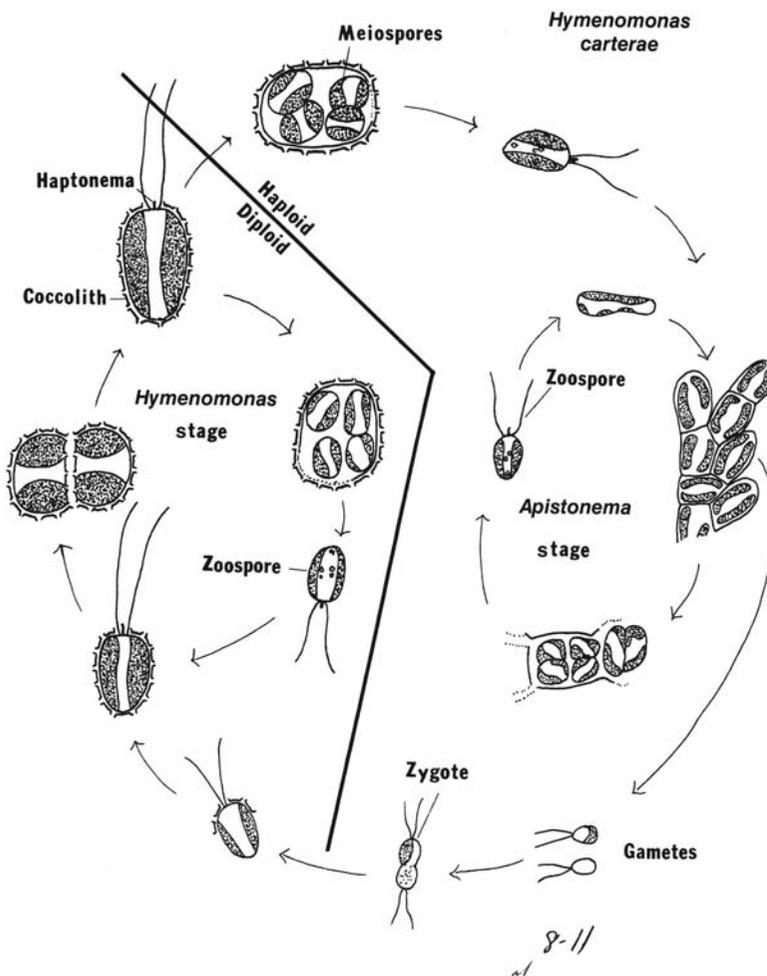


**Fig. 22.19** The probable life history of *Emiliana huxleyi*.  
(Adapted from Green et al., 1996; Klaveness, 1972.)

The life cycle of the coccolithophorid *Hymenomonas carterae* is more complex (Fig. 22.20). *H. carterae* has a haploid *Apistonema* stage ( $21 \pm 1$  chromosomes) that consists of a filamentous branched system of spherical or elongate cells. Cells of the *Apistonema* stage can give rise either to asexual swimmers, which form new *Apistonema* thalli, or to motile gametes. Each asexual swimmer has two long flagella and a cup-shaped chloroplast, and the cell is covered with uncalcified scales. Gametes can be distinguished from asexual swimmers by their smaller size and the reduced number or absence of chloroplasts. After fusion of two gametes, the resulting zygote develops coccoliths. The diploid *Hymenomonas* stage ( $42 \pm 1$  chromosomes) has an outer layer of coccoliths under which are several layers of organic scales. A

short haptonema is present between the flagella. The coccolith-bearing stage can perpetuate itself asexually by mitotic divisions. Under certain circumstances the protoplasts of *Hymenomonas* divide meiotically to produce four meiospores, which form haploid *Apistonema* thalli (von Stosch, 1967; Leadbeater, 1970; Parke, 1971).

The non-motile benthic stages of this order are resistant to fairly wide variations in the environment. The benthic stage of *Cricosphaera* sp. can survive temperatures of 35 to 40°C for an hour or more as well as deep freezing for periods up to 4 days. The same benthic phase is able to withstand a salinity up to the crystallization of the salt in solution. Normally benthic stages of *Cricosphaera* sp. grow near the high-water mark where they are presumably exposed to great variations in the

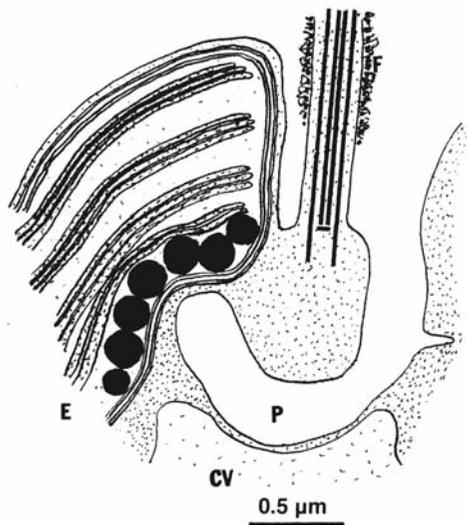


**Fig. 22.20** The life history of *Hymenomonas carterae*.

environment. The motile phase of *Cricosphaera* sp. will tolerate salinities only as low as 0.4% to 0.8% salt and as high as 4.5% to 5.0% salt (see Paasche, 1968, for a review).

### Pavlovales

The Pavlovales have cells with two unequal flagella with the haptonema arising between the flagella. In *Pavlova*, the two unequal flagella are attached some distance below the cell apex (Fig. 22.3(b)) (Green, 1967, 1976; van der Veer, 1969, 1976; Green and Manton, 1970). The longer flagellum is directed forward during swimming and is covered with fine hairs and dense bodies, whereas the short flagellum projects outward and can have fibrillar hairs on it. The flagella and haptonema are attached at the bottom of a depression (Green, 1973). A pit or canal passes from the bottom of the depression, under the base of the long flagellum, and terminates at the inner face of the eyespot (Fig. 22.21). The shape of the cells is variable, and there is a single two-lobed chloroplast with a pyrenoid. An eyespot is present inside the chloroplast. No sexual reproduction is known, and the cells propagate by longitudinal division in the motile state.



**Fig. 22.21** A drawing of a section through the apical area of *Pavlova granifera* showing the contactile vacuole (CV), eyespot (E), long flagellum (F), and pit (P). (After Green, 1973.)

The algae in the Pavlovales have unusual sterols with two hydroxyl groups called pavlovals (Ghosh et al., 1998).

### REFERENCES

- Anderson, O. R., Swanberg, N. R., and Bennett, P. (1983). Fine structure of yellow-brown symbionts (Prymnesiida) in solitary radiolaria and their comparison with similar Acantharian symbionts. *J. Protozool.* 30:718–22.
- Balch, W. M., Kilpatrick, K., Holligan, P. M., and Cucci, T. (1993). Coccolith production and detachment in *Emiliania huxleyi* (Prymnesiophyceae). *J. Phycol.* 29:566–75.
- Bhattacharya, D., and Ehling, J. (1995). Actin coding regions: gene family evolution and use as a phylogenetic marker. *Arch. Protistenk.* 145:155–64.
- Brennan, S. L., Lowenstein, T. K., and Horita, J. (2004). Seawater chemistry and the advent of biocalcification. *Geology* 32:473–6.
- Brown, R. M. (1969). Observations on the relationship of the Golgi apparatus to wall formation in the marine chrysophycean alga, *Pleurochrysis scherfferlii* Pringsheim. *J. Cell Biol.* 41:109–23.
- Chrétiennot-Dinet, M.-J., Giraud-Guille, M. M., Vaulot, D., Putaux, J.-L., Saito, Y., and Chanzy, H. (1997). The chitinous nature of filaments ejected by *Phaeocystis* (Prymnesiophyceae). *J. Phycol.* 33:666–72.
- Christensen, T. (1962). *Alger Botanik*, Vol. 2, No. 2. Copenhagen: Munksgaard.
- Corstjens, P. L. A. M., and Gonzales, E. L. (2004). Effects of nitrogen and phosphorus availability on the expression of the coccolith V-ATPase (subunit C) of *Pleurochrysis* (Haptophyta). *J. Phycol.* 40:82–7.
- Edvardsen, B., Eikrem, W., Green, J. C., Andersen, R. A., Moon-van der Stay, S. Y., and Medlin, L. K. (2000). Phylogenetic reconstructions of the Haptophyta inferred from 18S ribosomal DNA sequences and available morphological data. *Phycologia* 39:19–35.
- Faber, W. W., and Preisig, H. R. (1994). Calcified structures and calcification in protists. *Protoplasma* 181:78–105.
- Fistarol, G. O., Legrand, C., and Granéli, E. (2003). Allelopathic effect of *Prymnesium parvum* on a natural plankton community. *Mar. Ecol. Progr. Ser.* 255:115–25.
- Fournier, R. O. (1969). Observations on the flagellate *Diacronema vlkianum* Prauser (Haptophyceae). *Br. Phycol. J.* 4:185–90.
- Fujiwara, S., Tsuzuki, M., Kawachi, M., Minaka, N., and Inouye, I. (2001). Molecular phylogeny of the

- Haptophyta based in the *rbcl* gene and sequence variation in the spacer region of the Rubisco operon. *J. Phycol.* 37:121–9.
- Gallois, R. W. (1976). Coccolith blooms in the Kimmeridge Clay and origin of the North Sea Oil. *Nature* 259:473–5.
- Geisen, M., Billard, C., Broerse, A. T. C., Cros, L., Probert, I., and Young, J. R. (2002). Life-cycle associations involving pairs of holococcolithophorid species: intraspecific variation or cryptic speciation? *Eur. J. Phycol.* 37:531–50.
- Ghosh, P., Patterson, G. W., and Wikfors, G. H. (1998). Sterols of some marine Prymnesiophyceae. *J. Phycol.* 34:511–4.
- Gonzales, E. L. (2004). The proton pump of the calcifying vesicle of the coccolithophore, *Pleurochrysis*. In *Biom mineralization*, ed. E. Baeuerlein, pp. 217–8. Weinheim: Wiley-VCH.
- Green, J. C. (1967). A new species of *Pavlova* from Madeira. *Br. Phycol. Bull.* 3:299–303.
- Green, J. C. (1973). Studies in the fine structure and taxonomy of flagellates in the genus *Pavlova*. II. A freshwater representative, *Pavlova granifera* (Mack) comb. nov. *Br. Phycol. J.* 8:1–12.
- Green, J. C. (1976). Notes on the flagellar apparatus and taxonomy of *Pavlova mesolychnon* van der Veer, and on the status of *Pavlova* Butcher and related genera within the Haptophyceae. *J. Mar. Biol. Assoc. UK* 56:595–602.
- Green, J. C., and Hibberd, D. J. (1977). The ultrastructure and taxonomy of *Diacronema vlkianum* (Prymnesiophyceae) with special reference to the haptonema and flagellar apparatus. *J. Mar. Biol. Assoc. UK* 57:1125–36.
- Green, J. C., and Leadbeater, B. S. C. (1972). *Chrysochromulina parkae* sp. nov. (Haptophyceae) a new species recorded from S.W. England and Norway. *J. Mar. Biol. Assoc. UK* 52:469–74.
- Green, J. C., and Manton, I. (1970). Studies in the fine structure and taxonomy of flagellates in the genus *Pavlova*. I. A revision of *Pavlova gyrans*, the type species. *J. Mar. Biol. Assoc. UK* 50:1113–30.
- Green, J. C., and Pienaar, R. N. (1977). The taxonomy of the order Isochrysidales (Prymnesiophyceae) with special reference to the genera *Isochrysis* Parke, *Dicrateria* Parke and *Imantonia* Reynolds. *J. Mar. Biol. Assoc. UK* 57:7–17.
- Green, J. C., Course, P. A., and Tarran, G. A. (1996). The life-cycle of *Emiliania huxleyi*: A brief review and a study of relative ploidy levels analyzed by flow cytometry. *J. Marine Systems* 9:33–44.
- Greyson, A. J., Green, J. C., and Leadbeater, B. S. C. (1993). Structure and physiology of the haptonema in *Chrysochromulina* (Prymnesiophyceae). II. Mechanisms of haptonemal coiling and the regeneration process. *J. Phycol.* 29:686–700.
- Guillard, R. R. L., and Hellebust, J. A. (1971). Growth and production of extracellular substances by two strains of *Phaeocystis poucheti*. *J. Phycol.* 7:330–8.
- Haberman, K. L., Ross, R. M., Quetin, L. B., Vernet, M., Nevitt, G. A., and Kozlowski, W. (2002). Grazing by Antarctic krill *Euphausia superba* on *Phaeocystis antarctica*: an immunochemical approach. *Mar. Ecol. Progr. Ser.* 241:139–49.
- Hamm, C. E., Simson, D. A., Merkel, R., and Smetacek, V. (1999). Colonies of *Phaeocystis globosa* are protected by a thin but tough skin. *Mar. Ecol. Progr. Ser.* 187:101–11.
- Hansen, P. J., Nielsen, T. G., and Kaas, H. (1995). Distribution and growth of protists and mesozooplankton during a bloom of *Chrysochromulina* spp. (Prymnesiophyceae, Prymnesiales). *Phycologia* 34:409–16.
- Hawkins, E. K., and Lee, J. J. (2001). Architecture of the Golgi apparatus of a scale-forming alga biogenesis and transport of scales. *Protoplasma* 216:227–38.
- Hibberd, D. J. (1976). The ultrastructure and taxonomy of the Chrysophyceae and Prymnesiophyceae (Haptophyceae): A survey with some new observations on the ultrastructure of the Chrysophyceae. *Bot. J. Linn. Soc.* 72:55–80.
- Holligan, P. M., Viollier, M., Habour, D. S., Camus, P., and Champagne-Phillipe, M. (1983). Satellite and ship studies of coccolithophore production along a continental shelf. *Nature* 304:339–42.
- Igarashi, T., Satake, M., and Yasumoto, T. (1996). Prymnesium-2: a potent ichthyotoxic and hemolytic glycoside isolated from the red tide alga *Prymnesium parvum*. *J. Am. Chem. Soc.* 118:479–80.
- Inouye, I., and Kawachi, M. (1994). The haptonema. In *The Haptophyte Algae*, ed. J. C. Green, and B. S. C. Leadbeater, Systematics Assn. Special Vol. 51, pp. 73–89. Oxford: Clarendon Press.
- Janse, I., van Rijssel, M., van Hall, P. J., Gerswig, G. J., Gottschel, J. C., and Prins, R. A. (1996). The storage glucan of *Phaeocystis globosa* (Prymnesiophyceae) cells. *J. Phycol.* 32:382–7.
- Jordan, R. W., and Chamberlain, A. H. L. (1997). Biodiversity among haptophyte algae. *Biodiversity and Conservation* 6:131–52.
- Kawachi, M., and Inouye, I. (1994). Ca<sup>2+</sup> mediated induction of the coiling of the haptonema in

- Chrysochromulina hirata* (Prymnesiophyceae = Haptophyta). *Phycologia* 33:53–7.
- Kawachi, W., Inouye, I., Maeda, O., and Chihara, M. (1991). The haptonema as a food-capturing device: observations on *Chrysochromulina hirata* (Prymnesiophyceae). *Phycologia* 30:563–73.
- Klaveness, D. (1972). *Coccolithus huxleyi* (Lohm.) Kamptn. II. The flagellate cell, aberrant cell types, vegetative propagation and life cycles. *Br. Phycol. J.* 7:309–18.
- Kreger, D. R., and van der Veer, J. (1970). Paramylon in a Chrysophyte. *Acta Bot. Neerl.* 19:401–2.
- Leadbeater, B. S. C. (1970). Preliminary observations on differences of scale morphology at various stages in the life cycle of “*Apistonema-Syracosphaera*” sensu von Stosch. *Br. Phycol. J.* 5:57–69.
- Lechtreck, K.-F. (2004). An immunofluorescence study of the haptonema of *Chrysochromulina parva* (Prymnesiophyceae). *Phycologia* 43:635–40.
- Legrand, C., Johansson, N., Johnsen, G., Borsheim, K. Y., and Graneli, E. (2001). Phagotrophy and toxicity variation in the mixotrophic *Prymnesium patelliferum* (Haptophyceae). *Limnol. Oceanogr.* 46:1208–14.
- Linschooten, C., van Bleijswijk, J. D. L., van Emburg, P., de Vrind, J. P. M., Kempers, E. S., Westbroek, P., and de Vrind-de Jong, E. W. (1991). Role of light-dark cycle and medium composition in the production of coccoliths by *Emiliania huxleyi* (Haptophyceae). *J. Phycol.* 27:82–6.
- Manton, I. (1964). Further observations on the fine structure of the haptonema in *Prymnesium parvum*. *Arch. Mikrobiol.* 49:315–30.
- Manton, I. (1966). Further observations on the fine structure of *Chrysochromulina chiton*, with special reference to the pyrenoid. *J. Cell Sci.* 1:187–92.
- Manton, I. (1967a). Further observations on the fine structure of *Chrysochromulina chiton* with special reference to the haptonema, “peculiar” Golgi and aspects of scale production. *J. Cell Sci.* 2:265–72.
- Manton, I. (1967b). Further observations on scale formation in *Chrysochromulina chiton*. *J. Cell Sci.* 2:411–18.
- Manton, I. (1968). Further observations on the microanatomy of the haptonema in *Chrysochromulina chiton* and *Prymnesium parvum*. *Protoplasma* 66:35–53.
- Manton, I. (1972). Preliminary observations on *Chrysochromulina macra* sp. nov. *Br. Phycol. J.* 7:21–35.
- Manton, I., and Parke, M. (1962). Preliminary observations on scales and their mode of origin in *Chrysochromulina polylepis* sp. nov. *J. Mar. Biol. Assoc. UK* 42:565–78.
- Manton, I., and Peterfi, L. S. (1969). Observations on the fine structure of coccoliths, scales and the protoplast of a freshwater coccolithophorid. *Hymenomonas roseola* Stein, with supplementary observations on the protoplast of *Cricosphaera carterae*. *Proc. R. Soc. Lond. [B]* 172:1–15.
- Manton, I., Sutherland, J., and Oates, K. (1977). Arctic coccolithophorids: *Wigwammia arctica* gen. et sp. nov. from Greenland and arctic Canada. *W. annulifera* sp. nov. from South Africa and S. Alaska and *Calciarcus alaskensis* gen. et sp. nov. from S. Alaska. *Proc. R. Soc. Lond. [B]* 197:145–68.
- Marsh, M. E. (1994). Polyanion-mediated mineralization – assembly and reorganization of acidic polysaccharides in the Golgi system of a coccolithophorid alga during mineral deposition. *Protoplasma* 177:108–22.
- Marsh, M. E. (1996). Polyanion-mediated mineralization – a kinetic analysis of the calcium-carrier hypothesis in the phytoflagellate *Pleurochrysis carterae*. *Protoplasma* 190:181–8.
- Marsh, M. E. (2004). Biomineralization in coccolithophores. In *Biomineralization*, ed. E. Baeuerlein, pp. 198–215. Weinheim: Wiley-VCH.
- Medlin, L. K., Barker, G. L. A., Baumann, M., Hayes, P. K., and Lange, M. (1994). Molecular biology and systematics. In *The Haptophyte Algae*, ed. J. C. Green, and B. S. C. Leadbeater, Systematics Assn. Special Vol. 51, pp. 393–411. Oxford: Clarendon Press.
- Moestrup, Ø. (1994). Economic aspects: “blooms”, nuisance species, and toxins. In *The Haptophyte Algae*, ed. J. C. Green, and B. S. C. Leadbeater, Systematics Assn. Special Vol. 51, pp. 265–85. Oxford: Clarendon Press.
- Nielsen, M. V. (1995). Photosynthetic characteristics of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae) exposed to elevated concentrations of dissolved inorganic carbon. *J. Phycol.* 31:715–19.
- Outka, D. E., and Williams, D. C. (1971). Sequential coccolith morphogenesis in *Hymenomonas carterae*. *J. Protozool.* 18:285–97.
- Paasche, E. (1968). Biology and physiology of coccolithophorids. *Annu. Rev. Microbiol.* 22:71–86.
- Paasche, E. (2002). A review of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with particular reference to growth, coccolith formation and calcification-photosynthesis interactions. *Phycologia* 40:503–29.
- Parke, M. (1971). The production of calcareous elements by benthic algae belonging to the class Haptophyceae (Chrysophyta). *Proc. II Plank. Conf.*, pp. 929–38.

- Parke, M., and Manton, I. (1962). Studies on marine flagellates. VI. *Chrysochromulina pringsheimii* sp. nov. *J. Mar. Biol. Assoc. UK* 42:391–404.
- Parke, M., Manton, I., and Clarke, B. (1955). Studies on marine flagellates. II. Three new species of *Chrysochromulina*. *J. Mar. Biol. Assoc. UK* 34:579–609.
- Parke, M., Manton, I., and Clarke, B. (1956). Studies on marine flagellates. III. Three further species of *Chrysochromulina*. *J. Mar. Biol. Assoc. UK* 35:387–414.
- Parke, M., Lund, J. W. G., and Manton, I. (1962). Observations on the biology and fine structure of the type species of *Chrysochromulina* (*C. parva* Lackey) in the English Lake District. *Arch. Mikrobiol.* 42:333–52.
- Pringsheim, E. G. (1955). Kleine Mitteilungen über Flagellaten und Algen. I. Algenartige Chrysophyceen in Reinkultur. *Arch. Mikrobiol.* 21:401–10.
- Reynolds, N. (1974). *Imantonia rotunda* gen. et sp. nov., a new member of the Haptophyceae. *Br. Phycol. J.* 9:429–34.
- Rowson, J. D., Leadbeater, B. S. C., and Green, J. C. (1986). Calcium carbonate deposition in the motile (*Crystallolithus*) phase of *Coccolithus pelagicus*. *Br. J. Phycol.* 21:359–70.
- Savage, R. E. (1930). The influence of *Phaeocystis* on the migration of the herring. *Fish. Invest., Lond., Ser. II* 12:5–14.
- Shilo, M. (1967). Formation and mode of action of algal toxins. *Bacteriol. Rev.* 31:180–93.
- Simonsen, S., and Moestrup, Ø. (1997). Toxicity tests in eight species of *Chrysochromulina* (Haptophyta). *Can. J. Bot.* 75:129–36.
- Skovgaard, A., and Hansen, P. J. (2003). Food uptake in the harmful alga *Prymnesium parvum* mediated by excreted toxins. *Limnol. Oceanogr.* 48:1161–6.
- Solomon, C. M., Lessard, E. J., Keil, R. G., and Foy, M. S. (2003). Characterization of extracellular polymers of *Phaeocystis globosa* and *P. antarctica*. *Mar. Ecol. Progr. Ser.* 250:81–9.
- Tang, K. W. (2003). Grazing and colony size development in *Phaeocystis globosa* (Prymnesiophyceae): the role of the chemical signal. *J. Plankton Res.* 25:831–42.
- Tasch, P. (1973). *Paleobiology of the Invertebrates*. New York: John Wiley.
- van der Veer, J. (1969). *Pavlova mesolychnon* (Chrysophyta), a new species from the Tamar Estuary, Cornwall. *Acta Bot. Neerl.* 18:496–510.
- van der Veer, J. (1976). *Pavlova calceolata* (Haptophyceae), a new species from the Tamar Estuary, Cornwall, England. *J. Mar. Biol. Assoc. UK* 56:21–30.
- van der Wal, P., deVrind, J. P. M., deVrind-deJong, E. W., and Borman, A. H. (1987). Incompleteness of the coccosphere as a possible stimulus for coccolith formation in *Pleurochrysis carterae* (Prymnesiophyceae). *J. Phycol.* 23:218–21.
- van Rijssel, M., Hamm, C. E., and Gieskes, W. W. C. (1997). *Phaeocystis globosa* (Prymnesiophyceae) colonies: hollow structures built with small amounts of polysaccharides. *Eur. J. Phycol.* 32:185–92.
- von Stosch, H. A. (1967). Haptophyceae. In *Vegetative Forplanzung, Parthenogenese und Apogamie bei Algen*, ed. W. Ruhland, *Encyclopedia of Plant Physiology* 18:646–56.
- Young, J. R., Didymus, J. M., Bown, P. R., Prins, B., and Mann, S. (1992). Crystal assembly and phylogenetic evolution in heterococcoliths. *Nature* 356:516–18.
- Zapata, M., Jeffrey, S. W., Wright, S. W., Rodriguez, F., Garrido, J. L., and Clementson, L. (2004). Photosynthetic pigments in 37 species (65 strains) of Haptophyta: implications for oceanography and chemotaxonomy. *Mar. Ecol. Progr. Ser.* 270:83–102.

# Algae and the environment

It is possible to write whole books on the relationships between algae and the environment. In this chapter I have chosen a few subjects which have generated the most interest in the last decade.

## Toxic algae

Algae can be harmful in two basic ways (Hallegraeff et al., 2003).

- 1 Producing large populations in the aquatic environment** Large growths of some algae (e.g., the diatom *Chaetoceros* (Figs. 17.44, 17.45) or the prymnesiophyte *Chrysochromulina* (Fig. 23.1(c))) can clog the gills of fish and can be particularly a problem in aquaculture systems. Anoxic conditions, resulting in fish kills, can occur at the end of blooms of other algae (e.g., green algae) as the algae die and decompose.
- 2 Production of toxins** Some algae produce toxins that sicken and kill other organisms that prey on these algae. Indeed, this probably was the reason that these algae were selected for in the evolutionary process since it reduced predation by grazers (Gilbert, 1996). Filter-feeding shellfish can accumulate large quantities of these toxins as they filter the algae out of the water. Consumption of the shellfish by man, birds, and animals results in sickness and death. The algae that produce phycotoxins are:

### *Cyanophyceae* (cyanobacteria)

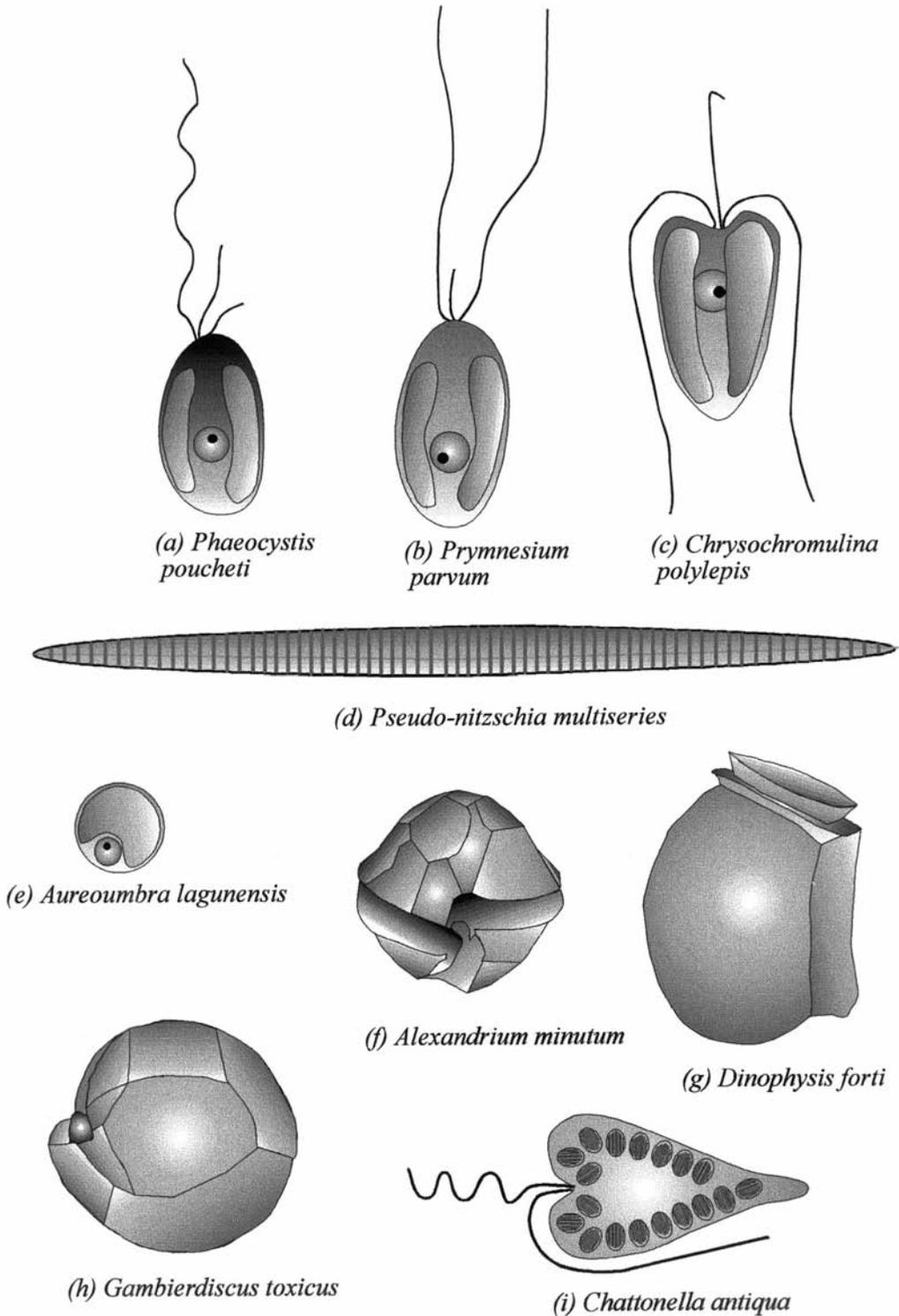
- **Neurotoxins** *anatoxin* (Fig. 23.2(c)) and *saxitoxin* (Fig. 23.2(c)) that block the transmission of

signal from neuron to neuron. These alkaloids (nitrogen containing compounds) bind to voltage-activated  $\text{Na}^+$ -channels and block influx of  $\text{Na}^+$ , thereby preventing the generation of an action potential (Shimizu, 2000).

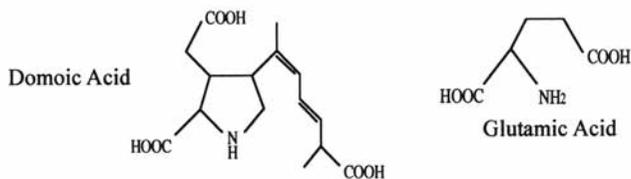
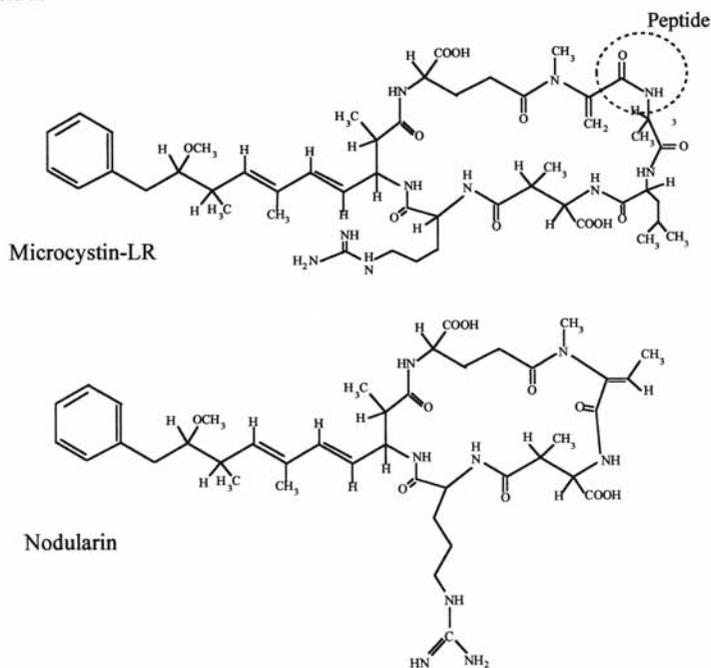
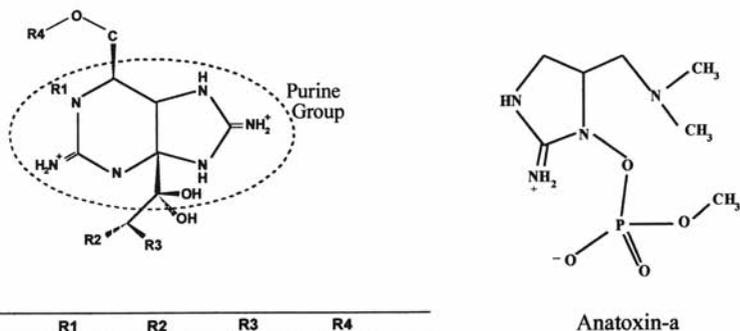
- **Hepatotoxins** *microcystin* (Fig. 23.2(b)) and *nodularin* (Fig. 23.2(b)) that are inhibitors of protein phosphatases 1 and 2A.

### *Dinophyceae* (dinoflagellates)

- **Diarrhetic shellfish poisoning** caused by *okadaic acid* (Fig. 23.3(e)), *macrolide toxins*, and *yessotoxins* (Fig. 23.4(f)). Okadaic acid and the related *dinophysistoxins* (Fig. 23.3(e)) are inhibitors of protein phosphatases. The free carboxyl groups bind to the catalytic site of the protein phosphatase. Yessotoxin may interfere with cyclic AMP in cells causing cytotoxicity (Cembella, 2003).
- **Ciguatera fish poisoning** caused by *ciguatoxin* (Fig. 23.4(f)), *maitotoxins* (Fig. 23.4(f)), and *brevetoxins* (Fig. 23.4(f)). All of these compounds are ion-channel disrupters, increasing the permeability of the cell membrane to positive ions and causing membrane depolarization. Brevetoxins require a lactone ring for activity. Ciguatoxin and brevetoxin bind voltage-sensitive channels, increasing the permeability of the cell membrane and causing membrane depolarization (Cembella, 2003). Maitotoxin initiates  $\text{Ca}^{2+}$ -channel activation, causing  $\text{Ca}^{2+}$ -influx and activation of calmodulin (Igarashi et al., 1999). This is followed by

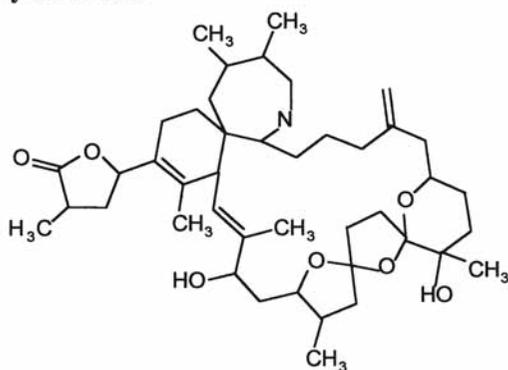
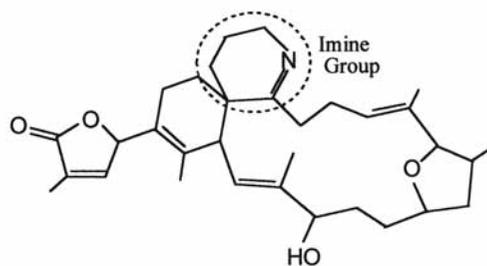
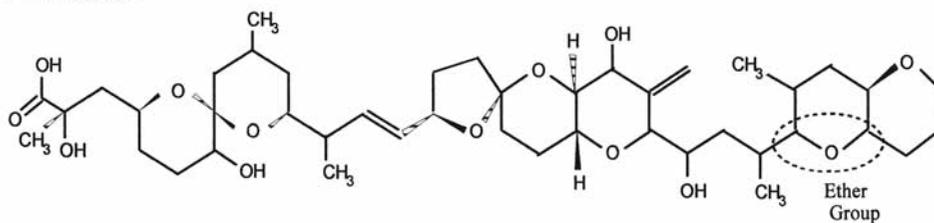
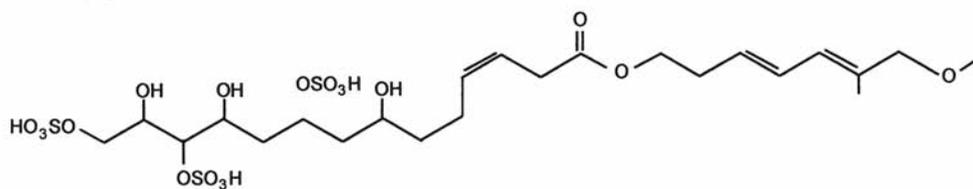
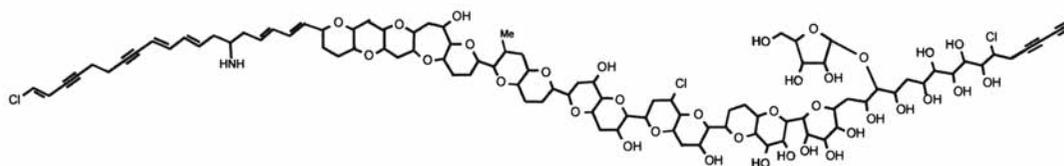


**Fig. 23.1** Examples of toxic marine algae.

**(a) Amino Acid Analogue****(b) Peptides****(c) Purine Derivatives**

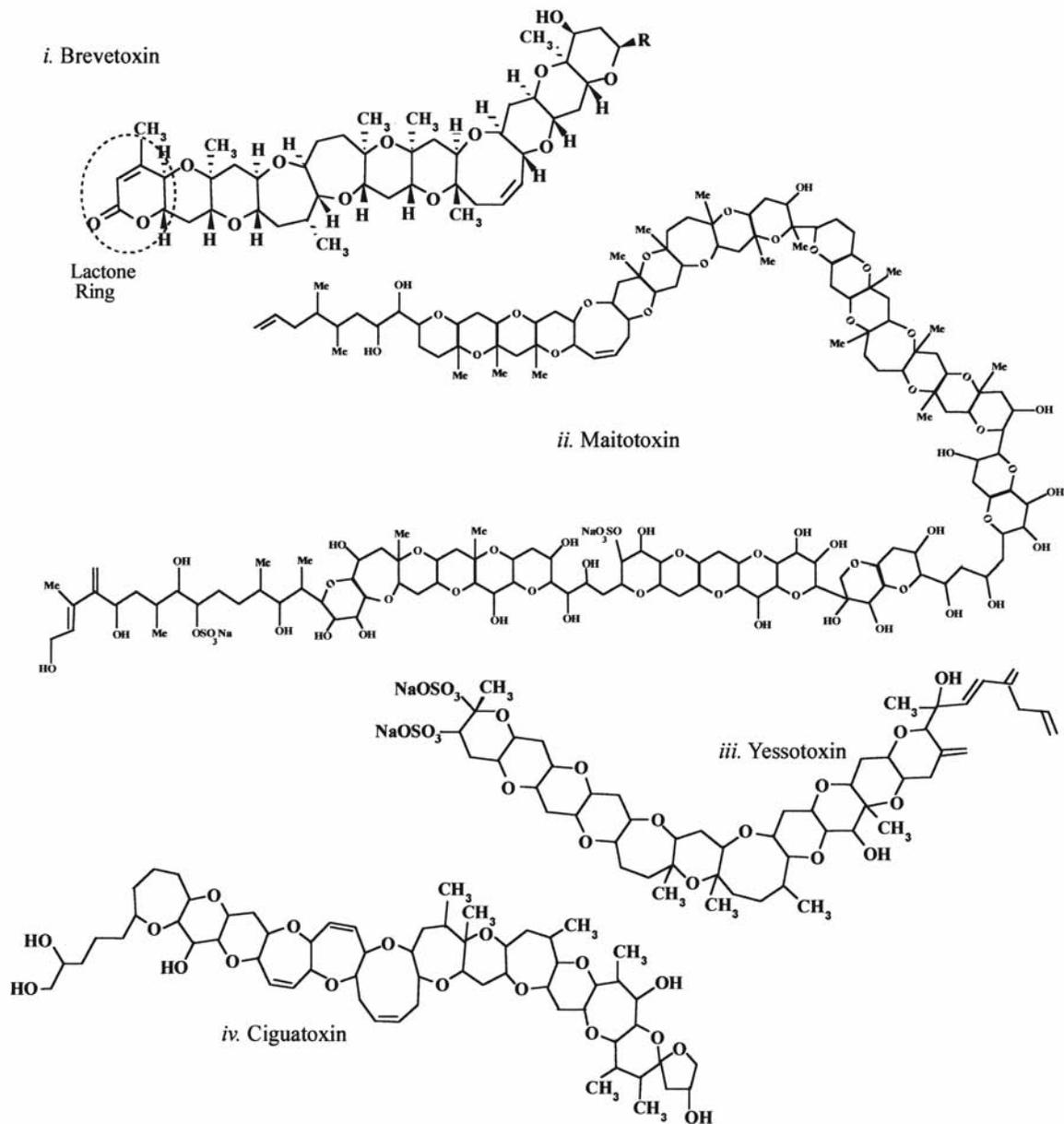
	R1	R2	R3	R4
<i>i.</i> Saxitoxin	H	H	H	CONH <sub>2</sub>
<i>ii.</i> Neosaxitoxin	OH	H	H	CONH <sub>2</sub>
<i>iii.</i> Gonyautoxin 1	OH	H	OSO <sub>3</sub>	CONH <sub>2</sub>

**Fig. 23.2** Chemical structure of the phycotoxins composed of amino acid analogues, peptides, and purine derivatives.

**(d) Cyclic Imines***i. Spirolide D**ii. Gymnodimine***(e) Non-nitrogen compounds containing linear and macrocyclic polyethers***i. Okadaic Acid**ii. Dinophysistoxin-4**iii. Prymnesin-2*

**Fig. 23.3** Chemical structure of phycotoxins composed of cyclic imines and non-nitrogen compounds containing linear and macrocyclic polyethers.

**(f) Nonnitrogen compounds containing ladder-frame polyethers**



**Fig. 23.4** Chemical structure of phycotoxins containing non-nitrogen compounds with ladder-frame polyethers.

promotion of phospholipase A2 activity and ultimately cell membrane disruption.

- **Paralytic shellfish poisoning** caused by *saxitoxin* (Fig. 23.2(c)) and approximately two dozen naturally occurring analogues (Fig. 23.2(c)). The saxitoxins bind to voltage-activated Na<sup>+</sup>-channels, blocking influx of Na<sup>+</sup> and preventing the generation of an action potential. Saxitoxins are retained primarily inside cells with very little excretion or leakage from cells. These highly water-soluble, but stable, compounds must be released during senescence or cell lysis, which coincides with the decline of a bloom (Cembella, 2003).
- **Spirolide poisoning.** This group of cyclic imines include the *spirolides* (Fig. 23.3(d)), *gymnodimine* (Fig. 23.3(d)), *pteriatoxin* and *pinnatoxins*. The imine ring gives the molecules their toxic properties. They are “fast-acting toxins”, causing death of mice within minutes after oral application.

#### *Bacillariophyceae (diatoms)*

- **Amnesic shellfish poisoning** caused by the neurotoxin *domoic acid* (Fig. 23.2(a)). Domoic acid is an amino acid that functions as a *glutamate agonist* (compound that has an affinity for a cell receptor), and in humans causes extensive depolarization in areas rich in glutamate receptors (e.g., the hippocampus). The biosynthesis of domoic acid is inducible and sustained only under stress conditions (Pan et al., 1998).

#### *Raphidophyceae (chloromonads)*

- **“Red tide” poisoning.** Members of the Raphidophyceae produce *brevetoxins* (Fig. 23.4(f)), neurotoxins that bind to voltage-activated Na<sup>+</sup>-channels causing rapid influx of Na<sup>+</sup>-ions and membrane depolarization.

#### *Prymnesiophyceae (haptophytes)*

- The phycotoxin *prymnesin* (Fig. 23.3(e)) produces hemolysis of red blood cells by disrupting the pores in the cell membrane, causing influx of positive ions and membrane depolarization.

Another way of classifying phycotoxins is by their chemical structure (Cembella, 2003):

- 1 *Amino acid analogues.* **Domoic acid** (Fig. 23.2(a)). This is a glutamate receptor agonist produced by the diatom *Pseudo-nitzschia* spp. (Figs. 17.27, 17.29, 23.1(d)).
- 2 *Peptides.* **Microcystins** (Figs. 23.2(b)) and **nodularin** (Fig. 23.2(b)). These phycotoxins are produced by cyanobacteria. Microcystin is formed by species of *Microcystis* (Figs. 2.48, 2.56(b)), *Anabaena* (Figs. 2.16, 2.18(d)), *Nostoc* (Figs. 2.46(b), (c), (d), 2.57(a)), *Nodularia* (Fig. 2.42(a)) and *Oscillatoria* (Figs. 2.19(a), (b), 2.34(a)), while nodularins are produced by species of *Nodularia* (Fig. 2.42(a)).
- 3 *Purine derivatives.* **Saxitoxins** (Fig. 23.2(c)), **gonyautoxins** (Fig. 23.2(c)), and **anatoxin** (Fig. 23.2(c)). There are about two dozen analogues of these molecules with their potency varying over several orders of magnitude. Saxitoxin has a LD<sub>50</sub> of 10 μg kg<sup>-1</sup>. The decarbamoyl derivatives have intermediate toxicity (decarbamoyl saxitoxin has a LD<sub>50</sub> of 20 μg kg<sup>-1</sup>) while the *N*-sulphocarbamoyl forms have a low toxicity (*N*-sulphocarbamoyl (B-toxin) has a LD<sub>50</sub> of 163 μg kg<sup>-1</sup>). Saxitoxins are produced by some cyanobacteria and by the dinoflagellates *Alexandrium* spp. (Figs. 7.35, 7.36, 23.1(f)), *Pyrodinium bahamense* (Fig. 7.34), and *Gymnodinium catenatum*. Anatoxin is produced by the cyanobacteria *Anabaena* (Figs. 2.16, 2.18(d)), *Aphanizomenon* (Fig. 2.18(b)), *Oscillatoria* (Figs. 2.19(a), (b), 2.34(a)), and *Trichodesmium* (Fig. 2.56(g)).
- 4 *Cyclic imines.* **Spirolides** (Fig. 23.3(d)) and **gymnodimine** (Fig. 23.3(d)). The spirolides are produced by the marine dinoflagellate *Alexandrium ostenfeldii* while gymnodimine is produced by the dinoflagellate *Karenia selliformis*.
- 5 *Non-nitrogen compounds containing linear and macrocyclic polyethers.* **Okadaic acid** (Fig. 23.3(e)), **dinophysistoxins** (Fig. 23.3(e)), and **prymnesium** (Fig. 23.3(e)). Okadaic acid and the related dinophysistoxins are produced by the benthic dinoflagellate *Prorocentrum* (Figs. 7.30(c), 7.55) and the planktonic forms of *Dinophysis* (Figs. 7.30(a), (b)). Prymnesium is formed by species of the haptophyte *Prymnesium* (Fig. 22.7).

6 Non-nitrogen compounds containing ladder-frame polyethers. **Brevetoxin** (Fig. 23.4(f)), **ciguatoxin** (Fig. 23.4(f)), and **yessotoxin** (Fig. 23.4(f)). Brevetoxins are formed by species of the dinoflagellate *Karenia* (*Gymnodinium*) (Figs. 7.19, 7.59) and by the raphidophytes *Chattonella* (Fig. 18.3) and *Heterosigma* (Fig. 18.1(a)). Ciguatoxins are produced by species of the tropical and subtropical benthic dinoflagellate *Gambierdiscus* (Figs. 7.6, 7.32). Yessotoxins are produced by the dinoflagellate *Prorocentrum*.

## Toxic algae and the end-Permian extinction

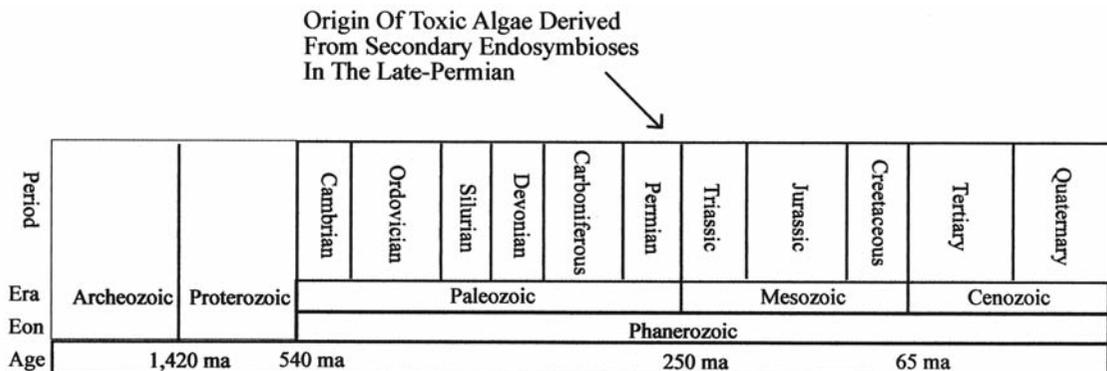
All eukaryotic toxic algae contain chloroplast endoplasmic reticulum around the chloroplast and have evolved through a secondary endosymbiosis. Algae derived from secondary endosymbioses evolved during the middle to late Permian Period (Medlin et al., 1997).

There was extinction of approximately 90% of marine species during the late Permian (about 270 million years ago) (Sepkoski, 1984; Erwin, 1993) (Fig. 23.5). Before this extinction, the Paleozoic oceans were dominated by epifaunal (attached to a substrate in the adult form) invertebrates that were filter feeders, sieving food particles out of the water, or passive carnivores. The Permian Period is demarcated from the Triassic by the extinction of

this fauna, and this boundary established the close of the Paleozoic era from the start of the Mesozoic era. The Mesozoic oceans comprise a different fauna with a much reduced number of filter feeders. The animals were more highly mobile with an expansion of predators and of a fauna that burrowed more deeply into the sediment.

Thus, the marine fauna that suffered the greatest extinction at the end of the Permian was attached to the seafloor in the adult form and filtered organic material from the water. In addition, those marine invertebrates with larvae that filtered plankton from the seawater had greater extinction rates than those invertebrates that did not feed on plankton (Christiansen and Fenchel, 1979; Jablonski and Lutz, 1983; Valentine and Jablonski, 1986). Filter feeding by sieving organic material from the seawater appears to be the one unifying characteristic of invertebrates that became extinct during the late Permian.

The evolution of toxic algae derived from secondary endosymbioses paralleled the decline of invertebrates during the end-Permian extinction. It appears that the attached filter-feeding invertebrates that characterized the Paleozoic seas were susceptible to the toxins produced by the newly evolved algae at the end of the Permian Period. This resulted in selective pressure that resulted in their decline and the evolution of a new fauna that was more mobile and less susceptible to the toxic algae in the marine environment.



**Fig. 23.5** Location of the end-Permian extinction in the geological time scale.

## Cooling of the Earth, cloud condensation nuclei, and DMSP

Algae adjust to changes in external salt concentration by adjusting the osmolarity of the cell protoplasm through the production of glycerol in freshwater algae (Fisher et al., 1994) or dimethylsulfoniopropionate (DMSP) in marine algae (Wolfe et al., 1997). The higher the external salt concentration, the greater the concentration of glycerol or DMSP in the protoplasm. DMSP and glycerol are used because they do not interfere with cellular function as do some organic salts (Keller and Korjeff-Bellows, 1996).

The production of DMSP by marine algae is an important factor in the formation of clouds and the temperature of the Earth's atmosphere. After a marine alga dies, DMSP is released into the ocean where it is broken down into the gas dimethyl sulfide (DMS) and propenoic acid (acrylic acid) (Fig. 23.6) (Liss et al., 1997). Dimethyl sulfide gas dissolves in the seawater and eventually some dimethyl sulfide escapes into the atmosphere. In the atmosphere, dimethyl sulfide is oxidized into methane sulfonic acid (MSA) and sulfuric acid. MSA and sulfuric acid have relatively low vapor pressures so they precipitate into atmospheric aerosols that produce cloud condensation nuclei. **Cloud condensation nuclei** are small particles of hygroscopic material on which liquid water condenses (Cox, 1997). This produces rain and cloud albedo. **Albedo** refers to the ability of clouds to reflect sunlight back out of the atmosphere. The more clouds the greater the reflectivity and albedo, and the less heating of the Earth (Charlson et al., 1987). This counters heating of the Earth by the greenhouse effect where anthropogenic (related to human activities) burning of fossil fuels has resulted in increased atmospheric carbon dioxide concentrations and heating of the Earth (Jones and Slingo, 1997).

Atmospheric sulfuric acid is also produced by burning of fossil fuels. Anthropogenic sulfuric acid in the atmosphere is mostly over land and in the Northern Hemisphere (Liss et al., 1997). In contrast, over the oceans, cloud condensation nuclei are produced mostly from dimethyl sulfide oxida-

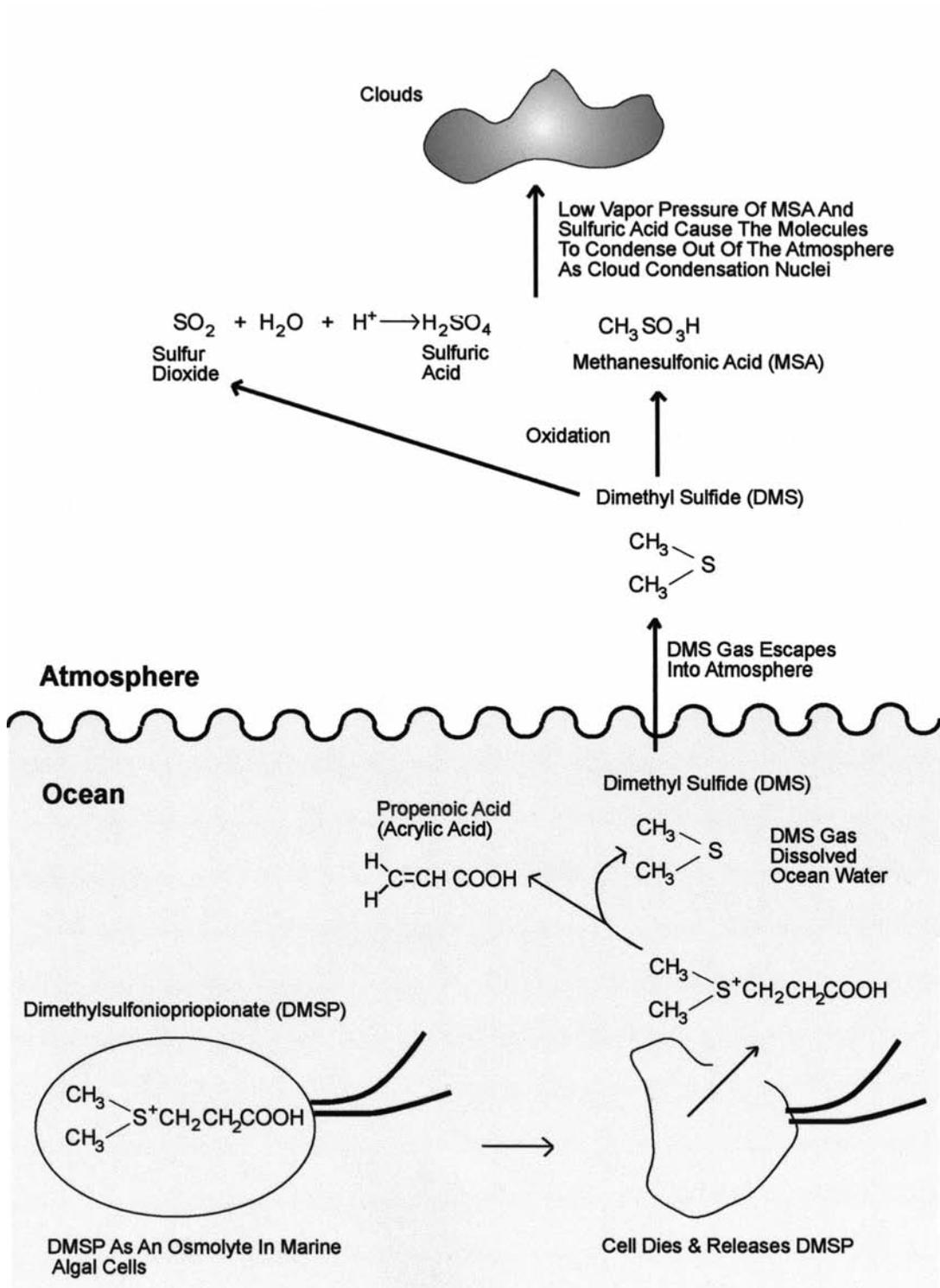
tion. Prymnesiophytes (haptophytes) and dinoflagellates produce more DMSP per cell than other marine algae (e.g., diatoms) and are the main contributors to the cloud condensation nuclei over oceans (Malin, 1996).

Marine flagellates may use DMSP (instead of glycerol) as an osmolyte because its degradation of DMSP produces propenoic (acrylic) acid. Acidifying seawater makes more carbon dioxide available for photosynthesis (see section on evolution of chloroplast endoplasmic reticulum). Although propenoic acid is metabolized by bacteria, the release of large quantities of propenoic acid from blooms of the prymnesiophyte *Phaeocystis* appears to prevent growth of other microorganisms in the water for some time after the bloom (Savage, 1930).

## Chemical defense mechanisms of algae

Herbivore grazing on macroalgae and predation by invertebrates on microalgae cause the greatest loss of algal biomass. Algae have evolved chemical defense mechanisms that can be defined as constitutive or inducible:

- 1 Constitutive defense.** The chemicals involved in defense are present all of the time. An example is the toxic "red tide" dinoflagellates that have reduced grazing by copepods (Wolfe, 2000). Coccolithophorids are another example. *Emiliana huxleyi* has an increased amount of dimethylsulfoniopropionate (DMSP) when it is grazed by invertebrates. DMSP lyase breaks down DMSP into dimethylsulfide and acrylate (Fig. 23.6). Water containing the latter compound is avoided by grazing protozoans (Strom et al., 2003).
- 2 Inducible defense.** The chemicals that function in defense are only produced when the alga is under pressure from a consuming organism. Chemicals are costly for the alga to produce so it is more economical for the algal cells to produce the chemicals only when in danger of being consumed. Examples of inducible defense chemicals are the increased production of phlorotannins by brown algae,



**Fig. 23.6** The mechanism by which DMSP produces clouds and cooling in the atmosphere.

and the increased production of halogenated furones by red algae, on grazing by invertebrates (Pavia et al., 2003).

Defense chemicals can also be divided into:

- **seriochemicals**, which act between individuals of the same species. **Pheromones** that function in sexual reproduction are examples of seriochemicals.
- **allelochemicals**, which act between members of different species. **Kairomones** and **synomones** are examples of allelochemicals (Cembella, 2003).

**Kairomones** are chemicals secreted by a predator that induce changes in the behavior, morphology, or life history of the prey.

- 1 Kairomones can be noxious compounds produced by the prey to act as feeding deterrents via olfactory or gustatory responses of the predator at the preingestive stage. Such contact signals could be sequestered on the surface of the prey cells or released into the medium. Often their main purpose would not be to intoxicate the predator, but to discourage “tasting” or to initiate rapid release of the prey following physical handling and capture. For example, grazing experiments exposing the tintinnid *Favella ehrenbergi* to cells of the toxic dinoflagellate *Alexandrium* resulted in the ciliate swimming in retrograde (avoidance) manner once a threshold of dinoflagellate cells was reached (Hansen et al., 1992).
- 2 Kairomones can be toxic compounds released from ingested cells that result in physical incapacitation or mortality of the predator. The prymnesiophyte alga *Phaeocystis* contains large amounts of  $\beta$ -dimethylsulfoniopropionate (DMSP). Ingestion of the alga releases the intracellular DMSP resulting in reduced grazing by the heterotrophic dinoflagellates *Amphidinium*, *Gymnodinium*, *Oxyrrhis*, and the ciliate *Coxiella* (Strom et al., 2003).
- 3 Kairomones can be “stealth compounds” of low acute toxicity to adult predators that lead to postdigestive reduction in fecundity (ability

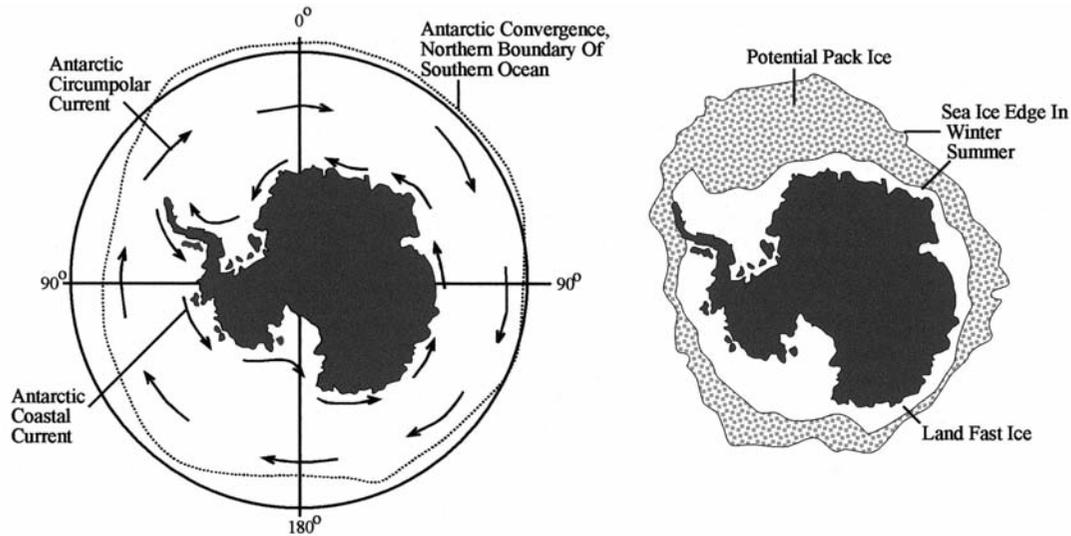
to produce offspring). Postdigestive deterrence would obviously be ineffective for the protection of the individual ingested cell, but group defense would be maintained on a community basis. For example, the ingestion of high concentrations of diatoms by copepods results in the low viability of eggs of the nauplii (larvae) of the copepods (Paffenhofer, 2002; Ianora et al., 2003).

**Synomones** are produced by prey species to attract predators of their predators at the next level of the food web.

## The Antarctic and Southern Ocean

The phytoplankton in the waters around Antarctica support a plethora of marine life. Easterly winds next to the Antarctic continent drive the **Antarctic Coastal Current** or **East Wind Drift** (Fig. 23.7). North of this, westerly winds drive one of the largest current systems of Earth, the **Antarctic Circumpolar Current** or **West Wind Drift** (Garrison and Siniff, 1986). This current flows unobstructed around Antarctica, transporting two to three times more water than the Gulf Stream. A region of upwelling occurs between the Antarctic Coastal Current and Antarctic Circumpolar Current. This area of upwelling pulls nutrient-rich deep water to the surface. This nutrient-rich water spills over all the Antarctic waters creating an environment for phytoplankton blooms. The **Southern Ocean**, comprising about 10% of the world ocean, occurs between the Antarctic continent and the outer edge of the Antarctic Circumpolar Current. There is an intense bloom of phytoplankton in the Southern Ocean during the austral (southern) summer that provides energy for marine food webs. Zooplankton, particularly the Antarctic krill (*Euphasia superba*), are abundant, providing a food source for vertebrate populations.

The sea ice surrounding the Antarctic continent can be divided into (1) **land-fast ice** that persists all year long and (2) **pack ice** that breaks up during the austral summer (December–March) (Fig. 23.7). There are two basic algal communities that exist in the ice in the Antarctic:



**Fig. 23.7** Drawings of currents (left) and sea ice (right) in the Southern Ocean surrounding the Antarctic continent.

bottom-dwelling algae on the bottom of the ice, and algae growing in the ice itself.

- 1 The **bottom-dwelling algae** are mostly diatoms such as *Nitzschia stellata* and *Amphiprora* sp. These bottom-dwelling diatoms occur principally on the bottom of land-fast ice. Pack ice, though, does not have a well-developed community of bottom-dwelling diatoms. This is probably because the bottom of pack ice is extensively grazed by the krill (Garrison et al., 1986). Observers on ice-breaking ships have seen swarms of krill on upturned ice floes or in holes of decaying ice floes.
- 2 **Algae growing in the ice.** The algae growing in the ice depend on the conditions of the ice. Initially, salts are excluded from the ice as the salt water freezes during the austral fall and winter. This results in brine inclusions in the ice with salinities as high as 10%. The brine inclusions contain cryo- and halotolerant algae that are able to tolerate the cold and high salinity. Dinoflagellates, chrysophytes, and the green *Mantoniella* are common in these brine inclusions (Stoecker et al., 1998). These brine algae peak and form resting spores before the melting of the surface ice dilutes the brine and produces hypersaline conditions and a new

population of algae. There is an abundance of nutrients available in the meltwater and diatoms such as *Nitzschia curta* proliferate. As pack ice melts, the algae in the ice are released to the water column where the algae form a seed population for the phytoplankton bloom that occurs during the austral summer (Smith and Nelson, 1986).

## The grand experiment

Man's activities have resulted in an increase in  $\text{CO}_2$  in the atmosphere and potential warming of the atmosphere of the earth due to the "greenhouse effect." This increase in  $\text{CO}_2$  in the atmosphere can be addressed in one of two ways, either by a reduction in the burning of fossil fuels or by removing the  $\text{CO}_2$  from the atmosphere.

John Martin of the Moss Landing Marine Laboratory in California put forth the hypothesis that iron availability limits phytoplankton production in nutrient-rich seas. He further suggested that it might be possible to fertilize the Southern Ocean (which has an abundance of unused nutrients) with iron, increase photosynthesis by plankton, and increase the flux of  $\text{CO}_2$  from the atmosphere to the deep ocean, which contains 60 times more  $\text{CO}_2$  than the atmosphere.

I first said this more or less facetiously at a Journal Club lecture at Woods Hole Oceanographic Institute in July 1988. I estimated that with 300 000 tons of Fe, the Southern Ocean phytoplankton could bloom and remove two billion tons of carbon dioxide.

Putting on my best Dr Strangelove accent, I suggested that with half a ship load of Fe, I could give you an ice age. Chisholm and Morel (1991)

Historically, there has been variation in the CO<sub>2</sub> concentration in the atmosphere. Man's activities have resulted in an increase in CO<sub>2</sub> concentration today to almost 350 parts per million (ppm). This is an increase from 200 ppm during the last ice age (glacial maximum, 18 000 years ago). The glacial minimum that preceded this, however, had an atmospheric concentration of 280 ppm, approximately the same as in 1900. The decrease in CO<sub>2</sub> concentration during the last glacial maximum is explained as follows. There was a fivefold increase in the arid areas of the earth, along with a 1.5 times increase in the winds. These two factors resulted in a 50-fold increase in the airborne dust particles. Since iron is the fourth most common element on Earth, the airborne dust contained a significant amount of iron, much of which was deposited in the oceans. This resulted in a threefold increase in photosynthesis and a decrease in CO<sub>2</sub> to around 200 ppm. This produced a decrease in the greenhouse effect, a cooling of the Earth, and an ice age (Martin, 1990).

The suggestion of adding iron to the Southern Ocean to reduce atmospheric CO<sub>2</sub> triggered a debate about whether we should engage in intentional large-scale intervention with the Earth's natural biogeochemical cycles. Martin put forward the following:

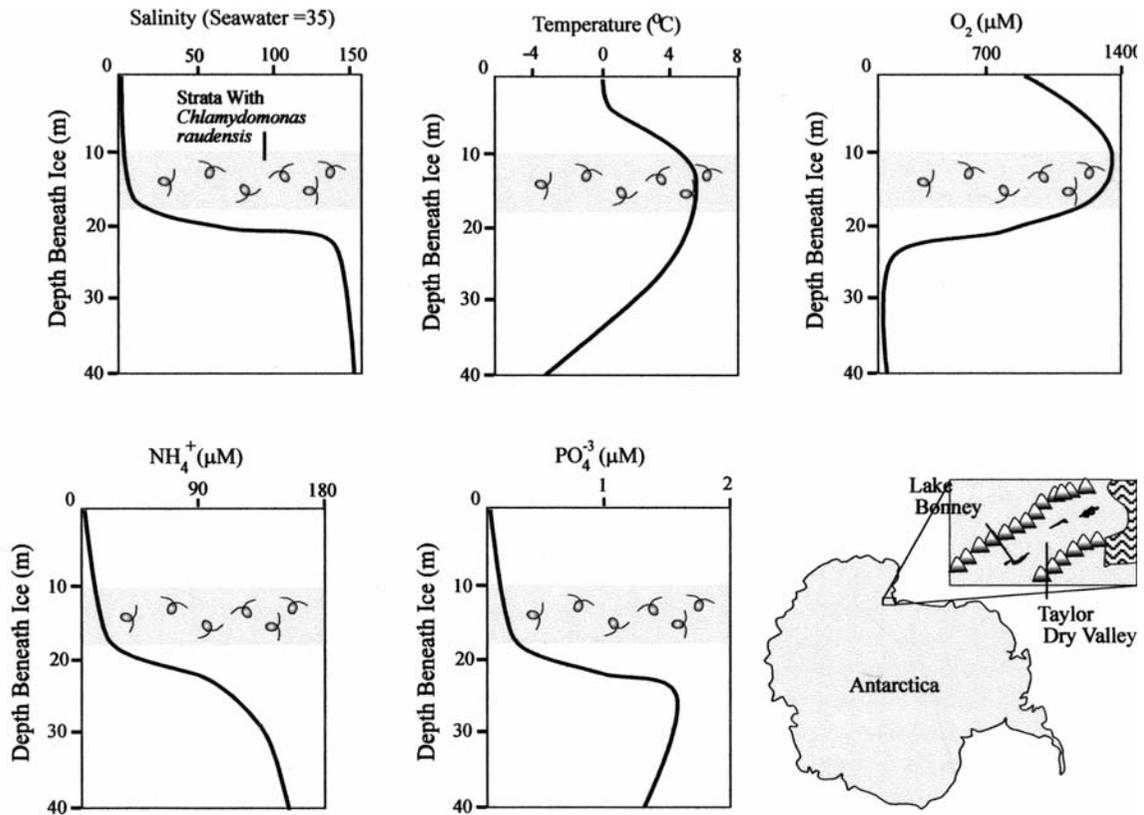
One could argue that this really is not such a new or weighty issue. After all, we have already changed dramatically the landscape of terrestrial ecosystems, we have converted forests to croplands, croplands to deserts, rivers to lakes and deserts to greenbelts. So why is there such a big fuss about the prospect of spreading some iron around the ocean? If we are inadvertently, but knowingly, changing the chemistry of the atmosphere through fossil fuel burning, why should we not change it purposely through iron fertilization or some other scheme? Chisholm and Morel (1991)

Ultimately it was decided to try a test of the hypothesis. John Martin orchestrated the scientific and logistical planning for a large-scale iron enrichment experiment in the open ocean, although his untimely death from cancer in 1993 prevented him from seeing the outcome. In mid-November 1993, the RV *Columbus Iselin* arrived 500 miles south of the Galapagos Islands with 480 kg of iron. The iron was pumped into the propeller wash as the vessel steamed to and fro across an 8 × 8 km field over 24 hours, raising the iron concentration from about 0.05 mM to about 4 mM. Water samples were taken and monitored for phytoplankton and nutrients, while a P-3 Orion airplane optically scanned the water for changes in phytoplankton pigments. The results showed that there was an increase in phytoplankton, although the increase was not as great as was predicted from laboratory cultures. This was probably due to increased grazing by zooplankton, which showed a 50% increase over the period (Wells, 1994). The experiment was repeated in 1995 (Coale et al., 1996) and again enrichment of photoplankton was obtained.

The experiment showed it is possible to remove 1000 to 100 000 tons of carbon from the atmosphere for each one ton of iron added to the Southern Ocean (Boyd, 2004; Dalton, 2002). The political realities of performing Fe enrichment in quantities high enough to significantly effect the atmospheric CO<sub>2</sub> concentration is on hold as the debate over the increase in CO<sub>2</sub> in the atmosphere, and global warming, continues.

## Antarctic lakes as a model for life on the planet Mars or Jupiter's moon Europa

The McMurdo Dry Valleys in Antarctica (Fig. 23.8) have one of the driest and coldest deserts on Earth and house the only permanently ice-covered lakes on our planet (Pocock et al., 2004). The headquarters of Captain Robert F. Scott (who perished with his party returning from the South Pole) was across McMurdo Sound from the dry valleys. In December 1903, Scott wrote, "It is worthy to record, too, that we have seen no living thing, not even a moss or lichen; all that we did



**Fig. 23.8** Profiles of water conditions from Lake Bonney, Taylor Dry Valley, Antarctica. The dominant alga, *Chlamydomonas raudensis*, occurs primarily between 10 and 17 meters depth. (Modified from Spigel and Priscu, 1996.)

find, far inland among the moraine heaps, was the skeleton of a Weddell seal, and how that came there is beyond guessing. It is certainly a valley of the dead; even the great glacier which once pushed through it has withered away” (Priscu, 1999). Average annual precipitation in these dry valleys is less than 10 cm and average air temperature is about  $-20^{\circ}\text{C}$ . Lake Bonney is in the Taylor Valley and has a permanent ice cover that prevents wind mixing of the column, resulting in vertical mixing only on the molecular scale; vertical mixing time is approximately 50 000 years. Stratification in Lake Bonney is the result of strong salinity gradients. The salinity of the lake varies from freshwater at the surface (from melting glaciers in the austral (Southern Hemisphere) summer) to hypersaline brine more

than five times seawater at the bottom. There are four months of darkness during the austral winter with algal cells generating energy from heterotrophy. During the austral summer the ice cover attenuates about 98% of light. Therefore, photosynthetic inhabitants of the water column below the ice are never exposed to saturating light levels and have adapted to a shade environment. *Chlamydomonas raudensis* is the dominant alga in this environment. This green alga is an obligate psychrophile because it does not grow above  $16^{\circ}\text{C}$ . *Chlamydomonas raudensis* lives in a discrete layer in the lake at depths of between 10 and 17 m, which is a transition zone between an oxygen-rich layer where  $\text{O}_2$  production exceeds respiratory  $\text{O}_2$  uptake and the deeper oxygen-deficient region (Fig. 23.8) (Pocock et al., 2004). The shade adaptation is reflected in its low chlorophyll *a/b* ratio (Morgan et al., 1998). Light above 680 nm is primarily absorbed by chlorophyll *a* while chlorophyll *b* absorbs shorter wavelengths (Fig. 1.13). Similarly there are low levels

of photosystem I to photosystem II; photosystem II utilizes light of wavelengths shorter than 680 nm while photosystem I utilizes light up to 700 nm wavelength. The permanent ice cover in these Antarctic lakes absorbs all light wavelengths above 600 nm (Pocock et al., 2004). The perennial ice overlying liquid water in these Antarctic lakes is similar to the situation on the moon Europa of Jupiter, and on Mars where permanently ice-covered lakes are thought to have existed between 3.1 and 3.8 billion years ago (Priscu et al., 1999).

### Ultraviolet radiation, the ozone hole, and sunscreens produced by algae

Ultraviolet radiation is customarily divided into three spectral regions: UV-C (200–280 nm), UV-B (280–320 nm), and UV-A (320–400 nm) (Banaszak and Trench, 1999). UV-C, with the shortest wavelengths, is the most potentially damaging to cells but it is almost entirely absorbed by ozone and other atmospheric gases. Biological weighing functions, which estimate the effect of each wavelength on a biological process, indicate the effect of UV-B is most severe. This is because aromatic amino acids (e.g., tyrosine, phenylalanine, tryptophan) strongly absorb UV-B at 280 nm. Therefore, proteins containing these amino acids are highly susceptible to photodestruction, particularly to splitting of disulfide bridges between cysteine residues (which control the tertiary structure of proteins) (Bischof et al., 2000).

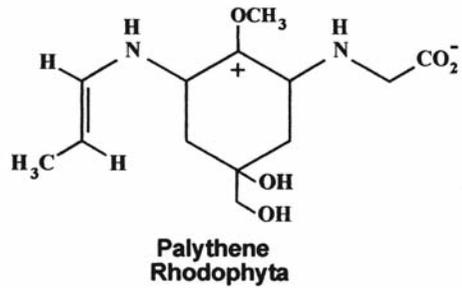
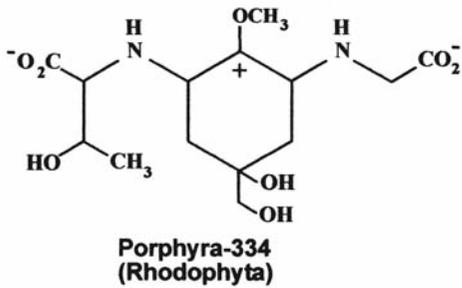
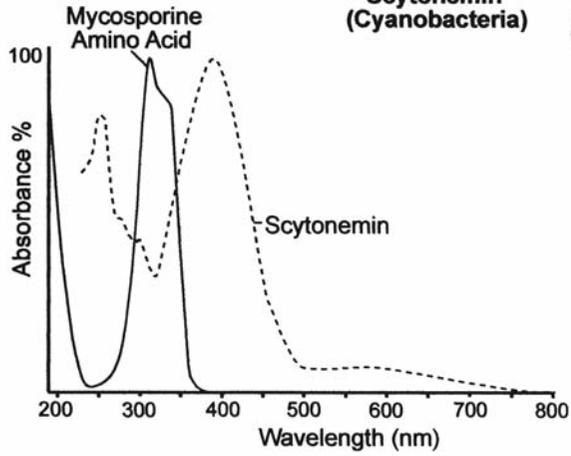
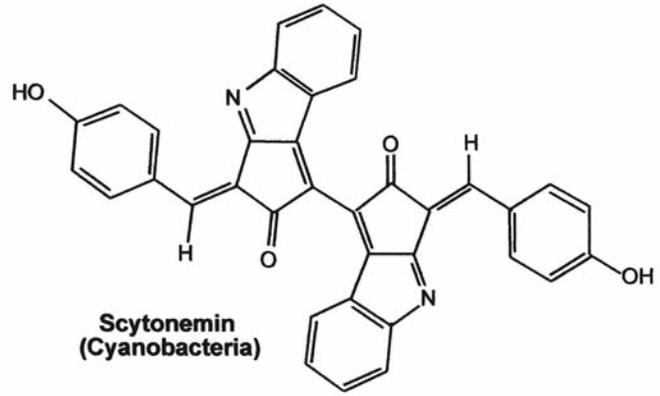
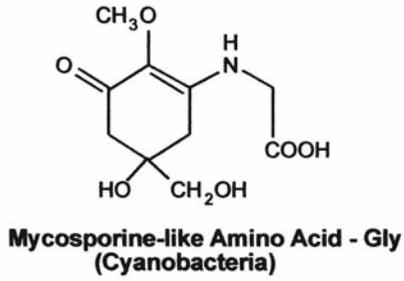
Recently there has been a depletion in the ozone layer, particularly in the polar regions, which has been at least partially attributed to chlorofluorocarbons. Springtime ozone reductions up to 60% compared with values 30 years ago have been recorded (Karsten et al., 1999). This has resulted in increased amounts of UV-B reaching the surface of the Earth.

The production of ultraviolet sunscreens by

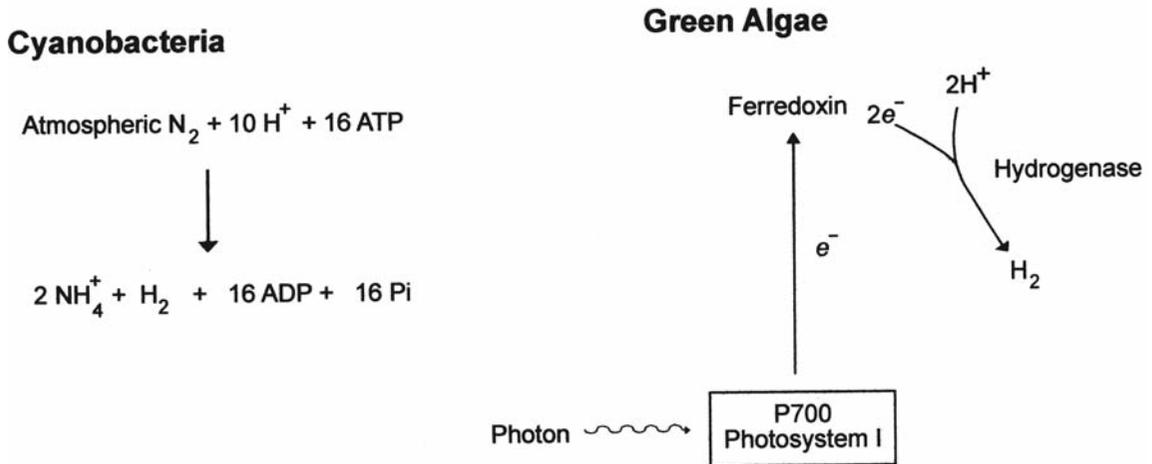
algae (especially those inhabiting shallow waters) is one mechanism the cells have evolved in dealing with damaging UV light. The most common of these UV-absorbing compounds are the mycosporine-like amino acids (MAAs) that absorb UV radiation between 310 and 360 nm (Karsten et al., 1999) (Fig. 23.9). Mycosporine is a generic term used to describe small, water-soluble, nitrogenous metabolites. The MAAs have amino groups conjugated to a cyclohexane chromophore (Fig. 23.9). The MAAs occur in many algal groups where they attenuate UV-B and reduce the harmful effects of the radiation on cells.

### Hydrogen fuel cells and hydrogen gas production by algae

Hydrogen fuel cells are an attractive and clean energy source for motor cars. Electrolysis of water is the most commonly mentioned method for generating hydrogen gas. Algae can also be induced to produce hydrogen gas. Cyanobacteria produce hydrogen as a byproduct of nitrogen fixation (Fig. 23.10). The nitrogenase enzyme, however, has a low catalytic turnover and has a high energy requirement, leading most investigators to reject it as a source of hydrogen gas (Ghiradi et al., 2000). The production of hydrogen gas by hydrogenases in green algae has been more extensively investigated (Melis et al., 2000; Ghiradi et al., 2000). In the green algae, photons of light are captured in the chlorophyll *a* in a reaction center (P700) of photosystem I, driving the potential by 1 V. Excitation of P700 results in the transfer of an electron to a membrane-bound form of ferredoxin, a 11.6 kdal iron-sulfur protein of the Fe<sub>4</sub>S<sub>4</sub> type. Hydrogenases in the green algae combine the electrons from ferredoxin with protons to produce hydrogen gas (Fig. 23.10). This is essentially an anaerobic reaction since the hydrogenase enzymes are inhibited by oxygen.



**Fig. 23.9** Some UV-B absorbing compounds that occur in algae. The absorbance spectrum of scytonemin and mycosporine amino acid is also shown. (Modified from Ehling-Schulz and Scherer, 1999.)



**Fig. 23.10** The chemical reactions that result in the formation of hydrogen gas in the cyanobacteria and green algae.

## REFERENCES

- Banaszak, A., and Trench, R. K. (1999). Ultraviolet sunscreens in dinoflagellates. *Protist* 152:93–101.
- Bischof, K., Hanelt, D., and Wiencke, C. (2000). Effects of ultraviolet radiation on photosynthesis and related enzyme reactions of marine macroalgae. *Planta* 211:555–62.
- Boyd, P. (2004). Ironing out algal issues in the Southern Ocean. *Science* 304:396–7.
- Cembella, A. D. (2003). Chemical ecology of eukaryotic microalgae in marine ecosystems. *Phycologia* 42:420–7.
- Charlson, R. J., Lovelock, J. E., Andreae, M. O., and Warren, S. G. (1987). Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature* 326:655–61.
- Chisholm, S. W., and Morel, F. M. M. (1991). What controls phytoplankton production in nutrient-rich areas of the open sea? *Limnol. Oceanog.* 36:1507–970.
- Christiansen, F. B. and Fenchel, T. M. (1979). Evolution of marine invertebrate reproductive patterns. *Theoret. Pop. Biol.* 16:267–82.
- Coale, K. H., Johnson, K. S., Fitzwater, S. E., Gordon, R. M., Tanner, S., Chavez, F. P., Ferioli, L., Sakamoto, C., Rodgers, P., Millero, F., Steinberg, P., Nightingale, P., Cooper, D., Cochlan, W. P., Landry, M. R., Constantinou, J., Rollwagen, G., Trasvina, A., and Kudela, R. (1996). A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. *Nature (Lond.)* 383:495–501.
- Cox, R. A. (1997). Atmospheric sulphur and climate – what have we learned? *Phil. Trans. Royal Soc., Biol. Sci.* 352:251–4.
- Dalton, R. (2002). Ocean tests raise doubts over use of algae as carbon sink. *Nature* 420:722.
- Ehling-Schulz, M. A., and Scherer, S. (1999). UV protection in cyanobacteria. *Eur. J. Phycol.* 34:329–38.
- Erwin, D. H. (1993). *The Great Paleozoic Crisis*. New York: Columbia University Press.
- Fisher, M., Pick, U., and Zamir, A. (1994). A salt-induced 60-kilodalton plasma membrane protein plays a potential role in extreme halotolerance of the alga *Dunaliella*. *Plant Physiol.* 106:1359–65.
- Garrison, D. L., and Siniff, D. B. (1986). An Antarctic perspective. *BioScience* 36:238–42.
- Garrison, D. L., Sullivan, C. W., and Ackley, S. F. (1986). Sea ice microbial communities in Antarctica. *BioScience* 36:243–50.
- Ghirardi, M. L., Zhang, L., Lee, J. W., et al. (2000). Microalgae: a green source of renewable  $\text{H}_2$ . *TIBTech.* 18:506–11.
- Gilbert, J. J. (1996). Effect of food availability on the response of planktonic rotifers to a toxic strain of the cyanobacterium *Anabaena flos-aquae*. *Limnol. Oceanog.* 41:1565–72.
- Hallegraeff, G. M., Andersen, D. M., and Cembella, A. D. (2003). *Manual on Harmful Marine Microalgae*. Paris: UNESCO Publishing.
- Hansen, P. J., Cembella, A. D., and Moestrup, Ø. (1992). The marine dinoflagellate *Alexandrium ostenfeldii*: paralytic shellfish toxin content, composition and toxicity to a tintinnid ciliate. *J. Phycol.* 28:597–603.

- Ianora, A., Poulet, S. A., and Miralto, A. (2003). The effects of diatoms on copepod reproduction: a review. *Phycologia* 42:351–63.
- Igarashi, T., Aritake, S., and Yasumoto, T. (1999). Mechanisms underlying the hemolytic and ichthyotoxic activities of maitotoxin. *Nat. Toxins* 7:71–9.
- Jablonski, D., and Lutz, R. A. (1983). Larval ecology of marine benthic invertebrates: paleobiological implications. *Biol. Rev.* 58:21–89.
- Jones, A., and Slingo, A. (1997). Climate model studies of sulphate aerosols and clouds. *Phil. Trans. Royal Soc., Biol. Sci.* 352:221–7.
- Karsten, U., Bischof, K., Hanelt, D., Tug, H., and Wiencke, C. (1999). The effect of ultraviolet radiation on photosynthesis and ultraviolet-absorbing substances in the endemic Arctic macroalgae *Devuleraea ramentacea* (Rhodophyta). *Physiol. Plantarum* 105:58–66.
- Keller, M. D., and Korjef-Bellows, W. (1996). Physiological aspects of the production of dimethylsulfoniopropionate (DMSP) by marine phytoplankton. In *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds*, ed. R. P. Kiene, P. T. Visscher, M. D. Keller, and G. O. Kirst, pp. 131–42. New York: Plenum Press.
- Liss, P. S., Hatton, A. D., Malin, G., Nightingale, P. D., and Turner, S. M. (1997). Marine sulphur emissions. *Phil. Trans. Royal Soc., Biol. Sci.* 352:159–67.
- Malin, G. (1996). The role of DMSP and DMS in the global sulfur cycle and climate regulation. In *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds*, ed. R. P. Kiene, P. T. Visscher, M. D. Keller, and G. O. Kirst, pp. 177–89. New York: Plenum Press.
- Martin, J. H. (1990). Glacial–interglacial CO<sub>2</sub> change. The iron hypothesis. *Paleoceanography*. 5:1–13.
- Medlin, L. K., Kooistra, W. H. C. F., Gersonde, R., Sims, P. A., and Wellbrock, U. (1997). Is the origin of diatoms related to the end-Permian mass extinction? *Nova Hedwigia* 65:1–11.
- Melis, A., Zhang, L., Forestier, M., Ghirardi, M. L., and Siebert, M. (2000). Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. *Plant Physiol.* 122:127–36.
- Morgan, R. M., Ivanov, A. G., Priscu, J. C., Maxwell, D. P., and Huner, N. P. A. (1998). Structure and composition of the photochemical apparatus of the Antarctic green alga *Chlamydomonas subcaudata*. *Photosynth. Res.* 56:303–14.
- Paffenhofer, G.-A. (2002). An assessment of the effects of diatoms on planktonic copepods. *Mar. Ecol. Progr. Ser.* 227:305–10.
- Pan, Y., Bates, S., and Cembella, A. D. (1998). Environmental stress and domoic acid production by *Pseudo-nitzschia*: a physiological perspective. *Nat. Toxins* 6:127–35.
- Pavia, H., Toth, G. B., Lindgren, A., and Aberg, P. (2003). Intraspecific variation in the phlorotannin content of the brown alga *Ascophyllum nodosum*. *Phycologia* 42:378–83.
- Pocock, T., Lachance, M.-A., Proschold, T., Priscu, J. C., Kim, S. S., and Huner, N. P. A. (2004). Identification of a psychrophilic green alga from Lake Bonney Antarctica *Chlamydomonas raudensis* Ettl. (UWO 241) Chlorophyceae. *J. Phycol.* 40:1138–48.
- Priscu, J. C. (1999). Life in the valley of the “dead”. *BioScience* 49:959.
- Priscu, J. C., Wolf, C. F., Taakacs, C. D., et al. (1999). Carbon transformations in a perennially ice-covered Antarctic lake. *BioScience* 49:997–1008.
- Savage, R. E. (1930). The influence of *Phaeocystis* on the migration of herring. *Fish. Investig., London, Ser. II* 12:5–14.
- Sepkoski, J. J. (1984). A kinetic model of Phanerozoic taxonomic diversity. III. Post Paleozoic families and mass extinctions. *Paleobiology* 10:246–67.
- Shimizu, Y. (2000). Chemistry and mechanism of action. In *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection*, ed. L. M. Botana, pp. 151–72. New York: Marcel Dekker.
- Smith, W. O., and Nelson, D. M. (1986). Importance of ice edge phytoplankton production in the Southern Ocean. *BioScience* 36:251–7.
- Spigel, R. H., and Priscu, J. C. (1996). Evolution of temperature and salt structure of Lake Bonney, a chemically stratified Antarctic lake. *Hydrobiologia* 321:177–90.
- Stoecker, D. K., Gustafson, D. E., Black, M. M. D., and Baier, C. T. (1998). Population dynamics of microalgae in the upper land-fast sea ice at a snow-free location. *J. Phycol.* 34:60–9.
- Strom, S., Wolfe, G., Slajer, A., Lambert, S., and Clough, J. (2003). Chemical defense in the microplankton. Inhibition of protist feeding by  $\beta$ -dimethylsulfoniopropionate (DMSP). *Limnol. Oceanogr.* 48:230–7.
- Valentine, J. W., and Jablonski, D. (1986). Mass extinctions: sensitivity of marine larval types. *Proc. Natl. Acad. Sci., USA* 83:6912–14.
- Wells, M. L. (1994). Pumping iron in the Pacific. *Nature (London)* 368:295–6.
- Wolfe, G. V. (2000). The chemical defense ecology of marine unicellular plankton: constraints, mechanisms, and impacts. *Biol. Bull.* 198:235–44.
- Wolfe, G. V., Steinke, M., and Kirst, G. O. (1997). Grazing-activated chemical defense in a unicellular alga. *Nature* 387:894–7.

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## Glossary

- abaxial:** located in a position away from the plant.
- acronematic flagellum:** a flagellum without hairs.
- acropetal:** toward the apex.
- action spectrum:** relative effectiveness of each stimulating light wave for the response rate.
- adaxial:** located on the side toward the main axis.
- adelphoparasite:** parasite that is closely related to the host.
- aerobic:** needing oxygen.
- aeroplankton:** air-borne microscopic organism.
- agar:** one or more polysaccharides containing sulfated galactose obtained from the walls of some red algae.
- agarophyte:** red algae used in the production of agar.
- agglutin:** chemical substance involved in the recognition of a gamete of the opposite strain.
- agglutination:** adherence of gametes of different mating types by their flagella tips.
- akinete:** thick-walled resting spore.
- alginates:** salts of polysaccharides composed of D-mannuronic and L-guluronic acids obtained from brown algae.
- alkalinity:** in water chemistry, the total quantity of base in equilibrium with carbonate or bicarbonate that can be determined by titration with strong acid. Alkaline waters have a high pH.
- allelochemical:** a chemical that functions between members of different species. Seriochemicals act between individuals of the same species.
- allelopathy:** injurious effects of one species on another.
- allomone:** a chemical excreted by a prey to avoid predation.
- alloparasites:** parasites not closely related to their host.
- allophycocyanin:** a blue biliprotein obtained from cyanobacteria and red algae.
- alpha granule:** protoplasmic structure containing myxophyceean starch in the cyanobacteria.
- alveoli:** membrane-bounded flattened vesicles or sacs underlying the plasma membrane in certain algae, particularly the dinoflagellates.
- amphiesma:** the plasma membrane and underlying flattened vesicles of dinoflagellates which, in some species, contain plates.
- amylopectin:** the storage polysaccharide in the cyanobacteria, composed of  $\alpha$ -1,4 glucoside linkages, with 1,6 linked side chains.
- amyloplast:** a colorless plastid containing starch.
- amylum star:** star-shaped aggregate of cells filled with starch that forms new plants in the stoneworts (Charales).
- anaerobic:** without oxygen.
- androsporangium:** sporangium that forms androspores.
- androspore:** spore that forms a dwarf male filament in the Oedogoniales (green algae).
- anhydrobiotic:** organisms that can withstand the removal of the bulk of their intracellular water for extended periods of time.
- anisogamy:** fusion of gametes that are unequal in size or physiology.
- antapical:** opposite from the apex.
- anterior:** the front end, forward.
- antheridium:** the sex organ in which the male gametes are formed.
- antherozoid:** male gamete.
- anthropomorphic:** related to man.
- anticlinal:** perpendicular to the circumference of the thallus.
- apical growth:** growth by means of an apical cell dividing to form the thallus beneath it.
- aplanogamete:** non-motile gamete.
- aplanospore:** non-motile spore.
- apochlorotic:** colorless.
- aponin:** Apparent Oceanic Naturally Occurring Cytolin. Chemicals that cause growth repression and mortality.
- aragonite:** orthorhombic crystals of calcium carbonate.
- areolae:** the chambers in the honeycomb arrangement of some diatom valves.
- asexual:** reproduction without the fusion of gametes.
- autospore:** aplanospore with the same shape as the parent cell.
- autotroph:** not needing an external source or organic compounds as an energy source. Energy is obtained from light or inorganic chemical reactions.
- auxiliary cell:** a cell that receives a nucleus from the zygote in the red algae.
- auxospore:** a resting cell in the diatoms that is commonly formed from the zygote.
- axenic culture:** a culture containing only one species.
- axoneme:** an axial array of (usually) nine outer doublet and two central microtubules.

- bacteriocin:** antibiotic secreted by cyanobacteria that kills related strains of cyanobacteria.
- baeocyte:** endospore produced by cyanobacteria.
- basal apparatus:** flagellar or ciliary apparatus exclusive of flagella/cilia.
- basal body (kinetosome):** cylindrical structure (ca. 0.2  $\mu\text{m}$  diameter) found at the base of a flagellum/cilium consisting of a continuation of the nine outer axonemal doublets (A,B) but with the addition of a C-microtubules to form triplets.
- basipetal:** toward the base.
- bathal zone:** ocean water over continental slope.
- benthic:** pertaining to any part of a lake or ocean bottom.
- benthos:** organisms living on, and attached to, the bottom of aquatic habitats.
- bilaterally symmetrical:** a plane through an object divides it into mirror image halves.
- biliprotein:** a red or blue pigment and attached bile (linear tetrapyrrole) chromophore in the cyanobacteria, cryptophytes, and red algae.
- biological carbon pump:** trapping of carbon dioxide in the deep levels of the ocean.
- bioluminescence:** emission of light by a living organism.
- biomass:** the amount at any one time of living organisms in a habitat.
- bisexual:** both sexes produced on the same individual.
- bisporangium:** a sporangium forming two meiospores in the red algae.
- bloom:** heavy growth of planktonic algae in a body of water.
- brackish:** saline water with a salinity less than that of seawater.
- budding:** exospore formation in the cyanobacteria.
- bulbil:** small plant formed on rhizoids in the stoneworts (charophytes).
- calcareous ooze:** bottom sediment in oceans composed of calcified remains of organisms.
- calcification:** deposition of calcium carbonate, usually in association with smaller amounts of other carbonates.
- calcite:** rhombohedral crystals of calcium carbonate.
- callose:** polysaccharide associated with pores in sieve cells.
- canal:** rigid opening in some flagellates.
- capsule:** extracellular mucilage.
- carotene:** oxygen-free, unsaturated, hydrocarbon carotenoid.
- carotenoid:** yellow, orange, or red hydrocarbon fat-soluble pigment.
- carpogonium:** female gametangium in the red algae.
- carposporangium:** carospore-producing sporangium derived directly, or indirectly, from the zygote in the red algae.
- carospore:** usually diploid spore produced by the carposporangium in the red algae.
- carosporophyte:** usually diploid generation in the red algae derived from the zygote, it forms carospores.
- carrageenan:** red algal polysaccharide (phycocolloid) similar to agar, but needing higher concentrations to form a gel.
- cellulose:** polysaccharide composed of  $\beta$ -1,4 linked glucose molecules that forms the main skeletal framework of most algal cells.
- cell wall:** a mostly rigid, often multilayered structure consisting of discrete microfibrillar polysaccharides embedded in an amorphous matrix composed of polysaccharides, lipids, and proteins, which together comprise an outermost layer of the cell proper.
- centric:** type of ornamentation arranged around a central point in the diatoms.
- centriole:** equivalent to a flagellar basal body.
- chemical ecology:** the relationship between the structure and function of metabolites and how these affect organisms in the environment, controlling the coexistence and coevolution of species.
- chemoautotroph:** an organism that obtains energy from oxidation of reduced inorganic compounds, and cell carbon primarily from carbon dioxide.
- chemotaxis:** the movement of a whole cell in response to a concentration gradient of a chemical substance. If it is toward higher concentration, it is positive; if away from a higher concentration, it is negative chemotaxis.
- chitin:** polysaccharide made up of repeating units of N-acetylglucosamine.
- chlorophyll:** fat-soluble, green, porphyrin-type pigment.
- chloroplast:** plastid with chlorophyll.
- chloroplast endoplasmic reticulum (chloroplast E. R.):** one or two membranes surrounding the chloroplast membrane.
- chlorosis:** degradation of photosynthetic pigments.
- chromatic adaption:** change in the proportions of different photosynthetic pigments enabling optimum absorption of the available wavelengths of light.
- chromoplast or chromatophore:** a chloroplast with some other color than green.
- chrysolaminarin or leucosin:** a liquid polysaccharide storage product composed principally of  $\beta$ -1,3 linked residues of glucose.

- ciliary/flagellar matrix:** the cytosol of the flagellum/cilium, often lacking structural detail.
- cilium (flagellum):** a long, cylindrical extension of a eukaryotic cell, bounded by the plasma membrane and containing an axoneme. A flagellum/cilium is a motility organelle that is mainly involved in cell movement by means of water propulsion, but can perform additional functions, such as feeding, mating, and sensory perception.
- cingulum or girdle:** transverse furrow in the dinoflagellates containing the transverse flagellum.
- circadian rhythm:** repeated sequence of events that occur about 24-hour intervals.
- circein:** female hormone secreted by oogonia in the green alga *Oedogonium*.
- cirri:** curled appendages on a zygote.
- clone:** the group of individuals derived from a single individual.
- coccolith:** spherical.
- coccolith:** calcareous scale or plate-like particle deposited on the surface membrane of some prymnesiophytes (the coccolithophorids), varying in complexity and surface decoration according to species.
- coenobium:** colony of algal cells in a specific arrangement and number that is fixed at the time of origin and is not subsequently augmented.
- coenocyte:** large multinucleate cell without cross walls, except where reproductive bodies are concerned.
- colony:** a group of unicells which cohere and remain together as a unit.
- commensalism:** a situation where one species benefits from an arrangement, while the other species does not benefit, nor does the second species suffer.
- compensation depth:** depth of water where there is sufficient light so photosynthesis equals respiration over a 24-hour period.
- compensation point:** light intensity at which respiration equals photosynthesis over a 24-hour period.
- conceptacle:** cavity in the thallus where gametangia are produced.
- conjugation:** fusion of two non-flagellated protoplasts.
- connecting band:** part of the girdle in diatoms.
- contractile vacuole:** vacuole fed by smaller vesicles that expels water and solutes rhythmically outside the cell.
- coralline:** reference to calcified algae.
- cornuate process:** horn-like wall extension.
- corona:** crown.
- Corps de Maupas:** a vesicular body in the cryptophytes used in digestion of unwanted cell components.
- cortex:** the outer portion or layer(s) of a protist cell, including the plasma membrane, but excluding secreted non-living structures that may lie outside the plasma membrane. The outer portion of an algal thallus.
- cosmopolitan:** occurring in many diverse places.
- costa:** an elongated, solid thickening of a diatom valve.
- crenulate:** wavy with small teeth or scallops.
- cribellum:** small sieve plate covering the pores in a cribrum in the diatoms.
- cribrum:** silicified plate with tiny pores covering the holes of the valve in the diatoms.
- cross fertilization:** the union of gametes from different thalli.
- cruciate:** cross-shaped.
- cryoplankton:** plankton of polar or cold regions.
- cryptobiotic crust:** accumulations of cyanobacteria, lichens, fungi, and/or mosses in desert soils.
- cryptoendolith:** organism that lives inside rocks.
- cryptostomata or cryptoblast:** flask-like opening in the thallus, with hairs.
- cuticle:** a thin hydrophobic layer deposited on the outside surface of the cell wall.
- cyanelle:** endosymbiotic cyanobacterium.
- cyanobacterocin:** an antibiotic produced by a cyanobacterium that inhibits growth of related cyanobacteria.
- cyanoglobulin:** myoglobin-like molecule capable of scavenging oxygen in heterocysts of cyanobacteria.
- cyanome:** host cell containing a cyanelle.
- cyanophage:** virus in cells of the cyanobacteria.
- cyanophycin granule:** polypeptide storage granule in the cyanobacteria.
- cyst:** a non-motile, often dehydrated, resistant, inactive dormant stage in the life cycle.
- cystocarp:** in the red algae, the carposporophyte and surrounding gametophytic tissue (pericarp).
- cytokinesis:** division of the cytoplasm usually right after karyokinesis (nuclear division).
- cytoskeleton:** intracellular network of protein filaments that is insoluble in non-ionic detergents.
- cytosome:** cell opening used for ingestion of food particles.
- dendricule:** short protoplasmic extension with no organelles.
- dendroid:** a type of non-motile colony that produces mucilage in one area, usually forming a stalk.
- detritus:** particulate organic matter.
- dextrotropic:** clockwise when seen from the cell apex (the opposite is *leiotropic*).

- diastole:** filling of a contractile vacuole (*systole* is the emptying).
- diatomaceous earth:** a mineral consisting of the remains of the silicified frustules of diatoms.
- diazocyte:** cell capable of fixing nitrogen.
- diazotroph:** organism capable of fixing atmospheric N<sub>2</sub> into ammonium.
- dichotomy:** division of a thallus into two equal branches.
- dictyosome:** stack of vesicles in a Golgi apparatus.
- diel:** occurring over a 24-hour period.
- diffuse growth:** type of growth where most of the cells in a thallus are capable of division.
- dioecious:** an organism that has male and female gametes borne on separate plants.
- diplobiontic:** having two separate stages in the life cycle.
- diplohaplont:** an organism having a separate multicellular diploid and multicellular haploid stage.
- diploid:** possessing two sets of chromosomes.
- diplont:** an organism in which the haploid stage of the life cycle consists of only the gametes.
- disc or thylakoid:** membrane-bound sac in the chloroplast.
- discobolocyst:** type of projectile in the Chrysophyceae.
- distichous:** arranged in two equal rows.
- distromatic:** thallus that is two cells thick.
- diurnal:** daily.
- dorsiventral:** flattened in one plane.
- dulse:** a preparation of *Rhodymenia* (red alga) used as food.
- ecad:** a form of a plant species produced in response to a particular habitat, the modifications not being inheritable.
- ecdysis:** shedding of the theca in the dinoflagellates.
- ecotype:** a locally adapted variant.
- ectoderm:** outer protoplasm next to the plasma membrane in the Charales.
- egg:** large non-motile female gamete.
- ejectosome:** type of projectile found in green algae and cryptomonads.
- electron-dense or electron-opaque:** term used to describe a material that absorbs electrons and appears dark in electron micrographs.
- electron-transparent:** term used to describe a material that does not absorb electrons and appears light in electron micrographs.
- eleutheroschisis:** parent cell wall completely cast off and new daughter cell walls formed.
- endemic:** occurring in a specific area.
- endochite:** inner wall of the oogonium in the Fucales (Phaeophyceae).
- endoderm:** inner protoplasm next to the vacuole in the Charales, capable of cytoplasmic streaming.
- endolithic:** living inside rock.
- endophyte:** plant living inside another plant.
- endosome:** nucleus in the euglenoids.
- endospore:** asexual spore produced by some cyanobacteria.
- endosymbiotic:** term that describes an organism living inside a host in a symbiosis.
- endozoic:** living inside an animal.
- enucleate:** without a nucleus.
- envelope:** a general term used variously for such structures as plasma membranes, pellicles, walls, sheaths, and gelatinous coverings.
- epicone:** part of the cell above the girdle in the dinoflagellates.
- epidermis:** outer layer of cells.
- epipellic:** growing on mud.
- epiphyte:** one plant living on another plant.
- epitheca:** larger of the two halves of a diatom frustule.
- epizoic:** living on an animal.
- epontic:** living on the bottom of ice.
- estuary:** the mouth of a river where tidal effects are evident, and where freshwater and seawater mix.
- euendolith:** an organism that bores into rock.
- eukaryotic or eucaryotic:** cells with a membrane-bounded nucleus.
- euphotic or photic zone:** water above the compensation depth.
- euryhaline (euryhaline):** tolerant of a wide salinity range.
- eurythermic:** tolerant of a wide temperature range.
- eutrophic:** term that described a body of water that receives large amounts of nutrients, usually resulting in a large growth of algae.
- exocite:** outer wall of an oogonium of the Fucales (Phaeophyceae).
- exotoxin:** toxin secreted into the medium.
- extant:** living today.
- extinct:** not living today.
- extracellular matrix:** mucilaginous glycoproteins external to the plasma membrane.
- eyespot:** red to orange area in a cell, composed of lipid droplets.
- facultative heterotroph or autotroph:** organism that is able to live as a heterotroph or an autotroph.
- facultative parasite or saprophyte:** organism that is able to live as a parasite or a saprophyte.

- false branching:** in the cyanobacteria, breakage of a trichome through a sheath, giving the appearance of a branch.
- fibulae:** in diatoms, small nodules in rows on the inside of the valve.
- filament:** in the cyanobacteria, one or more trichomes enclosed in a sheath.
- flagellar apparatus (kinetid):** an organellar complex consisting of one or more basal bodies/kinetosomes that may bear flagella/cilia, may have microtubular and fibrous roots associated with their bases, and may function in locomotion, feeding, sensation, and reproduction.
- flagellar/ciliary matrix:** the cytosol of the flagellum/cilium, often lacking structural detail.
- flagellar hairs:** filamentous appendages usually arranged in one or more rows but not covering the entire surface of a flagellum/cilium. There are two types of flagellar hairs. (1) Tubular flagellar hairs consisting of at least a hollow shaft (>15 nm diameter), often with a cylindrical shaft and one or more terminal filaments. (2) Non-tubular flagellar hairs, assembled in the Golgi apparatus and consisting primarily of carbohydrates, forming two rows along the length of the flagellum, and attached through the flagellar membrane to specific outer doublets.
- flagellar/ciliary roots:** fibrous, microtubular or amorphous structures originating at or near basal bodies/kinetosomes and terminating somewhere else in the cell but not at nearby basal bodies/kinetosomes.
- flagellar scales:** organic structures of discrete size and shape, often covering the whole surface of the flagellum/cilium and generally assembled in the Golgi apparatus.
- flagellate:** a unicell having at least one flagellum.
- flagellum (cilium):** a long, cylindrical extension of a eukaryotic cell, bounded by the plasma membrane and containing an axoneme. A flagellum/cilium is a motility organelle that is mainly involved in cell movement by means of water propulsion, but can perform additional functions, such as feeding, mating, and sensory perception.
- floridean starch:** red algal storage product composed of  $\alpha$ -1,4 and  $\alpha$ -1,6 linked glucose residues.
- floridoside:** primary product of photosynthesis in the Rhodophyta.
- foliose:** leaf-like.
- foramen:** chamber or hole.
- fragmentation:** type of asexual reproduction where a thallus breaks into two or more parts, each of which forms a new thallus.
- frustule:** silicified cell wall in the diatoms.
- fucoidin:** polysaccharide in the cell wall and mucilage of the brown algae composed of sulfated fucose units.
- fucosan or phaeophycean tannin:** a colorless acidic fluid in brown algae, giving a characteristic red color with vanillin hydrochloride.
- fucoserraten:** a sex attractant secreted by the eggs of *Fucus* brown algae.
- funori:** a commercially produced phycocolloid of red algae used as a glue.
- fusiform:** spindle-shaped.
- gametangium:** structure forming gametes.
- gamete:** cell capable of fusion with another to form a zygote.
- gametogenesis:** the formation of gametes.
- gametophyte:** plant generation that forms the gametes, usually haploid.
- gamone:** a chemical involved in the attraction of one gamete to another.
- gas vacuole:** a collection of gas vesicles.
- gas vesicle:** hollow cylindrical gas-filled structures in the Cyanophyta.
- generative auxiliary cell:** cell in the Rhodophyta forming the gonimoblast filaments.
- geotaxis:** movement of a cell away (negative geotaxis) or toward (positive geotaxis) gravity.
- girdle:** the transverse groove containing the transverse flagellum in the Dinophyta.
- girdle band:** part of a diatom wall where the theca overlap; a band of thylakoids running parallel under the chloroplast envelope.
- gliding:** active movement of an organism in contact with a solid substrate where there is neither a visible organ responsible for the movement nor a distinct change in the shape of the organism.
- globule:** male reproductive structure in the Charales.
- glycocalyx:** sticky polysaccharide secreted by the zygote in the Fucales (Phaeophyceae).
- glycogen:** a storage polysaccharide related to amylopectin and stains red-purple with iodine.
- glycopeptide or glycoprotein:** polysaccharide composed of sugars and amino acids or peptides.
- glycoside:** a polysaccharide composed of glucose.
- gonidium:** a cell that divides to form a daughter colony.
- gonimoblast:** usually diploid cells that form the carposporangia in the Rhodophyta.
- gonoid:** type of ornamentation that is dominated by angles in the diatoms.

- granum:** stack of thylakoids in a chloroplast.
- gullet:** anterior of invagination in euglenoids and cryptomonads.
- gyrogonite:** fossilized nucleole of the Charales.
- hair:** appendage on a flagellum; colorless elongate cell.
- haplobiontic:** having only one multicellular stage.
- haploid:** having one complete set of chromosomes.
- haplont:** an organism in which the only diploid stage is the zygote.
- hapteron or holdfast:** bottom part of an alga that attaches the plant to the substrate.
- haptonema:** appendage that arises between the flagella in the Prymnesiophyta.
- heleoplankton:** plankton that grow in marshy areas or in small ponds.
- hematochrome:** red or orange lipid bodies occurring outside the chloroplast.
- hermaphroditic or homothallic:** producing both male and female gametangia on the same thallus.
- heterocyst:** thick-walled, hollow-looking enlarged cell in the cyanobacteria.
- heterokont:** having flagella of unequal length.
- heteromorphic alternation of generations:** having haploid and diploid generations of different morphology.
- heterothallic:** producing male and female gametangia on different plants.
- heterotrichous:** term used to describe division of a plant into an erect and a prostrate part.
- heterotrophic:** needing an external source of organic compounds as an energy source.
- histone:** basic protein.
- holdfast:** part of an alga that attaches a plant to a substrate.
- holophytic or autotrophic:** needing only light and inorganic substances for growth.
- holozoic or phagocytic:** absorbing food particles whole into food vesicles for digestion.
- homothallic:** producing male and female plants on the same plant.
- hormogonium:** short pieces of a trichome in the cyanobacteria that become detached from the parent filament and move away by gliding, subsequently developing into new filaments.
- hyaline:** transparent.
- hydrophilic:** water-attracting.
- hydrophobic:** water-repelling.
- hypersaline:** greater than normal salinity.
- hypha:** long slender cell in the medulla of Laminariales (brown algae).
- hypnospore:** aplanospore with a greatly thickened cell wall.
- hypocone:** lower part of the cell in the Dinophyta, usually having a longitudinal sulcus.
- hyrogenous cells:** in the Rhodophyta, those cells under the carpogonium.
- hypolimnion:** water beneath the thermocline in thermally stratified water bodies.
- hypothallus:** lower part of the thallus composed of large cells in the coralline reds.
- hypotheca:** smaller half of a diatom frustule.
- hystrichospore or hystrichosphaerid:** fossilized resting spore in the Dinophyta.
- ichthyotoxin:** toxin that kills gill-bearing animals.
- intercalary:** in between two cells or tissues.
- intercalary bands:** bands between the valve and girdle band in the diatoms.
- internode:** part of axis between nodes.
- interstitial water:** that water trapped between particles of soil or mud.
- intertidal:** occurring between the low- and high-tide marks.
- intraflagellar transport:** the bidirectional movement of particles along the length of the flagellum between the axoneme and the flagellar membrane.
- inversion:** phenomenon in the green algae in which a colony turns itself inside out through a pore.
- iridescence:** the play of colors caused by refraction and interference of light waves at the surface.
- isoagglutination:** adhesion of flagella of the same sex when a mating-type substance of the opposite sex is added.
- isoenzyme:** enzymes having the same function but of a somewhat different structure.
- isogamy:** fusion of similar gametes.
- isokont:** cell with flagella of the same length.
- isomorphic alternation of generations:** generations that are morphologically alike.
- isthmus:** a passage connecting two bodies.
- kairomone:** a chemical that affects predator-prey interactions, inducing changes in prey behavior, morphology, or life history via compounds secreted by the predator.
- karyogamy:** fusion of two gamete nuclei.
- karyokinesis:** division of the nucleus.
- keel:** in pennate diatoms, an extension of the valve running lengthwise, similar to the keel of a ship.
- kelp:** a member of the Laminariales (brown algae); also used for the burnt ash of plants of the Laminariales.
- kerogen:** yellow-brown amorphous organic matter in sedimentary deposits.

- kinesin:** a mechanochemical protein capable of utilizing chemical energy from ATP hydrolysis to generate mechanical force.
- kinetic movement:** change in rate of movement. Ortho kinetic response is a decrease in swimming speed. Kline kinetic response is an increase in swimming speed.
- kinetid (flagellar apparatus):** an organellar complex consisting of one or more basal bodies/kinetosomes that may bear flagella/cilia, may have microtubular and fibrous roots associated with their bases, and may function in locomotion, feeding, sensation, and reproduction.
- klino kinetic response:** increase in swimming speed.
- kleptoplasty:** the capture of a chloroplast by a phagocytic organism.
- kombu (Japanese):** vegetable made from Laminariales (Phaeophyta).
- labiate process:** in diatoms, an appendage at the periphery of the valve through which mucilage may be secreted, functions in the movements of centric diatoms.
- laminarin:** food storage polysaccharide in the brown algae composed principally of  $\beta$ -1,3 linked glucose residues.
- lamine:** flat.
- laver or laver bread:** similar to Japanese *nori*, vegetable made from dried *Porphyra* (Rhodophyta).
- leiotropic:** counterclockwise when seen from the cell apex (opposite is dexiotropic).
- lentic:** related to a pond or lake.
- lessepsian migration:** migration of species through the Suez Canal. Derived from Ferdinand de Lesseps who designed the canal.
- leucosin or chrysolaminarin:** food storage polysaccharide of golden-brown algae composed mostly of  $\beta$ -1,3 linked glucose residues.
- leucoplast:** colorless plastid usually having a large number of starch grains and few thylakoids.
- list:** extension of the theca in the Dinophyta.
- lithophyte:** plant growing on rock.
- lithotrophic or autotrophic:** needing only light and/or inorganic compounds for growth.
- littoral zone:** zone from the water's edge to a water depth of about 6 m or the maximum depth of rooted vegetation, if any exists.
- loculus:** hexagonal chamber in the wall of diatoms.
- log phase of growth:** growth phase characterized by rapidly dividing cells.
- lorica:** an envelope around the protoplast, not attached to the protoplast as the wall is.
- lotic:** related to rivers or streams.
- luciferase:** enzyme that oxidizes luciferin.
- luciferin:** compound responsible for bioluminescence.
- lysosome:** single-membrane-bounded cytoplasmic particle containing destructive enzymes.
- macrandous:** species in the Oedogoniales (green algae) that do not produce dwarf male filaments.
- macroplankton:** plankton larger than 75  $\mu$ m.
- mäerl:** coralline red algae applied to soil to increase the pH of the soil.
- mannan:** polysaccharide composed of mannose residues.
- mannitol:** sugar alcohol,  $C_6H_{14}O_6$ ; primary product of photosynthesis in the brown algae.
- mantle or valve jacket:** part of a valve in the diatoms that is bent inward.
- maerl:** environment made up of coralline algae. Also refers to fertilizer manufactured by grinding up coralline algae.
- marine snow:** macroscopic organic aggregates (greater than 3 mm) in marine ecosystems produced by biological and chemical processes.
- marl:** deposits of calcium and magnesium carbonate.
- mastigoneme or hair:** filamentous appendage of a flagellum.
- mating-type reaction:** flagella adhesion between gametes of different sexes.
- mating structure:** a dense plate in the anterior part of the protoplasm of a gamete that determines its sex.
- medulla:** inner part of algal thallus, usually composed of packed colorless filaments.
- meiocyte:** a cell which undergoes meiosis.
- meiosis:** cell division in which the chromosome number is halved.
- meiosporangium:** structure in which spores are produced by meiosis.
- meiospore:** spore formed by meiosis.
- meristem:** dividing tissue that forms new cells.
- meristoderm:** dividing layer of cells in the brown algae.
- mesochite:** middle wall of an oogonium of the Fucales (brown algae).
- metachromatic granule:** protoplasmic body containing stored polyphosphate in the cyanobacteria.
- microaerophilic:** with small amounts of oxygen.
- microfibril:** crystalline anhydrous cellulose found in many algal cell walls.
- microfilament:** submicroscopic solid filament in protoplasm.
- micrometer ( $\mu$ m):**  $10^{-6}$  m, 1  $\mu$ m equals 1 micron.

- microtubule:** submicroscopic tubule in the protoplasm.
- mitosis:** nuclear division resulting in two daughter nuclei which are genetically identical to their parent.
- mitosporangium:** a sporangium in which spores are produced by mitosis.
- mitospore:** a spore produced as a direct result of mitosis.
- mixotroph** or **facultative heterotroph:** photosynthetic organism capable of using organic compounds in the medium.
- monoecious** or **homothallic:** having male and female gametangia borne on the same plant.
- monopodial:** having one main axis of growth.
- monosporangium:** a sporangium that forms a monospore in the Rhodophyta.
- monospore:** asexual spore that germinates to re-form the parent in the Rhodophyta.
- monostromatic:** a thallus only one cell thick.
- muciferous body:** a body, usually in the outer protoplasm of a cell, that discharges mucilage, usually explosively.
- mucilage canal:** canal present in some brown algae, composed of elongated cells in the cortex area that secrete mucilage.
- mucopetide:** polysaccharide of the walls of the cyanobacteria, composed of sugars and amino acids.
- multiaxial:** having an axis with a number of apical cells that give rise to a number of nearly parallel filaments.
- multicellular:** composed of many cells.
- multiseriate:** with more than one row of cells.
- mutualism:** a situation where two organisms benefit equally.
- myxophyccean starch:** storage polysaccharide of the cyanobacteria, similar to glycogen.
- nannandrous:** in the Oedogoniales (green algae), producing dwarf males.
- nannoplankton** or **nanoplankton:** plankton smaller than 75  $\mu\text{m}$  but larger than 2  $\mu\text{m}$ .
- nanometer (nm):**  $10^{-9}$  meter, 1 nm equals 10 angstrom units.
- necridium** or **separation disc:** a cell that dies in a trichome of the cyanobacteria resulting in the formation of a hormogonium from part of the trichome.
- nemathecium:** wart-like surface elevation containing the reproductive structures in the Rhodophyta.
- neritic region:** ocean water over the bottom that extends from the high-tide mark to a depth of 200 m.
- net plankton** or **macroplankton:** plankton larger than 75  $\mu\text{m}$ .
- niche:** the function of an organism within a community.
- nitrogen fixation:** the intracellular fixation of nitrogen gas from the atmosphere to ammonia in cyanobacteria.
- node:** part of the thallus that bears branches.
- nori** or **laver:** vegetable made from dried *Porphyra* (Rhodophyceae) in Japan, similar to laver bread.
- nucule:** female reproductive structure in the Charales.
- oceanic region:** open seas beyond 200-m bottom depth.
- oligotrophic:** term describing a body of water low in nutrients.
- ooblast** or **connecting filament:** a filament produced by the zygote that fuses with an auxiliary cell in the Rhodophyta.
- oogamy:** fusion of a large non-motile egg with a small motile sperm.
- oogonium:** single-celled female gametangium.
- oospore** or **zygospore:** thick-walled zygote with food reserves.
- opsin:** protein (apoprotein) part of a photoreceptor such as rhodopsin. The opsin is bound by a lysine group to the chromophore (colored compound) which is commonly retinal.
- organelle:** a membrane-bounded part of a cell.
- ortho kinetic response:** decrease in swimming speed.
- osmotrophic:** term describing a heterotrophic organism that absorbs organic molecules in a soluble form.
- ostiole:** an opening to the outside in a conceptacle.
- overtum:** phenomenon in a body of water in which the surface water becomes colder than the bottom water, causing the surface water to sink and resulting in a mixing of the water column.
- ovoid:** spherical (0.2–2 mm in diameter), concentrically laminated, carbonate grains that form by carbonate accretion in agitated, shallow tropical marine environments.
- ovum** or **egg:** non-motile large female gamete.
- palintomy:** repeated binary fission of a cell without an intermediate stage of nutrition and growth.
- pallium:** a pseudopod projection used for feeding in thecate heterotrophic dinoflagellates.
- palmelloid:** term describing a colony of an indefinite number of single, non-motile cells in a mucilaginous matrix.
- pantonematic** or **tinsel flagellum:** flagellum with hairs attached to the surface.
- papilla:** a small rounded protuberance.

- paraflagellar** or **paracrystalline body**: photoreceptor in the Eugleophyta consisting of a crystalline swelling in one of the flagella.
- paramylon**: storage polysaccharide composed of  $\beta$ -1,3 linked glucose molecules.
- paraphysis**: sterile structure found with sporangia or gametangia.
- parasite**: heterotrophic organism that derives nutrients from a living host.
- parasporangium**: sporangium producing more than one asexual spore in the Rhodophyta.
- paraxonemal body**: proteinaceous structure restricted to a certain area along the flagellum/cilium.
- paraxonemal rod**: long cylindrical structure (solid or hollow) that extends nearly the entire length of a flagellum/cilium, located between the axoneme and flagellar membrane, and usually connected to the axoneme and flagellar membrane by specific links.
- parenchyma**: a tissue formed of thin-walled living cells produced by division in three planes.
- parietal**: peripheral.
- parthenogenetic**: germination of an egg without fertilization to form a new plant.
- pedicel**: supporting structure of a reproductive tissue.
- peduncle**: tail-like process with a central core of microtubules.
- pelagic**: living in the open ocean or oceanic region; in some definitions, living at or near the surface of the open sea.
- pellicle**: proteinaceous outer covering in the Euglenophyta.
- pennate**: term describing type of ornamentation arranged on either side of a central line in the diatoms (bilateral symmetry).
- peptidoglycan**: polysaccharide composed of sugars and amino acids in the walls of the cyanobacteria.
- pericentral cell**: a small cell formed around a central axis.
- periclinal**: parallel to the circumference of the surface.
- peridinin**: a xanthophyll in dinoflagellate cells.
- periphyton**: organism attached to submerged vegetation.
- periplast**: outer cell covering in the Cryptophyta.
- perithallus**: outer part of the thallus in the coralline Rhodophyta.
- perizonium**: siliceous wall of auxospore in diatoms, consisting of multiple overlapping bands.
- phaeophycean tannin** or **fucosan**: colorless, acidic fluid in physodes in the brown algae.
- phagotrophic** or **holozoic**: ingesting solid food particles into a food vesicle for digestion.
- pheromone**: compound that functions as a sexual attractant or acts as a trigger for reproductive behavior.
- phialopore**: hole in an inverted daughter colony in the Volvocales (Chlorophyta).
- photic** or **euphotic zone**: depth of water above the compensation depth.
- photoautotrophic**: term describing an autotrophic plant that obtains energy from photosynthesis.
- photoheterotroph**: organism capable of using organic compounds as a source of carbon in the light but not in the dark.
- photokinesis**: change in the rate of movement (swimming speed or frequency of change of direction).
- photoperiodic time measurement**: ability of plants and animals to sense the season of the year by measuring the duration of the day or night in the natural environment and respond appropriately so as to adapt to seasonal changes in the environment.
- photophobic reaction**: cessation of movement, followed by a change in swimming direction in response to an increase or decrease in photon irradiance.
- photoreceptor**: the part of the cell that receives the stimulus in phototaxis, usually a dense area in a flagellar swelling.
- photosynthate**: organic product of photosynthesis.
- phototaxis**: movement that is affected by the direction and intensity of the light.
- phragmoplast**: wall formation by the coalescence of Golgi vesicles between spindle microtubules.
- phycobiliprotein** or **phycobilin**: water-soluble blue-green or pink pigment in the cyanobacteria, Rhodophyta, and Cryptophyta.
- phycobilisome**: an aggregation of phycobiliproteins on the surface of a thylakoid.
- phycobiont**: algal partner in a lichen.
- phycocolloid**: polysaccharide colloid formed by an alga.
- phycocyanin**: blue-green-colored phycobiliprotein.
- phycoerythrin**: pink-colored phycobiliprotein.
- phycomata**: walled cyst produced by unicellular green algae.
- phycophaein**: black oxidized phaeophycean tannins.
- phycoplast**: type of cell division in which the mitotic spindle disperses after nuclear division with the two daughter nuclei coming close together, another set of microtubules arising perpendicular to the former position of the microtubules of the mitotic spindle, and the new cell wall forming along these microtubules.
- physode**: vesicle containing phaeophycean tannins in the brown algae.

- phytochrome:** photoperiod-regulating chemical.
- phytoplankton:** plants that float aimlessly or swim too feebly to maintain a constant position against a water current.
- picoplankton:** plankton that will pass through a filter with pores 2  $\mu\text{m}$  in diameter but not through a filter with pores of 0.2  $\mu\text{m}$  in diameter.
- pili:** proteinaceous appendages on the surface of cyanobacterial cells.
- pit connection:** a continuous area between two red algal cells consisting of an aperture in a cross wall, a plug, and a plug cap.
- placoderm desmids:** desmids which have two semi-cells joined by a narrow isthmus (contrasted to a sacoderm desmid without semicells).
- plakea:** flat plate of cells in the Volvocales (Chlorophyta).
- plankton:** organisms that float aimlessly or swim too feebly to maintain a constant position against a water current.
- planogamete:** motile gamete.
- planospore:** motile spore.
- planozygote:** motile zygote.
- plasma membrane (plasmalemma):** the outermost living membrane of a cell.
- plasmodesma (plural plasmodesmata):** the minute cytoplasmic threads that extend through openings in cell walls and connect the protoplasts of adjacent living cells.
- plasmogamy:** fusion of protoplasm without fusion of nuclei (karyogamy).
- plastid:** double-membrane-bounded organelle usually containing the photosynthetic apparatus or some part of it.
- pleomorphic:** having more than one shape.
- plesiomorphic:** primitive evolutionary feature.
- plethysmothallus:** a stage composed of filaments or compacted filaments that can multiply itself by spores.
- plurilocular sporangium:** many-chambered sporangium in the brown algae, each chamber forming one swarmer.
- pneumatocyst or air bladder:** expanded part of thallus containing gases.
- polar nodule:** wall swelling near the end of a cell in diatoms.
- polyglucan granule:** protoplasmic structure containing the storage product in the cyanobacteria.
- polyhedral body:** protoplasmic structure in the cyanobacteria associated with DNA microfibrils; it may contain the carbon dioxide-fixing enzyme ribulose-1,5-bisphosphate carboxylase.
- polymorphic:** having more than one shape.
- polyol:** sugar alcohol.
- polyphosphate body:** protoplasmic structure containing stored phosphate in the cyanobacteria.
- polysiphonous:** term describing thallus made up of vertical files of parallel cells.
- polystichous:** a type of parenchymatous growth in the brown algae.
- pore:** a single hole.
- pore membrane:** part of wall over a loculus in diatoms.
- poroid:** pore occluded by a plate in diatoms.
- practical salinity unit (PSU):** conductivity of water relative to a standard KCl solution; seawater is about 35 PSU.
- primary producer:** photosynthetic plant.
- proboscis:** microtubules in the anterior part of a sperm, probably associated with the chemotactic response.
- procarp:** association of carpogonium and auxiliary cells in the Rhodophyta.
- process:** extension of a wall.
- productivity:** change in biomass per unit time.
- profundal zone:** part of a lake beneath the compensation depth.
- prokaryotic cell:** a type of cell lacking membrane-bounded organelles.
- prolamellar body:** a body composed of membrane-bounded connected tubules in dark-grown chloroplasts.
- promeristem:** a non-dividing apical cell controlling the division of a number of smaller promeristematic cells beneath it.
- propagulum:** branchlets that fall off and form new plants in the Sphacelariales (brown algae).
- properizonium:** secondary auxospore wall built of siliceous rings produced inside the primary scaly wall of diatoms.
- proplastid:** a small plastid that usually matures to a chloroplast or amyloplast.
- pseudocilia:** non-functional flagella.
- pseudoparenchyma:** densely packed filaments resembling parenchyma tissue.
- pseudoraphe:** unornamented area on a theca of diatoms where a raphe would occur.
- psychrophile:** organism able to grow at temperatures less than 15 °C.
- psychrotroph:** organism able to tolerate severe winter conditions and then grow in the warmer summer months.
- puncta:** opening in the frustule in diatoms, either a pore or a loculus.

- pusule:** a structure in the Dinophyta that is associated with the expulsion of excess protoplasmic water.
- pyrenoid:** proteinaceous area of the chloroplast associated with the formation of storage product.
- pyriform:** shaped like a pear.
- radial:** occurring around a central point.
- radially symmetrical:** when an object is cut in half, the two halves are superimposable by folding over at the plane of section.
- ramulus:** reproductive branch.
- raphe:** longitudinal slit in the valve of some diatoms.
- receptacle:** swollen tip of thallus containing conceptacles in the Fucales (brown algae).
- red tide:** water with a large number of dinoflagellates or other organisms that color the water red.
- reservoir:** large empty space at the bottom of a canal in some flagellates.
- resting cell:** cell with the same morphology as vegetative cells in diatoms, but with a large amount of lipid and reduced size of organelles.
- resting spore:** thick-walled cell resistant to unfavorable environmental conditions.
- reticulate:** net-like.
- retinal:** chromophore group (colored compound) that is bound by a lysine residue to the opsin (protein) part of a photoreceptor such as rhodopsin.
- rhabdosome:** an inclusion in dinoflagellates of the order Dinophysales that resembles a trichocyst but does not function as a projectile.
- rhizoid:** root-like filament without vascular tissue.
- rhizoplast:** flagellar root composed of microfibrils that are often contractile.
- rhizopodium:** long delicate cytoplasmic protrusion.
- rhizostyle:** flagellar root composed of microtubules and associated structures.
- rodolith (maerl):** bed of coralline algae that lack uncalcified joints.
- rhodopsin:** photoreceptor in many algal cells.  
Rhodopsin consists of the chromophore (colored compound) retinal bound to a protein (apoprotein).
- rib:** extension of the cell wall.
- saccoderm desmid:** a desmid without semicells (contrasted to a placcoderm desmid with semicells).
- saprophyte:** heterotrophic organism living off dead material.
- scale:** an organic or inorganic cell-surface element of variable geometry, distributed individually or arranged in a pattern sometimes forming an envelope around the cell.
- scintillon:** particle associated with bioluminescence.
- scytonemin:** pigment that accumulates in the sheaths of cyanobacteria acting as a sunscreen to reduce the amount of near ultraviolet (370–384  $\mu\text{m}$ ) reaching the protoplasm.
- separation disc or necridium:** a cell that dies in a trichome of the cyanobacteria, resulting in the separation of a hormogonium from part of the thallus.
- septum:** cross wall.
- seriochemical:** a chemical that acts between individuals of the same species. Allelochemicals function between members of different species.
- serrate:** toothed as in a saw.
- sessile:** lacking a stalk.
- seta or awn:** elongated hollow wall extension.
- sheath:** extracellular mucilage.
- siderophore:** secondary hydroxamate secreted by some cyanobacteria to solubilize external iron compounds.
- sieve cell:** cell in the medulla of the Laminariales (brown algae), involved in active transport of organic molecules.
- sieve membrane:** wall structure covering a loculus in diatoms.
- sieve plate:** end wall in a sieve cell with pores through which the cytoplasm is continuous (plasmodesmata).
- silica deposition vesicle:** vesicle in which silica is deposited.
- silicalemma:** membrane of a silica deposition vesicle.
- sinus:** the incision in the midregion of a desmid cell (green algae).
- siphonaceous or siphonous or coenocytic cells:** large multinucleate cells without cross walls except when reproductive bodies are formed.
- sirenine:** a sex attractant in *Ectocarpus* (brown algae).
- skeleton:** a hardened non-living, protective, or supportive structure, enclosed by, or attached to, cytoplasmic structures.
- slime layer:** extracellular mucilage.
- somatic:** vegetative.
- sorus:** cluster of reproductive bodies.
- sperm:** male gamete.
- spermatangium:** male gametangium forming one spermatium in the Rhodophyta.
- spermatogenesis:** formation of sperm.
- spermocarp:** zygote plus an enclosing layer of cells in *Coleochaete* (Chlorophyta).
- spicule:** a rod-like, spindle-shaped, stellate, or variously curved and ornamental structure, usually siliceous or calcareous, with blunt or tapered tips deposited individually on the cell surface, or distributed throughout the peripheral cytoplasm.

- spine:** a non-living, rod-like or tapered elongate structure attached to a scale, wall, or skeletal framework.
- spinulax:** very small wall extension.
- sporangium:** spore-producing structure.
- spore:** cell that germinates without fusing to form a new individual.
- spore mother cell:** a cell that divides to produce spores.
- sporeling:** young plant arising from a spore.
- sporocyte:** a cell that divides and gives rise to spores.
- sporogenesis:** the process of spore production.
- sporont:** free-living phase of parasitic dinoflagellates (compared with the parasitic or trophont state).
- sporophyte:** diploid plant that forms spores.
- stalk:** an elongate structure specifically formed to attach an organism to a substrate.
- standing crop:** the amount of biomass present at a specific time.
- starch:** storage polysaccharide composed of  $\alpha$ -1,4 and  $\alpha$ -1,6 linked glucose residues.
- statospore or cyst:** resting spore.
- stellate:** star-shaped.
- stenohaline:** able to tolerate only small changes in salinity.
- stenothermic:** able to tolerate only a small variation in temperature.
- stephanokont:** cell with a ring of flagella at one pole.
- stichidia:** specialized reproductive branches in *Polysiphonia* (red alga).
- stigma or eyespot:** group of pigmented lipid bodies that are associated with phototaxis.
- stipe:** organ between a holdfast and a blade.
- streptophyte:** refers to the grouping of stoneworts (Charales) and land plants (embryophytes).
- stria:** row of punctae (pores or loculi) in diatoms.
- stroma:** non-membranous part of a plastid.
- stromatolite:** rock-like deposition of carbonates and trapped sediments formed by cyanobacteria and diatoms.
- stud process:** short stubby wall extension.
- sublittoral zone:** in a lake the zone from the end of rooted vegetation (about 6 m) to the compensation depth; in the sea the zone from the lowest low-tide mark to 200-m depth.
- sufflatory cell:** cell to which the dwarf male filament attaches in *Oedogonium* (Chlorophyta).
- sulcus:** longitudinal groove in the hypococone of Dinophyta.
- supporting cell:** a cell that bears the carpogonial branch in some Rhodophyta.
- supralittoral zone:** zone above the high-tide mark in the ocean and above the standing-water mark in lakes, which receives splash during windy periods.
- suture:** area of fusion of two adjacent structures.
- swarmer:** motile cell.
- symbiosis or reciprocal parasitism:** two organisms living together to the mutual benefit of each.
- sympodial axis:** axis formed from successive dichotomous branches in which one branch is shorter than the other, giving the appearance of a simple stem.
- syncyanosis:** the symbiotic association between a cyanome and a cyanelle.
- syngamy:** fusion of gametes.
- synomone:** a chemical produced by a prey species that attracts predators of their predators at the next level in the food web.
- systole:** contraction of a contractile vacuole (opposite is diastole).
- tactic movement:** directed movement.
- taxon (plural taxa):** a taxonomic group.
- terrestrial:** growing on soil.
- test:** a hardened cell covering typically secreted by the organism, or built up of particles gathered from the environment, forming a protective barrier around the cell.
- tetrasporangium:** a sporangium producing four tetraspores, usually by meiosis.
- tetraspore:** spore formed in a tetrasporangium, usually by meiosis.
- tetrasporophyte:** usually diploid plant forming tetraspores in the Rhodophyceae.
- thallophyte:** plant lacking roots, stem, and leaves.
- theca:** outer covering of the Dinophyceae and some Chlorophyceae.
- thecal plate:** a plate in a vesicle under the plasmalemma in the Dinophyceae.
- thermal stratification:** phenomenon in a body of water in which the water is progressively colder with depth, resulting in no interchange of water between the bottom and top.
- thermocline:** layer in a thermally stratified lake where the temperature changes suddenly with depth.
- thermophiles:** organisms that grow at high temperatures.
- thermotaxis:** movement away from lower temperature (positive thermotaxis) or higher temperature (negative thermotaxis).
- thylakoid:** membrane-bound sac in a plastid.
- tinsel flagellum:** flagellum with hairs.
- trabeculae:** wall ingrowths in some coenocytic green algae.
- transitional region:** the most proximal (basal) part of a flagellum/cilium adjacent to the basal body/

- kinetosome, comprising matrix, axoneme, and flagellar/ciliary membrane.
- trellisoid:** type of uniformly arranged ornamentation without reference to a point or line in diatoms.
- trichoblast:** branching multicellular filament on a red alga.
- trichocyst:** projectile in the Dinophyceae and Raphidophyceae.
- trichogyne:** long colorless part of a carpogonium that receives the spermatium in the Rhodophyceae.
- trichome:** a row of cells without the sheath in the cyanobacteria.
- trichothallic:** term describing intercalary meristem producing a hair in one direction and the thallus in the other direction.
- trophocyte:** vegetative cell.
- trophont:** parasitic stage of dinoflagellates (compared with the sporont or free-living phase).
- trumpet hyphae:** drawn-out sieve cells wider at the cross walls than in the middle of the cells (Laminariales).
- uniaxial:** having a main axis consisting of a single row of usually large cells.
- unilocular sporangium:** sporangium composed of a single cell producing zoospores usually by meiosis.
- uniseriate:** having a single row of cells.
- unisexual:** having only one type of gametangium formed on one plant.
- upwelling:** an area of the ocean where nutrient-rich bottom water rises to the surface.
- uronic acid:** a type of monosaccharide.
- utricle:** inflated branchlet.
- valve:** part of the cell wall in diatoms, a valve plus a connecting band making up a theca.
- valve jacket or mantle:** part of the valve in diatoms that is bent inward.
- velum:** wall extension over a loculus in diatoms.
- volutin granule:** protoplasmic body containing stored polyphosphate.
- water column:** a vertical section of a body of water.
- whiplash flagellum:** flagellum without hairs on its surface.
- xanthophyll:** a carotenoid composed of an oxygenated hydrocarbon.
- xylan:** polysaccharide composed of xylose sugar residues.
- zoochlorellae:** Chlorophyta living inside invertebrate animals.
- zooplankton:** animal plankton.
- zoosporangium:** sporangium that forms zoospores.
- zoospore:** flagellated planospore.
- zoosporogenesis:** formation of zoospores.
- zooxanthellae:** non-green algal cells, usually Dinophyta, living inside invertebrates.
- zygospore:** thick-walled resting spore.
- zygote:** product of the fusion of two gametes.

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