Euglenozoa—Euglenophyceae

Euglenophyceae include mostly unicellular flagellates, although colonial species are common (Figures 1.1bi, 1.1bl, 1.1bm, 1.1bn, and 1.56). They are widely distributed, occurring in freshwater, but also brackish and marine waters, most soils and mud. They are especially abundant in highly heterotrophic environments. The flagella arise from the bottom of a cavity called reservoir, located in the anterior end of the cell. Cells can also ooze their way through mud or sand by a process known as metaboly, a series of flowing movements made possible by the presence of the pellicle, a proteinaceous wall that lies inside the cytoplasm. The pellicle can have a spiral construction and can be ornamented. The members of this division share their pigmentation with prochlorophytes, green algae, and land plants, since they have chlorophylls *a* and *b*, β- and γ-carotenes, and xanthins. However, plastids could be colorless or absent in some species. As in the Dinophyceae, the chloroplast envelope consists of three membranes. Within the chloroplasts, the thylakoids are usually in groups of 3, without a girdle lamella; pyrenoids may be present. The chloroplast DNA occurs

FIGURE 1.56 Unicell of *Euglena gracilis*. Scale bar: 10 µm.

as a fine skein of tiny granules. The photoreceptive system consisting of an orange eyespot located free in the cytoplasm and the true photoreceptor located at the base of the flagellum can be considered unique among unicellular algae. The reserve polysaccharide is paramylon, β-1,3-glucan stored in granules scattered inside the cytoplasm, and not in the chloroplasts such as the starch of the Chlorophyta. Though possessing chlorophylls, these algae are not photoautotrophic but rather obligate mixotrophic, because they require one or more vitamins of the B group. Some colorless genera are phagotrophic, with specialized cellular organelle for capture and ingestion of prey, while some other are osmotrophic. Some of the pigmented genera are facultative heterotrophic. Only asexual reproduction is known in this division. Euglenophyceae possess unique cellular and biochemical features that place these microorganisms closer to trypanosomes than to any other algal group.

ENDOSYMBIOSIS AND ORIGIN OF EUKARYOTIC PHOTOSYNTHESIS

The origin of cyanobacteria can be equated with the origin of aerobic photosynthesis around 2.8 billion years ago, in the Archaean Era of earth history. From that time to present, cyanobacteria have played fundamental roles in driving much of the ocean carbon, oxygen, and nitrogen fluxes, establishing a major turning point in the biogeochemistry of our planet. Prior to the appearance of these organisms, all photosynthetic organisms were anaerobic bacteria that used light to couple the reduction of carbon dioxide to the oxidation of low free energy molecules, such as H2S or preformed organics. Cyanobacteria developed a key characteristic that distinguished them from their photosynthetic evolutionary precursors, that is the ability to extract electrons from water releasing oxygen as a byproduct, with the help of two photosystems linked in series. Oxygenic photosynthesis exploits the energy of visible light to oxidize water and simultaneously reduces CO2 to organic carbon represented by (CH2O)*n*. Light energy is used as a substrate and chlorophyll *a* as a requisite catalytic agent.

Formally oxygenic photosynthesis can be summarized as

$$CO_2 + H_2O + \text{light} \xrightarrow{\text{Chlorophyll } a} (CH_2O)_n + O_2$$

Thanks to endosymbiosis, the photosynthetic trait was acquired in the founding group of photosynthetic eukaryotes, the Plantae or Archaeplastida, and spread to give rise to the wide variety of photosynthetic eukaryotes on earth today. The origin of plastids in eukaryotic cells about 1.8 billion years ago is one of the most profound effects of the endosymbiotic process. These organelles have been well established to have evolved

from a single so-called "primary endosymbiotic event" involving the uptake of a β-cyanobacterium by a heterotrophic/phagotrophic eukaryote, whose ancestors had already acquired a mitochondrion via endosymbiosis of a proteobacterium. Plastid genomes rarely encode more than 200 proteins, which represent a small fraction of the proteins required for full functionality, and an even smaller fraction of the few thousand proteins found in modern-day cyanobacteria. It is widely assumed that in the process of endosymbiosis, most endosymbiont genes were either lost together with the corresponding function or transferred to the host nucleus and merged into the chromosomes during the course of plastid integration. This migration of genes between two genomes is known as endosymbiotic gene transfer, a special case of horizontal gene transfer (HGT). Over time, some acquired targeting sequences that enabled their products synthesized on cytoplasmic ribosomes were targeted back to the organelle. In addition, some host genes acquired targeting sequences that enabled their products to be imported into the organelle. All these processes played a fundamental role in the integration of endosymbiont and host. "Primary plastids" are surrounded by two bounding membranes and are only found in three major eukaryotic photosynthetic groups of Plantae, that is, Glaucophyta, Chlorophyta, and Rhodopyta.

Glaucophyta occupy a key position in the evolution of plastids; in fact, the plastids of glaucophytes contain chlorophyll *a*, present in cyanobacteria, and retain the remnant of a Gram-negative bacterial peptidoglycan wall that would have been between the two membranes of the cyanobacterial ancestor symbiont. The retention of this ancestral character is absent in both green and red plastids.

Chlorophyta (green algae and plants) constitute the second lineage of primary plastids. The simple two-membrane system surrounding the plastid, the congruence of phylogenies based on nuclear and organellar genes, and the antiquity of the green algae in the fossil record all indicate that the green algal plastid is of primary origin. In these chloroplasts, chlorophyll *b* was synthesized as a secondary pigment, phycobiliproteins were lost, and starch was stored. Another hypothesis suggested that the photosynthetic ancestor of green lineage was a prochlorophyte that possessed chlorophylls *a* and *b* and lacked phycobiliproteins.

The green lineage played a major role in oceanic food webs and the carbon cycle from about 2.2 billion years ago until the end-Permian extinction, approximately 250 million years ago. It was this similarity to the pigments of plants that led to the inference that the ancestors of land plants (i.e., embryophytes) would be among the green algae and is clear that phylogenetically plants are a group of green algae adapted to life on land.

The plastids of Rhodophyta (red algae) constitute the third primary plastid lineage. Like the green algae, the red algae are also an ancient group in the fossil record, and some of the oldest fossils interpreted as being of eukaryotic origin are often referred to the red algae, although clearly these organisms were very different from any extant alga. Like those of green algae, the plastids of red algae are surrounded by two membranes. However, they are pigmented with chlorophyll *a*, phycobiliproteins, organized into phycobilisomes, and are distinguished by the presence of phycoerithrin. Phycobilisomes are relatively large extrinsic antennas, water-soluble, and attached to the surface of the thylakoid membrane. Thylakoids with phycobilisomes

do not form stacks like those in other plastids and consequently the plastids of red algae (and glaucophytes) bear an obvious ultrastructural resemblance to cyanobacteria.

Though the plastids of these three lineages are all primary plastids, that is, having only two membranes around them, they differ more noticeably in their light-harvesting machinery. Green algae and plants have membrane-intrinsic antenna proteins (members of the light-harvesting complex superfamily) that bind both chlorophylls *a* and *b* and are associated with both photosystems. Red algae have proteins of the same family that bind only chlorophyll *a* and are associated with photosystem I. Glaucophytes do not have this type of antenna, but along with the red algae they have phycobilisomes, extrinsic antennas which bind linear tetrapyrroles.

Molecular phylogeny supports two possible evolutionary scenarios for the branching order within Plantae, the red-first and glaucophyte-first hypotheses. The inability to unambiguously decide between these two using genome-wide analyses may be explained by the more than 1 billion years that have passed since primary endosymbiosis. The early period of Plantae evolution was likely characterized by a rapid radiation, reticulate evolution (by HGT) among taxa, and high rates of gene divergence, loss, and replacement that have diffused the evolutionary signal. This idea is supported by the findings regarding mitochondrial gene order and gene content that do not favor a particular order of branching.

As ancient as the primary endosymbiotic origin of plastid was (1.8 billion years ago), it is worth noting that cases of "recent" cyanobacterium–eukaryote endosymbiosis are known, for example, in the testate amoeba *Paulinella cromatophora* and the diatom *Rhopalodia gibba*. The photosynthetic consortium between *Paulinella* and an α-cyanobacterium was established 60 million years ago. This amoeba is surrounded by the cell wall called theca, which is composed of silica scales. Apart from typical eukaryotic organelles such as nucleus and mitochondria, it harbors two cyanobacterium-derived endosymbionts. The endosymbionts are photosynthetically active, deeply integrated with the host cell, and their genome has been reduced to one-third in comparison to their cyanobacterial ancestors. The pennate diatom *R. gibba* harbors endosymbionts closely related to extant cyanobacteria. Some of the closest free-living relatives of these so-called spheroid bodies are diazotrophic cyanobacteria of the *Cyanothece* sp. group. The spheroid bodies encode genes for nitrogen fixation and have the capacity to fix molecular nitrogen under light conditions only, unlike all other unicellular nitrogen-fixing cyanobacteria. Although the spheroid bodies are of cyanobacterial origin, they lack the typical photosynthetic pigmentation.

Plantae, that is, Glaucophyta, Chlorophyta, and Rhodopyta, represent just the beginning of the endosymbiosis story. In fact, while mitochondria originated once and have apparently never been lost, plastids have spread between eukaryotic lineages several times in events referred to as secondary and tertiary endosymbiosis, that is, the uptake and retention of a primary or secondary algal cell by another eukaryotic lineage.

Euglenozoa and Cercozoa derived from this primary plastid lineage by two separate secondary endosymbiosis acquiring the plastid by engulfing a green alga. Two independent lineages of green algae were captured by two distinct lineages of phagotrophic protists via secondary endosymbiosis; chloroplast genome analyses suggest that the chlorarachniophyte plastid is derived from a green alga belonging to the "core chlorophyte" group (Trebouxiophyceae, Ulvophyceae, Chlorophyceae), while the ancestor of the euglenophyte plastid is related to prasinophyte green algae. There is no strong similarity between the plastid of euglenids and chlorarachniophytes: euglenids have plastids bounded by three membranes and store paramylon in the cytosol, whereas chlorarachniophytes have plastids bounded by four membranes with a nucleomorph and store β-1,3-glucan in the cytosol.

Euglenozoa contains photoautotrophic species and species with different types of feeding strategies, osmotrophic (e.g., *Rhabdomonas*) or phagotrophic (e.g., *Peranema*). The plastids have been subsequently and independently lost in several branches within the phototrophic clade. Current knowledge on the phylogeny of euglenozoa implies that the secondary endosymbiotic event happened after the split of *Peranema*, but before the split of *Eutreptia* and *Eutreptiella*, which form the basal lineages of the phototrophic clade. Recently, a number of red algal origin genes have been identified in the photosynthetic *Euglena gracilis* as well as in the heterotrophic *Peranema.* It is likely that these genes have been acquired via eukaryote-to-eukaryote lateral gene transfer, giving rise to a complex pattern of genome mosaicism in euglenids. These genes may come from prey organisms, and the lineage of euglenids might have experienced a cryptic red algal plastid endosymbiosis before the current green algal plastid was established. Derived genes may have contributed to the

successful integration and functioning of the green algal secondary plastid in modern-day euglenids.

Multiple red algal-derived Calvin cycle genes have been detected also in Cercozoa (Chlorarachniophyta) nuclear genomes. One possible explanation is that these genes were transferred from red alga prey organisms via HGT. The prey organism might have been captured by and retained in an ancestral and probably nonphotosynthetic chlorarachniophyte as an endosymbiont, which was then replaced by a green algal endosymbiont, giving rise to the extant secondary plastid in Chlorarachniophyta.The plastid of cryptophytes, ochrophytes, dinoflagellates, and haptophytes likely arose from a single initial event of secondary endosymbiosis of a red alga with a nonphotosynthetic eukaryotic host.

The red alga eventually evolved chlorophyll *c* plastids and engaged in several subsequent higher-order endosymbiosis with phylogenetically diverse hosts (in an order still to be resolved), hence giving rise to all the extant photosynthetic lineages. The derivation of chlorophyll *c* containing plastids from the red algal lineage is still somewhat conjectural, but recent analyses of both gene sequences and gene content are consistent with this conclusion.

The cryptophytes were the first group in which secondary plastids were recognized, on the basis of their complex four-membrane structure. Like red algae, they have chlorophyll *a* and phycobiliproteins, but these are distributed in the intrathylakoidal space rather than in the phycobilisomes found in red algae, Glaucophyta, and Cyanophyta. In addition, cryptomonads possess a second type of chlorophyll, chlorophyll *c*, which is found in the plastids of the remaining red lineage clades, retain the nucleomorph, vestigial nucleus of their symbiont, and have starch the reserve polysaccharide of red algae in the periplastid space. Ochrophyta (including kelps, diatoms, chrysophytes, and related groups), Haptophyta (the coccolithophorids), and probably those dinoflagellates (Myzozoa) pigmented with peridinin, have chlorophylls *a* and *c*, along with a variety of carotenoids. Stacked thylakoids are found in those lineages that lack phycobilisomes.

The plastid situation in dinoflagellate is exceptionally complex; most of the phototrophic species harbor a plastid of red algal origin bound by three membranes and characterized by the carotenoid peridinin, chlorophylls *a* and *c*2. It has been speculated that the ancestral peridinin-containing chloroplast was replaced by one from a cryptophyte, a haptophyte, a diatom, or a green alga, that is, by additional endosymbiotic events, namely tertiary endosymbiosis in the first three cases, and serial secondary endosymbiosis for the green alga. All these symbiotic relationships have not reached the same level of permanence, and sometimes it is disputed whether the plastids are the traces of a stable symbiosis or rather they are kleptoplastids, that is, plastids obtained from ingested algal prey and retained, which may remain temporarily functional and be used for photosynthesis by the predator. In some cases, the endosymbiont is present in an almost unchanged form and was therefore established more recently (e.g., *Kryptoperidinium foliaceum*). In other cases, the symbiont has almost lost all organelles and only the chloroplast remains (e.g., peridinin-containing species, *Heterocapsa*, *Peridinium*), indicating an older, more well-established symbiosis.

Members of the Kareniaceae (*Karenia mikimotoi*, *Karlodinium micrum*) possess fucoxanthins as accessory pigment instead of peridinin; molecular data confirmed that the peridinin-containing chloroplast in this group has been superseded by a haptophyte-type-containing fucoxanthin. Other dinoflagellates such as *Dinophysis acuminata* have established a stable symbiosis with a cryptophyte symbiont, of which only the chloroplast remains, surrounded by three membranes. A diatom symbiont is present in a small group of closely related dinoflagellates named “dinotoms” consisting of only 10 species such as *Durinskia baltica*, *K. foliaceum*, *Peridinium quinquecorne*, and other species belonging to the genera *Galeidinium* and *Gymnodinium*. The endosymbionts have been shown to originate from three different diatom lineages, one pennate and two centric. Despite the loss of a distinctive cell wall, motility, and ability to divide mitotically, these endosymbionts have retained a

large nucleus with vast amount of DNA, a large volume of cytoplasm, separate from the host by a single membrane, and their own mitochondria in addition to the chloroplasts. Another unique symbiosis is present in the dinoflagellate genus *Lepidodinium*; members of this genus such as *L. viride* (Figure 1.1bh; courtesy of Francisco Rodriguez Hernandez) and *Lepidodinium chlorophorum* (formerly *Gymnodinium chlorophorum*) possess green-colored plastids containing chlorophylls *a* and *b*. The pigment composition, phylogenetic analyses of plastid-encoded genes, and a survey of nuclear genes encoding plastid-targeted proteins in *L. chlorophorum* clearly indicated that the ancestral *Lepidodinium* cells replaced the original Chl-*a* + *c*-containing plastids with the Chl-*a* + *b*-containing plastids of an endosymbiotic green alga belonging to the core chlorophytes. Only the symbiont chloroplast remains in these dinoflagellates. A schematic drawing of the hypothesis of the different symbiotic events is shown in Figure 1.57.

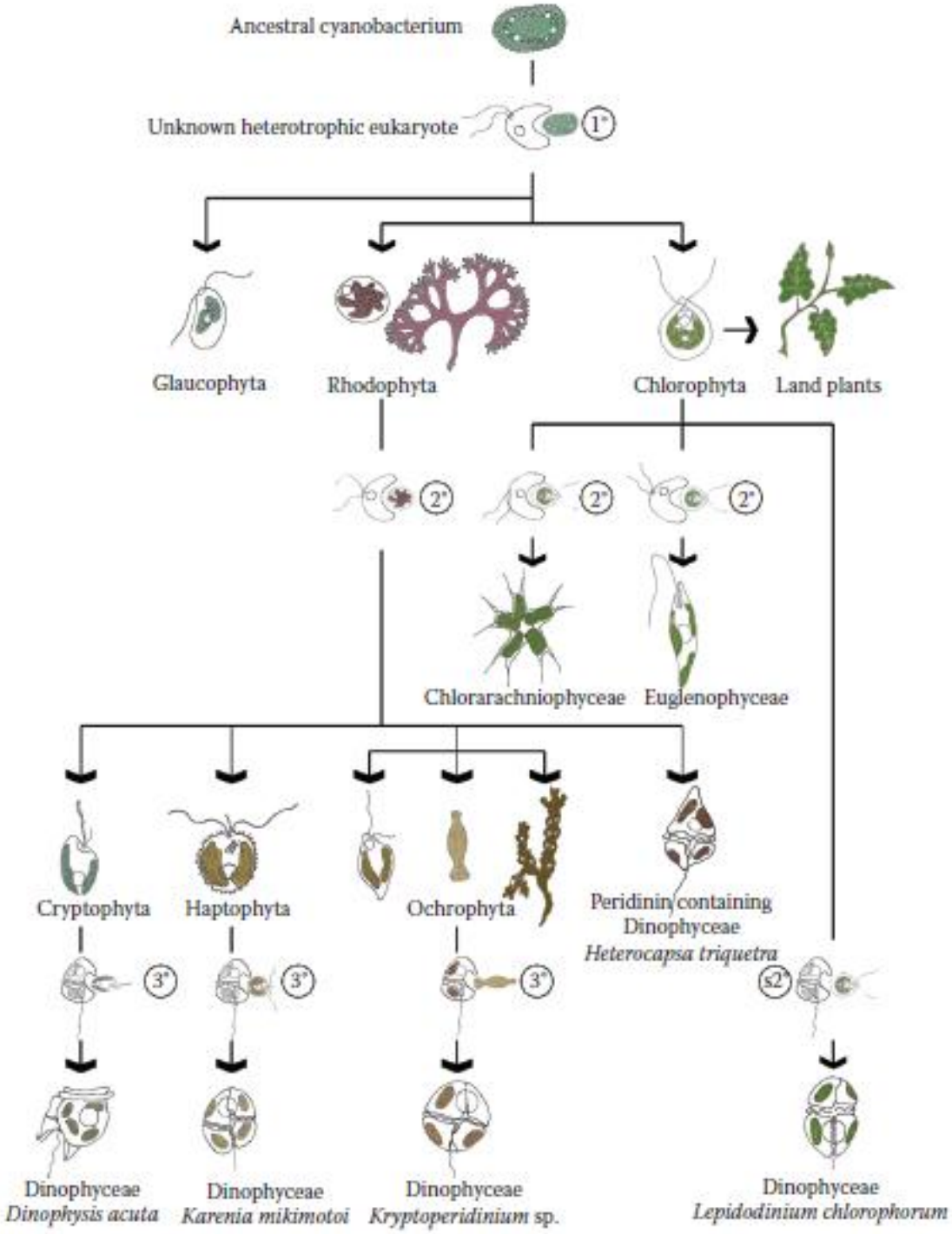

FIGURE 1.57 Hypothesis for algal evolution and endosymbiotic events. 1°, primary endosymbiosis; 2°, secondary endosymbiosis; s2°, serial secondary endosymbiosis; 3°, tertiary endosymbiosis.