Chapter 2

CYTOMORPHOLOGY AND ULTRASTRUCTURE

The description of the algal cell will proceed from the outside structures to the inside components. Details will be given only for those structures that are not comparable with analog structures found in most animals and plants. The reader is referred to a general cell biology textbook for the structure not described in the following.

Outside the Cell

Cell surface forms the border between the external world and the inside of the cell. It serves a number of basic functions, including species identification, uptake and excretion/secretion of various compounds, protection against desiccation, pathogens, and predators, cell signaling, and cell–cell interaction. It serves as an osmotic barrier, preventing free flow of material, and as a selective barrier for the specific transport of molecules. Algae, besides naked membranes more typical of animal cells and cell walls similar to those of higher plant cells, possess a wide variety of cell surfaces. The terminology used to describe surface structure of cells of algae is sometimes confusing; to avoid this confusion, or at least to reduce it, we will adopt a terminology mainly based on that of Preisig et al. (1994).

Cell surface structures can be grouped into four different basic types:

Simple cell membrane (Type 1)
Cell membrane with additional extracellular material (Type 2)
Cell membrane with additional intracellular material in vesicles (Type 3)
Cell membrane with additional intracellular and extracellular material (Type 4)

Type 1—Simple Cell Membrane

This cell surface consists of a simple or modified plasma membrane. The unit membrane is a lipid bilayer, 7–8-nm thick, rich with integral and peripheral proteins. Several domains exist in the membrane, each distinguished by its own molecular structure. Some domains have characteristic carbohydrate coat enveloping the unit membrane. The carbohydrate side chains of the membrane, glycolipids, and glycoproteins form the carbohydrate coat. Difference in the thickness of plasma membrane may reflect differences in the distribution of phospholipids, glycolipids, and glycoproteins (Figure 2.1).

A simple plasma membrane is present in the zoospores and gametes of Chlorophyceae (Chlorophyta), Xanthophyceae (Ochrophyta), and Phaeophyceae (Ochrophyta), in the zoospores of the Eustigmatophyceae (Ochrophyta), and in the spermatozoids of Bacillariophyceae (Ochrophyta). This type of cell surface usually characterizes very short-lived stages and, in this transitory naked phase, the naked condition is usually rapidly lost once zoospores or gametes have ceased swimming and have become attached to the substrate, since wall formation rapidly ensues. A simple cell membrane covers the uninucleate cells that form the net-like plasmodium of the Chlorarachniophyceae (Cercozoa) during all their life history. Most Chrysophyceae

occur as naked cells, whose plasma membrane is in direct contact with water, but in *Ochromonas*, the membrane is covered with both

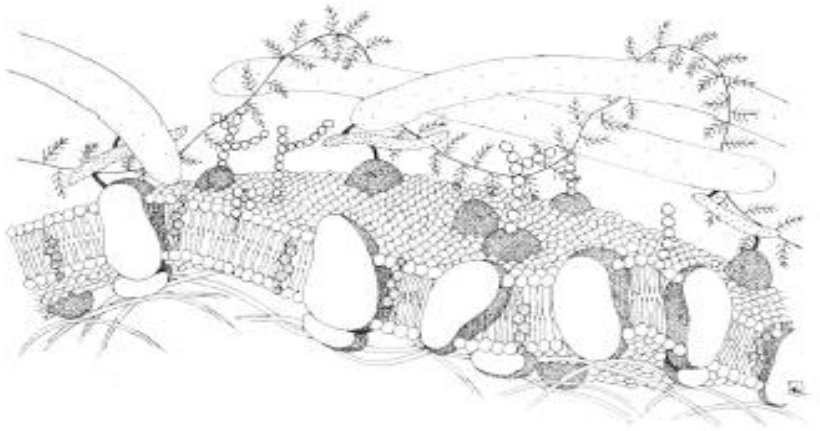

FIGURE 2.1 Schematic drawing of a simple cell membrane.

a carbohydrate coat and surface blebs and vesicles, which may serve to trap bacteria and other particles that are subsequently engulfed as food. The properties of the membrane or its domains may change from one stage in the life cycle to the next.

Type 2—Cell Surface with Additional Extracellular Material

Extracellular matrices occur in various forms and include mucilage and sheaths, scales, frustule, cell walls, loricas, and skeleta.The terminology used to describe this membrane-associated material is quite confusing, and unrelated structures such as the frustule of diatoms, the fused scaled covering of some prasynophyceae, and the amphiesma of dinoflagellates have been given the same name, that is, theca. Our attempt has been to organize the matter in a less confusing way (at least in our opinion).

Mucilages and Sheaths

These are general terms for some sort of outer gelatinous covering present in both prokaryotic and eukaryotic algae. Mucilages are always present and we can observe a degree of development of a sheath that is associated with the type of the substrate the cells contact (Figure 2.2). All cyanobacteria secrete a gelatinous material, which, in most species, tends to accumulate around the cells or trichome in the form of an envelope or sheath. Coccoid species are thus held together to form colonies; in some filamentous species, the sheath may function in a similar manner, as in the formation of *Nostoc* balls, or in development of the firm, gelatinous hemispherical domes of the marine *Phormidium crosbyanum*. Most commonly, the sheath material in filamentous species forms a thick coating or a tube through which motile trichomes move readily.

Sheath production is a continuous process in cyanobacteria, and variation in this investment may reflect different physiological stages or levels of adaptation to the environment. Under some environmental conditions, the sheath may become pigmented, although it is normally colorless and transparent. Ferric hydroxide or other iron or metallic salts as well as pigments originating within the cell may accumulate in the sheath. Only a few cyanobacterial exopolysaccharides have been defined structurally; the sheath of *Nostoc commune* contains cellulose-like glucan fibrils cross-linked with minor monosaccharides and that of *Mycrocystis flos-aquae* consists mainly of galacturonic acid, with a composition similar to that of pectin. Cyanobacterial sheaths appear as a major component of soil crusts found throughout the world, from hot desert to polar regions, protecting soil from erosion, favoring water retention and nutrient bio-mobilization, and affecting chemical weathering of the environment they colonize.

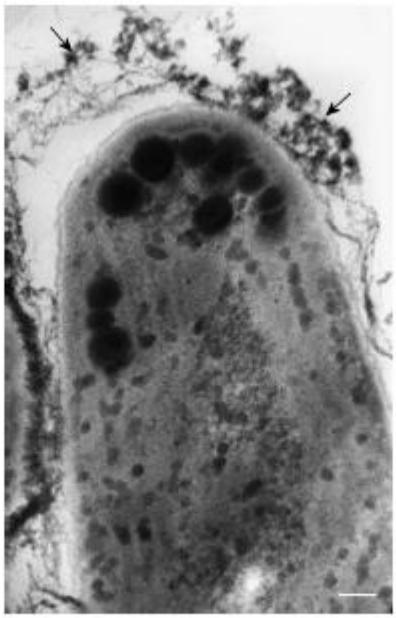

FIGURE 2.2 TEM image of the apical cell of *Leptolyngbya* spp. trichome in longitudinal section. The arrows point to the mucilaginous sheath of this cyanobacterium. Inside the cell, osmiophylic eyespot globules are present. Scale bar, 0.15 µm. (Courtesy of Prof. Patrizia Albertano.)

In eukaryotic algae, mucilages and sheaths are present in diverse divisions. The most common occurrence of this extracellular material is in the algae palmelloid phases, in which nonmotile cells are embedded in a thick, more or less stratified, sheath of mucilage. This phase is so called because it occurs in the genus *Palmella* (Chlorophyceae), but it is also present in other members of the same class, such as *Asterococcus* sp., *Hormotila* sp., and *Gloeocystis* sp. A palmelloid phase is also present in *Spirogyra* sp. (Zygnematophyceae), *Chroomonas* sp. (Cryptophyceae), *Gloeodinium montanum* vegetative cells (Dinophyceae) *Euglena gracilis* (Euglenophyceae) (Figure 1.22). Less common are the cases in which filaments are covered by continuous tubular layers of mucilages and sheath. It occurs in the filaments of *Geminella* sp. (Trebouxiophyceae). A more specific covering exists in

the filaments of *Phaeothamnion* sp. (Phaeothamniophyceae), since under certain growth conditions, cells of the filaments dissociate and produce a thick mucilage that surround them in a sort of colony resembling the palmelloid phase.

Scales

Scales can be defined as organic or inorganic surface structures of distinct size and shape. Scales can be distributed individually or arranged in a pattern sometimes forming an envelope around the cell. They occur only in eukaryotic algae, in the divisions of Ochrophyta, Haptophyta, and Chlorophyta. They can be as large as the scales of Haptophyta (1 μm), but also as small as the scales of Prasynophytes (Chlorophyta) (50 nm). There are at least three distinct types of scales: nonmineralized scales, made up entirely of organic matter, primarily polysaccharides, which are present in the Prasynophytes (Chlorophyta); scales consisting of calcium carbonate crystallized onto an organic matrix, as the coccoliths produced by many Haptophyta; and scales constructed of silica deposited on a glycoprotein matrix, formed by some members of the Ochrophyta.

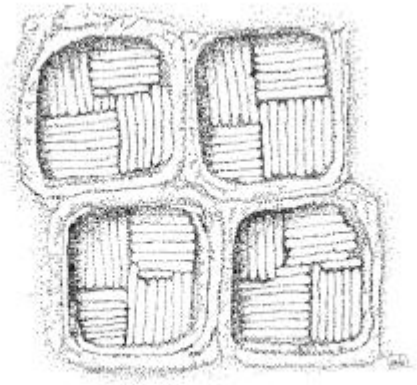

FIGURE 2.3 Box-shaped scales of the intermediate layer of *Pyramimonas* sp. cell body covering.

Most taxa of the Prasinophytes (Chlorophyta) possess several scale types per cell, arranged in 1–5 layers on the surface of the cell body and flagella, those of each layer having a unique morphology for that taxon. These scales consist mainly of acidic polysaccharides involving unusual 2-keto sugar acids, with glycoproteins as minor components. Members of the order Pyramimonadales such as *Pyramimonas* sp. exhibit one of the most complex scaly covering among the Prasinophytes. It consists of three layers of scales. The innermost scales are small, square, or pentagonal; the intermediate scales are either naviculoid or spider web-shaped or box-shaped (Figure 2.3); the outer layer consists of large basket or crown-shaped scales. It is generally accepted that scales of the Prasinophytes are synthesized within the Golgi apparatus; developing scales are transported through the Golgi apparatus by cisternal progression to the cell surface and released by exocytosis. In some genera such as *Tetraselmis* (Chlorodendrophyceae) and *Scherffelia* (Chlorodendrophyceae), the cell body is covered entirely by fused scales. The scales consist mainly of acidic polysaccharides. These scales are produced only during cell division. They are formed in the Golgi apparatus, and their development follow the route already described. After secretion, scales coalesce extracellularly inside the parental covering to form a new cell wall.

In the Haptophyta, cells are typically covered with external scales of varying degree of complexity, which may be unmineralized or calcified. The unmineralized scales consist largely of complex carbohydrates, including pectin-like sulfated and carboxylated polysaccharides, and cellulose-like polymers. The structure of these scales varies from simple plates to elaborate, spectacular spines and protuberances, as in *Chrysochromulina* sp. (Coccolithophyceae) (Figure 2.4), or to the unusual spherical or clavate knobs present in some species of *Pavlova* (Pavlovophyceae).

Calcified scales termed coccoliths are produced by the coccolithophorids, a large group of species within the Haptophyta. In terms of ultrastructure and biomineralization processes, two very different types of coccoliths are formed by these algae: heterococcoliths (Figure 2.5) and holococcoliths (Figure 2.6). Some life cycles include both heterococcolith- and holococcolith-producing forms. In addition, there are a few haptophytes that produce calcareous structures that do not appear to have either heterococcolith or holococcolith ultrastructure. These may be products of further biomineralization processes, and the general term nannolith is applied to them.

Heterococcoliths are the most common coccolith type, which mainly consist of radial arrays of complex crystal units. The sequence of heterococcolith development has been described in detail in *Pleurochrysis carterae* (Coccolithophyceae), *Emiliana huxleyi* (Coccolithophyceae), and the nonmotile heterococcolith phase of *Coccolithus pelagicus* (Coccolithophyceae). Despite the significant diversity in these observations, a clear overall pattern is discernible in all cases. The process commences with formation of a precursor organic scale inside Golgi-derived vesicles; calcification

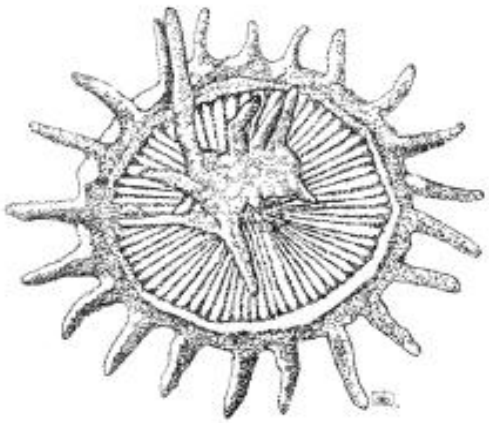

FIGURE 2.4 Elaborate body scale of *Chrysochromulina* sp.

occurs within these vesicles with nucleation of a protococcolith ring of simple crystals around the rim of the precursor base-plate scale. This is followed by growth of these crystals in various directions to form complex crystal units. After completion of the coccolith, the vesicle dilates, its membrane fuse with the cell membrane, and exocytosis occurs. Outside the cell, the coccolith joins other coccoliths to form the coccosphere, that is, the layer of coccoliths surrounding the cell (Figure 1.46). Holococcoliths consist of large numbers of minute morphologically simple crystals. Studies have been performed on two holococcolith-forming species, the motile holococcolith phase of *C. pelagicus* and *Calyptrosphaera sphaeroidea*. As the heterococcoliths, the holococcoliths are also underlain by base-plate organic scales formed inside Golgi vesicles. However, holococcolith calcification is an extracellular process. Experimental evidences revealed that calcification occurs in a single highly regulated space outside the cell membrane, but directly above the stack of Golgi vesicles. This extracellular compartment is covered by a delicate organic envelope or "skin." The cell secretes calcite that fills the space between the skin and the base-plate scales. The coccosphere

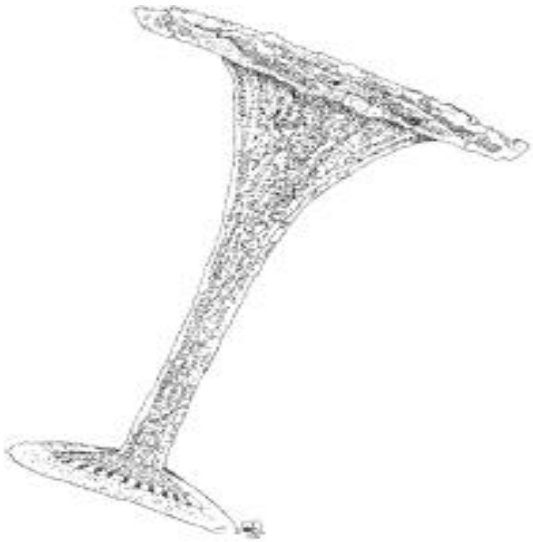

FIGURE 2.5 Heterococcolith of *Discosphaera tubifera*.

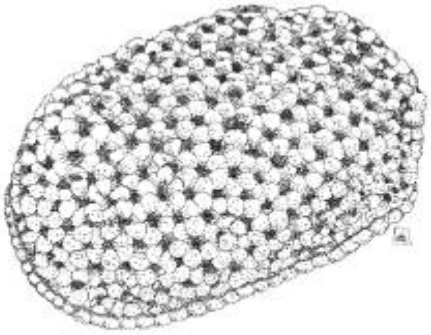

FIGURE 2.6 Holococcolith of *Syracosphaera oblonga*.

grows progressively outward from this position. As a consequence of the different biomineralization strategies, heterococcoliths are more robust than the smaller and more delicate holococcoliths.

Coccolithophorids, together with corals and foraminifera, are responsible for the bulk of oceanic calcification. Their role in the formation of marine sediment and the impact their blooms may exert on climate change will be discussed in Chapter 5.

Members of the Synurophyceae (Ochrophyta) such as *Synura* sp. and *Mallomonas* sp. are covered by armor of silica scales, with a very complicated structure. *Synura* scales consist of a perforated basal plate provided with ribs, spines, and other ornamentation (Figure 2.7). In *Mallomonas*, scales may bear long, complicated bristles (Figure 2.8). Several scale types are produced in the same cell, and deposited on the surface in a definite sequence, following an imbricate, often screw-like pattern. Silica scales are produced internally in deposition vesicles formed by the chrysoplast endoplasmic reticulum, which function as molds for the scales. Golgi body vesicles transporting material fuse with the scale-producing vesicles. Once formed, the scale is extruded from the cell and brought into correct position on the cell surface.

Frustule

This structure is present only in the Bacillariophyceae (Ochrophyta), commonly known as diatoms. The frustule is an ornate cell membrane made of amorphous hydrated silica, which displays intricate patterns and designs unique to each species. This silicified envelope consists of two overlapping valves, an epitheca and a slightly smaller hypotheca. Each theca is comprised of a highly patterned valve and one or more girdle bands (cingula) that extend around the circumference of the

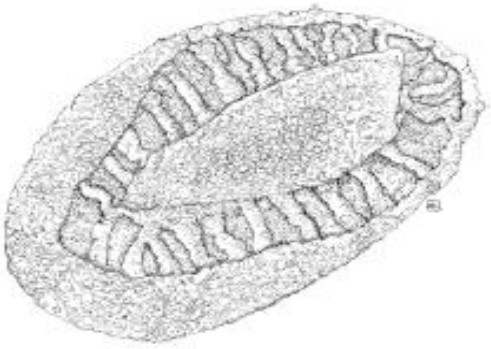

FIGURE 2.7 Ornamented body scale of *Synura petersenii*.

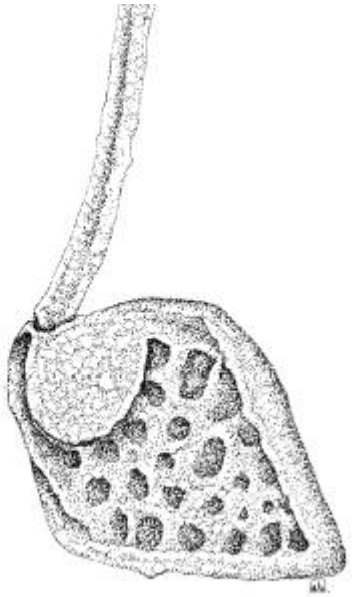

FIGURE 2.8 Body scale of *Mallomonas crassisquama*.

cell, forming the region of theca overlay. Extracellular organic coats envelop the plasma membrane under the siliceous frustule. They exist in the form of both thick mucilaginous capsules and thin tightly bound organic sheaths. The formation of the frustule occurs in the silica deposition vesicles, derived from the Golgi apparatus. The

vesicles eventually secrete their finished product onto the cell surface in a precise position.

Diatoms can be divided artificially in centric and pennate according to the symmetry of their frustule. In centric diatoms, the symmetry is radial, that is, the structure of the valve is arranged in reference to a central point (Figure 2.9). However, within the centric series, there also oval,

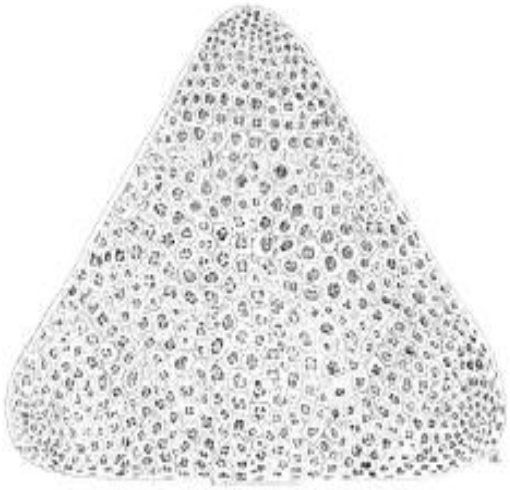

FIGURE 2.9 *Triceratium* sp., a centric diatom.

FIGURE 2.10 *Rhoicosphenia* sp., a pennate diatom.

triradiate, quadrate, and pentagonal variation of this symmetry, with a valve arranged in reference to two, three, or more points. Pennate diatoms are bilaterally symmetrical about two axes, apical and trans-apical, or only in one axis (Figure 2.10);

some genera possess rotational symmetry (Figure 1.48). Valves of some pennate diatoms are characterized by an elongated fissure, the raphe, which can be placed centrally, or run along one of the edges. At each end of the raphe and at its center, there are thickening called polar and central nodules. Addiction details in the morphology of the frustule are the stria, lines composed of areolae, pores through the valve that can go straight through the structure, or can be constricted at one side. Striae can be separated by thickened areas called costae. Areolae are passageways for the gases, nutrient exchanges and mucilage secretion for movement, and attachment to substrates or other cells of colony. Other pores, also known as portules, are present on the surface of the valve. There are two types of portules: fultoportulae (Figure 2.11), found only in the order Thalassiosirales, and rimoportulae (Figure 2.12), which are universal. The structure of the fultoportulae is an external opening on the surface of the valve extended or not into a protruding structure (Figure 2.11). The other end penetrates the silica matrix and is supported with 2–5 satellite pores. The portules, themselves, function in the excretion of several materials, such as the β-chitin fibrils. These fibrils are manufactured in the conical invaginations in the matrix, under the portule. This may be the anchoring site for the protoplast. The rimoportula is similar to the

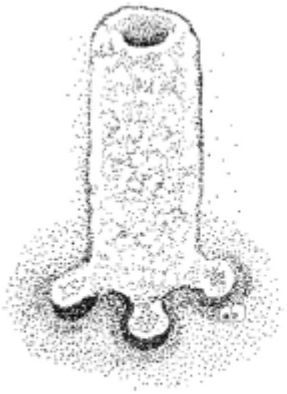

FIGURE 2.11 Fultoportula of *Thalassiosira* sp.

FIGURE 2.12 Rimoportula of *Stephanodiscus* sp.

fultoportulae, except that it has a simpler inner structure. The rimoportula does not have satellite pores in the inner matrix. However, the rimoportula does have some elaborate outer structures that bend, have slits, or are capped. Sometimes the valve can outgrow beyond its margin in structures called setae that help link adjacent cells into linear colonies as in *Chaetoceros* spp. or possesses protuberances as in *Biddulphia* spp., which allow the cells to gather in zigzag chains (Figure 2.13). In other genera such as *Skeletonema*, the valve presents a marginal ridge along its periphery consisting of long, straight spines, which make contact between adjacent cells, and unite them into filaments. Some genera also possess a labiate process, a tube through the valve with internally thickened sides that may be flat or elevated.

Diatoms are by far the most significant producer of biogenic silica, dominating the marine silicon cycle. It is estimated that over 30 million km2 of ocean floor are covered with sedimentary deposits of diatom frustules. The geological and economical importance of these silica coverings as well as the mechanism of silica deposition will be discussed in Chapter 4.

Cell Wall

A cell wall, defined as a rigid, homogeneous, and often multilayered structure, is present in both prokaryotic and eukaryotic algae.

In Cyanobacteria, the cell wall lies between the plasma membrane and the mucilaginous sheath; its fine structure is of Gram-negative type. The innermost layer, the electron-opaque layer or peptidoglycan layer, overlays the plasma membrane, and in most cyanobacteria its width varies between 1 and 10 nm, but can reach 200 nm in some *Oscillatoria* species. Regularly arranged discontinuities are present in the peptidoglycan layer of many cyanobacteria; pores are located in single rows on either side of every cross-wall and are also uniformly distributed over the cell surface. The outer membrane of the cell wall appears as a double track structure tightly connected

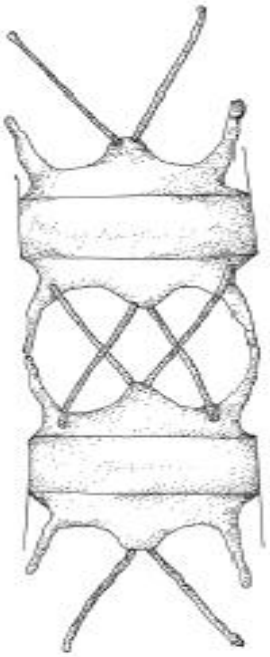

FIGURE 2.13 Cells of *Biddulphia* sp.

with the peptidoglycan layer; this membrane exhibits a number of evaginations representing sites of extrusion of material from the cytoplasm through the wall into the slime. The cell wall of genera such as *Prochloron* is comparable with that of the cyanobacteria in structure and contains muramic acid.

Eukariotic algal cell wall is always formed outside the plasmalemma and is in many respects comparable to that of higher plants. It is present in the Rhodophyta, Eustigmatophyceae (Ochrophyta) (Figure 2.14a and b), Phaeophyceae (Ochrophyta), Xanthophyceae (Ochrophyta), Chlorophyceae (Chlorophyta), and Charophyceae (Charophyta). Generally, cell walls are made up of two components, a microfibrillar framework embedded in an amorphous mucilaginous material composed of polysaccharides, lipids, and proteins. Encrusting substances such as silica, calcium carbonate, or sporopollenin may also be present. In the formation of algal cell walls, the material required are mainly collected into Golgi vesicles that then pass it through the plasma membrane, where enzyme complexes are responsible for synthesis of microfibrils, in a predeterminate direction.

In the Florideophyceae (Rhodophyta), the cell wall consists of more than 70% of water-soluble sulfated galactans such as agars and carrageenans, commercially very important in food and pharmaceutical industry, because of their ability to form gels.

In the Phaeophyceae (Ochrophyta), cell wall mucilagine is composed primarily of alginic acid; the salts of this acid have valuable emulsifying and stabilizing properties.

In the Xanthophyceae (Ochrophyta), the composition of the wall

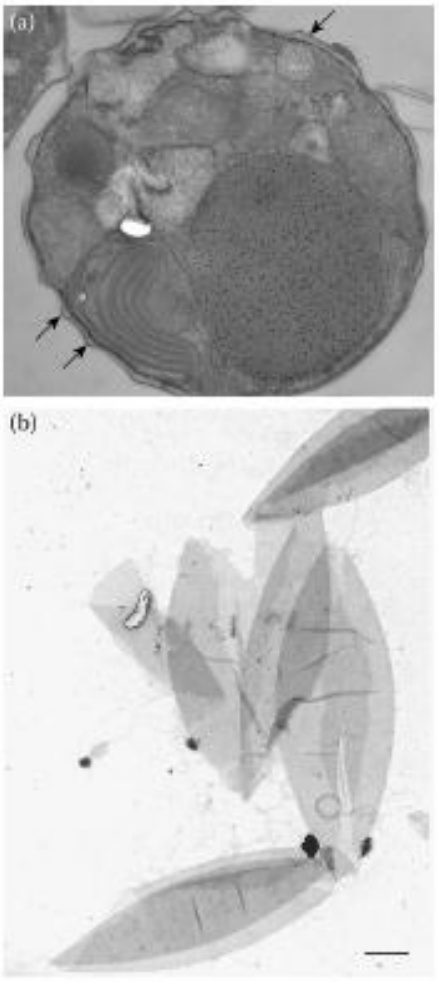

FIGURE 2.14 TEM image of *Nannochloropsis* sp. in transversal section: (a) arrows point to the cell wall and (b) negative staining of the shed cell walls. Scale bar, 0.5 µm.

is mainly cellulosic, while in the Chlorophyceae (Chlorophyta) xylose, mannose, and chitin may also be present in addition to cellulose. Some members of the Chlorophyceae (Chlorophyta) and Charophyceae (Charophyta) have calcified walls.

Lorica

These enveloping structures are present in some members of the class Chrysophyceae (Ochrophyta) such as *Dinobryon* sp., or *Chrysococcus* sp., and in some genera of the Chlorophyceae, such as *Phacotus*, *Pteromonas*, and *Dysmorphococcus*. These loricas are vase-shaped structure with a more or less wide apical opening, where the flagella emerge. These structures can be colorless, or dark and opaque due to impregnation of manganese and iron compounds. As can be expected, different shapes correspond to different species. In *Dinobryon* sp., the lorica is an interwoven system of fine cellulose or chitin fibrils (Figure 2.15). In *Chrysococcus* sp., it consists of imbricate scales. In *Phacotus*, the lorica is calcified, ornamented, and is composed of two cup-shaped parts that separate at reproduction. In *Pteromonas*, the lorica extends into a

projecting wing around the cell and is composed of two shell-like portions joined at the wings (Figure 2.16).

Skeleton

A siliceous skeleton is present in a small group of marine organisms called silicoflagellates, belonging to the class Dictyocophyceae (Ochrophyta). This skeleton is placed outside the plasma membrane; it is a three-dimensional structure resembling a flat basket, which consists of a system of branched tubular elements bearing spinose endings (Figure 1.51). The protoplast is contained inside the basket and has a spongy or frothy appearance, with a central dense region containing the nucleus and the perinuclear dictyosomes, and numerous cytoplasmic pseudopodia extending outward and containing the plastids. Sometimes a delicate cell covering of mucilage can be detected.

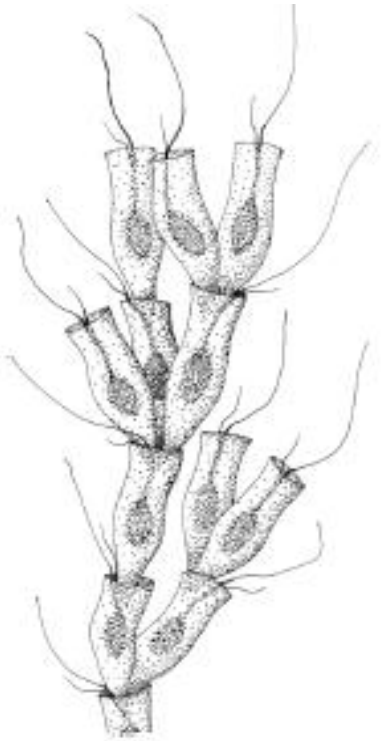

FIGURE 2.15 Tree-like arrangement of *Dynobrion* sp. cells showing their loricas.

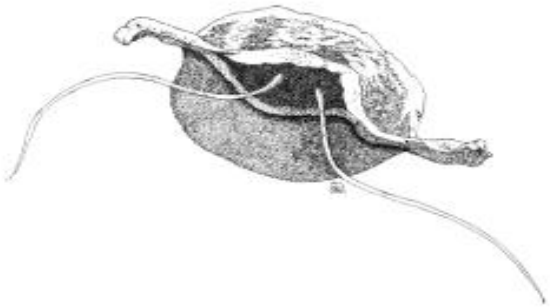

FIGURE 2.16 Lorica of *Pteromonas protracta*.