

Part 1

The Microalgal Cell with Reference to Mass Cultures

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The Microalgal Cell

Robert A. Andersen

Friday Harbor Laboratories, University of Washington, Friday Harbor, WA, USA
Provasoli-Guillard National Center for Marine Algae and Microbiota, Bigelow Laboratory for Ocean Sciences,
East Boothbay, ME, USA

Abstract

Microalgae are a diverse collection of microorganisms that conduct oxygen-evolving photosynthesis. Their biochemical diversity includes production of a wide array of carbohydrates, lipids, and proteins that are commercially valuable. Many produce several different morphologies, for example, flagellate, coccoid, and cyst stages. Many species are capable of sexual reproduction, some microalgae apparently having only asexual reproduction (e.g., *Chlorella*, *Nannochloropsis*). Algal ultrastructure is also diverse, paralleling their biochemical and physiological diversity. Many genomes of microalgae have been sequenced, and these are providing new insights into algal diversity. Genomic research has corroborated known endosymbiotic events and has revealed unknown, or cryptic, such events. Endosymbiosis has been a major factor in the production of algal diversity, and once it is better understood, this may be a practical means for producing new combinations of traits that have commercial application. The current state of algal taxonomy is summarized.

Keywords algae; carbohydrate; chloroplast; endosymbiosis; genome; lipid; morphology; physiology; phytoplankton; protein

1.1 INTRODUCTION

Algae are primarily oxygen-releasing photosynthetic organisms with simple body plans – no roots, stems, or leaves. Algae are usually aquatic organisms. They do not form a single monophyletic group and consequently cannot be easily defined. Although algae as a group are ubiquitous, individual species occupy specific habitats. Some algae are attached to a substrate like plants, some are motile like animals, some are simply suspended in water, some grow loosely on soil, trees, and animals, and some form symbiotic relationships with other organisms (e.g., corals, lichens). The internal cell structure of algae varies greatly. Microalgae lack complex multicellular structures that are found in seaweeds. The cyanobacteria or blue-green algae

have a prokaryotic cell structure and closely resemble bacteria. Eukaryotic algal cells have a nucleus and usually one or more chloroplasts; they also have mitochondria, Golgi bodies, endoplasmic reticulum, and other typical eukaryotic organelles. Despite the difficulty in presenting a clear definition for algae, thousands of books, scores of scientific journals, and numerous internet websites are dedicated solely to compiling our knowledge of algae (Lee, 2008; Graham et al., 2009).

1.2 GROSS MORPHOLOGY

Microalgae appear in a wide variety of shapes and forms. This morphological variation occurs not only among species but also among different life stages of the same

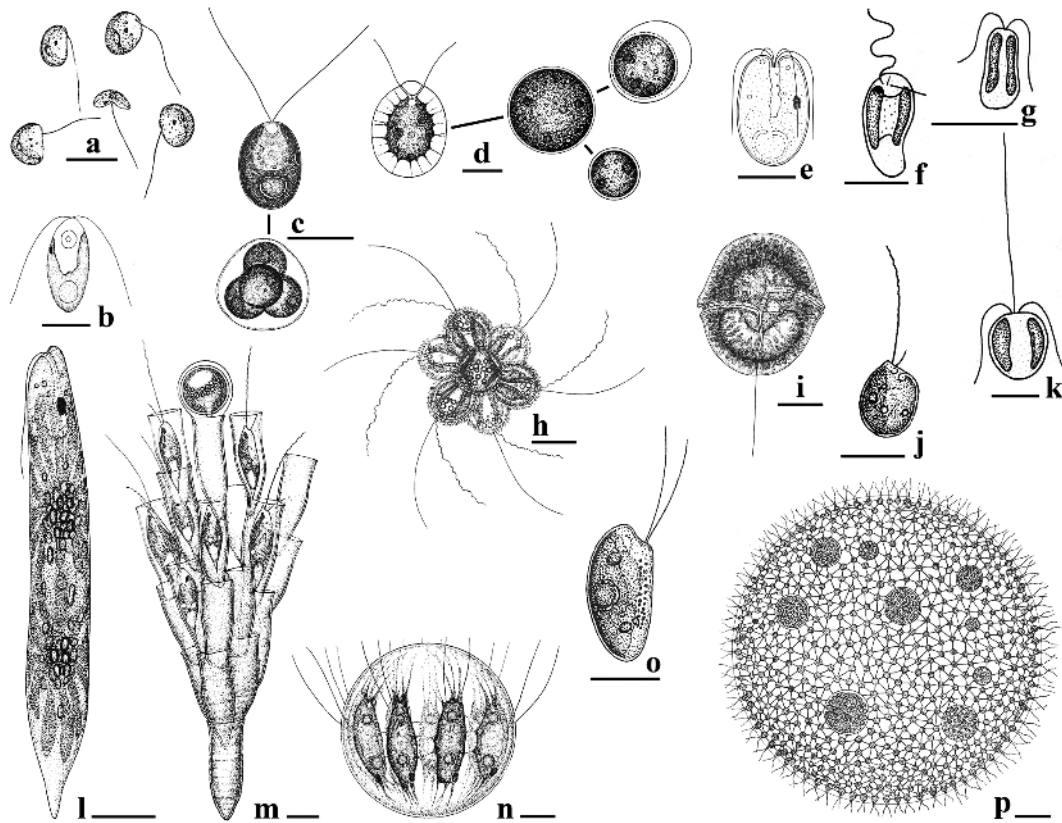


Figure 1.1. Flagellate algal diversity. (a) *Pedinomonas*, with one visible flagellum. Scale bar = 5 μm (from Skuja, 1956). (b) *Dunaliella*, with two equal flagella. Scale bar = 10 μm (from Bold & Wynne, 1985). (c) *Chlamydomonas*, showing the biflagellate cell and four nonflagellate cells. Scale bar = 10 μm (flagellate from Ettl, 1976; colony from Skuja, 1956). (d) *Haematococcus*, showing the flagellate cell and three nonflagellate cells. Scale bar = 10 μm (from Skuja, 1948). (e) *Tetrastelmis*, a quadraflagellate marine alga. Scale bar = 5 μm (from Throndsen, 1993). (f) *Pavlova*, with two unequal flagella and a very short haptoneema. Scale bar = 5 μm (after Throndsen, 1993). (g) *Isochrysis*, with two nearly equal flagella and two chloroplasts. Scale bar = 5 μm (after Throndsen, 1993). (h) *Synura*, a colony with cells attached in the center. Scale bar = 10 μm (from Skuja, 1956). (i) *Gymnodinium*, a dinoflagellate with a circling transverse flagellum and a trailing longitudinal flagellum. Scale bar = 25 μm (from Skuja, 1956). (j) *Ochromonas*, with two very unequal flagella. Scale bar = 10 μm (from Skuja, 1964). (k) *Chrysochromulina*, with a long haptoneema arising between the two flagella. Scale bar = 5 μm (after Throndsen, 1993). (l) *Euglena terricola*, a large cell with one flagellum emerging from a gullet. Scale bar = 10 μm (from Skuja, 1956). (m) *Dinobryon*, an arbuscular colony formed from loricas that surround each cell. Scale bar = 10 μm (from Skuja, 1964). (n) *Stephanosphaera*, a colony where cells are attached laterally. Scale bar = 10 μm (from Skuja, 1956). (o) *Rhodomonas*, a common marine biflagellate. Scale bar = 10 μm (from Skuja, 1948). (p) *Volvox*, a large colonial flagellate with reproductive cells inside the otherwise hollow colony. Scale bar = 35 μm (from West, 1904).

species. The common forms are defined with adjectives such as amoeboid, palmelloid (= capsoid), coccoid, filamentous, flagellate, and sarcinoid (Figs. 1.1 and 1.2). Scientists use morphological life forms when generally discussing algae and their stages; there are, however,

hundreds of thousands of algal species, and they do not always fit neatly into a few convenient categories. The first algae were morphologically simple organisms; today's simplest morphologies, however, are frequently the result of evolutionary reduction through which the algae are better

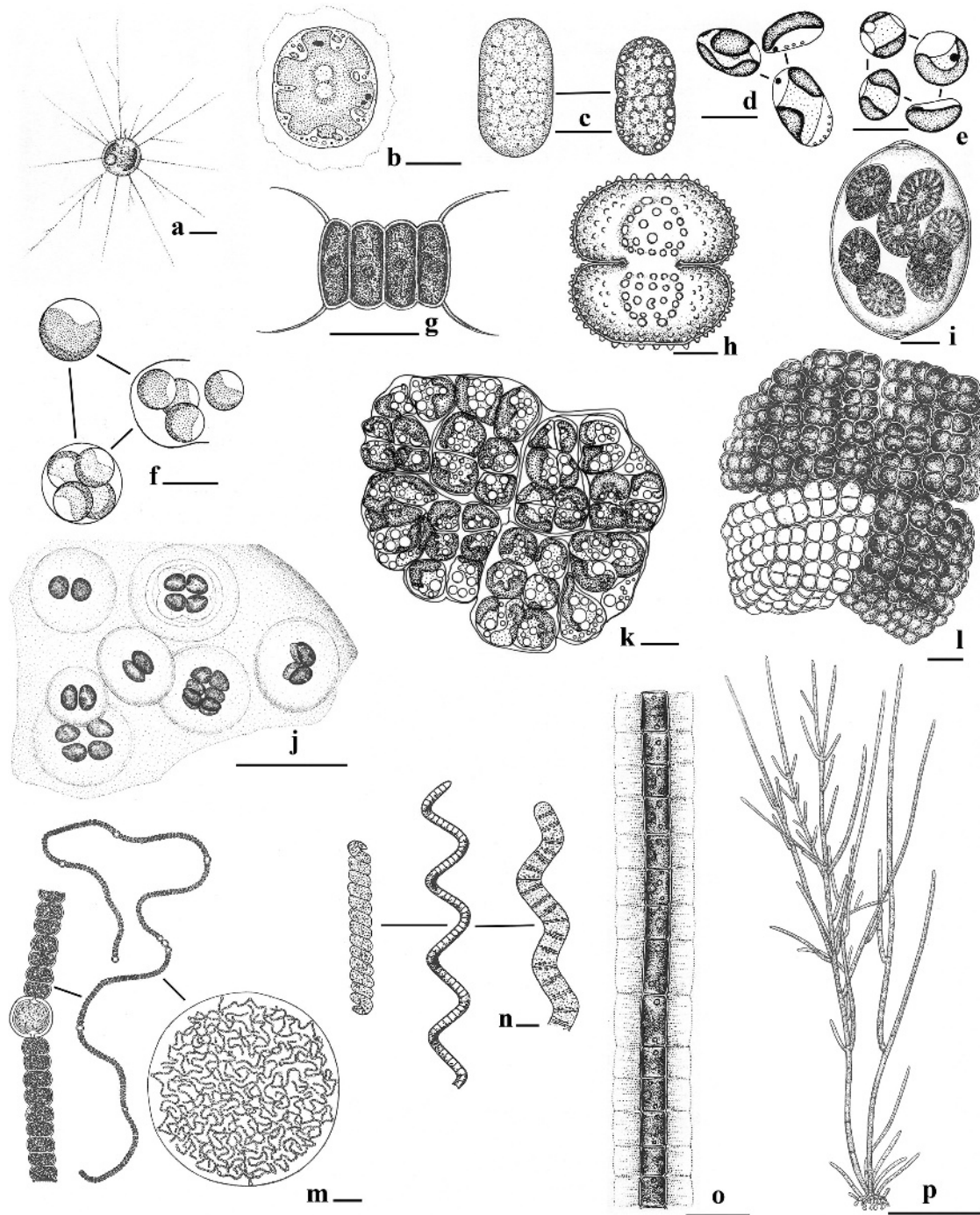


Figure 1.2. (Opposite)

able to survive because of their simplicity. In the following text, algal forms are treated from simple to complex, and this approach is strictly arbitrary (i.e., it does not reflect “primitive” vs. “advanced”).

Flagellates may be single cells where each cell is an independent organism propelled through water with one or more flagella (e.g., *Pedinomonas*, *Chlamydomonas*, *Gymnodinium*, *Ochromonas*, *Tetraselmis*) (Fig. 1.1). Several to many flagellate cells may be joined together to produce a motile colony (e.g., *Dinobryon*, *Synura*). Large colonies, such as *Volvox* (Fig. 1.1p), have hundreds of cells. Most flagellate cells have two flagella, but marine picoflagellates may have only one flagellum (e.g., *Micromonas* and *Pelagomonas*) while *Pyramimonas* may have up to 16 flagella per cell. Haptophyte algae usually have a haptonema positioned between the two flagella (Fig. 1.1k), and the haptonema can be used for attaching to surfaces or collecting particles of food. Many common flagellate algae also produce nonmotile stages, as shown for *Chlamydomonas* and *Haematococcus* (Figs. 1.1c and 1.1d). Changing the environmental conditions can induce these alternate stages, and the manipulation of stages can be used to advantage in commercial facilities.

Many microalgae have a nonmotile stage as the dominant life form, and in some cases, no motile cells are ever found in the life cycle (Fig. 1.2). Amoeboid algae (e.g., *Chlorarachnion*, *Chrysamoeba*, *Rhizochromulina*) slowly creep across substrates, including the marine snow particles in

oceans (Fig. 1.2a). Amoeboid cells may capture bacteria using pseudopods. Coccoid algae reproduce by autospores or zoospores, that is, mother cells undergo synchronized mitotic divisions and the number of daughter cells is fixed (e.g., 2, 4, 8, 16, 32). Single cells, such as *Nannochloropsis* are free, but commonly coccoid algae produce colonies (e.g., *Chlorella*, *Oocystis*, *Scenedesmus*) (Figs. 1.2d–g and 1.2i). Some, such as *Synechococcus* (Fig. 1.2c), exist today as single cells or weakly connected cells, but their ancestors were filamentous algae. Palmelloid algae have cells embedded within a gelatinous matrix; usually the cells are not physically connected to each other and only the gel holds them together. The gelatinous mass may be planktonic or attached to a substrate (Fig. 1.2j). The common flagellate *Pavlova* (Fig. 1.1f), for example, produces large palmelloid sheets when grown under certain culture conditions. Sarcinoid colonies result from equal cell division in three planes so that a cube is produced (*Chlorosarcina*; Fig. 1.2l). The oil-producing *Botryococcus* makes a crudely parenchymatous colony (Fig. 1.2k). Filaments are produced when cells attach end to end and form ribbon-like or chain-like assemblages. In their simplest form, filaments are unbranched and consist of a single row of cells (uniseriate) such as *Arthrospira/Spirulina* (Fig. 1.2n). Complexity develops with side branches (Fig. 1.2p) and multiple rows of cells (multiseriate). The cyanobacterium *Nostoc* forms large colonies that consist of uniseriate trichomes embedded in a soft, inner colonial gel matrix;

Figure 1.2. Diversity of nonflagellate algae. (a) *Chrysamoeba mikrokonta*, an amoeba with branching pseudopods. Scale bar = 5 μm (from Skuja, 1956). (b) *Porphyridium purpureum*, a single-celled red alga with a stellate chloroplast. Scale bar = 5 μm (from Hori, 1993b). (c) *Synechococcus aeruginosus*, the large freshwater type species of this cyanobacterium. Scale bar = 10 μm (from Geitler, 1932). (d) *Nannochloropsis salina* showing three elongate coccoid cells. Scale bar = 2 μm (from Andersen et al., 1998). (e) *Nannochloropsis oculata* showing four spherical coccoid cells. Scale bar = 2 μm (from Andersen et al., 1998). (f) *Chlorella vulgaris* showing a large single cell (top), four autospores (bottom), release of autospores (right). Scale bar = 10 μm (from Fott, 1959). (g) *Scenedesmus maximus*, showing four laterally connected coccoid cells. Scale bar = 10 μm (from Skuja, 1949). (h) *Cosmarium ornatum*, showing the typical semi-cell construction of desmids. Scale bar = 10 μm (from Skuja, 1956). (i) *Oocystis gigas* var. *incrassata* showing eight cells within the old mother cell wall. Scale bar = 20 μm (from Skuja, 1964). (j) *Phacomyxa sphagnicola*, a palmelloid alga showing vegetative cells within a colonial gelatinous matrix. Scale bar = 40 μm (from Skuja, 1956). (k) *Botryococcus braunii* showing cells in packets and numerous oil droplets in each cell. Scale bar = 10 μm (original). (l) *Chlorosarcina superba* showing a cuboidal colony. Scale bar = 10 μm (from Skuja, 1956). (m) *Nostoc planctonicum* showing an enlarged trichome (left), a long trichome, and the colony of trichomes. Scale bar = 5 μm (left, cells), = 25 μm (center, trichome), = 33 μm (colony) (from Geitler, 1932). (n) *Spirulina/Arthrospira*, showing different morphological forms of the spiraling trichome. Scale bar = 10 μm (left), = 18 μm (center), = 5 μm (right) (from Geitler, 1932). (o) *Ulothrix moniliformis*, an unbranched filament with a well-defined gelatinous sheath. Scale bar = 10 μm (from Skuja, 1956). (p) *Cladophora sterrocladia*, showing a typical branched filament shape. Scale bar = 250 μm (from Skuja, 1949).

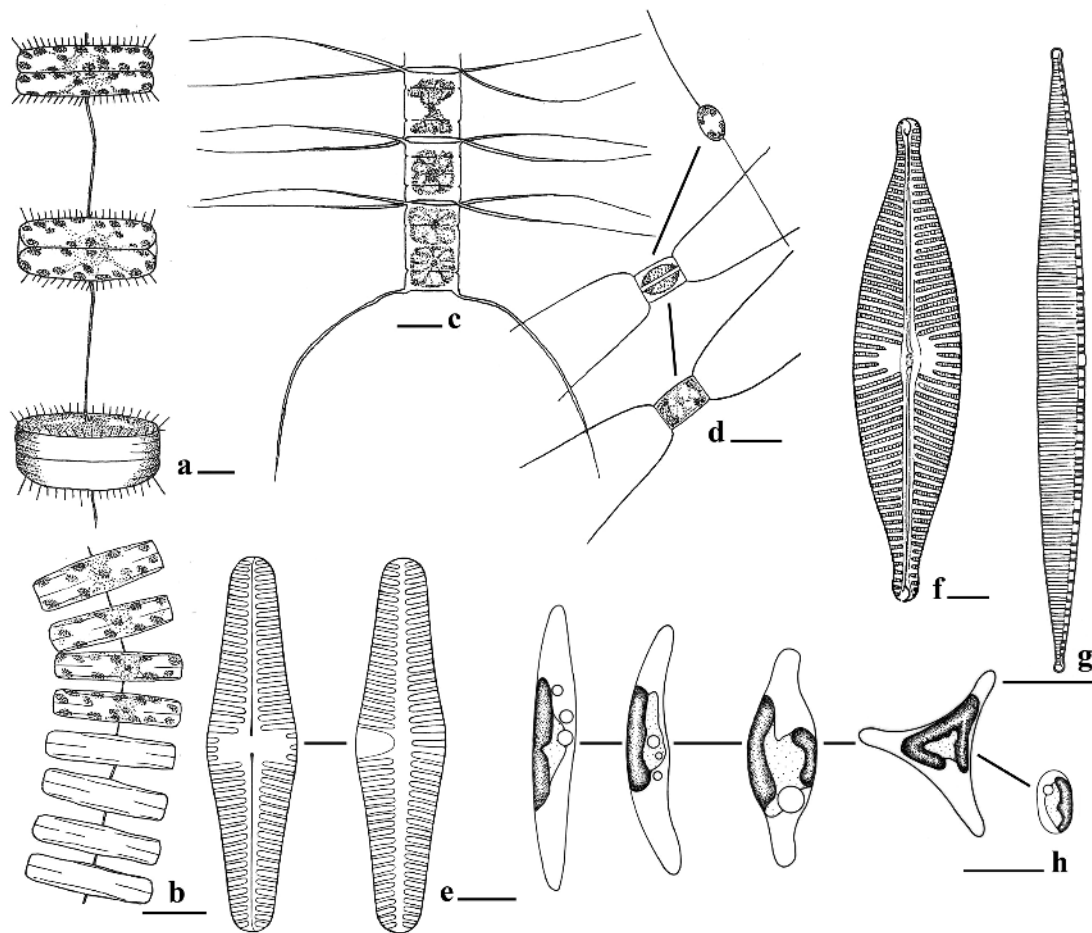


Figure 1.3. Diatom diversity. (a) *Thalassiosira decipiens*, showing three cells connected by a chitinous strand. Scale bar = 10 μm (from Hendy, 1964). (b) *Thalassiosira hyalina* showing a filament of cells, each cell with numerous chloroplasts. Scale bar = 20 μm (from Hendy, 1964). (c) *Chaetoceros pseudocrinium* showing cells connected by intertwined setae. Scale bar = 20 μm (from Hendy, 1964). (d) *Chaetoceros gracile*, a common single-cell species. Scale bar = 5 μm (from Hendy, 1964). (e) *Achnanthes lanceolata*, a monoraphid species with two central raphes on the left valve and no raphes on the right valve. Scale bar = 5 μm (from Patrick & Reimer, 1966). (f) *Navicula rhynchocephala* showing the central raphes and numerous striae. Scale bar = 5 μm (from Patrick & Reimer, 1966). (g) *Nitzschia linearis* showing the marginal raphe and numerous striae. Scale bar = 10 μm (Kalbe, 1980). (h) *Phaeodactylum tricornerutum* showing the common morphological shapes of the species. Scale bar = 10 μm (original).

the outer colony surface has a tough, leathery consistency (Fig. 1.2m).

Diatoms are essentially silica-walled coccoid cells that sometimes remain attached to form simple chains or filaments (Figs. 1.3a–1.3c). Diatoms are the most speciose group of algae, with estimates of up to one million or more species (Round et al., 1990). Diatoms have cell walls made of opaline silica, like that of window glass, and the

glass surfaces have numerous simple or complex “pores” that allow molecular exchanges between the cytoplasm and the environment (Figs. 1.4a and 1.4b). The cell has two valves that are held together by girdle bands. In a general sense, diatoms often categorized as centric or pennate; centric diatoms have valves that radiate from a central region (Figs. 1.3a–1.3d), whereas pennate diatoms have valves that are bilaterally symmetrical (Figs. 1.3e–1.3g).

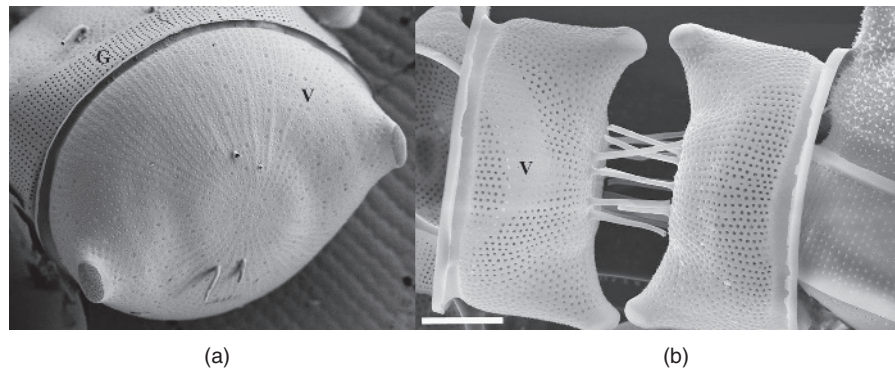


Figure 1.4. (a) *Odontella* sp. showing the variety of pores and structures on the valve (V) and girdle band (G) (original). (b) *Odontella aurita* showing strutted processes connecting two valves (V) (original). Scale bar = 10 μ m.

The valves and the connecting girdle bands have morphology mostly consistent within each species, thereby making it possible to identify diatoms based upon the markings of the silica walls.

1.3 SEXUAL REPRODUCTION

Sexual reproduction may increase cell numbers or produce resistant stages (e.g., zygospores) that greatly facilitate the geographic distribution for the species. The basic elements of algal sexual reproduction are similar to those of other eukaryotes; sperm and egg cells are formed, they fuse (syngamy) and zygotes are formed. However, many variations occur among algae (Hori, 1993a, 1993b, 1993c). For example, in the green algae *Pandorina*, there are not just male/female gametes – there are at least 30 mating types or syngens (Coleman, 2001). The sperm of pennate diatoms are amoeboid while the spermatids of red seaweeds are simple cells that drift in the oceans and reach a female oogonium by chance. The fusion of gametes is a complex process that involves signal transduction at receptor sites on flagella (Pan & Snell, 2000) or even the role of an actin cytoskeleton in nonflagellate spermatia (Wilson et al., 2003). A number of algal genera have no reports of sexual reproduction (e.g., *Chlorella*, *Euglena*, *Nannochloropsis*, *Porphyridium*), and there is debate whether these observations reflect the true absence of sex or the lack of thorough attempts to find sex. The ability to sexually reproduce has important biotechnological implications because breeding and selection can advance aquaculture just as breeding has advanced the improvement of plants and animals for agriculture. Conversely, if sex is not possible then a favorable asexual strain may be maintained indefinitely. That is, care should be taken to preserve the strain so that mutations do

not alter the traits; cryopreservation is a good technique for maintaining strains without change.

1.4 ULTRASTRUCTURE

The ultrastructure of algae is more diverse than that found among animal and plant cells. This reflects the broad phylogenetic diversity of algae, their adaptation to many environments, and 3.5 billion years of evolutionary change. The cyanobacteria have relatively simple cells (Fig. 1.5). A cyanobacterial cell contains many sheet-like thylakoids, and these thylakoids appear as parallel lines in thin sections viewed in the transmission electron microscope (TEM). The cells divide by fission, or pinching, that converts one larger cell into two smaller cells (Fig. 1.5a). The ultrastructure of eukaryotic cells is much more complex, their evolutionary history spans about 1.5–2.0 billion years, and the structures vary significantly within and among algal classes. Eukaryotes possess a number of organelles and these are important metabolic compartments that allow specialization (Martin, 2010). The general features of the eukaryotic algae will be described (Fig. 1.6).

1.4.1 Chloroplast

The chloroplast is the dominant organelle of eukaryotic algae, and the sheet-like thylakoids contain the membrane-bound pigments that capture light for photosynthesis. Thylakoid arrangement is consistent within algal groups but varies among groups. For example, charophycean plastids resemble plant chloroplasts and have distinct grana (stacks of thylakoids); haptophyte plastids have lamellae formed from three sheet-like thylakoids (Fig. 1.7); heterokont plastids are similar to haptophytes but have an outer sac-like girdle lamella surrounding the sheet-like lamellae. Many

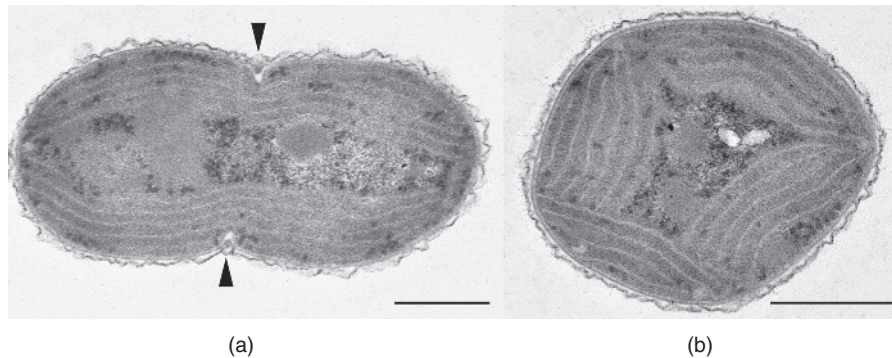


Figure 1.5. *Synechococcus* strain PCC7117. (a) Longitudinal section showing early cell division (arrowheads). (b) Transverse section, showing thylakoids. Scale bar = 500 nm (unpublished, courtesy of Daiski Honda).

plastids have a pyrenoid (Fig. 1.6). The pyrenoid is an accumulation of RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), the dominant protein involved in the Calvin cycle of photosynthesis. Interestingly, only green algae store their photosynthates within the chloroplast. Thus, green algae have starch grains inside the plastid, but for all other algae, carbohydrate or lipid storage is outside the plastid (e.g., between the plastid and the chloroplast ER in cryptophytes) (Ball et al., 2011). Lipid bodies are

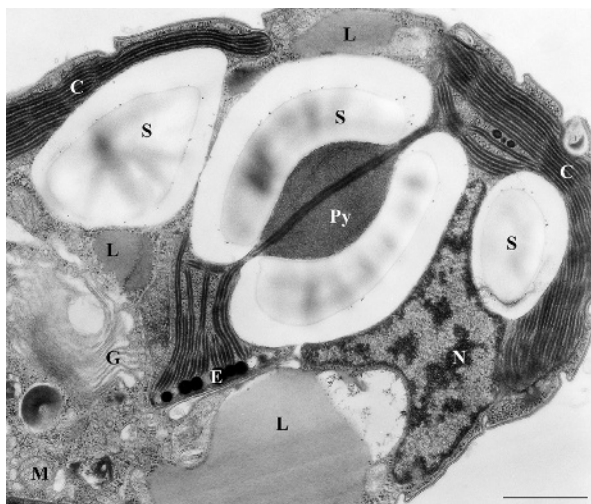


Figure 1.6. *Chroomonas mesostigmatica*. Transmission electron micrograph (TEM). C, chloroplast; E, eyespot; G, Golgi body; L, lipid body; M, mitochondrion; Py, pyrenoid; S, starch granule. Scale bar = 600 nm (unpublished, courtesy of Robert E. Lee).

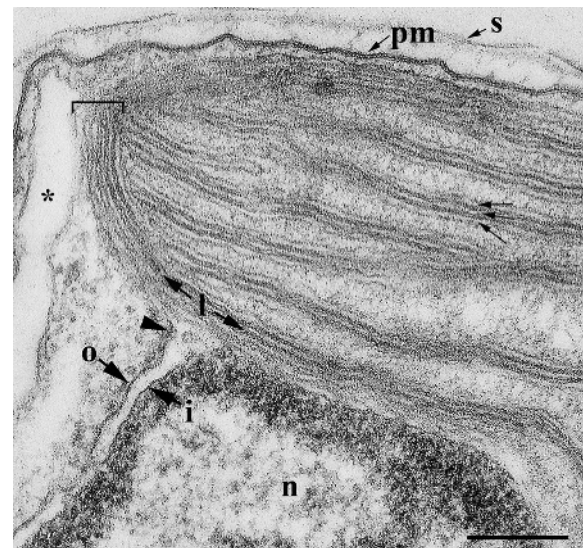


Figure 1.7. *Phaeocystis* TEM showing a secondary plastid. The lamellae (l) are composed of three thylakoids (arrows); the chloroplast is surrounded by four membranes (see bracket region with four membranes plus a lamella). The outer chloroplast membrane is continuous with the outer membrane (o) of the nuclear envelope, which is easily distinguished from the inner membrane (i) of the nucleus (n). The plasma membrane (pm) is visible, the peripheral endoplasmic reticulum is visible (*) and scales (s) surround the cell. Scale bar = 250 nm (original).

also common, and their appearance is usually related to the physiological or environmental conditions of the algae, for example, the number and size of lipid bodies increase when the cells are under high light stress or nutrient starvation. The chloroplast contains its own DNA, typically circular chromosomes; however, the DNA encodes for just a small number of genes because most genes have been transferred to the nucleus. The dinoflagellate plastid genome often has many small rings of DNA. Many chloroplast genomes have been sequenced (for review, see Green, 2011).

1.4.2 Mitochondrion

Mitochondria diversity is also greater among algae than for animals and plants. Green and red algae have mitochondrial cristae that are flattened like those of plants or animals. However, the euglenoids have disc-like cristae, and the haptophyte, heterokont, and dinoflagellate algae have cristae that are tubular in shape. For recent comments on protistan mitochondria, see Shiflett & Johnson (2010).

1.4.3 Nucleus and mitosis

The nucleus of many algae is similar to that found in plants or animals; however, significant differences occur. The typical euglenoid nucleus always has condensed chromosomes, and the dinoflagellate nucleus has unique chromosomes that visually resemble a stack of coins. Great diversity exists for nuclear division or mitosis (see Lee, 2008; Graham et al., 2009). The mitotic spindle may be formed inside a persistent nuclear envelope, the spindle microtubules may penetrate through a persistent but perforate envelope or the nuclear envelope may break down like typical plants and animals. A highly reduced nucleus, named the nucleomorph, remains as evidence of secondary endosymbiosis in the cryptophytes and chlorarachniophytes; for a review, see Moore & Archibald (2009).

1.4.4 Golgi body and endoplasmic reticulum

Organelles such as the Golgi body and endoplasmic reticulum are generally similar in structure to those of other eukaryotes. Algae use these organelles to produce organic, silicate, or calcium carbonate scales as well as flagellar hairs and other structures.

1.4.5 Vacuoles

Eukaryotic cells may possess one or more types of vacuoles. Cells with rigid and complete cell walls often have a vacuole that is at least analogous to the typical plant vacuole and the vacuole functions to maintain a positive osmotic pressure that in turn maintains cell and organismal rigidity. Some organisms, especially heterokont algae, use vacuoles for

storage products or byproducts derived from degradation or remodeling of subcellular compartments, particularly under stress. These algae produce low-molecular-weight carbohydrates (laminarin, chrysolaminarin) in the cytosol, and because the molecules are small (e.g., 20–40 glucose residues) they affect the osmolarity of the cell. To avoid a surge in osmotic pressure, these small carbohydrates are kept within specialized vacuoles. The heterokont and haptophyte algae commonly store oils in their cells. The contractile vacuole is an osmoregulatory organelle in freshwater algae that removes osmotic water from cells. Cells with true walls develop a positive osmotic pressure such that the wall keeps the protoplasm from bursting. However, the protoplasm of freshwater naked, thecate, or loricate cells will expand as water enters the cell by osmosis. If no water is removed, these cells will burst. Contractile vacuoles are rare in marine organisms because the saltwater is more or less isotonic with the protoplasm.

1.4.6 Flagella and eyespots

Swimming algae are propelled by eukaryotic flagella. The flagellar axoneme, like that of animals and many non-flowering plants, has the nine pairs plus two microtubules arrangement. The diatom axoneme, however, has a 9 + 0 arrangement (Manton & von Stosch, 1966) and the same arrangement occurs in algae whose flagella are not used for swimming (e.g., *Tetraspora*, Lembi & Herndon, 1966). Furthermore, the basic flagellum in many algae is enhanced or modified. The cryptophytes, typically, have bipartite flagellar hairs on both flagella; heterokont algae have tripartite flagellar hairs on the immature flagellum (bipartite in *Pelagomonas*, absent in some *Pinguiphyceae*), and some euglenoids produce hair-like scales (Bouck et al., 1978; Kugrens et al., 1987; Andersen et al., 1993; Kawachi et al., 2002). Flagellar hairs change the swimming direction, that is, if the hairs are present, the cell swims forward, but if the hairs are removed, the cell swims backwards (Sleigh, 1989). Flagella in certain groups also possess organic scales (e.g., *Synura*). Paraxonemal rods are found in the flagella of certain algae (e.g., euglenoids, dinoflagellates, dictyochophytes). The paraxonemal rod is contractile in dinoflagellates but is noncontractile in *Euglena*.

The flagellum of a swimming cell exerts considerable force on the cell body and, therefore, the flagellum is anchored securely in the cell. The flagellum undergoes a transition to form the basal body inside the cell and the basal bodies are anchored with microtubular and fibrous roots. These structures, in turn, either constitute or attach to the cytoskeleton (see Andersen et al., 1991; Moestrup, 2000). Flagella also undergo a maturation process. When

a flagellum first forms from a nascent basal body, it is termed the immature flagellum. When the cell divides, the immature flagellum is retracted, and it grows out again as a mature flagellum; this process is termed flagellar transformation (Melkonian et al., 1987; Wetherbee et al., 1988).

Eyespots, or stigmata, are found on many swimming cells. The eyespot is red and represents a specialized lipid accumulation that is associated with a flagellum. The eyespot, often in concert with a paraflagellar body in the flagellum, functions by providing phototaxis for the swimming cell (Kreimer, 1994, 1999; Jékely, 2009). That is, the cell detects the direction of incident light and swims either toward or away from the light source.

1.4.7 Cell walls and coverings

The cell wall is a robust structure that completely encloses the cytoplasm and allows the cell to increase its turgor pressure without bursting. Cell coverings, such as thecae, loricas, scales, and coccospheres surround the cell body; they provided protection but the cell must remain osmotically balanced with the surrounding environment. Therefore, flagellate or amoeboid cells cannot have a true cell wall. Algae with true cell walls undergo cell division in two ways. If the mother cell wall is largely retained and a new wall partitions the mother cell into two daughter cells, this is termed desmoschisis. These organisms can develop a tough, rigid thallus because the cells are strongly bound by their walls. The brown, green, and red seaweeds – the macroscopic algae – form walls by desmoschisis. Alternatively, the mother cell wall can be completely dissolved or discarded and the daughter cells must each produce an entirely new cell wall; this is termed eleuteroschisis. Some organisms, like *Chlorella*, maintain a somewhat digested and expanded mother cell wall, and this old wall holds the daughter cells together as a colony.

The biochemical composition of cell walls varies amongst algal groups. The cyanobacteria have a peptidoglycan wall, often with associated layers or fibrils. This wall is a rich source of protein, and *Spirulina* is sold as a health food rich in protein. The cell walls of macroscopic red algae consist of a cellulose microfibrillar scaffold that is impregnated with polymers of sulfated galactans and various mucilages. Agars and carrageenans (highly sulfated) are commercially extracted from red algal walls and they are used as thickeners and emulsifiers in a variety of applications. Green algal cell walls may be composed of cellulose, hemicellulose, pectic compounds, and glycoproteins.

Thecae are thin organic coverings surrounding most, but not all, of the cell. *Chlamydomonas* and thecate (armored) dinoflagellates are organisms with thecae. The composition

of the theca varies, but often it has a cellulose microfibrillar infrastructure. Loricas are similar to thecae but, typically, there is a greater space between the cell and the lorica. Loricas are also frequently mineralized and may appear yellow or red in color. Scale composition may be organic (cellulosic) materials, silicate glass, or calcium carbonate crystals. There are other cell coverings such as the pellicle of euglenoids, the periplast of cryptophytes, and a wide variety of mucilaginous excretions.

Strong cell walls may be advantageous if the cells pass through pumps or strong mixing devices; however, these walls are often difficult to crack open when trying to extract cellular contents. Walls also impede genetic transformation. Conversely, the sheer forces of pumps easily damage naked, scaled, or thecate cells, but it is easier to extract their contents or to employ genetic transformation techniques. Organisms with mineralized walls may require special growth conditions, for example, diatoms require silica. The mineralization process can also be used to advantage, for example, coccolithophorid algae make scales with CaCO_3 , and they can be used as a CO_2 bioscrubber for carbon sequestration.

1.5 BIOCHEMICAL ASPECTS

Large biomolecules are classified into broad major groups (e.g., carbohydrates, lipids, nucleic acids, and proteins). These groups are similar to those found in other living organisms, but again the algae provide an exceptional diversity of biomolecules. This diversity has interested commercial companies in recent years, and as a consequence algae are grown for the express purpose of harvesting these compounds – many examples are found in this book. There are extensive publications on algal biochemistry, and the precise biochemical product can be manipulated to some degree by altering the growth conditions (Chapter 7; Hu, 2004; Beer et al., 2009). For the purpose of this chapter, a few examples will be provided.

1.5.1 Carbohydrates

The carbohydrate storage product in many algae is starch or a starch-like product (e.g., green and red algae, cryptophytes, dinoflagellates). These starches have a primary α -1,4-linked glucan molecular backbone, and typically the backbone chain has α -1,6-linked side chains (Viola et al., 2001; Ball et al., 2011). The starches are large molecules (i.e., colloidal particles or larger particles) and starch grains are easily visible in a light microscope. Another group of algae utilize a β -1,3-linked glucan backbone (e.g., heterokont algae, haptophytes, euglenoids). The degree of polymerization varies significantly for these laminarin

and paramylon products. The smaller molecules, such as chrysolaminarin, consist of fewer than 30 glucose residues, and therefore to avoid osmotic problems, the molecules are maintained in vacuoles. At the other end of the spectrum, large paramylon grains, such as those found in euglenoids, are easily visible in a light microscope.

1.5.2 Lipids

The diversity of algal lipids is also extensive (Wood, 1984) and the cellular lipid composition can be manipulated (Hu et al., 2008; Wang et al., 2009), that is, under low nitrogen conditions (e.g., in the stationary phase), cells carry out photosynthesis and produce lipids from photosynthetically fixed carbon (e.g., 3-phosphoglycerate). Fatty acid and sterol diversity are found in cellular membranes among algal groups; these lipids are more difficult to extract than the lipids accumulated as lipid bodies or oil droplets. Of the membrane lipids, galactolipids (e.g., monogalactosyldiacylglycerol and digalactosyldiacylglycerol) are the major constituents of thylakoid membranes on which photosynthetic machinery reside (Hu et al., 2008). *Nannochloropsis* accumulates significant amounts of membrane-bound eicosapentaenoic acid (EPA) (Khozin-Goldberg & Iskandarov, 2011). A wide range of algae produce lipids as storage products (i.e., oleaginous algae), and frequently the lipids can be observed as oil droplets in cells (Fig. 1.2k). These lipids are largely polyunsaturated fatty acids (PUFAs), including the omega-3 PUFAs arachidonic acid (AA), docosahexaenoic acid (DHA), and EPA. The heterokont algae (e.g., *Chaetoceros*, *Nannochloropsis*, *Pinguicoccus*) and the haptophytes (e.g., *Pavlova*, *Isochrysis*) typically use oil droplets as a storage product, especially when their carbohydrate storage is chrysolaminarin like. The *Pinguiphyceae* store EPA in large quantities (Kawachi et al., 2002), and DHA is stored in many haptophyte algae (Guschina & Harwood, 2006; Khozin-Goldberg & Iskandarov, 2011). General textbooks often report that green algae store starch, but a considerable number of chlorophyte green algae (e.g., *Scenedesmus*, *Chlorella*) store oils under stress (Guschina & Harwood, 2006). Other chapters in this book address algal lipids in detail.

1.5.3 Proteins

Algal proteins are also exceptionally diverse, and research in specific areas, such as photosynthesis, has shown that proteins can be manipulated by environmental changes (Grossman et al., 1995). Cyanobacteria have peptidoglycan cell walls and, therefore, are an excellent source of proteins, that is, 40–60% of the dry weight is protein (e.g., *Arthrospira/Spirulina*, *Synechococcus*) (Becker,

2007). Green algae are also good sources (e.g., *Chlorella*, *Scenedesmus*); *Euglena gracilis* as well as *Porphyridium* produce up to 30–60% protein by dry weight. Protein-rich cells are often actively growing/dividing cells (log phase) and, therefore, differ from stationary phase lipid-rich cells. Furthermore, some organisms sequester nitrogen when it is available in the environment and they store the excess nitrogen in proteins; when nitrogen becomes limited, they digest these storage proteins to release the nitrogen. Many algae produce pyrenoids (Fig. 1.6), which are accumulations of the enzyme RuBisCO (Kuchitsu et al., 1988). The enzyme plays a crucial role in photosynthetic carbon fixation, but the pyrenoid accumulations are also a rich source of nitrogen that can be tapped when nitrogen become deleted in the environment. In a similar way, cryptophytes store nitrogen in phycobiliproteins.

1.6 BIODIVERSITY

The diversity of algae is amazing at several levels. Species diversity is measured by the number of described species and there is general agreement that many species have not yet been described (Andersen, 1992; Norton et al., 1996). Diatoms have been called the “insects” of the algal world because there may be millions of diatom species and because they are ubiquitous in distribution. Conversely, the glaucophytes and dictyochophytes are groups with very few species, and they may be relic groups left over from times past. Algal diversity may also be measured in terms of biochemical pathways, ecological roles, endosymbiotic genomes, morphology, reproductive strategies, and so forth. For example, the nontraditional, unusual, and even unique biochemical pathways and products of algae are described in other chapters of this book. The recent discovery of cryptic endosymbiotic genomes is significant, for example, predominately green algal genes in diatoms (Moustafa et al., 2009). Endosymbiotic events, and even horizontal gene transfers, have been major genetic mixing pots that have shuffled genomes, created gene duplications, and allowed for gene replacements. These have contributed significantly to algal diversity at all levels.

1.7 EVOLUTION AND SYSTEMATIC BIOLOGY

1.7.1 Evolutionary origins

Fossil prokaryotic cyanobacteria have been found in sediments approximately 3.8 billion years ago, and at least since this date oxygen-releasing photosynthesis has occurred on earth. Not only were these early algae efficient autotrophs, they produced so much free oxygen that it fundamentally changed life on earth (Falkowski & Knoll, 2007).

Ancient Precambrian stromatoliths first appeared about 3.5 billion years ago, and they are fossil remnants of massive cyanobacterial growths in ancient times. Stromatoliths are still formed, but they are quite rare (e.g., Shark Bay, Australia).

The origin of eukaryotes is not precisely known, but may have occurred about 2 billion years ago (Knoll et al., 2006). Currently, there is much debate about the eukaryotic origin; analyses of entire genomes are providing both questions and answers (Foster et al., 2009; Gribaldo et al., 2010; Koonin, 2010). We do know that the eukaryotic algae are not a single evolutionary lineage, and therefore algae per se are not a monophyletic group. However, the plastid profoundly defines eukaryotic algae from nonphotosynthetic protists (e.g., “protozoa” and “aquatic fungi”), and the original plastid genome traces back to a single primary endosymbiotic event (Keeling, 2010). That is, about 2 billion years ago, a nonphotosynthetic eukaryote engulfed a cyanobacterium; rather than digesting it as food, the eukaryote “enslaved” the cyanobacterium cell. Over time, the enslaved cell became a chloroplast, and its existence became deeply entwined within the host cell. Chloroplast division synchronized with host cell division; genes were transferred from symbiont to host genomes and optimized biochemical reactions inside the host cytosol; and the structure and pigmentation of the plastid evolved. For a recent review of chloroplast pigments (chlorophylls, carotenoids), see Roy et al. (2011).

1.7.2 Cyanobacteria

The cyanobacteria, or blue-green algae, are the oldest group of algae (Fig. 1.5). Originally, their classification was based strictly on gross morphology (Geitler, 1932). However, both electron microscopy and molecular phylogenetic analysis have shown that the traditional morphological groups are not monophyletic groups, for example, filaments have arisen independently several times. Cytologically and biochemically, the cyanobacteria are similar to bacteria. Most cyanobacteria possess chlorophyll *a*, phycocyanin and phycoerythrin as light-harvesting molecules, but chlorophylls *b* and *d*, as well as divinyl derivatives of chlorophylls *a* and *b*, are found in a few organisms (e.g., *Acaryochloris*, *Prochloron*, *Prochlorococcus*). The storage product is typically cyanophycean starch, a predominantly α -1,4-linked polyglucan. Ecologically, cyanobacteria are autotrophs that photosynthesize and release oxygen, thus they share this ecophysiology with eukaryotic algae. Some species are commercially valuable (e.g., *Arthrospira*), some produce toxins that can taint and poison drinking water (e.g., *Microcystis*), but most are innocuous organisms that

are ecologically significant but rarely recognized. Remarkably, *Prochlorococcus*, a tiny oceanic picoplankton (0.5–0.8 μm in diameter), is the most abundant living organism on the planet (Chisholm et al., 1992).

1.7.3 Eukaryotic super groups

The phylogenetic relationships of the eukaryotic algae were rarely considered in the light microscopy era (Fritsch, 1935). The electron microscopy era was dominated by the discovery of new ultrastructural diversity and the description of new classes; nevertheless, new evolutionary relationships began to emerge. Recently, molecular biology and phylogenetic analysis have contributed significantly toward our understanding of relationships. Multigene analysis and genomic/proteomic analyses have helped recover deep branch relationships (Baldauf, 2003). Consequently, we have some emerging super groups of algae although there remains considerable debate (Hackett et al., 2007; Bodyl et al., 2009; Reeb et al., 2009; Baurain et al., 2010; Burki et al., 2010; Parfrey et al., 2010; Green, 2011).

1.7.3.1 Algae with primary plastids

It is generally accepted that the glaucophyte, green, and red algae form a monophyletic group sometimes called the *Archaeplastida*; recent genomic data supports the monophyly of these three lineages that contain double-membrane-bound plastids (Price et al., 2012). The glaucophytes may be more ancient because they have cyanelle-type photosynthetic organelles with peptidoglycan cell walls, and they maintain the enzyme fructose biphosphate aldolase. The green algae are a deeply divided lineage, with one branch containing many common organisms (e.g., *Chlamydomonas*, *Chlorella*, *Ulva*, *Volvox*) and another branch (e.g., *Klebsormidium*, *Spirogyra*, *Chara*) giving rise to plants. The evolutionary history of the red algae is more complex than first imagined (see Section 1.7.6), and furthermore, red algae have been captured and converted to plastids by secondary endosymbiotic events.

1.7.3.2 Algae with secondary plastids

Secondary endosymbiotic events were once viewed as frequent occurrences (Leedale, 1974), but the recent literature argues for very few events (Keeling, 2010). The primary morphological change is the occurrence of a secondary plastid that typically has one or two additional membranes just outside the two chloroplast envelope membranes (Fig. 1.7). One secondary endosymbiosis involving a red algae must have occurred early in the evolution of eukaryotic life, and the ancestors diversified to form what is sometimes called the chromalveolates (Cavalier-Smith,

1999). This group is composed of the stramenopiles, alveolates, and rhizaria (SAR) as well as the cryptophytes and haptophytes. There is growing consensus for SAR: stramenopiles include the heterokont algae, oömycetes, and thraustochytrids; alveolates consist of the apicomplexans, ciliates, and dinoflagellates; rhizarians are largely heterotrophic amoebae but the group includes photosynthetic chlorarachniophytes and *Paulinella*. There is less support for adding the Cryptophyceae and Haptophyceae to the SAR to form the chromalveolates (Bodř et al., 2009; Parfrey et al., 2010) even though they all contain chlorophyll *c*; cryptophytes produce flagellar hairs similar to those of heterokont algae, and haptophytes have chloroplasts and storage products nearly identical with heterokont algae.

The chlorarachniophytes and euglenoids also became algae by two independent secondary endosymbioses. However, rather than a red algal symbiont, it was a green alga (see Keeling, 2010). These two groups are small, but they do have some unusual biochemical and physiological attributes that are contributed by their host cell evolutionary ancestors.

1.7.3.3 Algae with tertiary plastids

Dinoflagellates have added another level of complexity. Dinoflagellates began with a plastid derived by red algal secondary endosymbiosis, but many dinoflagellates abandoned photosynthesis in favor of a phagotrophic existence (see Section 1.7.8). In several independent cases, nonphotosynthetic dinoflagellates regained photosynthesis by tertiary endosymbiosis; the symbionts in these varied cases were a diatom, a haptophyte, a cryptomonad, or a green alga (Hackett et al., 2004).

1.7.3.4 Cryptic endosymbioses

The role of endosymbiosis in the evolution of algae is apparently even more complex because genomic analyses reveal cryptic endosymbioses. For example, Moustafa et al. (2009) found that most plastid genes in diatoms had a green algal origin. This was completely unexpected and suggests that evolutionary biologists must exercise caution when trying to unravel the early history of eukaryotes. Thus, over a billion years of evolutionary time, the occurrence of endosymbiotic events, horizontal gene transfers, extinction of intermediary forms, and perhaps additional yet undiscovered factors have contributed to the complex evolutionary history of eukaryotic algae.

1.7.4 Glaucophyte algae

Glaucophytes are sometimes considered among the most basal of eukaryotic algae because their photosynthetic

cyanelles are like a cyanobacterial cell (e.g., chlorophyll *a*, phycocyanin, phycoerythrin). This “preplastid” is surrounded by a peptidoglycan cell wall and has other biochemical features characteristic of prokaryotes. The algae are rarely encountered, but the common species can be found in acid bogs.

1.7.5 Green algae

Green algae have chlorophylls *a* and *b*, and most do not have accessory light-harvesting pigments (for exceptions, see Prasinophyceae). The storage product is typically starch. Green algae are deeply divided into two groups, the chlorophytes and charophytes. While this division was recognized about 40 years ago based upon ultrastructural features (Pickett-Heaps & Marchant, 1972; Mattox & Stewart, 1984; Stewart & Mattox, 1984), gene sequence data as well as chloroplast and nuclear genome data continue to support this deep divergence (Timme & Delwiche, 2010). However, the relationships within each of the two lineages remain somewhat uncertain. While the scaly flagellate *Mesostigma* is probably the closest known living green alga to the divergence of the two groups (Rodríguez-Ezpeleta et al., 2007), the chlorophycean lineage has several named classes that are paraphyletic in many phylogenetic analyses (e.g., Chlorophyceae, Mamiellophyceae, Prasinophyceae, Trebouxiophyceae, Ulvophyceae). The phylogeny within the charophyte lineage also is debated, particularly about which group is most closely related to land plants.

1.7.6 Red algae

Red algae contain chlorophyll *a* and phycobilisomes (pigment complexes with allophycocyanin, phycocyanin, and phycoerythrin) that are located on the surface of unstacked thylakoid membranes. Red algae are unique among eukaryotes in lacking both flagella and centrioles during their entire life cycle. Even 10 years ago, red algal diversity was considered more or less framed, if not finalized. However, studies on unicellular red algae have shown that the base of the red algal tree is very diverse (Yoon et al., 2006). Seven classes are currently recognized. Unicellular Cyanidiophyceae, which thrive in acidic hot springs, are positioned at the base of the red algae; the Porphyridiophyceae and Rhodellophyceae are also unicellular. Among the 6000 red algal species, 5800 belong to the Florideophyceae, which includes the large and commercially valuable seaweeds (e.g., *Eucheuma*, *Gelidium*, *Gracilaria*).

1.7.7 Heterokont algae

Heterokonts are perhaps the most diverse major group of algae, and they currently consist of about 16 classes. They

vary from the giant kelps to picoplanktonic open ocean species and include the ubiquitous diatoms. The group consists of three subgroups: Clade S1, Clade S2, and Clade S3 (Yang et al., 2012).

Clade S1 is composed of the Aurearenophyceae, Chrysomerothyceae, Phaeophyceae, Phaeothamniothyceae, Raphidophyceae, Schizocladophyceae, and Xanthophyceae. Morphologically, they range from tiny coccoid single cells to giant kelps. The carotenoid pigments are also diverse; some groups have the antheraxanthin–violaxanthin light-harvesting carotenoid cycle, others have the diatoxanthin–diadinoxanthin cycle, and the Raphidophyceae have both types (Bjørnland & Liaaen-Jensen, 1989; Mostaert et al., 1998). This group shares some morphological features with the nonphotosynthetic stramenopiles, and may be the earliest diverging group of the three clades.

Clade S2 includes the golden algae (*Chrysophyceae*, *Synurophyceae*), the Eustigmatophyceae, and the oil-producing algae (*Pinguiphyceae*). These algae are distinguished in antheraxanthin–violaxanthin light-harvesting carotenoid cycle, and most species have two flagella with well-developed microtubular root systems. Eustigmatophyceae: these algae lack a chloroplast girdle lamella and include the economically important *Nannochloropsis*. *Pinguiphyceae*: this class uses EPA as a storage product, and some members have odd flagellar features (Kawachi et al., 2002). *Chrysophyceae* and *Synurophyceae*: these two classes were originally distinguished based upon a series of features (Andersen, 1987), but molecular studies suggest they may be recombined. *Synchromophyceae*: this odd group of marine amoebae has only two species, but there are some interesting links with *Chlamydomyxa*, *Leukarachnion*, and *Chrysophyceae* (Grant et al., 2009).

Clade S3 includes the diatoms (*Bacillariophyceae*), the bolidomonads and Parmales (*Bolidophyceae*), the silicoflagellates, pedinellids, and *Rhizochromulina* (*Dictyochophyceae*) and the *Pelagophyceae* (oceanic picoplankters, brown tide organisms, coastal macroalgae). The group is distinguished morphologically by a reduced flagellar apparatus (Saunders et al., 1995), biochemically by light-harvesting carotenoids belonging to the diatoxanthin–diadinoxanthin cycle (Bjørnland & Liaaen-Jensen, 1989), and molecularly with multiple genes (Yang et al., 2012). The diatoms are essentially single cells, sometimes held together in chains, and the unifying character is their siliceous cell walls (Round et al., 1990). Diatoms are rapidly growing organisms that often produce large amounts of oil, but they require silica for growth. Originally, the bolidophytes were limited to two picoflagellates, but the

silica-scaled Parmales are an alternate stage according to a recent molecular study (Ichinomiya et al., 2011). Bolidophytes are closely related to diatoms. The dictyochophytes are a small but distinct group that is largely composed of marine organisms. The pelagophytes are marine algae, including open ocean picoplankters (e.g., *Pelagococcus*, *Pelagomonas*), coastal brown tide organisms (*Aureococcus*, *Aureoumbra*), and several benthic macrophytes forming gelatinous colonies (e.g., *Chrysocystis*, *Chrysoreinhardia*).

1.7.8 Dinoflagellates

These algae are predominately swimming organisms, and they occur in both freshwater and seawater. Typically, one flagellum circles the cell in a cingulum while the second flagellum extends along a groove to beyond the cell posterior and pushes the cell. Some dinoflagellates have an armored cell covering made of thecal plates. Many dinoflagellates lack thecal plates, and they are termed naked dinoflagellates. Both thecate and naked cells exist as photosynthetic, heterotrophic, and mixotrophic organisms. Photosynthetic cells typically use chlorophyll c_2 and c_3 as well as peridinin, but dinoflagellates with tertiary endosymbionts (e.g., *Dinophysis*, *Karenia*, *Kryptoperidinium*, *Lepidodinium*) have other pigments. Photosynthetic dinoflagellates frequently grow very slowly, often with a photosynthetic rate that barely outpaces respiration; however, dinoflagellates, may grow rapidly forming blooms in some cases (Smayda & Reynolds, 2001; Heil et al., 2005).

1.7.9 Haptophytes

The haptophytes are almost exclusively marine and brackish water organisms. Most species have an unusual appendage – the haptonema – that may be used for attaching to substrates or for capturing food. *Isochrysis* and *Pavlova* have been important for aquaculture hatcheries because they grow rapidly, provide rapid and healthy growth for shellfish, are easy to maintain in large volume cultures, and produce significant amounts of PUFAs (Patil et al., 2007). Some haptophytes produce calcium carbonate scales termed coccoliths, and this group is therefore called coccolithophores. Huge oceanic coccolithophore blooms occur and when cells sink to the bottom, they deposit significant amounts of calcium carbonate into ocean sediments (François et al., 2002; Balch et al., 2010).

1.7.10 Cryptophytes

These red, brown, and green (rarely blue) flagellates are very common in freshwater and coastal seas. They have several unusual features. For example, the nucleomorph is

a reduced nucleus that remains from a secondary endosymbiosis, one or both flagella have bipartite tubular hairs, and the cell is covered with a periplast consisting of special plates (Fig. 1.2). They utilize chlorophylls *a* and *c* as well as phycobilins for harvesting light used in photosynthesis; the photosynthetic storage product is starch. Recent phylogenetic studies show a molecular relationship between the cryptophytes and haptophytes; however, morphologically and biochemically, the two groups have little in common.

1.7.11 Euglenoids

These green flagellates are largely freshwater species that occur in puddles, bogs, ponds, lakes, and rivers; they are often abundant in waters with high ammonia or urea content. *Colacium* is an attached euglenoid that lives in the cloaca of frogs and there are a few parasitic taxa (e.g., *Euglenomorpha*, *Kawakine*). The photosynthetic euglenoids are evolutionarily related to trypanosomes and other parasitic organisms. They have a secondary endosymbiotic “green” plastid, and they utilize chlorophylls *a* and *b*; however, their storage product is a β -1,3-linked glucan, paramylon. The cell is covered with pellicular strips, and the common *Euglena* and *Phacus* frequently change cell shape by a process termed metaboly.

1.7.12 Chlorarachniophytes

These are a small group of marine algae that occur primarily in coastal waters, but the flagellate *Bigelowiella* is common in the Sargasso Sea and other oligotrophic open ocean waters. *Chlorarachnion* and *Gymnochloris* are amoeboid organisms, but others such as *Lotharella* and *Partenskyella* have dominant coccoid forms for at least some species. The “green” plastid resulted from a secondary endosymbiosis, the photosynthetic pigments are chlorophyll *a* and *b*, and the storage product is a β -1,3-linked glucan (McFadden et al., 1997).

1.7.13 Other photosynthetic alga-like organisms

In addition to the well-known algae, there are organisms that are alga like, if not algae. *Paulinella chromatophora* was described long ago (Lauterborn, 1895); the amoeboid cell is surrounded by a lorica composed of silica scales, and the typical vegetative cell has two cyanelle-like or plastid-like photosynthetic organelles. *Paulinella* belongs to the largely amoeboid group, Rhizaria. *Paulinella* is very exciting because it arose via primary endosymbiosis that occurred only 60 million years ago; the other primary endosymbiosis that led to all other plastids having occurred about 2 billion years ago. The genomes of this organism have been sequenced and considerable research is being

carried out to understand how a recent endosymbiosis operates (Marin et al., 2005; Yoon et al., 2009; Reyes-Prieto et al., 2010; Mackiewicz et al., 2011).

Hatena arenicola is exciting because it is in the very early stage of secondary plastid formation (Okamoto & Inouye, 2005). *Hatena* contains a *Pyramimonas*-like cell and photosynthesis occurs via the *Pyramimonas*-like chloroplast. However, when *Hatena* undergoes cell division, the *Pyramimonas*-like plastid does not divide – the synchrony of host and endosymbiont divisions has not been established. Therefore, one of the *Hatena* daughter cells contains the plastid-like algae and the other daughter cell must find a *Pyramimonas* cell and engulf it (Okamoto & Inouye, 2006).

The picobiliphytes were primarily described from environmental gene sequences, although some epifluorescence images were provided (Not et al., 2007). Yoon et al. (2011) isolated single cells and sequenced the genomes for three individual cells. Their results show that the picobiliphytes are not algae; rather, picobiliphytes are phagotrophic flagellates that eat cryptophyte-like prey. These, therefore, are not an algal group; they are heterotrophic flagellates that eat algae.

Finally, we have the description of *Roombia truncata*, another colorless flagellate. Okamoto et al. (2009) have shown that *Roombia*, cryptophytes, haptophytes, katablepharids, telonemids, centrohelids, and possibly the so-called picobiliphytes form a clade, the Hacrobia. This study helps us to understand how the algal groups have evolved, and from a more practical viewpoint, it should lead to experiments where various organisms can be recombined to produce new, valuable organisms for commercial purposes.

1.8 ECOLOGY

Algae commonly grow in water, but certain species grow on rocks, soils, snow, plants, and even animals (e.g., sloths); they also grow inside plants, rocks, and ice. Algae are common symbionts living in lichens, ciliates, corals, flatworms, and other animals (Round, 1981; Reisser, 1992). Algae contribute approximately half of the photosynthetic productivity on earth, most of the production occurring in the oceans (Falkowski & Raven, 2007). This seems unbelievable because plants are obvious and abundant on land. However, there are two important factors. The oceans cover 71% of the earth’s surface and 66% is open oceans where only phytoplankton exists. Secondly, there is a fundamental difference between land plants and oceanic phytoplankton (Andersen, 2008). Land plant cells divide and accumulate over months, years, centuries, and even millennia in the case of giant sequoia trees; therefore, biomass is obvious

and visible. Conversely, when phytoplankton cells divide, on average one of the daughter cells is eaten or dies; cells do not accumulate into visible biomass. Other marine life seems more abundant (e.g., jelly fishes, crustaceans, fishes, whales, sea birds) because the phytoplanktonic biomass is accumulating in these marine animals.

While most algae are oxygen-releasing photosynthetic organisms (Larkum et al., 2003), there are many examples of algae without chloroplasts. For example, approximately half of the dinoflagellates are heterotrophic organisms unable to carry out photosynthesis. Algae, such as *Ochromonas*, are mixotrophic organisms; that is, they have chloroplasts and photosynthesize, but they also engulf bacteria and other particles that are digested in food vacuoles. Finally, we assume that all algae are capable of taking up occasional sugar molecules, amino acids, vitamins, and other organic molecules; this process of moving molecules across the plasma membrane is termed osmotrophy. In some cases, for example, *Schizochytrium*, osmotrophy is utilized for mass culture.

Seasonal succession is an interesting ecological process for microalgae in nature. Some algae will suddenly increase in numbers, but soon other algae rapidly increase and replace them. The succession of algal species is repeated at approximately the same time each year and events such as the spring diatom bloom are predictable. For most species, it is not known where organisms reside during the slack times; some produce cysts or other resting stages, but many seemingly disappear, perhaps their numbers so reduced that they became nearly impossible to find them. Succession may eventually be an important factor for large outdoor polyculture ponds where algae are continuously grown and harvested.

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