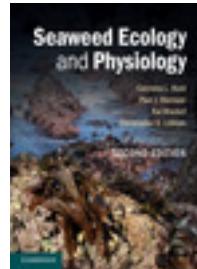


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Seaweed Ecology and Physiology

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Chapter

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Seaweed thalli and cells

1.1 Introduction: the algae and their environments

1.1.1 Seaweeds

The term “seaweed” traditionally includes only macroscopic, multicellular marine red, green, and brown algae. However, each of these groups has microscopic, if not unicellular, representatives. All seaweeds at some stage in their life cycles are unicellular, as spores or gametes and zygotes, and may be temporarily planktonic (Amsler and Searles 1980; Maximova and Sazhin 2010). Some remain small, forming sparse but productive turfs on coral reefs (Hackney *et al.* 1989) while others, such as the “kelps” of temperate reefs, can form extensive underwater forests (Graham *et al.* 2007a). Siphonous algae such as *Codium*, *Caulerpa* and *Bryopsis* that form large thalli are, in fact, acellular. The prokaryotic Cyanobacteria have occasionally been acknowledged in “seaweed” floras (e.g. Setchell and Gardner 1919; Littler and Littler 2011a). They are widespread on temperate rocky and sandy shores (Whitton and Potts 1982) and are particularly important in the tropics, where large macroscopic tufts of Oscillatoriaceae and smaller but abundant nitrogen-fixing Nostocaceae are major components of the reef flora (Littler and Littler 2011a, b; Charpy *et al.* 2012). Benthic diatoms also form large and sometimes abundant tube-dwelling colonies that resemble seaweeds (Lobban 1989). An ancient lineage of (mostly) deep-water green algae, the Palmophylloales, that includes *Verdigellas* and *Palmophyllum*, have a palmelloid organization with complex thalli built from an amorphous matrix

with a nearly uniform distribution of spherical cells (Womersley 1971; Zechman *et al.* 2010). On a smaller scale are the colonial filaments of some simple red algae, such as *Stylonema* (previously *Goniotrichum*). A “seaweed” is therefore problematic to precisely define: here “seaweed” refers to algae from the red, green, and brown lineages that, at some stage of their life cycle, form multicellular or siphonous macrothalli. In this book we shall consider macroscopic and microscopic marine benthic environments and how seaweeds respond to those environments.

The algae are evolutionarily diverse, but are related to one another through the endosymbiotic events that gave rise to plastids. The traditional classification of seaweeds as “red”, “green”, and “brown” is still fitting, but our understanding of how these groupings arose and their relatedness to each other and other eukaryotes has been transformed over the past 20 years as our understanding of endosymbiosis has grown (e.g. Walker *et al.* 2011). The evolutionary origin of the algae continues to be the subject of considerable research effort and debate (e.g. Brodie and Lewis 2007; Archibald 2009; Keeling 2010; Yoon *et al.* 2010; Burki *et al.* 2012; Collén *et al.* 2013). The taxonomic position of a species can be viewed as a “working hypothesis”, and as such is subject to change as new information arises (Cocquyt *et al.* 2010). Unraveling algal evolution is complex because, in addition to the multiple endosymbiotic events, there are other complicating events such as horizontal (lateral) gene transfer (HGT; see Brodie and Lewis 2007). Knowledge of the relatedness of the different seaweed groups, and their relations to

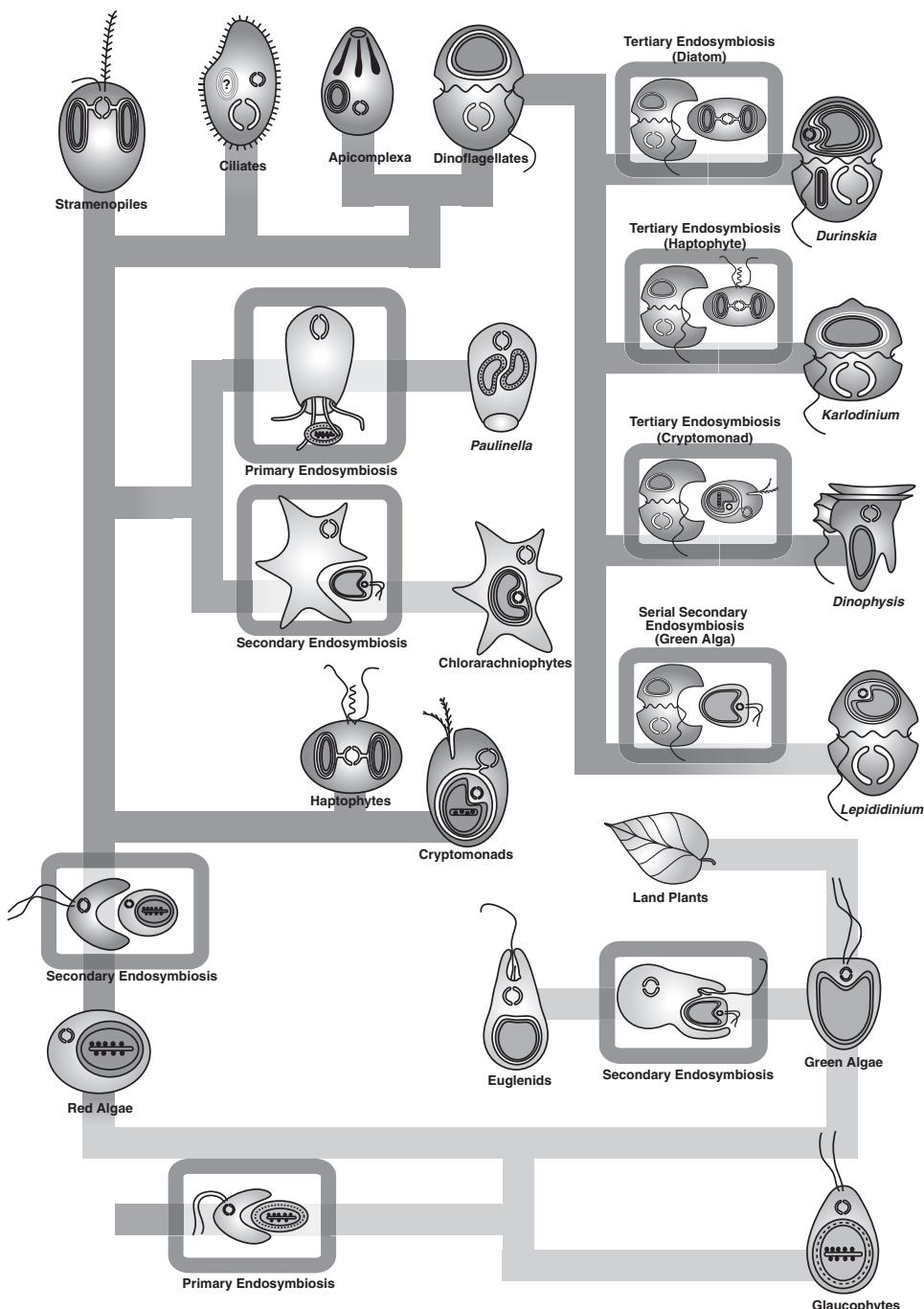


Figure 1.1 Schematic view of plastid evolution in the history of eukaryotes. The various endosymbiotic events that gave rise to the current diversity and distribution of plastids involve divergences and reticulations whose complexity has come to resemble an

other eukaryotes, is helpful in predicting aspects of their physiology and ecology.

There are several hypotheses on how algal plastids have arisen. A leading hypothesis is that a primary endosymbiotic event (~1.5 billion years ago), in which a free-living cyanobacterium was engulfed and incorporated within a heterotrophic eukaryote, gave rise to three major lineages: (1) the glaucophytes, (2) the green lineage in which the green algae are ancestral to the terrestrial plants, and (3) the red lineage which includes the red seaweeds (Yoon *et al.* 2004; Keeling 2010; Fig. 1.1). But there may have been more than one primary event, and the glaucophytes could have arisen separately from the green and red lineages (see Graham *et al.* 2009). At least three secondary endosymbiotic events (eukaryote + eukaryote) have occurred. It is fairly certain that two separate secondary events involving unicellular green algae gave rise to the euglenoids and chlorarachniophytes (reviewed by Keeling 2009, 2010; Fig. 1.1). Less clear, however, are the secondary endosymbiotic event(s) involving unicellular red algae (Burki *et al.* 2012). The chromalveolate hypothesis proposed by T. Cavalier-Smith (1999) suggests that a single secondary endosymbiotic event involving a red alga gave rise to six lineages (Fig. 1.1): ciliates, dinoflagellates, apicomplexa, haptophytes, cryptomonads, and the stramenopiles (heterokonts), with the first three belonging to the Alveolata. The chromalveolate hypothesis is “highly contentious” but considered by Keeling (2009) and others as the “hypothesis to beat”. At the time of writing (2013),

the consensus is that the stramenopiles and Alveolata group with Rhizaria, forming the “SAR” clade; the haptophytes form a closely related sister group to the SAR clade, and the position of the cryptomonads is equivocal (Walker *et al.* 2011; Burki *et al.* 2012). Within the stramenopiles, the unicellular diatoms share a common ancestor with the multicellular brown seaweeds (Phaeophyceae; Patterson 1989a; Andersen 2004). However, phylogenies based on carbon storage and cell wall polysaccharides suggest that the stramenopiles arose separately from the Alveolates, and that a related, but distinct, red algal plastid was incorporated into an ancestral stramenopile in a second endosymbiotic event (Michel *et al.* 2010a, b). The dinoflagellates arose from tertiary or serial secondary endosymbioses (Fig. 1.1). As new information arises, and new molecular and bioinformatic techniques are added to the existing repertoire, hypotheses on eukaryotic evolution and speciation will continue to develop.

Ocean vegetation is dominated by the algae. No mosses, ferns, or gymnosperms are found in the oceans, and only a few angiosperms (the seagrasses) occur in marine habitats. That there are relatively few marine angiosperms may reflect the problems of adaptation to the sea, including ion regulation and pollination (Ackerman 1998). The water column is chiefly the domain of the phytoplankton, but populations of floating seaweeds that have been detached from the substratum are common and provide an important mechanism of dispersal (sec. 3.3.7). Intertidal rocky shores are abundantly covered with a macrovegetation

Caption for Figure 1.1 (cont.) electronic circuit diagram. Endosymbiosis events are boxed, and the lines are shaded to distinguish lineages with no plastid (dark gray), plastids from the green algal lineage (light gray) or the red algal lineage (mid-gray). At the bottom is the single primary endosymbiosis leading to three lineages (glaucophytes, red algae, and green algae). On the lower right, a discrete secondary endosymbiotic event within the euglenids led to their plastid. On the lower left, a red alga was taken up in the ancestor of chromalveolates. From this ancestor, haptophytes and cryptomonads (as well as their non-photosynthetic relatives such as katablepharids and telonemids) first diverged. After the divergence of the rhizarian lineage, the plastid appears to have been lost, but in two subgroups of Rhizaria, photosynthesis was regained: in the chlorarachniophytes by secondary endosymbiosis with a green alga, and in *Paulinella* by taking up a cyanobacterium (many other rhizarian lineages remain non-photosynthetic). At the top left, the stramenopiles diverged from alveolates, where plastids were lost in ciliates and predominantly became non-photosynthetic in the apicomplexan lineage. At the top right, four different events of plastid replacement are shown in dinoflagellates, involving a diatom, haptophyte, cryptomonad (three cases of tertiary endosymbiosis) and green alga (a serial secondary endosymbiosis). Most of the lineages shown have many members or relatives that are non-photosynthetic, but these have not all been shown for the sake of clarity. (From Keeling, 2010, reproduced with permission.)

that is almost exclusively seaweeds, although in western North America surf grass (*Phyllospadix* spp.) is an exception. Seaweed surfaces themselves are colonized by benthic microalgae and bacteria, with which they may have intimate ecological relationships, and seaweed microstages grow on and within larger seaweeds. Muddy and sandy areas have fewer seaweeds, because most species cannot anchor there, though some siphonous greens (e.g. some species of *Halimeda*, *Caulerpa*, and *Udotea*) produce penetrating, root-like holdfasts that also serve in nutrient uptake (Littler *et al.* 1988). In such areas, seagrasses become the dominant vegetation, particularly in tropical and subtropical areas (Larkum *et al.* 2006). There is also a paucity of freshwater macroalgae. Freshwater red and brown algae are represented by relatively few genera and species, and Ulvophyceae are also scarce with only a few genera (e.g. *Cladophora*) having penetrated fresh waters (Wehr and Sheath 2003).

Most seaweeds are multicellular most of the time. What does this imply for physiological ecology? Multicellularity confers the advantage of allowing extensive development in the third dimension of the water column. Such development can be achieved in other ways, however. Siphonous green algae form large multinucleate thalli that are at least technically single cells (acellular rather than unicellular), supported by turgor pressure (*Valonia*), ingrowths of the rhizome wall (trabeculae) in *Caulerpa*, or interweaving of numerous narrow siphons (*Codium*, *Avrainvillea*) (Fig. 1.2). Colonial diatoms, both tube-dwelling and chain-forming, also build three-dimensional structures, as do zooxanthellae (dinoflagellates) in association with corals. Multicellular algae often grow vertically away from the substratum; this habit brings them closer to the light, enables them to grow large without extreme competition for space, and allows them to harvest nutrients from a greater volume of

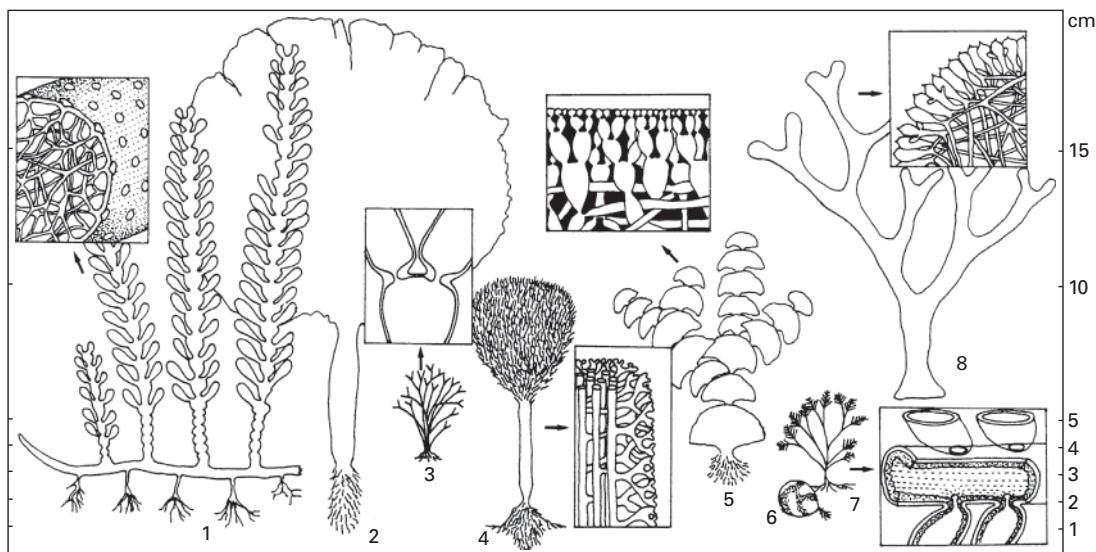


Figure 1.2 Thallus morphology and construction in siphonous green algae. Thalli drawn to scale; insets (not to scale) show principles of construction: (1) *Caulerpa cactoides*: network of trabeculae. (2) *Avrainvillea gardineri*: tightly woven felt of filaments. (3) *Chlorodesmis* sp.: bush of dichotomously branched siphons, constricted at the bases of the branches (inset). (4) *Penicillus capitatus*: calcified siphons form a multiaxial pseudotissue in the stem (inset), but separate to form bushy head. (5) *Halimeda tuna*: segmented, calcified thallus of woven medulla and cortical utricles (inset). (6) *Halicystis* stage of *Derbesia*, a single ovoid cell (shown at gametogenesis). (7) *Bryopsis plumosa* gametophyte: pinnately branched free siphons. (8) *Codium fragile*: interwoven uncalcified siphons form multiaxial branches. (From Menzel 1988, with permission of Springer-Verlag, Berlin.)

water. On the other hand, there are creeping filamentous algae, even endophytic and endolithic filaments (e.g. *Entocladia*), as well as crustose algae such as *Ralfsia*, and *Porolithon*, that do not grow up into the water column. Support tissue usually is not necessary for this upward growth, because most small seaweeds are slightly buoyant, and the water provides support. Support tissue is metabolically expensive, however strength and resilience are required to withstand water motion. Some of the larger seaweeds (e.g. *Pterygophora*) have stiff, massive stipes, but others (e.g. *Hormosira*) employ flotation to keep them upright. Many of the kelps and fucoids have special gas-filled structures, pneumatocysts (Dromgoole 1990; Raven 1996), whereas in other seaweeds (e.g. erect species of *Codium*) gas trapped among the filaments achieves the same effect (Dromgoole 1982).

A second important feature of multicellularity is that it allows division of labor between tissues; such division is developed to various degrees in seaweeds. Nutrient (and water) uptake and photosynthesis take place over virtually the entire surface of the seaweed thallus, in contrast to vascular land plants. Differentiation and specialization among the vegetative cells of algal thalli range from virtually nil (as in *Ulothrix*, where all cells except the rhizoids serve both vegetative and reproductive functions), through to *Porphyra* [many species of this genus are now treated in other genera, with most being in *Pyropia*; Sutherland *et al.* 2011; see sec. 10.2] and *Ulva* whose blades are morphologically simple but are differentiated into regions with distinct physiologies (e.g. Hong *et al.* 1995; Han *et al.* 2003), to the highly differentiated photosynthetic, storage, and translocation tissues in a variety of organs, including stipe, blades, and pneumatocysts, that occur in fucoids and kelps (Graham *et al.* 2009). Of course, no seaweed shows the degrees of differentiation seen in vascular plants. Even in vascular plants, the cells are biochemically more general than animal cells: the organs of vascular plants (stems, leaves, roots, flowers) all contain much the same mix of cells, whereas animal organs each contain only a few specialized cell types. The low diversity of cells in a seaweed thallus means that each cell is physiologically and biochemically even more general than vascular plant cells.

The evolution of multicellularity entails the co-ordinated growth of cells, which, in turn, requires cell-to-cell communication. The detection of genes coding for receptor kinases (signaling molecules that are found in all multicellular eukaryotes) in *Ectocarpus*, and their absence in the related unicellular diatoms, suggests that these molecules were a pre-requisite for multicellularity. Another pre-requisite is cell-cell adhesion via a sticky extracellular matrix. Integrin-related proteins that have a key role in cell adhesion in animals are also present in *Ectocarpus*, but not in diatoms (Cock *et al.* 2010a). In the red seaweeds, pit plugs are considered a vital step in the evolution of multicellularity, by providing structural integrity within the otherwise loosely packaged cells of pseudo-parenchymatous construction (Graham *et al.* 2009, p. 319; Gantt *et al.* 2010).

1.1.2 Environmental-factor interactions

Benthic algae interact with other marine organisms, and all interact with their physico-chemical environment. As a rule, they live attached to the seabed between the top of the intertidal zone and the maximum depth to which adequate light for growth can penetrate. Among the major environmental (abiotic) factors affecting seaweeds are light, temperature, salinity, water motion, and nutrient availability. Among the biological (biotic) interactions are relations between seaweeds and their epiphytic bacteria, fungi, algae, and sessile animals; interactions between herbivores and seaweeds (both macroalgae and epiphytes); and the impact of predators, including humans. Each propagule contains the genetic information that will allow the maturing seaweed to develop a phenotype that is suited to its environment: in fact, there can be a high degree of phenotypic plasticity even within a genetically uniform population grown under the same environmental conditions (Fig. 1.3). Individual patterns of growth, morphology, and reproduction are overall effects of all these factors combined.

An organism's physico-chemical environment, consisting of all the external abiotic factors that influence the organism, is very complex and constantly varying. In order for us to discuss or study it, we need to reduce it

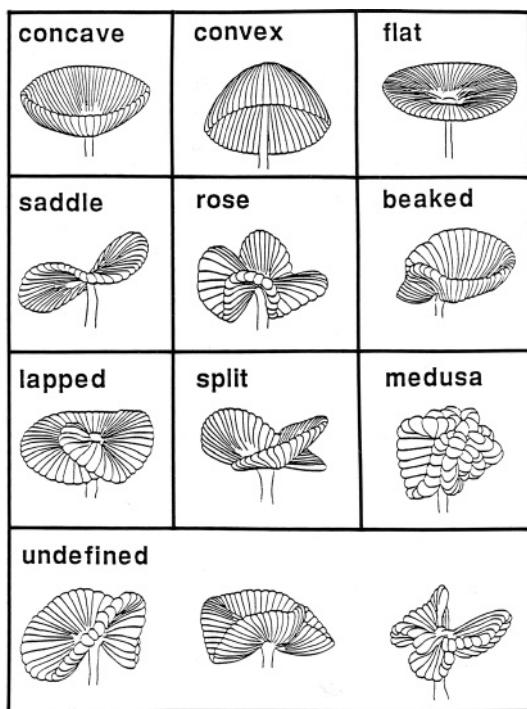


Figure 1.3 Variation in cap morphologies of *Acetabularia acetabulum*, the progeny of which were raised in the same experimental conditions. “Concave” included a minor variant (“concave-bell”) in which the rim of a concave cap was flattened. “Convex” are mirror images of concave. “Flat” caps are usually perpendicular to the stalk. “Saddles” have two opposing quadrants of the cap curved up and the other two down. In “beaked”, one or both halves of the cap are addressed and parallel to the stalk. “Split” caps have rays that are not all fused so that the cap is divided into halves, quarters or sixths. In “lapped”, the rays adjacent to the two that had not fused overlap each other. “Rose” and “medusas” are the most convoluted cap shapes. “Undefined” are caps which combine two or more of the above morphologies. (From Nishimura and Mandoli 1992, reproduced with permission.)

to smaller parts, to think about one variable at a time. And yet, each of the environmental “factors” that we might consider – temperature, salinity, light, and so forth – is really a composite of many variables, and they tend to interact. Most importantly, the organization of life is now best understood as constitutive hierarchies (Mayr 1982, p. 65), in which at each new level or system

there are emergent properties that are not predictable from study of the component parts. This is most evident in comparing the properties of individuals (say, humans) to the properties of the next level of components (organ systems, e.g. nervous system), but it also works upward from the individual through populations, communities, ecosystems, and the biosphere. It has major implications when we attempt to predict community or ecosystem properties from studies at the species (population) level, as we usually must. The following paragraphs are intended to paint the big picture, before we go on to study it pixel by pixel.

Factor interactions can be grouped into four categories: (1) multifaceted factors, (2) interactions between environmental variables, (3) interactions between environmental variables and biological factors, and (4) sequential effects.

1. Many environmental factors have several components that do not necessarily change together (i.e. multifaceted factors). Light quality and quantity, which are important in photosynthetic responses and metabolic patterns, both change with depth, but the changes depend on turbidity and the nature of the particles. In submarine caves, light quantity diminishes with little change in quality. Natural light has the additional important component of day length, which influences reproductive states. Salinity is another complex factor, of which the two chief components are the osmotic potential of the water and the ionic composition. Osmotic potential affects water flow in and out of the cell, turgor pressure, and growth, while the concentrations of Ca^{2+} and HCO_3^- affect membrane integrity and photosynthesis, respectively. Hydrodynamic forces affect thallus survival and spore settlement on wave-swept shores, and water motion also has important effects on the boundary layers over seaweed surfaces and thus on nutrient uptake and gas exchange. Nutrients must be considered not simply in their absolute concentrations but also in the amounts present in biologically available forms; concentrations of trace metals may create toxicity problems, particularly in polluted areas. Pollution, as a factor, may include not only the toxic effects of component chemicals but also an increase in

turbidity, hence a reduction in irradiance. Emersion often involves desiccation, heating, or chilling, removal of most nutrients (except CO₂), and, frequently, changes in the salinity of the water in the surface film on the seaweeds and in the free space between cells.

2. Interactions among environmental variables are the rule rather than the exception. Bright light is often associated with increased heating, particularly of seaweeds exposed at low tide. Light, especially blue light, regulates the activities of many enzymes, including some involved in carbon fixation and nitrogen metabolism. Temperature and salinity affect the density of seawater, hence the mixing of nutrient-rich bottom water with nutrient-depleted surface water. Thermoclines can affect plankton movements, including migration of the larvae of epiphytic animals. Temperature also affects cellular pH and hence some enzyme activities. The seawater carbonate system and especially the concentration of free CO₂ are greatly affected by pH, salinity, and temperature, while the availability of ammonium is pH-dependent, because at high pH the ion escapes as free ammonia. Water motion can affect turbidity and siltation as well as nutrient availability. These are examples of one environmental variable affecting another. There are also examples of two environmental variables acting synergistically on seaweed; for instance, the combination of low salinity and high temperature can be harmful at levels where each alone would be tolerable. In some seaweeds, the combined effects of temperature and photoperiod regulate development and reproduction.
3. Interactions between physico-chemical and biological factors are also the rule rather than the exception. The environment of a given seaweed includes other organisms, as we have seen, with which the seaweed interacts through intraspecific and interspecific competition, predator-prey relationships, associations with parasites and pathogens, and basiphyte-epiphyte relationships. These other organisms are also affected by the environment, as are their effects on other organisms. Moreover, other organisms may greatly modify the

physico-chemical environment of a given individual. Protection from strong irradiance and desiccation by canopy seaweeds is important to the survival of understory algae, including germlings of the larger species. Organisms shade each other (and sometimes themselves) and have large effects on nutrient concentrations and water flow. Other interactions stem from the way the biological parameters, such as age, phenotype, and genotype, affect a seaweed's response to the abiotic environment, as well as the effects that organisms have on the environment. The chief biological parameters that condition a given seaweed's response to its environment are age, reproductive condition, nutrient status (including stores of N, P, and C), and past history. By "past history" is meant the effects of past environmental conditions on seaweed development. Genetic differentiation within populations leads to different responses in seaweeds from different parts of a population. The seasons can also affect certain physiological responses, aside from those involved in life-history changes; these responses include acclimation of temperature optima and tolerance limits.

4. Finally, there are factor interactions through sequential effects. Nitrogen limitation may cause red algae to catabolize some of their phycobiliproteins, which will in turn reduce their light-harvesting ability. In general, any factor that alters the growth, form, or reproductive or physiological condition is apt to change the responses of the seaweed to other factors both currently and in the future. A good example of a sequential effect, and also biotic-abiotic interaction, was seen by Littler and Littler (1987) following an unusual flash flood in southern California. Intertidal urchins (*Strongylocentrotus purpuratus*) were almost completely wiped out, but the persistent macroalgae suffered little damage from the freshwater. Subsequently, however, there was a great increase in ephemeral algae (*Ulva*, *Ectocarpaceae*) because of the reduction in grazing pressure. The complexity of the interactions of variables in nature often confounds interpretation of the effects even of "major" events, such as El Niño warm-water periods (Paine 1986; sec. 7.3.7).

Testing the effects of the various factor interactions described above requires a multifaceted approach that includes quantitative field observations, field manipulations and targeted laboratory experiments; for each approach a rigorous experimental design is essential so that the appropriate statistical analyses can be applied to detect differences among experimental treatments. In laboratory experiments, usually one variable is tested at a time, and all other factors are held constant, or at least equal in all treatments. Experiments in which two (or occasionally three) factors are varied are possible but the number of culture vessels required for independent replication of treatments can be technically difficult to achieve especially with large seaweeds: it is important to avoid pseudoreplication in both laboratory and field experiments (Hurlbert 1984). It is also important to understand how field manipulations can confound results, and to include appropriate controls. For example, Underwood (1980) criticized some field experiments designed to determine the effects of grazer exclusion because the fences and cages used to keep out grazers also affected the water motion over the rock surface and provided some shade. Furthermore, field studies that use correlation analyses to elucidate whether an environmental factor causes a specific biological pattern (e.g. growth, onset of reproduction) can be misleading because the key environmental factors that regulate seaweed biological processes are themselves tightly correlated, for example light, temperature, and nitrate concentration. As Schiel and Foster (1986, p. 273) explain ‘‘The existence of patterns and abundance of species constitutes evidence that these physical factors and biological interactions may affect the structure of these communities. They do not at the same time, however, demonstrate the importance or unimportance of these factors in producing observed patterns.’’

1.1.3 Laboratory culture versus field experiments

Several considerations confound the interpretation of field reality via laboratory studies. First, while laboratory studies provide much more controlled conditions than are found in nature, they are limited in some

important ways and contain some implicit assumptions, such as the following: (1) High nutrient levels common in lab experiments do not alter the seaweeds’ responses to the factor under study. (2) The reactions of seaweeds to uniform conditions (including the factor under study) are not different from their responses to the factor(s) under fluctuating conditions. To a certain extent these assumptions are valid. Culture media can be very rich in nutrients, to compensate for lack of water movement and exchange, but it is unlikely that this substitution can give precisely the same results. Other culture conditions are also generally optimal, except for the variable under study, and the results may not elucidate the behavior of seaweeds in the field, which are subject to competition and often suboptimal conditions (Neushul 1981). Another important difference between laboratory and field is that in culture, species usually are tested in isolation, away from interspecific competition and grazing. Furthermore, culture conditions are uniform (at least on a large scale), whereas in nature there often are large and unpredictable fluctuations in the environment (e.g. Gorospec and Karl 2011). Microscale heterogeneity in culture conditions should not be overlooked (Allen 1977; Norton and Fetter 1981). In the culture flask, one cell may shade another, and cells form nutrient-depleted zones around them, creating a mosaic of nutrient concentrations through which cells pass. In the field, scale also needs to be considered at the large end – for instance, the amount of space needed for a patch of a given alga to establish itself (Schiel and Foster 1986). In essence, for both field and laboratory experiments, informed decisions must be made on the experimental conditions that are provided, and it is important to be aware that these conditions will affect the outcome and interpretation of the results.

Second, the timescale over which an experiment is conducted affects the interpretation of the data (Raven and Geider 2003). In short-term physiological experiments (seconds to minutes), a single factor can be varied (e.g. different levels of UV-B radiation) and a response (e.g. the production of reactive oxygen species) is measured. This physiological response is at the level of *regulation* i.e. the up- or down-regulation

of pre-existing enzymes, and reveals the physiological potential of that organism to respond to an immediate environmental change. In medium-term experiments (hours to days) *acclimation* to new environmental conditions may occur. Acclimation involves gene expression, and the synthesis of new proteins such as enzymes. *Adaptation* to particular environmental factors occurs over a longer timescale (up to millennia) and is a mechanism for speciation (sec. 7.1).

Third, when a single species occurs in widely different latitudes or longitudes, its physiology and ecology may be quite different. For many topics, only one study or a few studies have been done, and a phenomenon demonstrated in a particular alga under certain conditions will not necessarily turn out to be the same in other algae or under other conditions. In Australia, for example, the kelp *Ecklonia radiata* dominates across 3000 km of coastline, from the southeast to southwest. However, the morphology and ecology of *Ecklonia* on the east coast is very different to *Ecklonia* on the west and south coasts, with the result that different coastal management plans are required for these different regions (Connell and Irving 2009). Equally, very few natural populations or communities have been studied often enough to assess how much variability is present from place to place (ecotypic variation). The kelp beds of southern California are exceptional in that they have been repeatedly analyzed by different people along the coast since the 1960s (Steneck *et al.* 2002; Graham *et al.* 2007a). For *Macrocystis*, there is no typical kelp bed; environmental parameters differ from one kelp bed to another, and parameters such as specific growth rate versus nitrogen supply vary among populations (Kopczak *et al.* 1991).

In this first chapter we shall review the foundations of seaweed construction, cell biology, molecular biology and genetics on which any understanding of seaweed physiological ecology must rest. In Chapter 2, we continue this review by tracing the development of seaweed thalli from gametes and spores to reproductive individuals. In both these chapters, we build upon the fundamental information on seaweed anatomy and development that is described in algal text books, particularly van den Hoek *et al.* (1995) and Graham *et al.* (2009).

1.2 Seaweed morphology and anatomy

1.2.1 Thallus construction

Diversity of thallus construction in algae contrasts strongly with uniformity in vascular plants. In the latter, parenchymatous meristems (e.g. at the shoot and root apices) produce tissue that differentiates in a wide variety of shapes. For seaweeds, parenchymatous construction is prevalent only in the brown orders. For example, in kelps, fucoids, and Dictyotales, this mode of construction has given rise to internal and morphological complexity (Fig. 1.4). The larger seaweeds, especially Laminariales and Fucales, have several different tissue and cell types, including photosynthetic epidermis, cortex, medulla, sieve tubes, and mucilage ducts (Graham *et al.* 2009). The ontogeny of the parenchyma in the Dictyotales (Fig. 1.4d-m) has been followed in detail by Gaillard and L'Hardy-Halos (1990), who cite many sources, and by Katsaros and Galatis (1988). However, the great majority of seaweeds either are filamentous or are built up of united or corticated filaments. Large and complex structures can be built up this way, for example *Codium amplivesiculosum* (previously *C. magnum*) can reach several meters long (Dawson 1950). Cell division may take place throughout the alga, or the meristematic region may be localized. If localized, it is most commonly at the apex, but may be at the base or somewhere in between (intercalary).

A simple filament consists of an unbranched chain of cells attached by their end walls and results from cell division only in the plane perpendicular to the axis of the filament. Unbranched filaments are uncommon among seaweeds; examples are *Ulothrix* and *Chaetomorpha*. Usually, some cell division takes place parallel to the filament axis to produce branches (*Cladophora*, *Ectocarpus*, *Antithamnion*; see Fig. 1.17). Filaments consisting of a single row of cells (branched or not) are called uniseriate. Pluriseriate filaments, i.e. two or more rows of cells, are seen in genera such as *Blidingia*, *Bangia*, and *Sphacelaria* (Fig. 1.4a; Graham *et al.* 2009). Branches need not grow out free, but may creep down the main filament, forming cortication, as seen in *Ceramium* (Fig. 1.5a) and *Ballia*. In some of the

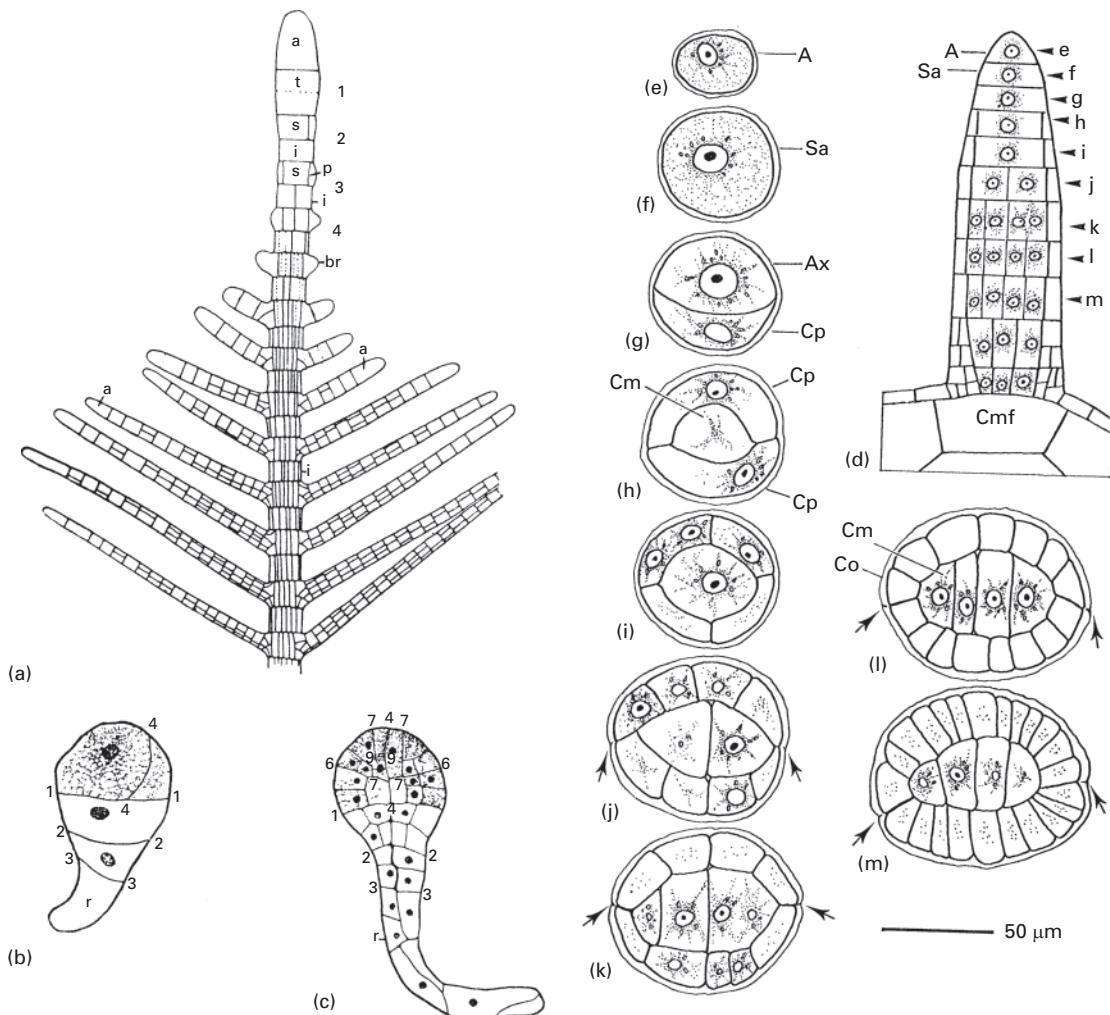


Figure 1.4 Parenchymatous development in seaweeds. (a) *Sphaerelaria plumula* apex showing first transverse division (t), followed by pairs of cells (i, s), of which s forms branches, but i does not. (b, c) *Fucus vesiculosus* germination showing successive cell divisions (numbered) (divisions 5 and 8 in the plane of the page). (d-m) *Dictyota*: development of parenchyma; (d) long section through adventive branch, showing locations of cross sections at each level (diagrammatic); (e-m) serial cross sections to show sequence of periclinal divisions. Arrows indicate junction between original two pericentral cells (first shown in h). For the sake of clarity, the proportions of the cells were changed; the adventive branch is actually half as long and twice as wide as shown. A, apical cell; Sa, subapical cell; Ax, axial cell; Cp, pericentral cell; Cm, medullary cell; Co, cortical cell. (Parts a-c from Fritsch 1945, based on classical literature; d-m from Gaillard and L'Hardy-Halos 1990, with permission of Blackwell Scientific Publications.)

larger Rhodomelaceae, such as *Laurencia* and *Acanthophora*, the cortication becomes so extensive that the origin of the structure is obscured. Pseudoparenchymatous construction is when neighboring filaments adhere to one another and form a structure that looks very much like parenchymatous bodies (Graham *et al.* 2009). A detailed study by Kling and Bodard (1986) of axis development in *Gracilariaopsis longissima* (previously *Gracilaria verrucosa*) (uniaxial) showed how complex, and difficult to interpret, pseudoparenchymatous growth patterns can be (compare Fig. 1.5g–n with the parenchymatous construction of *Dictyota* Fig. 1.4d–m).

Many of the larger seaweed thalli are multiaxial, produced by the adhesion of several filaments. This is particularly common among the red algae (Fig. 1.5d–f) (van den Hoek *et al.* 1995; Graham *et al.* 2009). Multiaxial construction is most readily seen in the less tightly compacted thalli of *Nemalion* or *Liagora*. The contrast between multiaxial and uniaxial growth can be seen within thalli of *Dumontia contorta* (previously *Dumontia incrassata*) (Fig. 1.5d–f), in which bases are multiaxial, but upper branches are uniaxial (Wilce and Davis 1984). The adhesion of filaments can also produce a pseudoparenchymatous crust (*Peyssonnelia*, *Neoralfsia*) or blade (*Anadyomene*; Fig. 1.5b,c). Many siphonous green algae, including *Halimeda* and *Codium*, are formed by the interweaving of numerous filaments (Fig. 1.2). In the Corallinaceae, multiaxial apical growth forms the hypothallus (in crusts) or central medulla (in erect forms), while intercalary meristems on the lateral branches form the epithallus and perithallus (cortex in erect axes) (Cabioch 1988).

Cell division in two planes can result in a monostromatic sheet of cells, as in *Monostroma*. *Ulva* spp. are distromatic and develop from a uniseriate filament that becomes a pluriseriate filament, which in turn can become either a hollow tube (e.g. *Ulva intestinalis*, previously *Enteromorpha intestinalis*) or a two-layered blade (e.g. *Ulva lactuca*). Interestingly, for both *Monostroma* and *Ulva* the development of a thallus depends on the presence of epiphytic bacteria (Matsuo *et al.* 2005; sec. 2.6.2).

Plasmodesmata are a feature shared by land plants and parenchymatous green and brown seaweeds, and they connect neighboring cells allowing cellular communication (Raven 1997a). The red seaweeds, however,

do not exhibit parenchymatous construction, nor do they have plasmodesmata. Characteristic of florideophycean red seaweeds are pit connections with pit plugs (Peuschl 1989). Primary pit plugs are granular protein masses that literally “plug the hole” that is left following incomplete cell division. Secondary pit plugs can form between cells of different filaments within a pseudoparenchymatous structure; they can also form between individual germlings as part of the coalescence process that gives rise to the chimeric organization that is common in red seaweed (Santelices *et al.* 1999 – see below). Although less common, pit connections and plugs do occur in the Bangiophyceae. For example in *Pyropia yezoensis* (previously *Porphyra yezoensis*), they are present in the filamentous sporophyte phase (conchocelis) but absent in the bladed gametophyte (Ueki *et al.* 2008).

1.2.2 The Littler functional-form model

The construction of the thallus has importance for developmental physiology. Similar morphologies can be constructed in different ways; the overall morphology is important to ecological physiology. Among different algal classes, certain morphologies are repeated, which, as noted by Littler *et al.* (1983a), indicates convergent adaptations to critical environmental factors. On the other hand, species face divergent selection pressures: those favoring more productive, reproductive, and competitive thalli, versus those favoring longevity and environmental resistance (Littler and Kauker 1984; Russell 1986; Norton 1991). Many seaweeds show a variety of morphologies within one life history (see Chapter 2). Heterotrichous seaweed with crustose bases and erect fronds within one generation (e.g. *Corallina*) and heteromorphic seaweeds with crustose/filamentous and frondose generations (e.g. *Scytoniphon*) (Fig. 2.2) are both common. How can we assess the significance of morphology when we are faced with convergence between classes on the one hand and diversification within species on the other hand?

The functional-form model was advanced by Littler and Littler (1980) and subsequently tested extensively by both themselves and others. The model has also

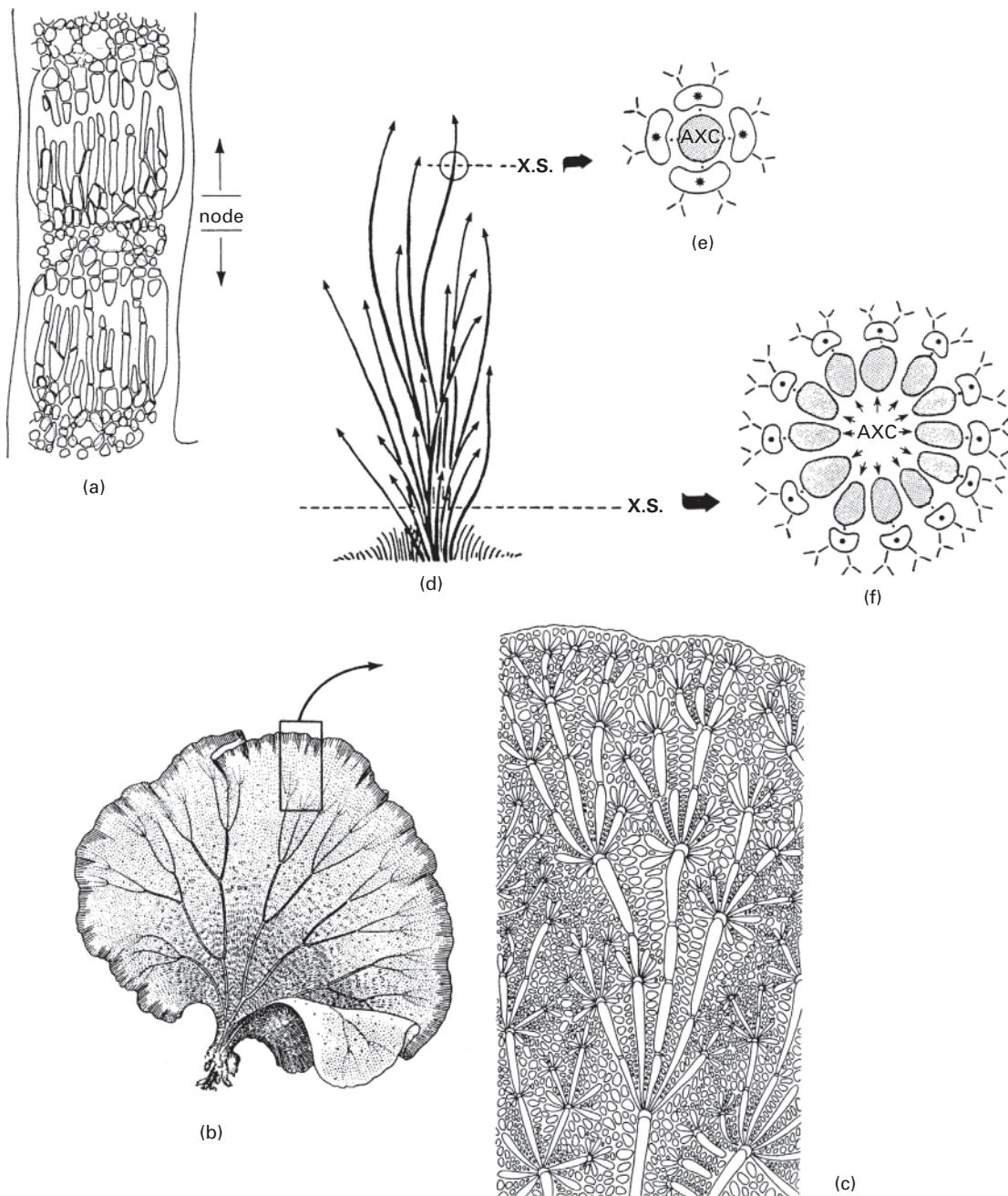
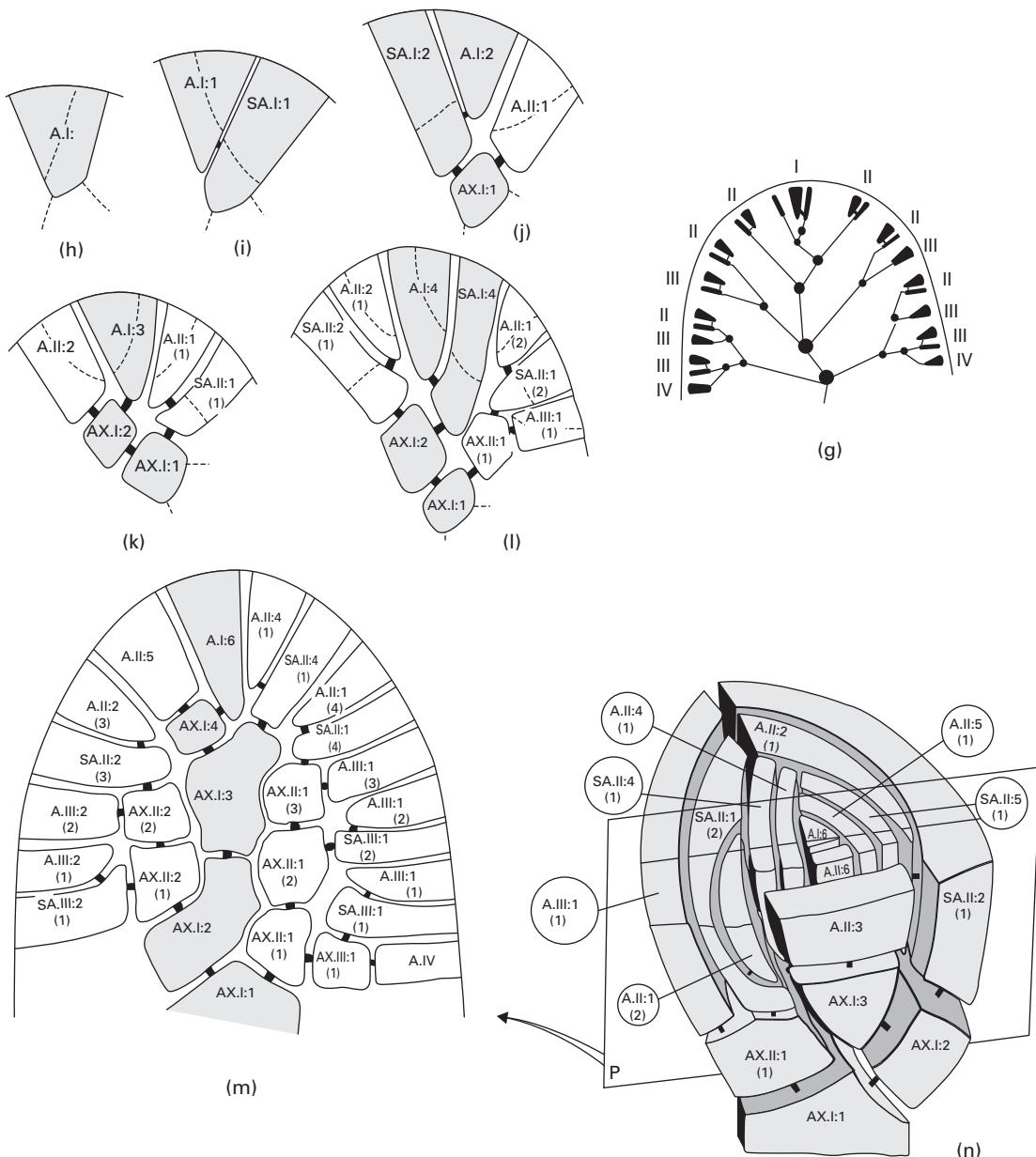


Figure 1.5 Filamentous thallus construction. (a) Small portion of a *Ceramium* axis with cortication growing upward and downward from a node between axial cells. (b, c) Formation of blade-like thallus from filaments in *Anadyomene stellata* ($b, \times 1.82$; $c, \times 13.65$). (d–f) Growth of *Dumontia contorta* (previously *Dumontia incrassata*) showing schematically the axial filaments and apical cells (arrows); cross section in the uniaxial part of the thallus near the tip (e) shows a single axial cell (AXC) surrounded by four pericentral cells (*) that have in turn produced cortical cells; (f) cross section through base shows multiaxial construction with a core of axial cells, each with one pericentral cell.



Caption for Figure 1.5 (cont.) (g-n) Apical growth of *Gracilaria longissima* (previously *Gracilaria verrucosa*). (g) A primary apical cell (I) occurs at the tip of the main axis, and secondary apical cells (II, III, etc.) occur at the tips of lateral filaments. (h-m) Division of the apical cell (A.I), shown by dotted line in (h), gives rise to a subapical cell (SA.I: 1) and a new apical cell (A.I:1)(i). In (i-j), the subapical cell is shown dividing to form an axial cell (AX.I:1) and a secondary apical cell (A.II:1), while the new apical cell (A.I:1) cuts off another subapical cell (SA.I:2) and becomes A.I:2. The lineages can be traced further with the help of the pit connections (represented as dark bars between cells). (n) The three-dimensional arrangement is complex because the apical cell divides on three faces. P is the plane of the vertical section in (m). (Part a from Taylor 1957; band c from Taylor 1960, with permission of University of Michigan Press; d-f from Wilce and Davis 1984, with permission of *Journal of Phycology*; g-n from Kling and Bodard 1986, with permission of *Cryptogamie: Algologie*.)

Table 1.1 Functional-form groups of macroalgae

Functional-form group	External morphology	Internal anatomy	Texture	Sample genera
Sheet group	Thin, tubular, and sheetlike (foliose)	Uncorticated, one to several cells thick	Soft	<i>Ulva, Pyropia, Dictyota</i>
Filamentous group	Delicately branched (filamentous)	Uniseriate, multiseriate, or lightly corticated	Soft	<i>Centroceras, Polysiphonia, Chaetomorpha, Ectocarpus</i>
Coarsely branched group	Coarsely branched, upright	Corticated	Fleshy-wiry	<i>Laurencia, Chordaria, Caulerpa, Penicillllus, Gracilaria</i>
Thick, leathery group	Thick blades and branches	Differentiated, heavily corticated, thick-walled	Leather, rubbery	<i>Laminaria, Fucus, Udotea, Chondrus</i>
Jointed calcareous group	Articulated, calcareous, upright	Calcified genicula, flexible intergenicula with parallel cell rows	Stony	<i>Corallina, Halimeda, Galaxaura</i>
Crustose group	Prostrate, encrusting	Calcified or uncalcified parallel rows of cells	Stony or tough	<i>Lithothamnion, Ralfsia, Hildenbrandia</i>

Source: Littler *et al.* (1983b), with permission of *Journal of Phycology*.

been modified by Steneck and Dethier (1994), and by Balata *et al.* (2011) who propose a system that has 35 functional groupings, compared to Littler and Littler's 6. The Littler and Littler (1980) model holds that the functional characteristics of seaweeds, such as photosynthesis, nutrient uptake, and grazer susceptibility, are related to form characteristics, such as morphology and surface-area:volume (SA:V) ratios (Table 1.1). One can thus set up predictions of function from an examination of form. For example, the sheet group are predicted to have high rates of growth, photosynthesis and nutrient uptake, low resistance to herbivory, and low competitive ability. Functional groupings have been used to test hypotheses relating to algal primary production and nutrient uptake, resistance to herbivory, tolerance to physiological stress, and successional stage of communities (reviewed by Padilla and Allen 2000). Functional form in relation to light harvesting and nutrient uptake are discussed in secs. 5.3.2 and 5.7.2, respectively.

Functional-form models have proven valuable in predicting physiological rates, because nutrient and inorganic carbon uptake are strongly related to surface-area:volume ratio (e.g. Taylor *et al.* 1999). There is a trend of declining physiological rates and

specific growth rate from group 1 to group 6 (e.g. Fig. 5.25). However, power-scaling approaches can be equally useful as predictors of net photosynthesis, respiration, and growth (Enríquez *et al.* 1996; de los Santos *et al.* 2009). On a tropical reef, the productivity of the unicellular and filamentous components of a turf-forming community could be determined accurately without knowledge of the individual species (Williams and Carpenter 1990). However, functional groups are less successful in predicting the susceptibility of seaweeds to herbivores, and successional stage (Padilla and Allen 2000, Table 1). Also, categorizing specific morphologies is not always simple because there are no sharp boundaries between some groups. For example, 15 and 20% of species could not be allocated to a functional group in the studies of Phillips *et al.* (1997) and de los Santos *et al.* (2009), respectively.

The allocation of species to particular functional groups requires little taxonomic expertise, and as such it can be an attractive method of examining ecosystem biodiversity and detecting long-term changes (Collado-Vides *et al.* 2005; Balata *et al.* 2011). Phillips *et al.* (1997) compared functional groups and full taxonomic classification as methods of detecting shifts in seaweed communities along a wave-exposure gradient. The

functional grouping method was less able to detect differences between communities, and resulted in a substantial loss of biodiversity information. In the Florida Keys, USA, four genera of calcareous green seaweeds (*Halimeda*, *Udotea*, *Penicillus*, and *Rhipocephalus*) fall into the same functional group (jointed, calcareous), but a 7-year study revealed that each species had very different seasonal patterns in abundance. Once again, grouping the different genera into one functional group lead to a loss of information (Collado-Vides *et al.* 2005). However, using their expanded functional-form model, Balata *et al.* (2011) were able to detect differences between Mediterranean seaweed assemblages that were exposed to different environmental stressors. In summary, functional groups have proven useful in assessing seaweed metabolic processes, but further testing is required if they are to be rigorously applied to other aspects of ecology and biodiversity (Padilla and Allen 2000; for further discussion on functional form and grazing see sec. 4.3.2).

1.2.3 Unitary, clonal, and coalescing seaweeds, and modular construction

In the 1970s, terrestrial plant ecologists distinguished between “unitary plants” (also termed “aclonal” and “non-clonal”) which have leaves and roots connected to a main axis and grow predominantly in the vertical direction and “clonal plants” which can spread laterally and vegetatively over the soil surface; these distinctions equally apply to seaweeds (reviewed by Santelices 2004a; Scrosati 2005). Unitary seaweeds originate from unicellular propagules (haploid or diploid), have just one axis that grows vertically from the holdfast, tend to have morphological and physiological differentiation, and do not produce ramets (defined below). Examples include canopy-forming seaweeds such as *Durvillaea antartica*, *Saccharina latissima* (previously *Laminaria saccharina*), and *Lessonia nigrescens*, and also smaller seaweeds such as *Fucus* species, *Ulva taeniata* and *Colpomenia tuberculata* (Santelices 2004a; Scrosati 2005). Clonal seaweeds are defined by Scrosati (2005) as those for which the “holdfast produces a number of fronds vegetatively, each

frond having the potential capacity for autonomous life if it becomes physically isolated from the rest while remaining attached to the substrate by an original portion of holdfast”. *Genet* is a “genetic individual” defined as “the free-living individual that develops from one original zygote, parthenogenetic gamete or spore and produces ramets vegetatively during growth” (Scrosati 2002a). A *ramet* is the smallest potentially physiologically independent unit of the genet or “any algal fragment with the ability to reattach to the substratum and develop as a new individual” (Collado-Vides 2002a). Seaweeds exhibiting clonal growth include *Mazzaella parksii* (previously *M. cornucopiae* and *Iridaea cornucopiae*), *Caulerpa*, and *Ascophyllum nodosum*. Clonal seaweeds can be further categorized as either coalescing clonal (e.g. *Mazzaella parksii*) or non-coalescing clonal e.g. *Pterocladiella capillacea* (previously *Pterocladia capillacea*) (Scrosati 2005). The genets of some clonal seaweeds establish cellular connections and coalesce to form chimeras¹ (Fig. 1.6a). Coalescence is widespread in the red seaweeds, but rare in the other seaweed phyla (Santelices *et al.* 1996, 1999; Santelices 2004a).

Both unitary and clonal seaweed groupings contain examples of modular construction. The term “module” can refer to any part of an organism that is a reiterated unit. *Fucus* species, for example, are unitary modular because each branch and associated apical cell is repeated as a result of growth, whereas *Laminaria* species are unitary non-modular because each individual has only one meristem and this pattern is not repeated within an individual. *Gelidium* species are examples of clonal-modular seaweeds, in which the branched ramets are the repeated units, whereas *Mazzaella* is clonal non-modular because each ramet is unbranched (Ricardo Scrosati, pers. comm.).

Until the early 2000s, most physiological and ecological research has considered seaweeds as unitary

¹ The terms “chimera” and “genetic mosaic” are often used interchangeably but this usage is incorrect. Both are genetically heterogeneous (i.e. not homogenous), but mosaics are more common as they arise from intrinsic genetic variations caused by for example somatic mutations whereas chimeras are the result of genetic mixing by genetically distinct individuals (Santelices 2004b).

organisms, including in the functional-form models of sec. 1.2.2. Santelices (2004a) suggests that the way in which unitary, clonal, and coalescing organisms interact and respond physiologically and morphologically to their abiotic and biotic environment will be different. For example, the number of erect axes formed from

basal crusts of *Gracilaria chilensis* and *Mazzaella laminarioides* increases with the number of spores forming the coalescence, and within the first 60 days of life, the chimeric “individuals” grow faster than the unitary ones (Fig. 1.6b): such differences are likely to influence resource acquisition in the field (Santelices *et al.* 2010).

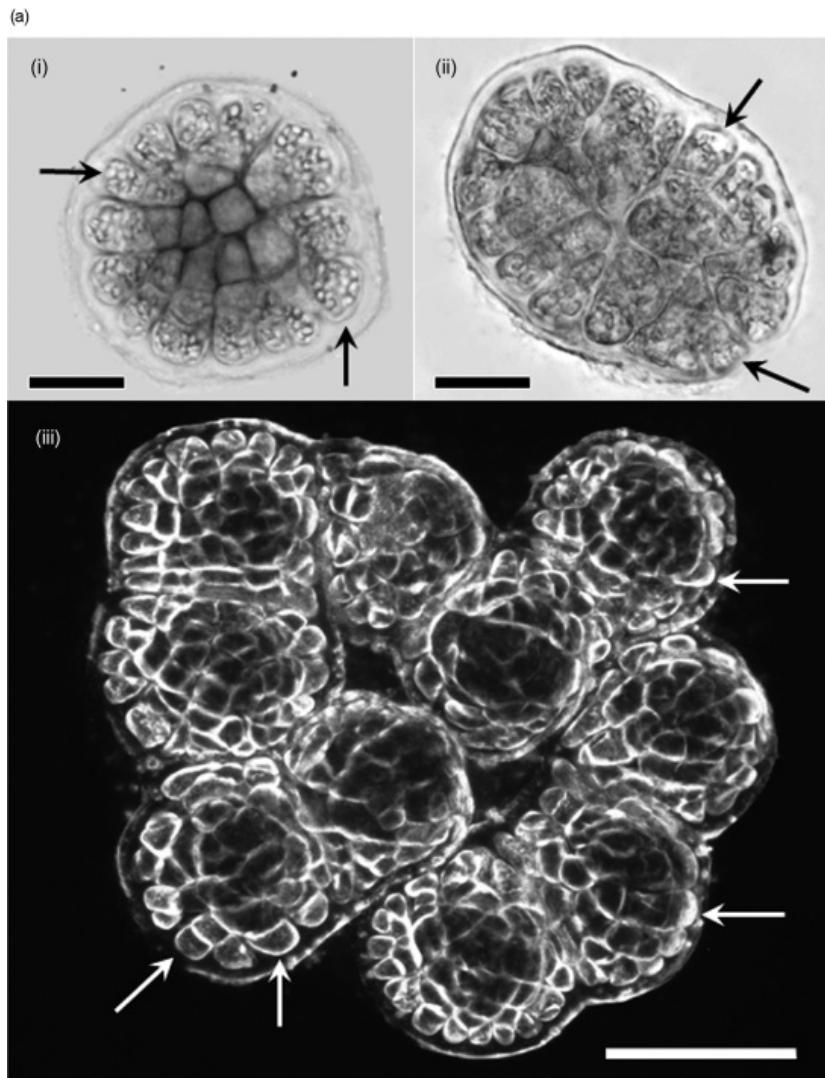


Figure 1.6 (a) Sporelings of *Gracilaria chilensis* formed by one (i), two (ii), and 10 (iii) spores. Observe the proportional reduction in free marginal cells with increasing number of fusions. Arrows indicate free marginal cells. Scales are 50 µm in (i) and

For species diversity, many indices are based on the numbers of individuals but an individual clonal-modular organism can be very large and cover a considerable surface area of substrate (Santelices 1999; see sec. 3.5.1). The ability of seaweeds to coalesce also raises the question of what constitutes an individual (Santelices 1999). Furthermore, self-thinning rules also do not apply equally to clonal versus unitary seaweed (Scrosati 2005; sec. 4.2.3). There is clearly a need for a more holistic model that combines the traditional functional-form groupings of Littler and Littler (1980) with developing theories on modularity and coalescence (e.g. Santelices 2004a; Scrosati 2005).

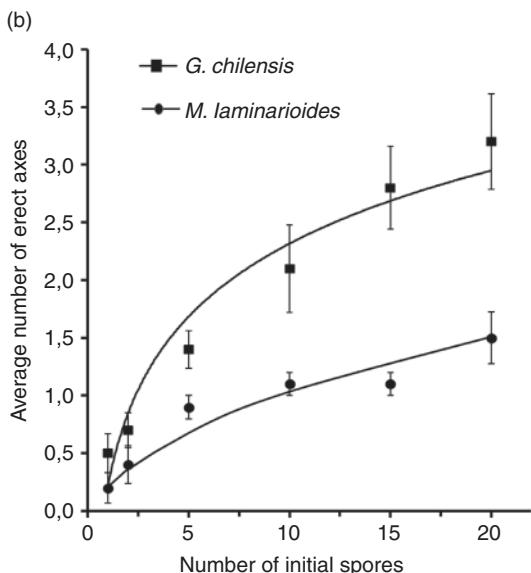
1.3 Seaweed cells

Although there is interaction between the morphology of the whole seaweed and the environment, the physiological responses to the environment, as well as the mechanisms by which the overall morphology

is generated, occur within the individual cells (Niklas 2009). Cells are protected by walls and membranes, and are compartmentalized with membrane-bound organelles, and it is through these membranes and walls that contact with the environment must take place. The structure and composition of cell components thus provide a necessary background to the study of physiological ecology.

Certain components and functions of algal cells are similar to (though not necessarily identical to) the systems worked out in other organisms (e.g. rats or bacteria). Mitochondrial structure and function, genetic material and its translation into proteins, and membrane structure are fundamental features of eukaryotic cells. Other cell components are distinctive in the algae; these include cell wall composition and structure, flagellar apparatus, the cytoskeleton, and the thylakoid photosystem structure. See Pueschel (1990), Van den Hoek *et al.* (1995), Larkum and Vesk (2003), Katsaros *et al.* (2006) and Graham *et al.* (2009) for reviews of algal cytology; see Buchanan *et al.* (2000) and Beck (2010) for reviews of higher plant cell biology.

Algal cells also contain unique structures, many of which contain bioactive secondary metabolites. Brown algal cells characteristically contain physodes (Fig. 1.7), phlorotannin-containing vesicles that fulfill a wide range of roles at the cellular and organismal level including cell wall formation, wound healing (sec. 2.6.4), adhesion of propagules to the substrate (sec. 2.5.2), protection from UV radiation (sec. 7.6), herbivore deterrents (sec. 4.4) and detoxifying metals (9.3.3) (Schoenwaelder 2002). The *corps en cerise* (cherry bodies), specific to *Laurencia* species, are storage vesicles for halogenated compounds which are trafficked to the cell surface where the released contents act as herbivore deterrents and anti-foulants (Salgado *et al.* 2008). “Gland cells” common in the red algae also contain secondary metabolites which defend against bacteria (Paul *et al.* 2006a; sec. 4.2.2).



Caption for Figure 1.6 (cont.) (ii) and 100 µm in (iii). (b) Average number of erect axes differentiated by 30-d sporelings of *Gracilaria chilensis* and *Mazzaella laminariooides* as a function of the number of initial spores forming the sporeling. (From Santelices *et al.* 2010, reproduced with permission.)

1.3.1 Cell walls

Cell walls do not merely provide rigidity. They are essential to cell growth and developmental processes, such as axis formation in zygotes and branching in

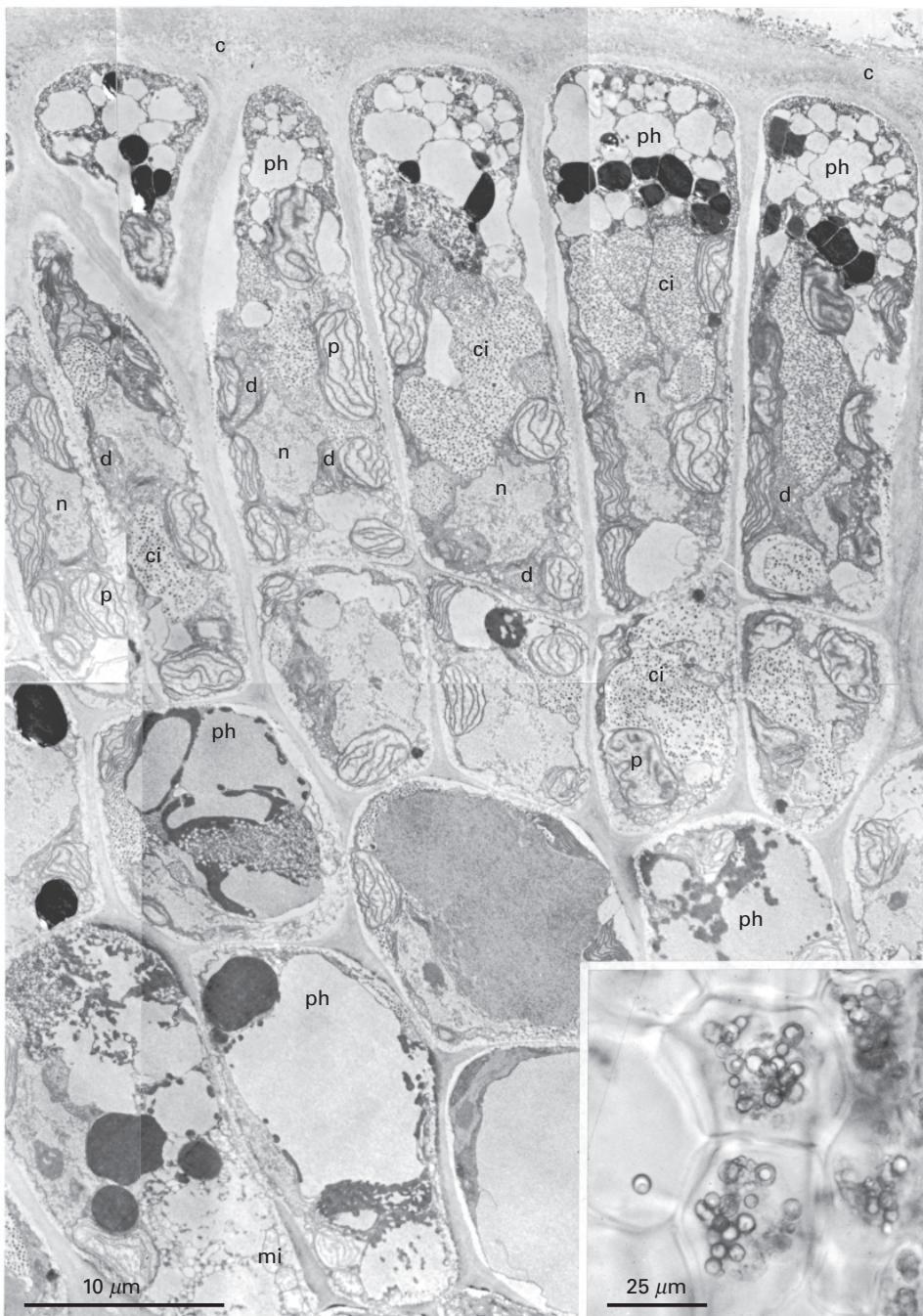


Figure 1.7 Cross section of the fucoid *Cystoseira amentacea* var. *stricta* Montagne (previously *Cystoseira stricta*) showing differentiation of tissues. The cells at the top of the view are the outer, meristodermal cells; those at the bottom are promeristematic. Inset shows fresh section stained with caffeine to reveal physodes; c, cuticle; ci, iridescent body; d, Golgi body; mi, mitochondrion; n, nucleus; p, chloroplast; ph, physode. (From Pellegrini 1980, with permission of The Company of Biologists.)

growing seaweed. Walls are crucial in mating, in the release and adhesion of reproductive cells, and as the outermost surface of many algae they are the first line of defense against pathogens and grazers (secs. 4.2.2 and 7.8). The abundance of matrix material relative to fibrillar components, the extensive sulfation, and the extensive intercellular matrix are characteristics of seaweeds that suggest environmental adaptations (e.g. to wave force and desiccation) (Kloareg and Quatrano 1988; see secs. 5.5 and 8.3.1). Cell walls also contain structural proteins, which are well studied in terrestrial plants and unicellular green algae (e.g. review by Casab 1998) but have proven difficult to extract and characterize in red seaweeds (Deniaud *et al.* 2003). For *Palmaria palmata* the composition of structural proteins differed between blades that were soft versus rigid, indicating a role in “cell development and specialization” (Deniaud *et al.* 2003). So important are cell walls that Szymanski and Cosgrove (2009) consider it “more useful to think of the wall as another cell organelle . . . and regulated by cytoplasmic and membrane processes that control pH, ion activities, reactive oxygen species, the concentration of metabolites, enzyme content and structural components”.

Since the early days of electron microscopy, plant cell walls have been viewed as a meshwork of cellulose microfibrils in an amorphous matrix (Mackie and Preston 1974). There is a bewildering array of matrix polysaccharides in algae and considerable research effort has been exerted to identify and catalogue these, largely because of their potential commercial importance (Vreeland and Kloareg 2000; see sec. 10.3, 10.5 and 10.6). The biosynthetic pathways of polysaccharides are not fully understood, especially for brown seaweeds (Charrier *et al.* 2008), although putative pathways have been identified for *Ectocarpus* based on gene content; the next challenge is to rigorously test gene function (Michel *et al.* 2010a; sec. 5.5.2). The fibrillar components of algal walls are made of cellulose, β -(1,4)-D-mannan, β -(1,3) or (1,4)-D-xylan and, while they constitute just a small component (5–15%) of the wall dry weight, they are essential in providing tensile strength to the cells (Tsekos 1999; Lechat *et al.* 2000). The hypothetical model of algal wall structure

advanced by Kloareg and Quatrano (1988, Fig. 1.8a) has changed little (Michel *et al.* 2010a), and a similar but more detailed model has been proposed for green genus *Ulva* (Lahaye and Robic 2007; see sec. 5.5.2). Nevertheless, since the mid-1990s, the application of freeze-fracture electron micrograph and molecular biological techniques have resulted in substantial progress in understanding the sophisticated cellular machinery responsible for the synthesis of cellulose microfibrils and their assembly within plant and algal cell walls (reviewed by Tsekos 1999; Doblin *et al.* 2002; Saxena and Brown 2005).

Cellulose microfibrils are created by terminal complexes (TCs), comprised of cellulose synthases, which move through the cell membrane manufacturing microfibrils in a two-step process. First UDP-glucose is polymerized into β -1,4-linked glucan chains and second the chains are crystalized together into microfibrils (Tsekos 1999; Saxena and Brown 2005; Roberts and Roberts 2009). In higher plants, TCs are “rosettes” of six subunits but in the red, green, and brown seaweeds the TCs are linear. For example, the TCs of the brown seaweed *Pelvetia* are organized in a single line with 10–100 subunits, whereas those of the red *Pyropia yezoensis* are 2–3 rows deep. The structure of the TC shapes the dimensions and morphology of the cellulose microfibrils (see both Table 1 and Fig. 7 in Tsekos 1999). For instance, there are two forms of red algal microfibrils, either a “squarish” rectangular parallelepiped or “flat and ribbon-like” orthogonal structure. Cellulose synthase genes have been sequenced for *P. yezoensis* and *Ectocarpus siliculosus* and they are being used to unravel the evolutionary origins of cellulose synthesis (Roberts and Roberts 2009; Michel *et al.* 2010a).

Cellulosic cell walls are made of layers of parallel cellulose microfibrils. The organization of these microfibrils is determined by the route that the TCs take as they move through the cell membrane. In terrestrial plants, this route is guided by the cortical microtubules, but much less is known for seaweeds except that in *Fucus* zygotes F-actin provides the roadmap (Bisgrove and Kropf 2001). In some genera, such as *Chaetomorpha* and *Siphonocladus*, the microfibrils in successive layers are oriented at steep angles to each

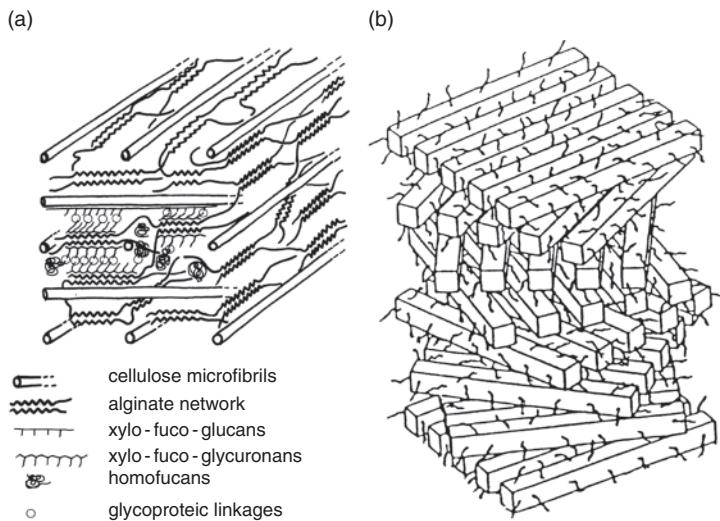


Figure 1.8 Algal cell wall construction. (a) Brown algal wall showing fibrillar and matrix components. (b) Cell wall with helicoidal stack of hemicellulose molecules, as found in some green algae. The backbone of each molecule is represented by a rod, and the flexible side chains by squiggles. (Part a from Kloareg *et al.* 1986, with permission of Butterworth and Co.; b from Neville 1988, with permission of Academic Press, Inc.)

other (90° = orthogonal). In other algae, or in certain walls, including aplanospores of *Boergesenia forbesii*, eggs of *Silvetia compressa* (formerly *Pelvetia fastigiata*), zygotes of *Fucus serratus*, and vegetative walls of *Spongomorpha arcta* and *Boodlea coacta*, the angle changes much more slowly, giving a helicoidal arrangement typical of higher plants (Fig. 1.8b). In many algal walls, however, the microfibrils in each layer have no preferential orientation (Kloareg and Quatrano 1988; Tsekos 1999). Most red algae, for instance, have microfibrils randomly distributed within each layer although there are exceptions in which they are parallel (*Spermothamnion johannis*, *Polysiphonia denudata* (previously *P. variegata*), and *Herposiphonia secunda* f. *tenella* (previously *Herposiphonia tenella*) (Tsekos 1999).

Cellulose is the fibrillar material throughout the brown algae and most of the reds, but it is not the only fibrillar structural polysaccharide in algal walls as xylans also form microfibrils, and mannans form short rods. Xylans and mannans are particularly common in unicellular and coenocytic members of the Chlorophyta but, compared to cellulose, they have been little

studied (Dunn *et al.* 2007; Fernández *et al.* 2010). Some seaweeds feature a biochemical alternation of generations in which different ploidy levels have different fibrillar or matrix polysaccharides. For instance, the diploid thallus of *Acetabularia* and *Codium* has mannans, and yet the walls of reproductive phases are mostly cellulose (Kloareg and Quatrano 1988). *Pyropia yezoensis* blades produce xylan while the filamentous sporophyte produces cellulose (Tsekos and Reiss 1994). No reason for these biochemical differences between generations has been deduced.

Most red seaweeds have an outer, multilayered, proteinaceous cuticle covering their surface (Craigie *et al.* 1992) that may confer protection against herbivore grazing, desiccation, and bacterial degradation (Hanic and Craigie 1969; Gerwick and Lang 1977; Estevez and Cáceres 2003). The iridescence typical of some species including *Chondrus crispus* gametophytes and *Mazzaella* is the result of a thick multilaminated cuticle, in which many thin layers of alternating higher and lower refractive indices produce interference, as in a soap bubble. *C. crispus* sporophytes have fewer laminae (3–7) compared to

gametophytes (6–14) and they are irregularly arranged, explaining why sporophytes of this species are not iridescent (Craigie *et al.* 1992). The utricles of the green seaweed *Codium vermilara* also have a cuticle, but its structure has yet to be detailed (Fernández *et al.* 2010). Other algae are well known for impregnating their walls with calcium carbonate, and these seaweeds may be vulnerable to ocean acidification (see secs. 6.5.3, 7.7 and Essay 4, Chapter 7). Martone *et al.* (2009) were the first to discover lignin and secondary cell walls in the calcifying red seaweed *Calliarthron cheilosporioides*, both characteristics of terrestrial plants, previously unknown in seaweeds.

The complexity and molecular specialization of wall surfaces are being revealed by the use of monoclonal antibodies and related techniques (Vreeland *et al.* 1987; Eardley *et al.* 1990; Vreeland *et al.* 1992; see Jelinek and Kolusheva 2004 for a review of methods). Different parts of a thallus have different wall structures. The high proportion of polyguluronic acid in adhesive alginate is well known (Craigie *et al.* 1984; Vreeland and Laetsch 1989; Vreeland *et al.* 1998; and see sec. 5.5.2). The difference between rhizoidal and thallus poles has been detected even in germinating zygotes and regenerating protoplasts, again using antibodies to different carbohydrate fractions (Boyen *et al.* 1988). In a detailed study of *Fucus serratus* sperm, Jones *et al.* (1988) were able to distinguish several regions, including the tip of the anterior flagellum (crucial in egg recognition; sec. 2.4), the mastigonemes on the anterior flagellum, and the sperm body. Localization of certain wall components also occurs during zygote germination, when carbohydrates are directed from their Golgi body to the appropriate piece of wall. The actin/Arp2/3 network is involved in this process in *Fucus* (see sec. 2.5.3).

1.3.2 Cytoplasmic organelles

Plastids and mitochondria are cellular organelles that originated via endosymbiosis of once free-living cyanobacteria (Fig. 1.1) and alpha-proteobacteria, respectively. As they became assimilated, most of their genes (90–95% for plastids) were transferred to the host nucleus, making them reliant on the host for

essential gene products although some essential proteins are still made by the plastid (see below). These products are coded by nuclear DNA, synthesized in the cytoplasm and then imported into the organelles. This process is facilitated by transit peptides (TOC and TIC for plastids, and TIM and TOM for mitochondria), which are terminal peptides that attach to the nuclear-encoded pre-proteins and act as an address label that is recognized by membrane component(s) of the target cellular organelle. Once they have crossed organellar membranes, the transit peptides are cleaved (Reyes-Prieto *et al.* 2007; Graham *et al.* 2009; Weber and Osteryoung 2010; Delage *et al.* 2011). Thus, the once free-living prokaryotic cells became semi-autonomous organelles, but they retain many characteristics of their free-living ancestors. For example, both organelles divide by binary division but the genes for organelle division are now encoded in the nucleus which regulates division, explaining why plastids are unable to replicate in cell-free suspensions (Grant and Borowitzka 1984; Miyagishima and Nakanishi 2010).

Although most of the DNA associated with plastids and mitochondria was lost to the host's nucleus, the cpDNA and mtDNA that remains (within the nucleoids and ribosomes of the respective organelles) codes for essential core metabolic functions of the cells. The nucleus controls gene expression in organelles, termed antegrade signaling, and in return the organelles exert some control on nuclear gene expression by sending "retrograde" signals (Nott *et al.* 2006). Mitochondrial genome sizes of seaweeds are 25 836 bp for *Chondrus crispus*, 36 753 bp for *Porphyra purpurea* and 36 392 bp for *Fucus vesiculosus* (see Table 1 in Barbrook *et al.* 2010). The mitochondrial genomes include "core" protein-coding genes that are involved in oxidative phosphorylation and translation, and also RNA genes coding for large (LSU) and small (SSU) subunits of ribosomal RNA (rRNA). The cpDNA of almost all photosynthetic organisms contains a "core of genes" that are responsible for photosynthesis, including genes for photosystem I and II, cytochrome b6f, ATP synthase, RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) and components of LSU and SSU (see Table 3 in Barbrook *et al.* 2010). Plastid genomes of several seaweeds have been fully sequenced,

including the floridiophyte red seaweeds *Gracilaria tenuistipitata* (183 883 bp) (Hagopian *et al.* 2004), *Calliarthron tuberculosum* (178 981 bp), *Chondrus crispus* (180 086 bp), and *Grateloupia lanceola* (188 384 bp) (Janouškovec *et al.* 2013), the bangialean red seaweeds *Porphyra purpurea* (191 028 bp; Reith and Munholland 1995) and *Pyropia yezoensis* (191 954 bp; Smith *et al.* 2012), the brown seaweeds (*Ectocarpus siliculosus* (139 954 bp) and *Fucus vesiculosus* (124 986 bp) (Le Corguillé *et al.* 2009), and the greens *Bryopsis hypnoides* (153 429 bp) (Lü *et al.* 2011), *Acetabularia* which has the largest cpDNA of all photosynthetic organisms (1 500 000 bp; Mandoli 1998a), and *Codium fragile* which has the smallest at 89 000 bp (reviewed by Simpson and Stern 2002).

The mode of plastid and mitochondrion inheritance from parent to offspring varies between the seaweed phyla, and with the mode of reproduction (oogamy, anisogamy, and isogamy). For brown seaweeds that are oogamous, including *Fucus vesiculosus*, *Saccharina angustata* (formerly *Laminaria angustata*), and *Alaria esculenta*, mitochondria and plastids are inherited maternally (Motomura 1990; Kraan and Guiry 2000; reviewed by Motomura *et al.* 2010). Sperm plastids in *Saccharina angustata* zygotes remain small and do not divide, although they do survive, whereas mitochondria are enclosed in endoplasmic reticulum and digested in lysosomes (Fig. 1.9a) (Motomura 1990). For the isogamous brown seaweeds *Ectocarpus siliculosus* and *Scytoniphon lomentaria*, plastids are inherited from either

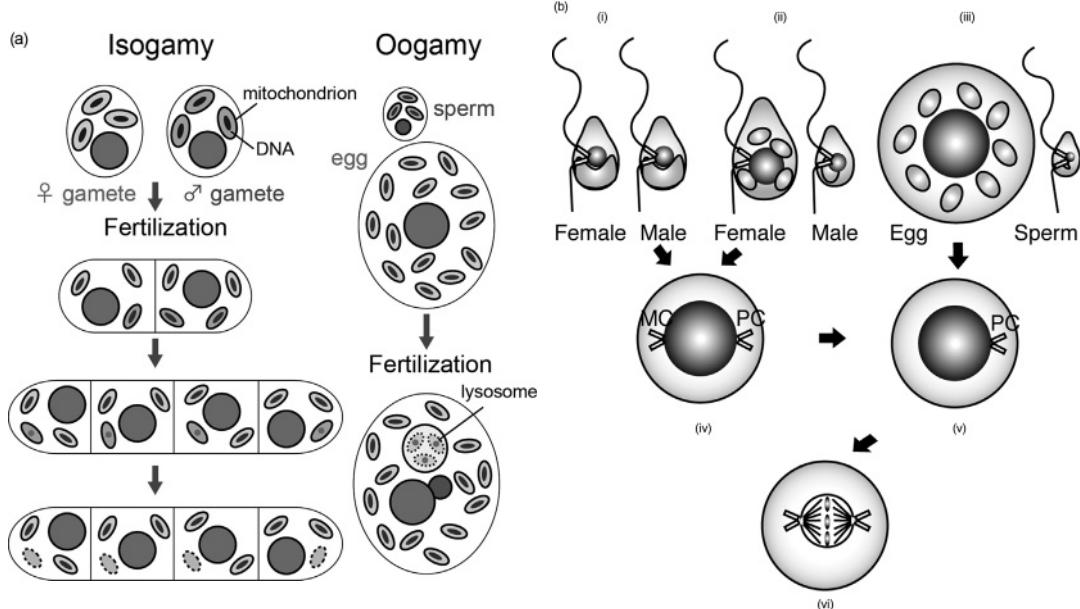


Figure 1.9 (a) Diagram of the cytoplasmic inheritance of mitochondria in isogamy (*Scytoniphon lomentaria*) and oogamy (*Saccharina angustata* – previously *Laminaria angustata*) in brown algae. In isogamy, mitochondrial DNA (or mitochondria) is selectively eliminated from the four-celled sporophyte after fertilization. In oogamy, sperm mitochondria are digested in the lysosome soon after fertilization. (b) Schematic representation of paternal inheritance of centrioles in fertilization of brown algae. (i) Isogamy, (ii) anisogamy, (iii) oogamy. In isogamy and anisogamy, the female gamete attracts the male gamete via sexual pheromones after settling. (iv) Immediately after fertilization, the zygote has two pair of centrioles (= flagellar basal bodies) derived from male and female gametes. (v) Subsequently, the maternal centrioles selectively disappear. (vi) Just before mitosis, the paternally derived centrioles duplicate and each pair of centrioles locates to the opposite pole of the spindle. MC, maternal centrioles; PC, paternal centrioles. (Part a from T. Motomura *et al.* 2010, Figure 1; b from Nagasato 2005, Figure 1, reproduced with permission.)

parent (biparentally) whereas mitochondria are maternally inherited. However, the timing of male mtDNA degradation in isogamous browns differs from that of oogamous species: mtDNA from male and female gametes survives in the offspring until the four-cell stage, at which time the male mtDNA is selectively broken down – the mechanism for this selective process is unknown (Peters *et al.* 2004a; Kimura *et al.* 2010). In all brown seaweeds, centrioles are inherited from the male gamete although, again, the timing of female centriole degradation differs between reproductive modes (Fig. 1.9b): for oogamous browns, the female centrioles disappear during oogenesis and the male centrioles are subsequently introduced as flagella basal bodies while in anisogamous and isogamous reproduction, centrioles from both parents are present in the zygote, but the maternal pair are subsequently degraded (Nagasato 2005).

Maternal inheritance of both plastids and mitochondria is common for green algae. For *Bryopsis maxima* and *Derbesia tenuissima* the male cpDNA and mtDNA degenerate during sperm gametogenesis (Lee *et al.* 2002). For *Acetabularia caliculus* and *Dictyosphaeria cavernosa* degeneration occurs in the zygote, following fertilization (reviewed in Lee *et al.* 2002). There are exceptions, however, and for *Ulva compressa*, the cpDNA is from mt+ (mt = mating type), whereas for some crosses of different genetic lines, mtDNA can be inherited from mt+, mt-, or both (Kagami *et al.* 2008; Miyamura 2010).

Plastids

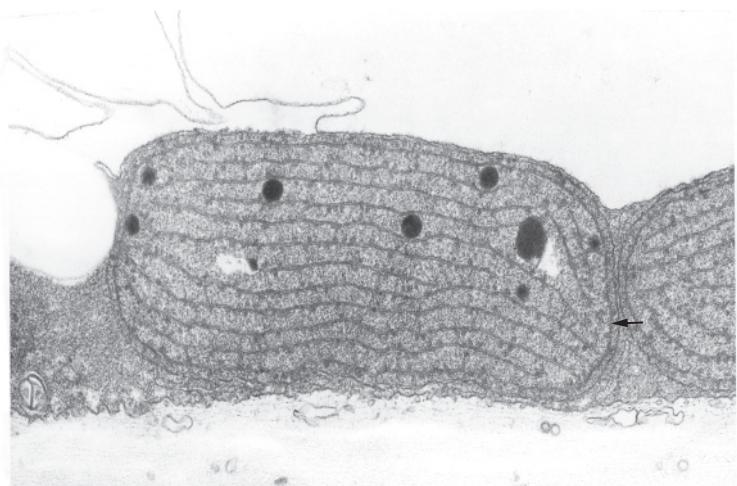
Plastid diversity in eukaryotes is remarkable and reflects the numerous gains, losses, and replacements via endosymbiosis (Howe *et al.* 2008; sec. 1.1), and the wide diversity of plastids and their various functions are reviewed by Wise (2007). The term “chloroplast” has historically been used to describe plastids from all algal lineages and this usage is still common. However, it is also used to refer specifically to the plastids of higher plants and green algae that contain chlorophylls *a* and *b* (Purton 2002; Howe *et al.* 2008), and other terms exist, e.g. rhodoplast for red algal plastids (Wise 2007). Here, we follow Graham *et al.* (2009) and use

“plastid” as a general term to encompass the light-harvesting plastids of the red, green, and brown algal lineages. The plastids of green and red algae have two outer membranes as a result of primary endosymbiosis, while secondary endosymbiosis resulted in the 3–4 membranes typical of brown algae (Fig. 1.10; Larkum and Vesk 2000; Archibald 2009; see sec. 5.3.1 and Fig. 5.8). Some siphonous green algae (*Caulerpa*, *Halimeda*, *Udotea*, and *Avrainvillea*) have colorless amyloplasts in addition to plastids, which are used for starch storage (van den Hoek *et al.* 1995). In terrestrial plants, amyloplasts are involved in gravitropism (Palmieri and Kiss 2007), but such a role in algae has not been reported except for their role in orientation of regenerating rhizoids in *Caulerpa* (sec. 2.6.4).

Photosynthetic algal cells contain one or more plastids (some *Acetabularia* species may have 10^7 – 10^8 per giant cell). In thick thalli, medullary cells that are shaded from light and blocked from rapid gas exchange by overlying cortical cells usually lack plastids or have vestigial plastids. Plastids have characteristic shapes that are useful for taxonomy; they may be discoidal, stellate, band-shaped, or cup-shaped (Larkum and Barrett 1983; van den Hoek *et al.* 1995; Graham *et al.* 2009). All have photosynthetic pigments in thylakoids (and red algae also have phycobiliproteins that occur on thylakoids), and the arrangements of thylakoids are taxonomically significant (Larkum and Vesk 2003; Su *et al.* 2010; see sec. 5.3.1 and Fig. 5.8). Red algal thylakoids are single while brown algae typically have three per lamella, and in green algae they range from two to many. Some plastids in the more advanced Florideophycidae have a peripheral thylakoid just inside the plastid envelope (Fig. 1.10a), and brown algal plastids have endoplasmic reticulum tightly associated on the outside, termed periplastidal endoplasmic reticulum (PER) (Fig. 1.10b). Some plastids have a pyrenoid (again there are characteristic shapes) comprising chiefly RuBisCO; in others, this key Calvin cycle enzyme is dispersed in the matrix (Tanaka *et al.* 2007). Some pyrenoids (e.g. those of *Bryopsis maxima*) are also the sites of nitrate reductase (Okabe and Okada 1990).

The differences among the shapes and arrangements of plastids are used as key characters to assess phylogenetic relations (although in some cases

(a)



(b)



Figure 1.10 Algal plastids. (a) Plastid of the red alga *Osmundea spectabilis* (previously *Laurencia spectabilis*) showing parallel single thylakoids and one thylakoid (arrow) surrounding the others, just inside the plastid membrane. (b) Plastid of a brown alga (*Fucus* sp.) showing characteristic triple thylakoids, the genome (G), and endoplasmic reticulum (ER) surrounding the organelle. Scale: 1 µm (Courtesy of Dr T. Bisalputra.)

similarities may represent convergent evolution). For example, a specific organization of stellate plastids of brown seaweeds are a key character that, in combination with molecular phylogenetics, led Peters and Clayton (1998) to establish the brown algal order Scytothamnales. Although the significance to physiology is not entirely clear, differences in plastid shape and sizes may reflect different evolutionary response to the reduction in light absorbance by “packaged” pigments, as compared to pigments in uniform solution (the “package effect”) (Osborne and Raven 1986; Dring 1990; sec. 5.3.2)

The plastids of siphonous green algae are unusual in that they have much greater autonomy than those of other algae and higher plants (Lü *et al.* 2011). For example, if the protoplasm of *Bryopsis* is experimentally squeezed out of the cell (or sucked out by herbivores), the plastids can aggregate and form a special integument around themselves. This extra membrane encloses a small amount of cytoplasm. Isolated plastids from *Codium* and *Caulerpa* do not swell or burst in distilled water. The integument may prevent the plastids of these species from being digested when they are eaten by saccoglossan mollusks (sea slugs), thus allowing the plastids to continue photosynthesis in a type of symbiotic relationship (termed kleptoplasty) with the animal (Grant and Borowitzka 1984; sec. 4.5.3).

Plastids may migrate within a cell. Dramatic diel migration of plastids takes place in *Halimeda* (Drew and Abel 1990). More than 100 plastids from each surface utricle pass along cytoplasmic strands through narrow constrictions into medullary filaments. They end up below the carbonate exoskeleton (Fig. 1.11), leaving the plant looking bleached. Inward migration is triggered by the onset of darkness (at any time of the day). Outward migration begins before dawn, apparently on an endogenous rhythm. Endogenously controlled plastid movements are also evident in the formation of new *Halimeda* segments. A proto-segment of colorless filaments is first formed and then, at night, plastids stream into the new segment, aided by microtubules and microfilaments, which becomes fully green within 3–5 h (Larkum *et al.* 2011). In the intertidal species *Dictyota*, plastid movements are a mechanism of photoprotection, with plastids moving away from the

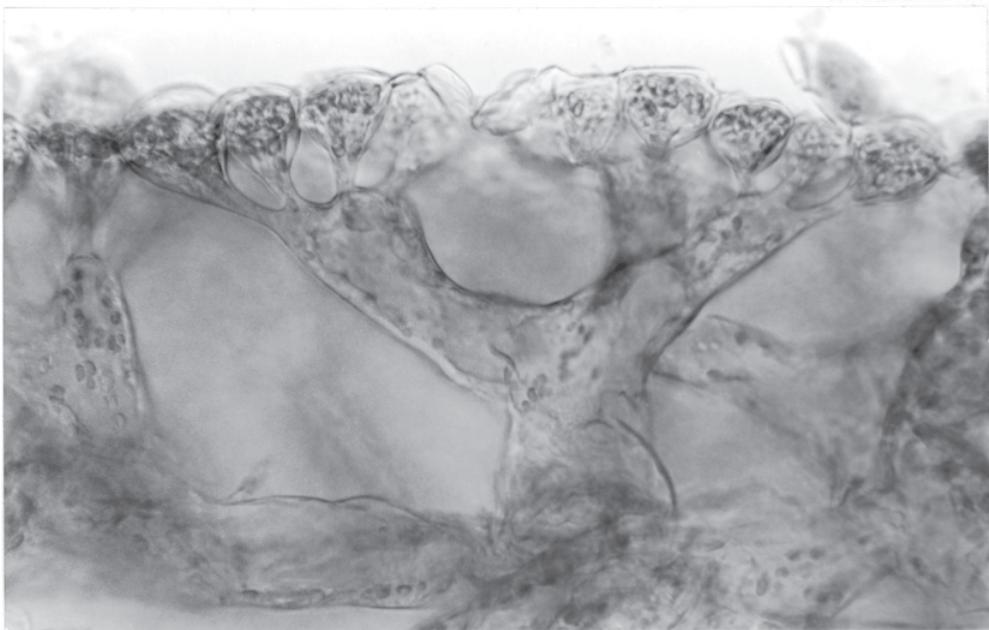
light during midday (Hanelt and Nultsch 1990, 1991). In *Caulerpa*, amyloplasts are even more mobile than plastids and are transported on microtubules, whereas plastids are moved by the actin-myosin system (Menzel and Elsner-Menzel 1989).

1.3.3 Cytoskeleton and flagella apparatus

The cytoskeleton in algal cells plays fundamental roles in mitosis, cytokinesis, karyokinesis, polarity, and morphogenesis in zygotes and vegetative cells, organelle trafficking (including plastids and physodes) and cytoplasmic streaming, cell growth, flagella apparatus, and wound healing (e.g. Menzel 1994; Fowler and Quattrano 1997; Schoenwaelder and Clayton 1999; Katsaros *et al.* 2006; Bisgrove 2007). In algae, the cytoskeleton is composed of microtubules (MTs, ~25 nm diameter) and filamentous actin (F-actin) microfibrils (~5–7 nm), which are assembled and disassembled from their component protein subunits of tubulin and actin respectively (e.g. Hable *et al.* 2003; Taiz and Zieger 2010). Fucoid zygotes have been studied extensively as a model system for fertilization, polarization, and cell division because of their large size, accessibility and the apolar nature of the egg (sec. 2.5.3). The cytoskeleton of green seaweeds is also well studied, especially *Acetabularia*, but the picture is less complete for red seaweeds although the application of immunolabelling and confocal microscopy to *Griffithsia japonica*, *Aglaothamnion oosumense*, and protoplasts of *Palmaria palmata* has revealed details of its organization (Garbary and McDonald 1996; Kim *et al.* 2001a; Le Gall *et al.* 2004), and actin genes and their expression have been reported for *Pyropia yezoensis* (Kitade *et al.* 2008).

An example of the way the cytoskeleton shapes cells is seen in the development of cysts in *Acetabularia* (Menzel 1994; Mandoli 1998b; Mine *et al.* 2008). During vegetative growth (Fig. 1.12), bundles of actin microfibrils are arranged along the axis of the cell (Fig. 1.13). After the cap has formed, the diploid primary nucleus undergoes one round of meiosis then divides mitotically into several thousand haploid “secondary” nuclei. The nuclei migrate along the actin microfibrils to the rays of the cap, where cyst formation

(a)



(b)

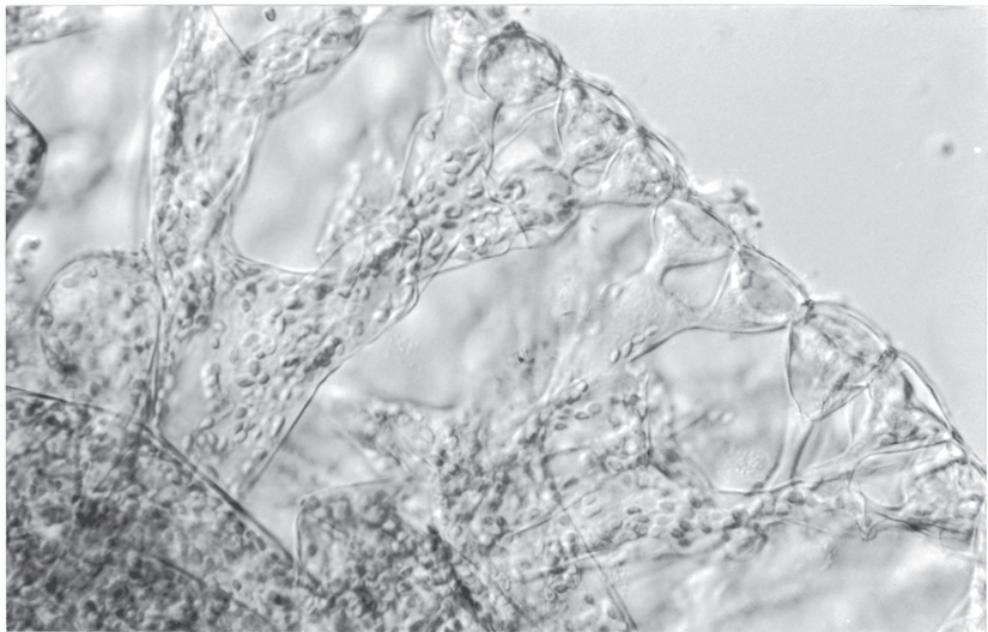


Figure 1.11 Migration of plastids in *Halimeda*. (a) Daytime cross section shows surface (primary) utricles packed with plastids. (b) Nighttime section shows that the chloroplasts have migrated below the calcified layer into the secondary utricles and medullary filaments. (From Drew and Abel 1990, with permission of Walter de Gruyter and Co.)

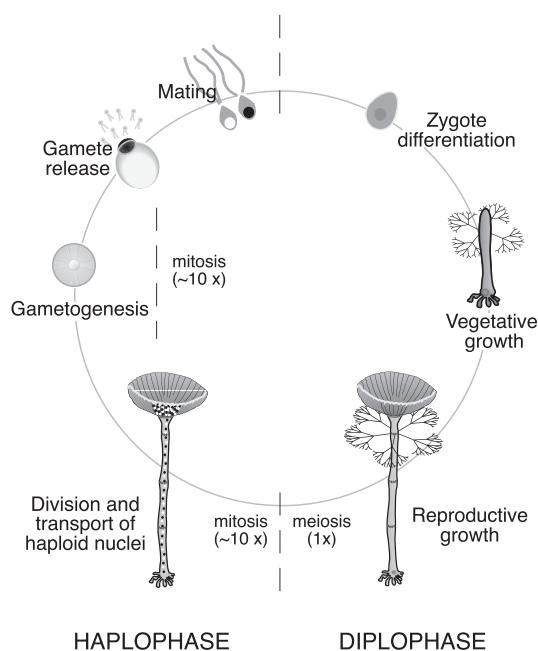


Figure 1.12 The basics of the haploid and diploid portions of the life cycle of *Acetabularia acetabulum*. Major portions of the life cycle that comprise a unit of development, i.e. all of gametogenesis, are united by an arc subtending the relevant label. For the sake of clarity, cartoons of the organism are not to scale. (From Mandoli 1998a, reproduced with permission.)

occurs. Then the actin network, which served as an organellar transport system in the vegetative phase, begins to disintegrate; once this is complete, the position of the secondary nuclei becomes fixed and cyst formation ensues (Fig. 1.13). The entire surface of each secondary nucleus acts as a microtubule organizing structure. The microtubules radiate, then draw organelles, including a single plastid, towards the nucleus and finally the nucleus, organelles and some cytoplasm become enclosed in a cyst. The shape of the developing cyst is determined by cytokinetic actin rings. In the final stage, the rings contract and cyst walls form (Fig. 1.13).

Brown and green seaweeds have flagella-bearing motile cells at some stage of their life cycle whereas red seaweeds do not. This difference between algal phyla is linked to the presence of centrioles in green

and brown seaweeds (and animals), but in red algae along with angiosperms and higher fungi, centrioles have been lost (Azimzadeh and Marshall 2010). Centrioles act as microtubule organizing centers during mitosis and are essential in flagella synthesis (Azimzadeh and Marshall 2010; Kitagawa *et al.* 2011). In flagella the centrioles are known as “basal bodies”, and their role is to synthesize the flagella at the cell membrane surface. Basal bodies comprise of a central cartwheel that has a hub from which nine spokes radiate, and each spoke joins via a pinhead to one of the nine triplet microtubules (Fig. 1.14a). There is a transition zone between the basal body and flagellum. The flagella themselves consist of a 9 + 2 arrangement of microtubules, collectively called the axoneme (Fig. 1.14b), a structure that is highly conserved among flagella-possessing eukaryotes (Ginger *et al.* 2008; Marande and Kohl 2011). The nine outer tubules are doublets (A- and B-tubules), connected by the protein nexin, and flagella movement is brought about by the actions of dynein motor proteins that are attached to the A-tubule (see Chapter 18 of Lodish *et al.* 2008).

The basal bodies of flagella are joined by striated fibers and are anchored into the cytoskeleton by four microtubular rootlets (one pair with two tubules, and one pair with three to five tubules). One of these rootlets anchors the “eyespot” in position (in those cells that have them). The flagella also have striated “system II” roots reaching back around the nucleus (van den Hoek *et al.* 1995). The two sets of striated fibers are involved in Ca^{2+} -dependent contractions and are made of centrin, an acidic phosphoprotein with a molecular weight of about 20 000. The centrin protein family is one of ~350 ubiquitous “eukaryotic signature proteins” that are highly conserved and critical for eukaryotic cell function (Salisbury 2007).

The mechanism of flagella synthesis, the intraflagella transport (IFT) system, was first identified in the unicellular green microalga *Chlamydomonas reinhardtii*, which is a model organism for flagella/cilia structure and function (reviewed by Cole 2003; Vincensini *et al.* 2011). Flagella are manufactured from more than 500 component proteins (representing over 3% of the *Chlamydomonas* genome) that are synthesized within the cell cytoplasm, and then enter the

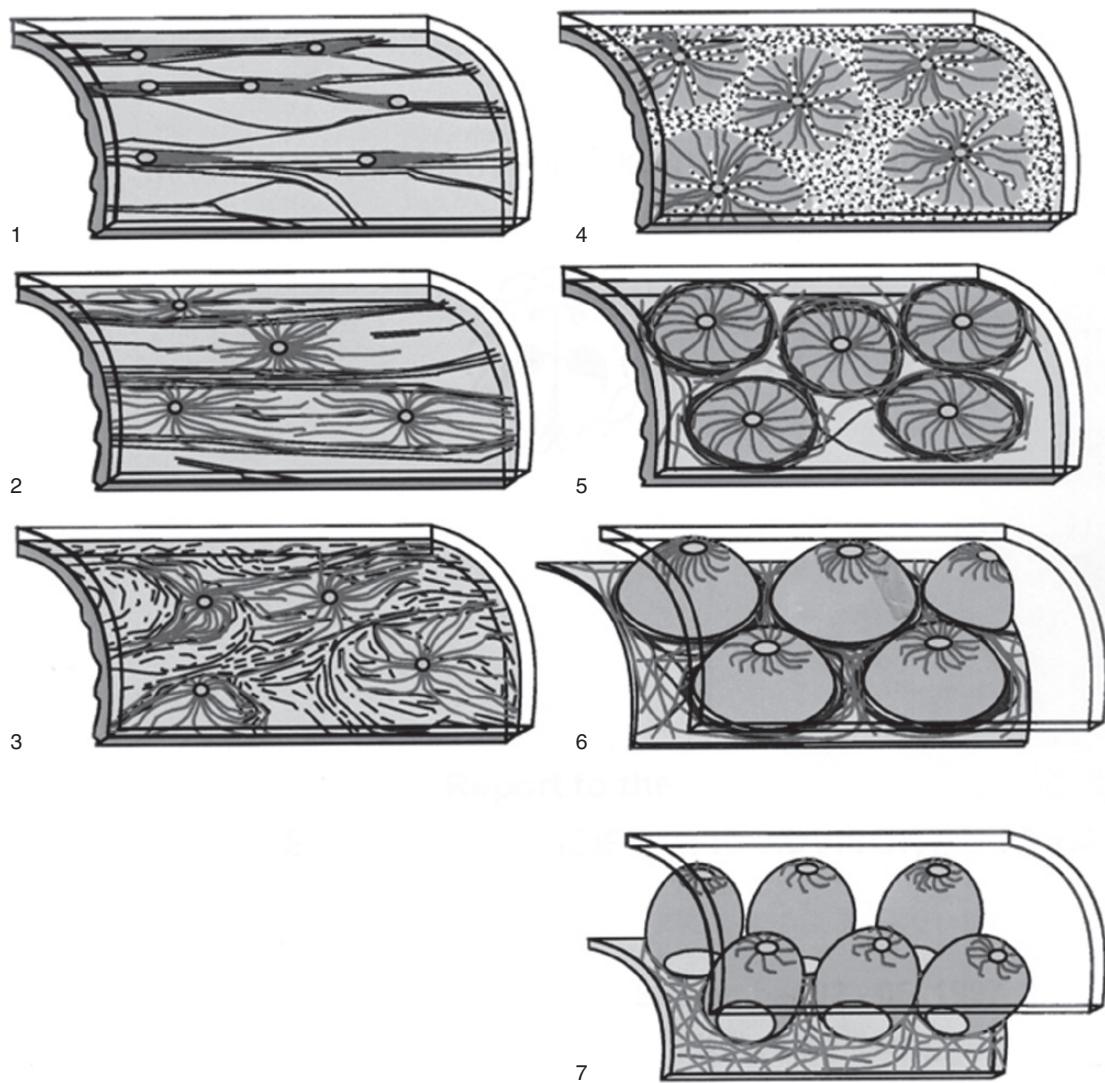
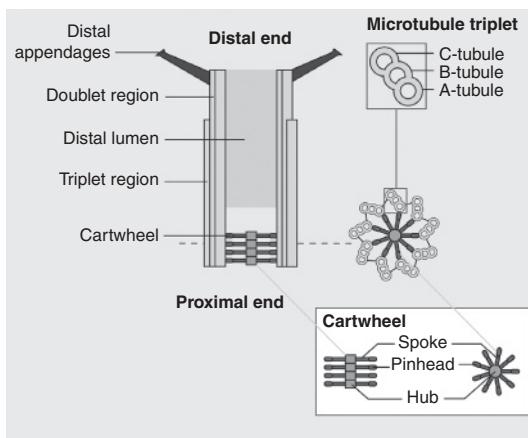


Figure 1.13 Schematic representation of cyst morphogenesis in *Acetabularia* in seven stages. Microtubules = dark gray lines; actin filament bundles = black lines; nuclei = light gray circles. (1) Migration of secondary nuclei along actin cables in the cap rays. (2) Beginning of immobilization of the nuclei and the extension of radial perinuclear microtubule systems. (3) Breakdown of actin cables causing irregular contractile events in the cytoplasm. Nuclear positions become rearranged. (4) Actin breakdown completed. Maximal radial expansion of perinuclear microtubules, gathering of chloroplasts and other organelles in disks around each nucleus. (5) Microtubules have become fragmented at their distal ends and the fragments gave rise to a second peripherally located microtubule-system, cytokinetic actin rings have formed around each domain. (6) Cyst domains begin to bulge out, actin rings contract. (7) Advanced state of contraction of the actin rings. Cyst protoplasts are being shaped. Note counter clockwise bending of perinuclear microtubules. This configuration eventually gives rise to microtubule band of the lid-forming apparatus. (From Menzel 1994, reproduced with permission.)

(a)



(b)

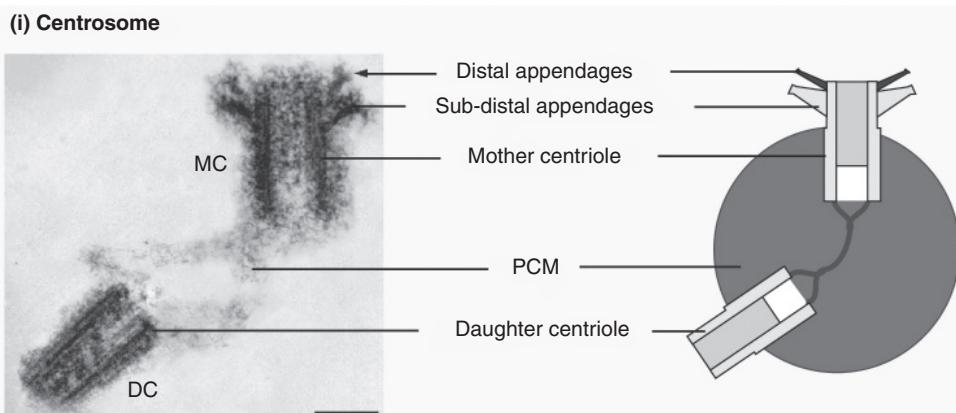
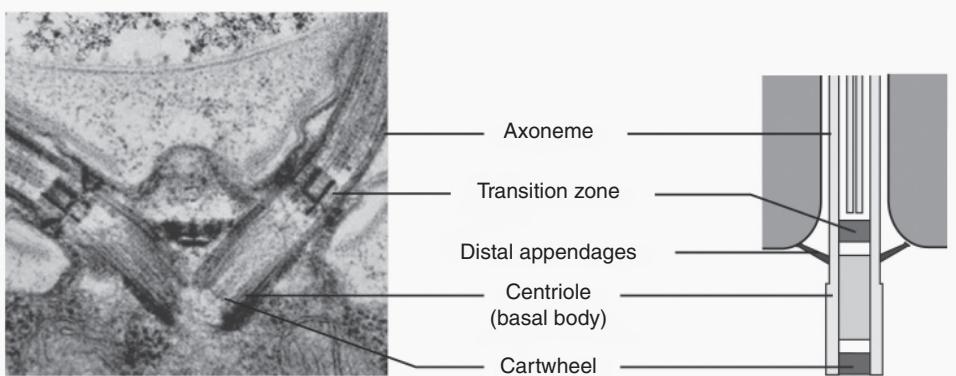
(i) Centrosome**(ii) Cilia/flagella**

Figure 1.14 (a) Centriole structure. Centrioles are microtubule arrays composed of nine triplets of microtubules organized around a cartwheel structure. The triplets are connected to the cartwheel through the A-tubule, the first to assemble during (cont. over)

flagella through the basal body; these proteins can be regarded as the “cargo” of the IFT system (Fig. 1.15). At the basal body, the cargo proteins are “loaded” onto IFT particles which are then conveyed by motor proteins (kinesins) along the outer doublet microtubule to the construction site at the flagella tip (anterograde IFT). The cargo is unloaded and used to extend the flagella. Another group of motor proteins, dyneins, moves the IFT particles back to the flagella base (retrograde IFT), where they are re-loaded (Fig. 1.15; Marande and Kohl 2011). The IFT system also regulates flagellum length (see Vincensini *et al.* 2011).

Flagella of seaweed microstages have two key roles: locomotive and sensory. Locomotion is important for getting gametes together and helping propagules swim to the seabed (sec. 2.5.1). For brown seaweeds, the anterior flagellum bears hairs (mastigonemes), and pulls the gametes through the water, while the smooth, posterior flagellum acts as a steering rudder (Jékely 2009; Fig. 1.16). Flagella also act as specialized recognition and adhesion organelles during mating (sec. 2.4) and selection of a suitable substratum for settlement (sec. 2.5.1).

Most lineages of algae contain species with pigmented eyespots (stigmata) on their motile cells, but their structure, position, and function differs between groups (reviewed by Hegemann 2008; Jékely 2009). The eyespots of motile seaweed cells are patches of lipid droplets, orange or red because of associated carotenoid pigments. Those of green algae are located

on the outermost region of the plastid, directly under the plastid membranes, and those of brown algae are closely associated with a swelling at the base of the posterior flagellum (Fig. 1.16; Kawai *et al.* 1990, 1996; Jékely 2009). The term eyespot is misleading because the stigmata themselves do not detect light (Jékely 2009). Their role is to focus light onto the photoreceptor, either directly (like a lens) in the brown seaweeds (Kreimer *et al.* 1991) or by constructive interference by stacked lipid layers (something like iridescence) in the green algae (Melkonian and Robenek 1984; Kreimer 2001). Eyespots also shade the adjacent photoreceptors when the swimming cells are in particular orientation relative to a light source, thereby providing a directional signal. The close association of eyespots with the microtubular rootlets of the flagella, and their placement relative to the flagella, are critical for co-ordinating phototactic swimming behavior of motile cells of green and brown seaweeds (Hegemann 2008; Miyamura *et al.* 2010).

Two photoreceptor proteins (channelrhodopsin 1 and 2) on the outer surface of the plastid of *Chlamydomonas* perceive light and a “photoreceptor current” is generated. This current is mostly carried by Ca^{2+} but also H^+ and K^+ . Flagella currents are subsequently generated when the photoreceptor current reaches a critical level, and this results in an adjustment in the plane, pattern, and frequency of the flagella beating (Hegemann 2008). For the motile gametes of brown

Caption for Figure 1.14 (cont.) centriole assembly and the only complete microtubule in a triplet. The B- and C-tubules are incomplete microtubules. In vertebrates and in *Chlamydomonas*, the C-tubule is shorter than the A- and B-tubules and the distal end of the centriole is thus formed by doublet microtubules. The cartwheel is formed by a central hub from which emanate spokes terminated by a pinhead structure that binds the A-tubule of the microtubule triplet. The very distal end of the centriole is decorated by nine-fold symmetric distal appendages (or transition fibers) required for anchoring the centrioles at the plasma membrane when they act as basal bodies. (b) (i) In animal cells, centrioles form the core structure of the centrosome, the main microtubule-organizing center. Quiescent cells ($G0$) or proliferating cells in the G1 phase of the cell cycle contain a single centrosome. The centrosome is formed by one mature centriole, the mother centriole (MC), and one non-mature centriole, the daughter centriole (DC), linked together and surrounded by a protein matrix called the pericentriolar material (PCM). In vertebrates, the mother centriole is decorated by two sets of ninefold symmetrical appendages: the distal and sub-distal appendages, required for ciliogenesis and for the stable anchoring of microtubules at the centrosome, respectively. The distal appendages are observed throughout eukaryotes, whereas the sub-distal appendages are only found in animal centrosomes. (ii) In animals as well as in most other eukaryotes, centrioles are also required for the assembly of cilia/flagella. Centrioles, often referred to as basal bodies in this case, dock to the plasma membrane through their distal appendages and template the assembly of the nine outer microtubule doublets of the axoneme, the cytoskeletal core of cilia/flagella. A distinct structure called the transition zone separates the basal body from the axoneme. Shown are electron micrographs of the *Chlamydomonas* flagellar apparatus. (From Azimzadeh and Marshall 2010, reproduced with permission.)

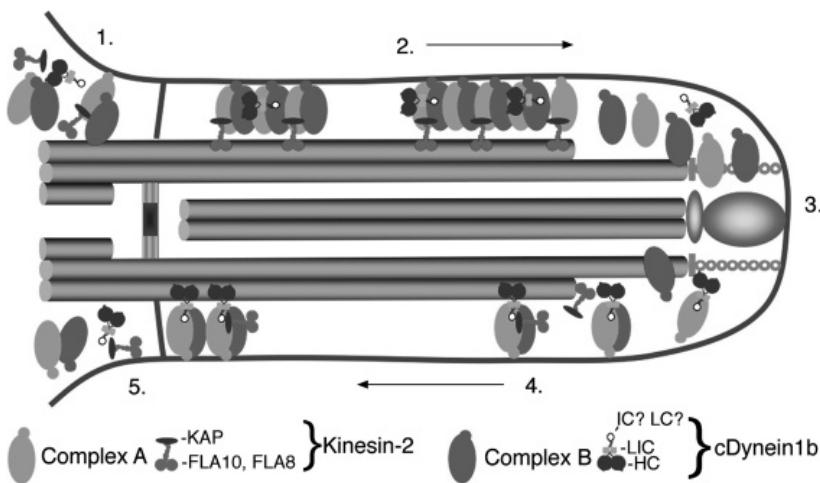


Figure 1.15 Intraflagellar transport. (1) Gathering of IFT particles and motors in the peribasal body region. (2) Kinesin-2-mediated anterograde transport of IFT complexes and inactive c Dynein1b. (3) Dissociation of IFT complexes. (4) Active cDynein1b transports everything back into the cell body. (5) IFT components are recycled to the cell body. cDynein1b: Cytoplasmic dynein1b; IC: Intermediate chain; IFT: intraflagellar transport; LC: Light chain. (From Marande and Kohl 2011, whose figure was modified from Pedersen *et al.* 2006, reproduced with permission.)

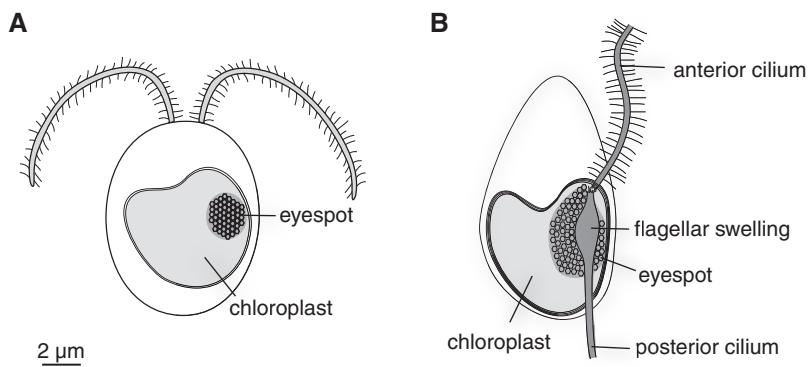


Figure 1.16 Location of eyespot in relation to flagella and chloroplasts in (a) a green alga and (b) a heterokont zoospore. Scale bar = 2 μm. (From Jékely 2009, reproduced with permission.)

seaweeds, the flagella swelling, upon which the stigma focuses light, is involved in photoreception, and also the posterior flagellum which contains at least two fluorescent compounds (a flavin and a pterin) that cause it to autofluoresce (Kawai *et al.* 1996; Fujita *et al.* 2005). Swimming *Ectocarpus* gametes, and other brown and green algal motile cells, roll as they swim, and when they are moving at a sufficient angle to the

light, the photoreceptor receives flashes of light as the cell rolls. This stimulation is thought to cause the posterior flagellum to beat, acting as a rudder. When the cell is swimming parallel to the light, the photoreceptor is continually shaded by the cell (Kawai *et al.* 1990). The action spectrum of *Ectocarpus* phototaxis has peaks in the blue region (Kawai *et al.* 1990, 1996). Algal photoreceptors are discussed further in sec. 2.3.3.

Although red algal spores have no flagella, some are motile. The spores of 21 species of red seaweeds have “amoeboid gliding or shuffling movements” and for archeospores of *Pyropia pulchella* (formerly *Porphyra pulchella*) the movements are driven by the actin-myosin motility system (Pickett-Heaps *et al.* 2001; Ackland *et al.* 2007).

1.3.4 Cell growth

Cell growth is driven by water influx and is restricted by the cell wall. Plant and seaweed cells are normally turgid, because water tends to flow into them by osmosis (sec. 7.4). The layers of fibrils in the wall (sec. 1.3.1) resist swelling and stop net water influx. In terrestrial plants, cell growth is achieved by locally controlled loosening (yielding) of the cell wall in unison with water influx (reviewed by Szymanski and Cosgrove 2009); the role of expansins and auxins in cell growth is reviewed by Choi *et al.* (2008) and Perrot-Rechenmann (2010), respectively. Compared to terrestrial plants, there has been very little work on the physiological and molecular mechanisms underpinning algal cell expansion and growth but the finding, using bioinformatics, that *Ectocarpus* appears to lack known families of enzymes that are thought to be involved in cell wall expansion (cellulases, expansins, and alginate lyases) may stimulate new research in this field (Michel *et al.* 2010a).

Garbury and Belliveau (1990) list four modes of cell growth: (1) uniform throughout the wall which is typical of green plants; (2) localized in the tip of the cell with the remainder of the cell remaining rigid, for example the apical dome of the apical cell of *Pyropia yezoensis* sporophytes, and fucoid zygotes (Tsekos 1999); (3) band deposition typical of many red seaweeds, and (4) diffuse wall deposition, characteristic of the red algal orders Arcochaetales and Ceramiales. Some of the mechanisms involved in the expansion of the apical cell have been elucidated for the filamentous sporophytes of *P. yezoensis* (reviewed by Tsekos 1999). The linear terminal complexes (TCs) are more abundant in the tips and, along with the Golgi apparatus, are responsible for synthesizing the new cell wall. Wall expansion is a dynamic process, with Golgi vesicles trafficking synthetic cell wall materials while also

delivering lytic enzymes that loosen the wall, allowing it to stretch; however, the wall thickness remains stable as new wall material is laid down by the TCs and Golgi apparatus.

Species that feature localized growth are useful as experimental material (e.g. Garbury *et al.* 1988; Fig. 1.17). The location of cell growth can be followed by labeling existing cell wall polysaccharides with a fluorescent stain such as Calcofluor White M2R (a brightener at one time used in laundry detergents) (Waaland 1980; Belliveau *et al.* 1990). If cell growth occurs by extension of existing wall material, the dye will be uniformly diluted. If, on the other hand, cell growth occurs by localized synthesis of new wall, dark bands will appear on the cells when seen under ultraviolet (UV) light, because the new wall will not be stained.

Intercalary cell extension in some Ceramiales, studied by Waaland and Waaland (1975), Garbury *et al.* (1988), and others, takes place through localized additions of wall material at each end of the cell (Fig. 1.17). The number and locations of the bands are characteristic of a species. In the *Antithamnion* illustrated, there is a strong basal growth band and a small apical band in axial cells, and only a basal band in determinate laterals. The location of band growth in this species is under apical control: if, for instance, the apex of a main axis is removed, the main growth band in those axial cells will switch to the other end of the cell, remaining basal relative to the nearest apex on an indeterminate lateral. This is an example of apical dominance (sec. 2.6.1). Cell growth may follow or be followed by cell division (sec. 1.3.5). Meristematic cells divide and grow repeatedly; other cells may stop growth and enter a stage of differentiation.

1.3.5 Cell division

Cell replication consists of two processes that do not necessarily happen together: nuclear division (karyokinesis) and cell division (cytokinesis). The brown algae, some green algae, and the Bangiophycidae have uninucleate cells, but coenocytic algae have many nuclei in cells, and thus karyokinesis and cytokinesis may be separated. In an unusual case in *Ascophyllum nodosum*, cell division can occur without mitosis,

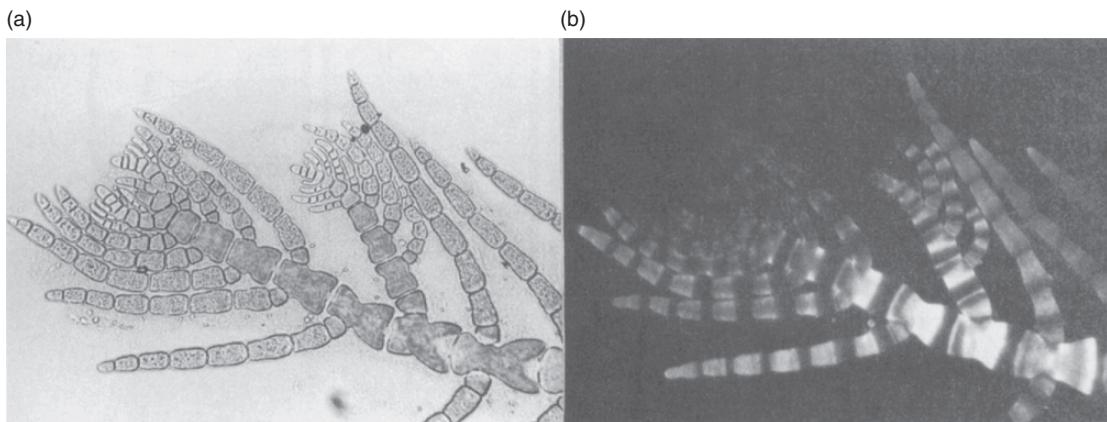


Figure 1.17 Cell growth in *Antithamnion defectum* visualized with Calcofluor White, as seen in bright field (a) and under UV light (b). A main axis with apical cell bears one indeterminate lateral and several determinate laterals. Under UV, dark bands of new, unstained wall are visible. The main axial and indeterminate lateral cells have two growth bands, the determinate laterals only one. Notice also the pit connections in the main axis. (From Garbary *et al.* 1988, reproduced by permission of the National Research Council of Canada from the *Canadian Journal of Botany*, vol. 66.)

perhaps a form of programed cell death (Garbary *et al.* 2009).

The cytological details of cell division have been studied particularly in the green algae, where the eight types of cell division that are recognized can be used as a taxonomic tool (van den Hoek *et al.* 1988, 1995). For the green seaweeds, the Ulvophyceae are characterized by having a persistent nuclear membrane (closed mitosis) and persistent telophase spindle microtubules. In coenocytic taxa (Dasycladales, Bryopsidales, Cladophorales, as defined by van den Hoek *et al.* 1988), mitosis is not immediately followed by cytokinesis. In uninucleate taxa (Ulvales, Codiolales), a cleavage furrow forms across the cell, and Golgi-derived vesicles are added to create the new cell wall. In the division of the apical cell of *Acrosiphonia*, more of the nuclei are partitioned to the apical cell than to the sub-apical cell; the apical cell remains meristematic, whereas the other cell rarely divides again (Kornmann 1970).

The application of new techniques for preparing samples for electron microscopy has allowed more accurate pictures of cytokinesis in brown seaweeds. Here, the mitotic spindle is more similar to that of animals than terrestrial plants and during mitosis one centrosome is at each mitotic pole (Fig. 1.18a). Spindle microtubules spread out from the centrosome

(Motomura and Nagasato 2004), and a small polar fenestration (pore) forms in the nuclear membrane, which otherwise remains intact until anaphase (Graham *et al.* 2009). The cytokinetic plane is set by the centrosomal position. The centrosomes act as microtubule organizing centers (MTOC), but there are no cortical microtubules in brown algae (Fig. 1.18a). For most, cytokinesis involves an outgrowth of the cell partition membrane, but *Sphaerelaria* is an exception in which the plasma membrane becomes furrowed (note that previously this mechanism was considered the norm) (Katsaros *et al.* 2009; Motomura *et al.* 2010; Nagasato *et al.* 2010). For *Silvetia babingtonii*, the new cell partition membrane is formed by the flat plate cisternae (FC, unique to brown algae) together with Golgi vesicles (GVs), both of which accumulate at the future cytokinetic plane (Fig. 1.18b). They then fuse, forming an extended flat cisterna (EFC), and additional GVs supply fucoidin to the EFC which forms a membranous network (MN). The MN in turn develops into a membranous sac (MS), alginate deposition within the sacs begins, then the gaps between the sacs disappear and a continuous cell partition membrane is formed. Finally, cell wall materials including cellulose are deposited within the cell partition membrane and a new cell wall is formed (Nagasato *et al.* 2010).

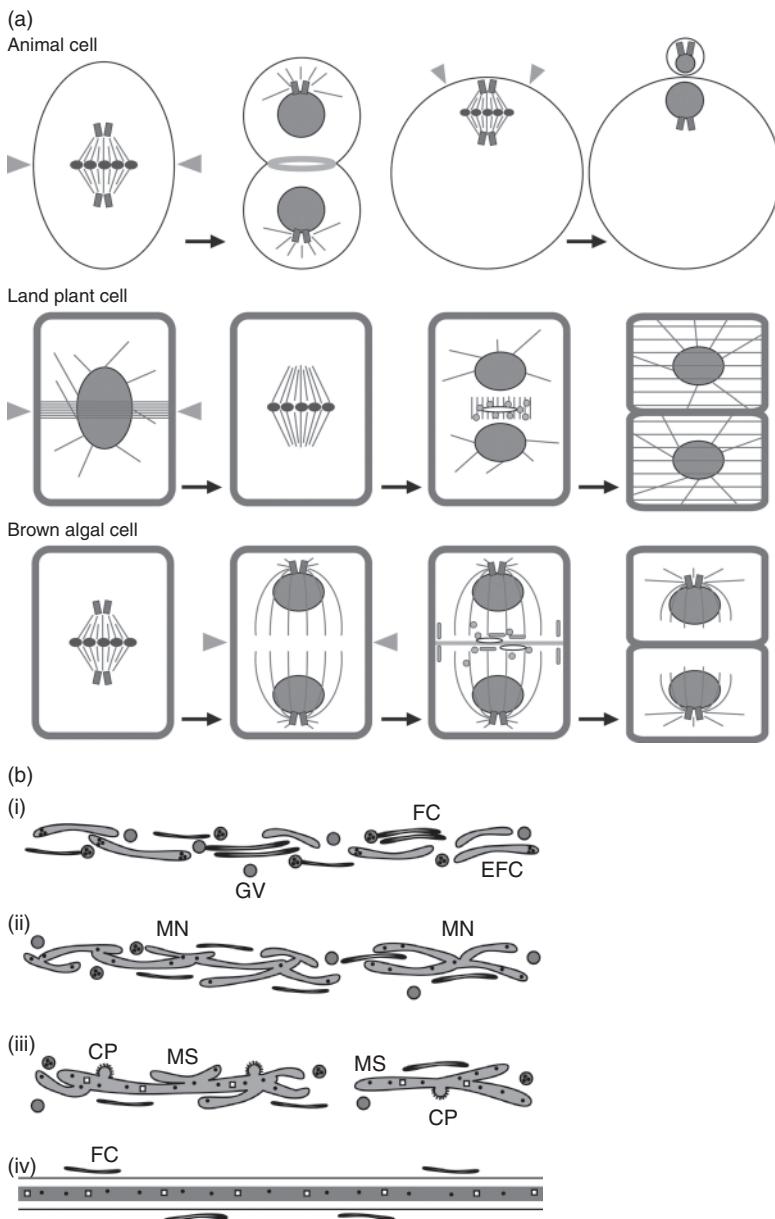


Figure 1.18 (a) Cytokinetic patterns of animals, land plants, and brown algae. In animal cells, the cytokinetic plane is determined by the position of the spindle (arrowheads), and is adapted during polar body formation. Cytokinesis proceeds via a contractile ring of actin (gray band). In land plant cells, there is no centrosome, and cortical microtubules (MTs) are well developed. The cytokinetic plane is determined by the microtubular preprophase band (arrowheads). Cytokinesis proceeds by outgrowing of a cell plate, which is mediated by the phragmoplast. Golgi vesicles participate in cell plate formation. In brown algal cells, a centrosome exists as a definite microtubule organizing center (MTOC), and no cortical MTs are observed. The cytokinetic plane is determined by the position of two centrosomes after mitosis (arrowheads). Cytokinesis proceeds by outgrowing of

Red algae are notable for the extensive evagination of the nuclear envelope that occurs during mitosis and for their nuclear associated organelles (NAOs, previously termed “polar rings”), that substitute for centrioles as microtubule organizing centers. As for the browns, they exhibit closed mitosis except for polar fenestrations, and cytokinesis begins with characteristic centripetal furrowing of the plasma membrane (Graham *et al.* 2009; Ueki *et al.* 2009).

Mitosis frequently occurs on a diurnal rhythm, with most cell division taking place at night (e.g. Austin and Pringle 1969; Kapraun and Boone 1987; Makarov *et al.* 1995; Kuwano *et al.* 2008). Two mechanisms controlling diurnal patterns of cell division have been identified in seaweed. The first is an endogenous circadian clock, in which the diurnal cycle of cell division continues even when the light/dark cue is removed, i.e. seaweeds are grown under constant light or darkness (see sec. 2.3.3 and 5.7.2 for other examples of endogenous rhythms). This has been reported for some brown, e.g. *Laminaria* and *Pterygophora* (Lüning 1994; Makarov *et al.* 1995), and red, e.g. *Porphyra umbilicalis* (Lüning *et al.* 1997), seaweeds. The molecular mechanisms underlying circadian clocks have been well studied in terrestrial plants, the unicellular green algae *Chlamydomonas*, and cyanobacteria (e.g. Harmer 2009; Johnson 2010; Schulze *et al.* 2010), but not yet in seaweeds.

The second mechanism, “circadian gating”, was first identified in a seaweed by Kuwano *et al.* (2008) for *Ulva compressa* (formerly *Enteromorpha compressa*). Here, the light-dark cycle drives cell-cycle progression. The gate for cell division is situated in G1 and is opened only following a specific time in the dark; during darkness a “dark-induced substance” accumulates within a

cell until a critical level is reached, at which time the “gate opener” is triggered and cells can enter S-phase and undergo mitotic division. Cells must also be at a critical size to enter mitosis; if they are too small they will not undergo mitosis even if the gate is open, but if a cell grows fast enough during the dark phase, they can undergo a second round of cell division. No endogenous clock is involved in this mechanism, because in continuous light or dark, cell division ceases immediately. Moreover, if the timing of the light-dark cycle is changed, cell division is immediately re-synchronized, which is too fast for regulation by an endogenous clock. The question of how widespread gating control is within the three seaweed phyla requires further testing (Kuwano *et al.* 2008).

1.4 Molecular biology and genetics

1.4.1 Advances in seaweed molecular biology

The “molecular revolution” has had a profound impact on most aspects of seaweed research (Essay 1, Fig. 1), initially for taxonomy, phylogenetics, and biogeography, and increasingly for ecology and physiology. Early applications to seaweeds included the sequence data for the small subunit of cytoplasmic ribosomal RNA for *Costaria costata* by Bhattacharya and Druehl (1988) to evaluate its relatedness to other organisms. Electrophoretic patterns of plastid DNA were used to assess populations and species over geographic areas (Goff and Coleman 1988) and to address kelp phylogeny (Fain *et al.* 1988), and plastid genome sequences were generated for *Griffithsia pacifica* and

Caption for Figure 1.18 (cont.) cell partition membranes, which is mediated by the actin plate (gray band). Golgi vesicles and flat cisternae participate in cell partition membrane formation. (b) Schematic diagram of transitional membrane configuration during cytokinesis in brown algae. (i) Fusion of GV to FCs transforms FCs into EFCs. GVs put fucoidan into FCs. Dots show the accumulation of fucoidan. (ii) Fusion of EFCs and supply of GVs produce MN. (iii) MN grows into MS with disappearance of gaps in the MN. MSs appear in patches. Clathrin-coated pits (CP) are detected on the MS. Alginate indicated by open squares begin accumulating. (iv) MSs become a continuous new cell partition membrane. Crystalline cell wall material deposits within it. EFC, expanded flat cisterna; FC, flat cisterna, GV, Golgi-derived vesicle; MN, membranous network, MS, membranous sac. (Part a from Motomura *et al.* 2010; b from Nagasato *et al.* 2010, reproduced with permission.)

Essay 1 Molecular techniques and their profound impact on contemporary phycological studies

Gary W. Saunders

I expect that few grow up anticipating a career as a systematist, let alone the seaweed variety. It certainly wasn't the case for me. Growing up along the coast of Nova Scotia, I developed a deep and enduring love of marine biology – that part of the path was obvious. During undergraduate studies, I was required to take a botany course, phycology, as it was widely referred to at the time, and was thus the logical choice. Much of the course was spent scuba diving and identifying algae – I was hooked! Moving to graduate studies under the supervision of Dr Jack McLachlan, I was tasked with resolving the life history of *Rhodophysema georgei* in light of exciting discoveries for another species of this genus (DeCew and West 1982). Exploring the literature surrounding my research, I started speculating on the relationships among the taxa under study and without conscious effort morphed into the realm of systematics. A pivotal moment, while pontificating my views on acrochaete evolution, occurred when Dr Christine Maggs (completing postdoctoral studies in the lab), asked “have you read Kylin?” – “ch who”... He is now, of course, iconic to me.

Leading to the early 1980s, Kylin's system of classification had worked so well for the majority of red algae that it, regrettably, attained universal acceptance, impeding efforts for reform. In this light, the words of Papenfuss (1958), in his review of Kylin's (1956) exceptional volume on red algal systematics, ring ironic – “As a former student of Professor Kylin, I know that the highest reward that he would have liked for his labors would be, for this, his last work, soon to become obsolete as a result of the intensive studies certain to be inspired by it.”

It was during this time of universal acceptance that I endeavored to transfer certain algae from the “primitive and ancestral” Acrochaetales to the “more derived” Palmariales. Needless to say, my manuscript was repeatedly rejected. Combining naivety and the arrogance-of-youth, I took the outcome personally – the old guard blocking the work of a young up-start. Turns out this was not the case, it was the state of red algal systematics at the time.

A few had succeeded in rendering change. In proposing the family Palmariaeae, Guiry (1974) departed from the axiomatic features of female reproductive anatomy and post-fertilization to emphasize tetrasporangial development. Guiry (1978) later argued that there was little save cruciate tetrasporangia, a state reported for species in all of Kylin's orders, to ally the Palmariaeae to the Rhodymeniales, and the Palmariales was proposed. Shortly thereafter Van der Meer and Todd (1980) published on a new life-history type and Pueschel and Cole (1982) provided ultrastructural observations supporting Guiry's proposals (Saunders and Kraft 1997). Pueschel and Cole further recognized a number of segregate orders thus implementing the first major revisions to Kylin's system. What frustrated me in reading these works is why Guiry had taken over 4 years to establish the order Palmariales and Pueschel had limited phylogenetic speculation in his manuscript to a short paragraph in which a very putative association between what were then thought to be divergent orders was outlined. I was fortunate through conferences and other communications to acquire from both of these colleagues explanations for these perceived shortcomings. Indeed it was not personal, for these two exceptional scientists were also not able to publish fully, or in a timely manner, their ideas on red algal evolution. Something new was needed, a tool so strong that critics would have to provide justification for rejecting new ideas that reached deeper than “it's simply wrong”.

Near the end of my MSc studies I attended a seminar by Dr Linda Goff. She talked about comparative genomics, that by looking at the DNA of organisms and comparing it, we could understand evolutionary relationships. The remainder of my path was clear – I had to learn and apply these tools to my systematic hypotheses.

I packed my cultures and moved to the lab of Dr Louis Druhl, the only place in Canada at that time applying molecular tools to macroalgae, and outlined my exciting research agenda, explained the excellent culture resources that I had amassed – this was to be our finest moment! Apparently not sharing my enthusiasm, the response was “we work on kelp here”. And so it was. I collected kelp, and set about learning the tools of the trade. As it turns out, kelp are very interesting.

Techniques were primitive in the early days and 2 years (a day's task now) were dedicated to generating eight small subunit ribosomal DNA (SSU) sequences for kelp in an effort to confirm relatedness in the face of what was also a widely established system of classification. However, the SSU was too conservative to resolve relationships among most genera. All was lost, or was it? At this point another valuable aspect of molecular data was presented – they can be used to estimate past divergence dates. Using molecular clock analyses we predicted that the derived kelps shared a common ancestor as recently as 16, and at most 30, million years ago in contrast to the 200 million years postulated for the group at that time (Saunders and Druhl 1992). This result was exciting, and of course controversial, but it did match with paleontological (Estes and Steinberg 1988) and paleoceanographic (Lüning and tom Dieck 1990) data. Most importantly, publication could not be blocked by the “it's simply wrong” argument (although some tried). Molecular clock analyses have matured greatly since those fledgling efforts and have provided numerous insights into the timing of past events for which detailed fossil records are lacking, which is the case for most algal lineages (e.g. Silberfeld *et al.* 2010).

Although the previous was exciting, the task of confirming that the system of classification for the kelps was natural had not been accomplished. One of the strengths of molecular tools is that an entire genome worth of characters is available for

Essay 1 (cont.)

exploration. If the first gene tested is too conservative, simply try something more variable. A second common marker in use at the time was the internal transcribed spacer region (ITS) of the ribosomal cistron. We had progressed to PCR by that point, and were even experimenting with direct sequencing of the amplicons (i.e. foregoing the tedious and time-consuming cloning steps), which allowed me to generate these data in just under a year. Although a bit too variable across all of the advanced kelp, the ITS did allow for “state-of-the-art” (methods that are now largely considered unacceptable) phylogenetic analyses of kelp evolutionary relatedness (Saunders and Druehl 1993). Something apparently went horribly wrong. Why was *Lessoniopsis*, a paradigm representative of the Lessoniaceae resolving deeply in the Alariaceae? Was the molecular systematics dream just a farce as advocated by critics at the time? On the exposed coast of British Columbia, Louis elucidated the anomaly for me – *Lessoniopsis*, it turns out, has the splitting of the Lessoniaceae, and the paired sporophylls of the Alariaceae. In this light, it was hard to comprehend how kelp classification had become so firmly accepted. Indeed the taxonomists who established the system, Setchell and Gardner (1925), commented “the tribe of the Lessoniopseae might perhaps be placed with equal propriety either under Lessoniaceae or under Alariaceae, since the sole genus, monotypic, has the characters of each of these families”. The shortcomings fully acknowledged, in essence a challenge put forth to resolve the conundrum, overlooked as the classification rooted. Kelp systematics remain a passion and I still have students working on various aspects, notably a recent multi-gene phylogenetic study (Lane *et al.* 2006) that, although more robust and taxon-rich, supported the conclusions of our rudimentary study.

Molecular tools have had an impact on many questions outside the realm of phylogenetics. Owing to the observation in culture, less commonly from nature, of putative intergeneric hybrids of different kelps (see Druehl *et al.* 2005b, Table 1 for a summary), a myth was perpetuating that kelp could interbreed freely, and that traditional species concepts may not apply. This concept never sat well with my way of thinking and, indeed, other possible hypotheses to explain these morphologically anomalous individuals (putative hybrids), by direct development from female (parthenogenesis) and male (apogamy) gametophytes, were largely ignored (with notable exceptions; see Druehl *et al.* 2005b). We designed species-specific primers to test the parentage of putative culture-reared hybrid individuals (Fig. 1a) with the result that only one individual was truly a hybrid. In this case the hybrid was between the species *Saccharina angustata* and *S. japonica* (as *Laminaria* spp.), which are very closely allied. As discussed in Druehl *et al.* (2005b), promiscuity among kelp genera, although exciting speculation, is more myth than reality and failed the molecular test.

It is not news that identifying algal collections to known species (even for experienced systematists) is a frustrating task. Difficulties arise from key commonalities among algal species, viz. simple morphologies, rampant convergence, phenotypic plasticity in response to environmental conditions, incompletely resolved life histories (alternations of heteromorphic generations), and abundant cryptic species (Saunders 2005, 2008; Lindstrom 2008; Le Gall and Saunders 2010). We have thus come to rely increasingly on molecular tools for the resolution of species (e.g. Saunders and Lehmkul 2005) and for assigning cryptic field specimens to known species (e.g. Lane and Saunders 2005; Fox and Swanson 2007). Under the label DNA barcoding (Hebert *et al.* 2003) a substantial database of COI-5P sequences (as well as other markers) is being established to facilitate the rapid identification of any biological specimen (see www.boldsystems.org). Ultimately this endeavor will provide scientists and managers worldwide with a powerful ally in the important task of species identification. An obvious practical outcome will be the ability to identify rapidly and accurately introduced species to an area. A key initiative of my research group is to complete a contemporary floristic account of the marine macroalgae of Canada using the DNA barcode as a preliminary screening tool of species diversity and distribution (e.g. Saunders 2008, 2009; Lane *et al.* 2007; Kucera and Saunders 2008; McDevit and Saunders 2009). With over 9800 COI-5P sequences generated for various red and brown algal collections, we have uncovered well over 100 new records or species in the Canadian flora. This minimally represents an increase of c. 10%, which means that if you walk on a beach in Canada and pick up 10 different algal species, on average, one of them is not currently known to science, or at least

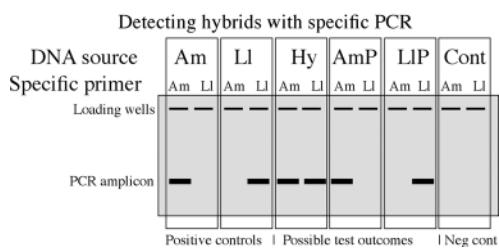


Fig. 1(a) ITS sequences vary among species facilitating the design of specific PCR primers. In the example here, primers for *Alaria marginata* (Am) and *Lessoniopsis littoralis* (LL) are reciprocally tested (positive controls) to confirm specificity. For progeny of hybrid cross experiments (or field-collected putative hybrids) there are three possible outcomes: Hy – both markers give positive amplification indicating hybridization; AmP – parthenogen of *Alaria*; or LIP – parthenogen of *Lessoniopsis*. Neg cont – is the negative control (no DNA added to the PCR reaction) and should have no amplification. (Original Saunders figure.)

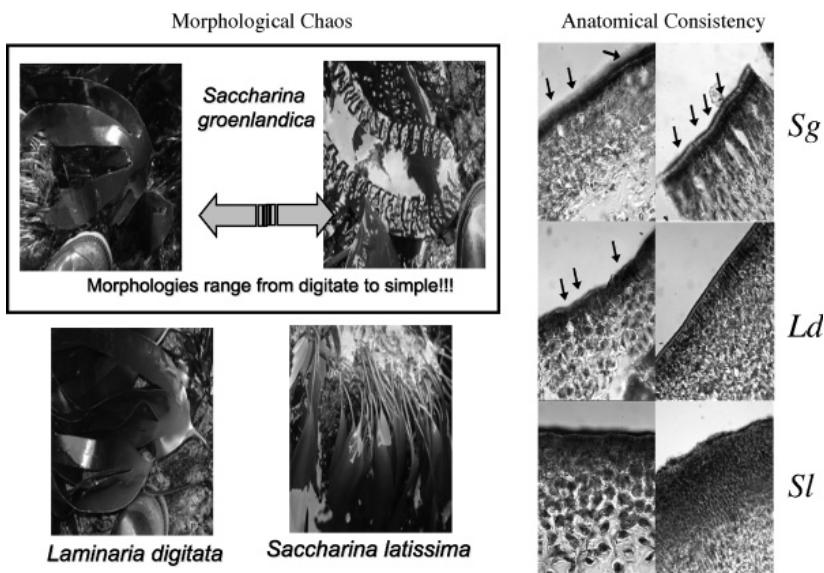
Essay 1 (cont.)

Fig. 1(b) DNA barcoding has uncovered an additional kelp species, the Pacific *Saccharina groenlandica* (*Sg*), in Eastern Canada, which masquerades as either *L. digitata* (*Ld*) or *S. latissima* (*Sl*) depending on the local environment. In agreement with the molecular data, the presence or absence of mucilage ducts (arrows) in the blade and/or stipe distinguish these three species. (Modified from McDevit and Saunders 2010.)

to the Canadian flora. In short, molecular data are completely changing perspectives of algal biodiversity and biogeography. These discoveries are not limited to small fuzzy reds and browns, even kelp species have gone undetected. McDevit and Saunders (2010) uncovered *Saccharina groenlandica*, currently considered a Pacific species, widely throughout the Atlantic Provinces, as well as in Hudson Bay. Whereas this seaweed can take on the gross morphology of *Laminaria digitata* or *Saccharina latissima* depending on the local environment, there are key anatomical differences in support of the molecular results (Fig. 1b).

And what of those cultures for species that putatively belonged to the Palmariales rather than the Acrochaetales? Following graduate school, I moved to Melbourne in search of mentorship with a significant figure in traditional red algal systematics, Dr Gerry Kraft. We embarked on a substantial phylogenetic investigation of many red algal lineages, generally emphasizing the Gigartinales sensu lato (see Saunders and Kraft 1997; Saunders and Hommersand 2004; Le Gall and Saunders 2007). During those critically formative years, I had the pleasure of accomplishing many objectives with regards to red algal systematics, explore side projects dealing with a variety of chromophytic lineages (in collaboration with Dr Robert Andersen), and, yes, generate data and complete analyses with regards to my MSc work. In 1995, we published a phylogenetic study in which the molecular data supported my view of red algal evolution (Saunders *et al.* 1995). Many lessons were learned along the way, adventures explored and discoveries made. To the graduate students reading this essay, do not subscribe to dogma, challenge the paradigms, and work hard to test your hypotheses. Remember, research is not about vindication, or about being right; the objective is to advance your chosen field and make contributions to the body of knowledge that is science. I have no idea how long my own contributions will stand, only that eventually some will give way to new views and data as should be expected, indeed encouraged, in science. I am, however, certain that future revisions will inevitably involve molecular data, the profound impacts of these powerful tools sure to dominate systematics and other aspects of phycological research into the future.

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Pyropia yezoensis (Li and Cattolico 1987, Shivji 1991): today complete plastid genome maps have been assembled for various seaweeds (e.g. Fig. 1.19). Molecular methods have proven invaluable in clarifying the identity of species with extreme morphological variation such as the Laminariales (Essay 1) and Fucales (e.g. *Durvillaea*, Fraser *et al.* 2009), and species within genera such as *Pyropia* and *Porphyra*.

that have few obvious morphological features to distinguish them from one another (Sutherland *et al.* 2011). In biogeography, molecular methods have helped to resolve the origin of genera such as *Fucus* which was thought to be Atlantic because of the high species diversity there, but Coyer *et al.* (2006a) show a North Pacific origin, with *Fucus distichus* as the ancestral form. “DNA barcoding” is an approach to

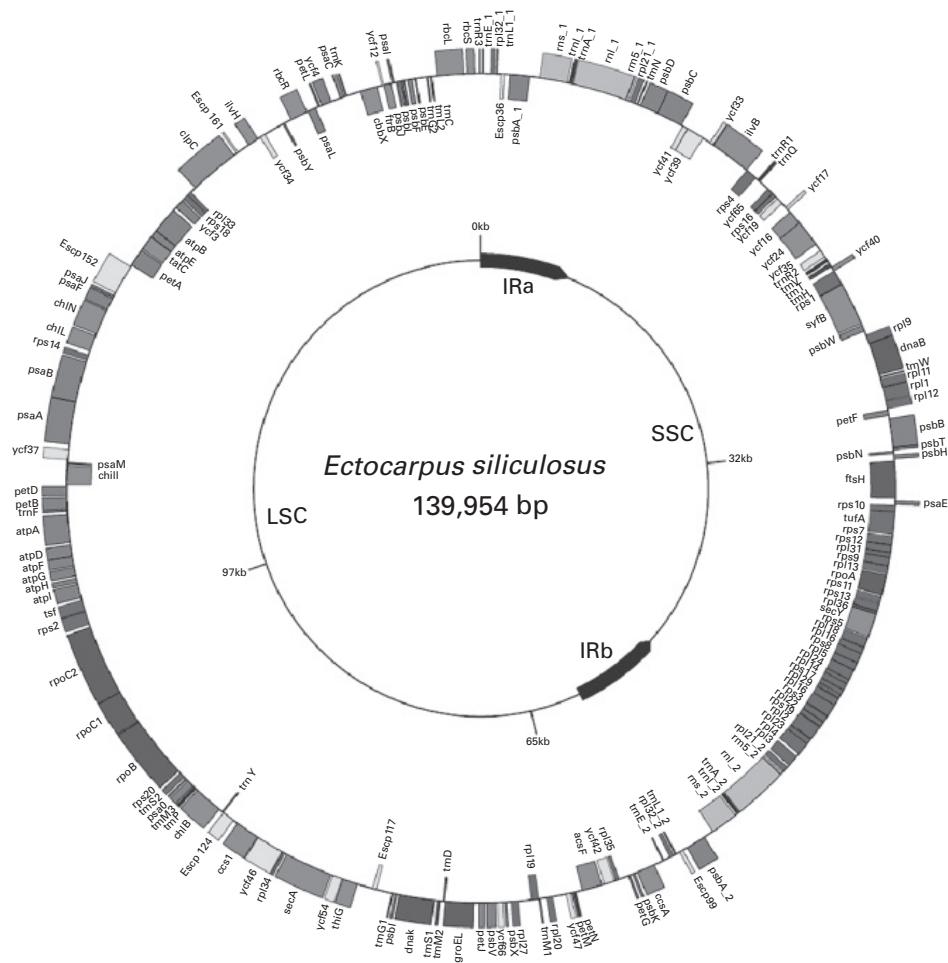


Figure 1.19 Plastid genome maps of *Ectocarpus siliculosus* and *Fucus vesiculosus*. Genes on the outside of the circles are transcribed clockwise, whereas those on the inside counter clockwise. Annotated genes are shaded according to the functional categories shown in the legend and the tRNA genes are indicated by the single-letter code of the corresponding amino acid. Abbreviations: IR, inverted repeats; SSC, small single-copy region; LSC, large single-copy region. (From Le Corguillé *et al.* 2009, reproduced with permission.)

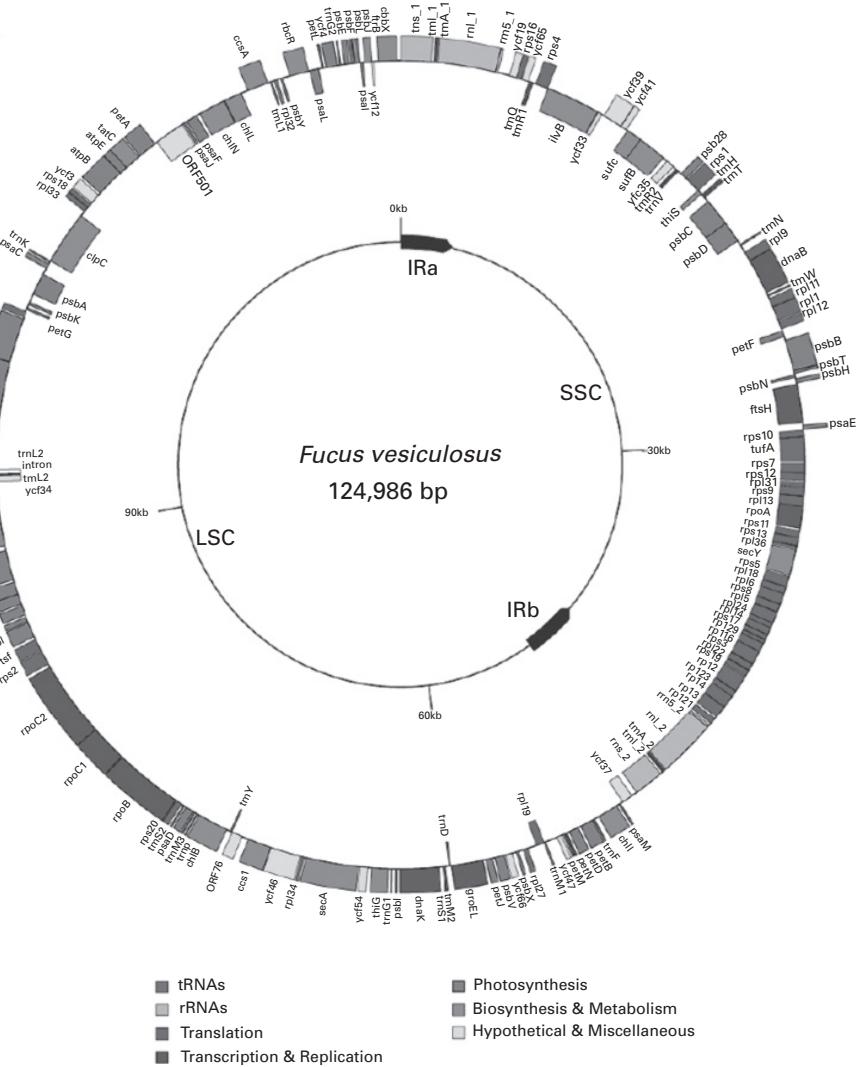


Figure 1.19 (cont.)

identifying species, based on gene sequences, and provides a rapid way of cataloging species; the mitochondrial gene cytochrome oxidase (Cox 1) used for animals has been applied to red algae (Saunders 2005). Combining molecular markers from the nuclear, plastid, and mitochondrial genomes has improved the resolution of phylogenies of problematic genera such as *Gracilaria* (Pareek *et al.* 2010), and

almost complete genome sequences are available for several seaweeds (Cock *et al.* 2010a; Collén *et al.* 2013), and genetic maps have been assembled (Heesch *et al.* 2010). The application of various molecular biological tools, including potential pitfalls, are discussed in Graham *et al.* (2009 Chapter 5) and Cock *et al.* (2010b).

We start this section by reviewing some recent advances in seaweed molecular genetics (the “omics”)

that are particularly relevant to seaweed physiological ecology, and the basis for the selection of model seaweeds. We then move on to classical genetic studies that used color mutants and cross-breeding to elucidate inheritance patterns, and then focus on non-Mendelian transmission of genetic material and hybridization.

Genomics is the study of an organism's entire genome and is a powerful tool that can tell us which genes are potentially available for use by an organism. Cells perceive external stimuli and respond by switching the appropriate genes on and off; gene expression is mostly regulated at the level of transcription (*transcriptomics*) and controlled by transcription factors (TFs) which function as "switches" (Rayko *et al.* 2010). For example, Collén *et al.* (2006) used Expressed Sequence Tags (ESTs) to compare the genes expressed by *Chondrus crispus* exposed to desiccation stress with non-desiccated controls and thereby identify putative genes involved in desiccation tolerance (Essay 5, Chapter 7). In their transcriptomic study of *Ectocarpus siliculosus*, Dittami *et al.* (2009) have begun to unravel the molecular mechanisms that underpin regulation and acclimation to physiological stress. Gene expression is not static, however (Clark *et al.* 2010). For instance, in an EST study of *Sargassum aquifolium* (formerly *Sargassum binderi*), genes coding for alginate synthesis were not detected: these genes must be present in *Sargassum* but it seems that at the time of collection alginate synthesis was not taking place due to slow rates of growth (Wong *et al.* 2007). The absence of an expressed gene should therefore be interpreted with due caution (Clark *et al.* 2010).

Genes code information for protein synthesis, and *proteomics* studies which proteins are available and their cellular functions. This is an emerging area of research for seaweeds, with *Gracilaria* being one of the first seaweeds in which proteome annotation has been attempted (Wong *et al.* 2006). Proteins in turn synthesize metabolites and the study of metabolite production is termed *metabolomics*. Metabolic profiling in combination with gene expression analysis is a powerful technique that can be used to link physiological processes to the underlying genetic control. Gravot *et al.* (2010) used these techniques for *Ectocarpus siliculosus* to examine the effect of CO₂ and O₂

concentrations over a diurnal cycle on key metabolites such as citrate, glutamine, mannitol, and the expression of genes for carbonic anhydrase.

Several model seaweeds have been selected for genomic studies; this approach allows resources to be focused on understanding in great depth a particular organism, and the tools developed can be subsequently applied to other species. Peters *et al.* (2004b) proposed *Ectocarpus siliculosus* as the model brown seaweed, and its genome was the first seaweed to be fully sequenced (Cock *et al.* 2010). The criteria for *Ectocarpus* as model seaweed were: short life cycle (2 months), small and easy to grow in the laboratory, reproductive traits and life cycles are well known from classical studies (especially Dieter Müller's work), genetic crosses can be easily carried out, and it has a relatively small genome size (214 Mbp) so that sequencing was faster than for other candidate brown algae (~650 Mbp for the kelp *Laminaria digitata* and 1095 Mbp for *Fucus serratus*). The red seaweeds selected for genome projects are *Porphyra umbilicalis* (~270 Mbp) (Gantt *et al.* 2010; Chan *et al.* 2012a), and *Chondrus crispus* (105 Mbp) which represent the two major evolutionary lines of red seaweed (Florideophyceae and Bangiophyceae). *C. crispus* is the first red seaweed genome to be fully sequenced (Collén *et al.* 2013). Pearson *et al.* (2010) propose *Fucus* as a model seaweed for ecological genomics. *Ulva* is an obvious candidate for a model green seaweed (Waaland *et al.* 2004), as is *Acetabularia*, which is already a model system for nuclear-cytoplasmic interactions (Mandoli 1998a; sec. 1.4.3).

The complete sequencing of the *Ectocarpus* and *Chondrus* genomes has led to some exciting discoveries (see *New Phytologist*, Volume 188(1), 2010; Collén *et al.* 2013). Despite its simple morphology, isomorphic life history and small genome size, *Ectocarpus* is an "advanced" brown alga, most closely related to the Laminariales, and has 16 256 protein-coding genes (Cock *et al.* 2010a). Genes coding for 23 enzymes were discovered that may enable *Ectocarpus* to grow epiphytically on kelp by protecting it from kelp defensive systems. It has a large family of genes coding for reactive oxygen species, thought to be adaptive to the extreme environmental fluctuations typical of the

intertidal zone (see sec. 7.1). *Chondrus* has a rich diversity of genes, with 52% of those discovered being previously unknown. There were surprisingly few genes (12) responsible for starch biosynthesis, and some of the cellulose synthases are ancient, having been acquired before the primary endosymbiotic event (Collén *et al.* 2013). Genomic studies thus offer the opportunity for studying eukaryotic evolution, the molecular basis for adaptation to stressful environments, and novel metabolic pathways (Gantt *et al.* 2010; Kamiya and West 2010; Collén *et al.* 2013).

1.4.2 Seaweed genetics

Seaweed genetics has lagged behind that of unicellular algae and terrestrial plants, but the discovery of color mutants in the red algae in the 1970s substantially advanced our understanding of mating systems. However, such breeding experiments fell out of favor for some ~20 years, largely because of the time-consuming nature of crossing experiments, their limited applications in answering some genetic questions, and a strong interest in developing molecular techniques that could be applied to seaweed genetics for example, sex determination of *Gracilaria* (Martinez *et al.* 1999) and heterosis (hybrid vigour) in *Gelidium* (Patwary and van der Meer 1994). The field has come full circle, with scientists now using an integrative approach that combines classical genetic and molecular tools (e.g. Yan and Huang 2010).

Breeding experiments by J. P. van der Meer and co-workers using color mutants of red seaweeds, particularly *Gracilaria tikvahiae*, shed much light on mechanisms of genetic inheritance in seaweeds (reviewed by Kain and Destombe 1995). These studies began with the discovery of two spontaneous green mutants in gametophyte populations raised from spores (van der Meer and Bird 1977), which allowed a study of Mendelian inheritance. The two green mutants, with reduced phycoerythrin, were stable, different from each other, and recessive. Besides a rainbow of color mutants, van der Meer also accumulated morphological and reproductive mutants (van der Meer 1986a, 1990). Some of the mutations are located

in the plastid DNA and show non-Mendelian inheritance: Tetrasporophytes have the phenology of the maternal gametophyte (Fig. 1.20) (van der Meer 1978). Color mutants have been used to study the genetics of other *Gracilaria* species (see Plastino *et al.* 2003), other red seaweeds including *Champia parvula* and *Chondrus crispus* (Steele *et al.* 1986), *Porphyra purpurea* (Mitman and van der Meer 1994), *Pyropia yezoensis* (Niwa *et al.* 2009), and life histories (e.g. van der Meer and Todd 1980; Maggs and Pueschel 1989) including spore coalescence (Santelices *et al.* 1996).

Color mutants of *Gracilaria* sp. were also used to show the existence of mitotic recombination. Crossing-over of chromosomes normally occurs in meiosis but can also take place during mitosis, with the result that one daughter cell in a heterozygous diploid gets both copies of one gene (wild type, +, in the example illustrated), while the other cell gets both copies of the mutant gene (*grn*) (Fig. 1.21; van der Meer and Todd 1977). The sex-determining gene (*mt^m*/*mt^f*) is also involved in the recombination, so that the color patches become diploid male and female gametophyte tissue and produce diploid gametes.

In addition to the primary sex-determining locus (*mt*), there is a second sex-determining gene regulating the dioecious condition in *Gracilaria tikvahiae*. A spontaneous bisexual mutant (*bi*) produced strange results in crosses, except with normal haploid females, and even then the F1 had females, males, and bisexual individuals in a 2:1:1 ratio, suggesting that the mutation is expressed only in males (van der Meer *et al.* 1984). The bisexual allele cannot substitute for the female allele *mt^f*, and subsequent analysis (van der Meer 1986b) has suggested that the *bi* + allele actually represses expression of female-specific genes. The significance of the *bi* mutation is that it allows the production, by selfing, of homozygous diploids. Bisexuality (mixed-phase reproduction) is also common in haploid generations of other red seaweeds, but here mitotic recombination is not responsible because it can occur only in diploids. In *Dasyiphonia chejuensis* (Ceramiales), for example, haploid individuals can form tetrasporophyte, male and female reproductive structures. Here sexuality is

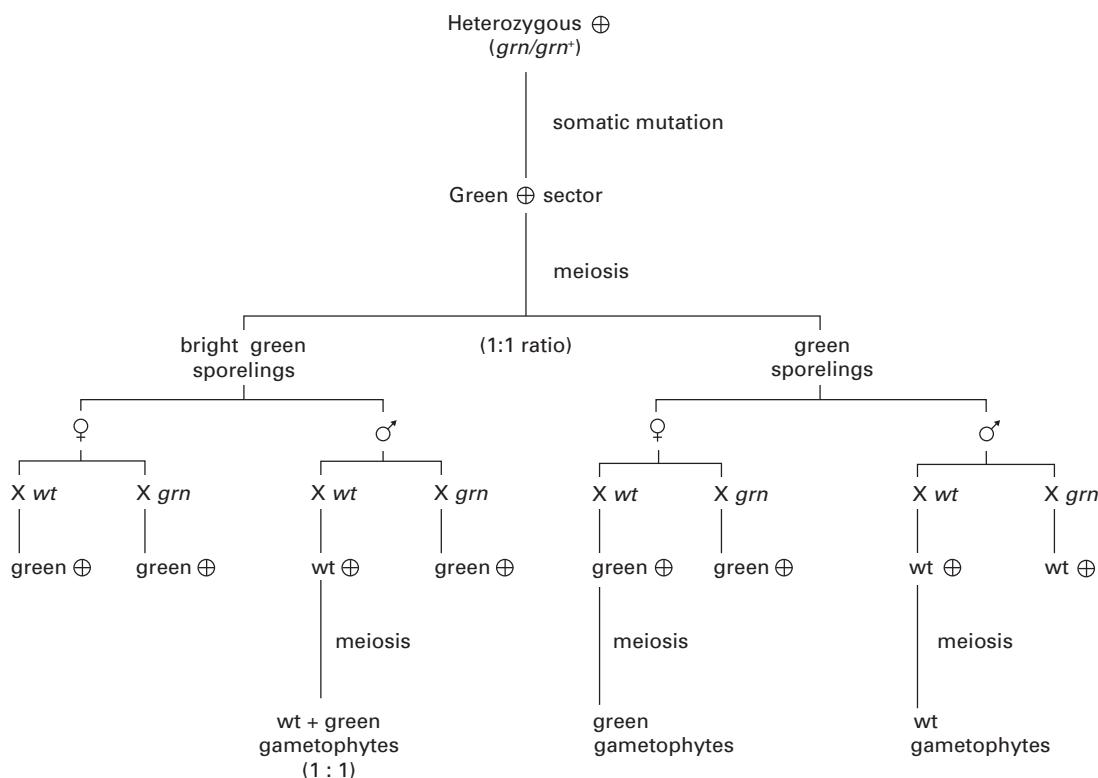


Figure 1.20 Non-Mendelian inheritance of a green somatic mutation in *Gracilaria tikvahiae*: wt, wild-type color (phenotype), grn⁺, the mutation for green color and its normal allele; ⊕, tetrasporophyte. (From van der Meer 1978, with permission of *Phycologia*.)

thought to be “controlled by multiple alleles at complex mating-type loci”, compared to the two alleles (*mt* and *bi*) for *Gracilaria* (Choi and Lee 1996).

Unstable mutants of *Gracilaria tikvahiae* also occur (van der Meer and Zhang 1988). These may be the result of transposable (genetic) elements (transposons) during genome rearrangement. Insertion of a transposon disrupts the gene function, and removal restores it. The temporary change may be visible as an unstable mutation. Some transposons are autonomous, that is, they control their own insertion and excision. Transposons and/or retrotransposons have since been discovered in *Ectocarpus siliculosus* (Cock *et al.* 2010a; Dittami *et al.* 2011) and *Pyropia yezoensis* (Peddigari *et al.* 2008). In *Ectocarpus*, transposons were among the most variable

gene sequences detected, and they may be important in enabling genetic adaptation of seaweeds to environmental stress (Dittami *et al.* 2011). The genome size of *Chondrus crispus* appears to have increased rapidly within the last 300 000 years due to the action of transposable elements (Collén *et al.* 2013).

In addition to the classical (Darwinian) theories on the vertical transfer of genetic information, that is from parents to progeny, there is increasing awareness that other processes have also shaped the speciation of organisms. Horizontal gene transfer (HGT) is the movement of genes between species, via endosymbiosis in eukaryotes, and in prokaryotes via the viral transduction of genes between organisms. For example, genes coding for trehalose synthesis by *Ectocarpus* were

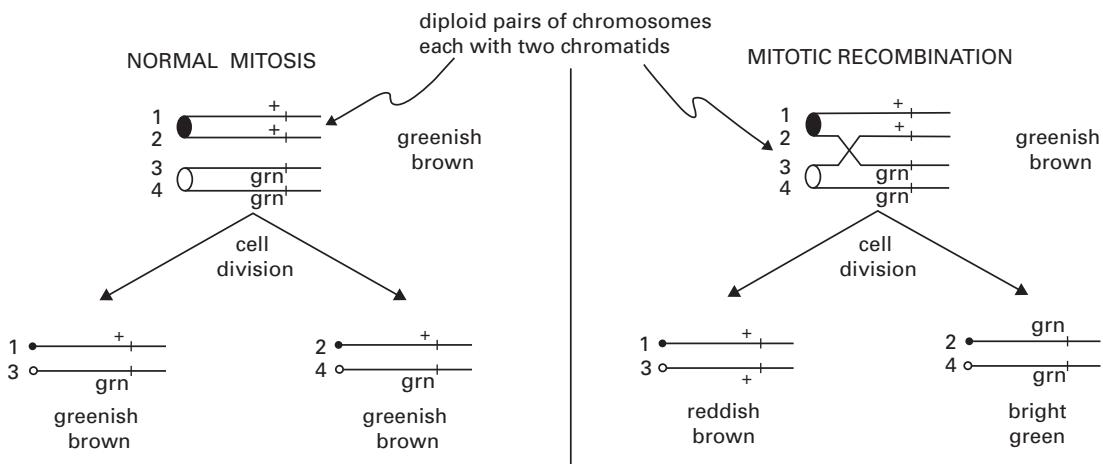


Figure 1.21 Mitotic recombination (right) compared to normal mitosis in heterozygous, diploid tetrasporophytes of *Gracilaria tikvahiae*. Diploid pairs of chromosomes are shown, each with two bivalents; the chromatids are numbered 1–4; +, wild-type color gene; grn, green mutant gene. (From van der Meer and Todd 1977, reproduced by permission of the National Research Council of Canada from the *Canadian Journal of Botany*, vol. 55.)

vertically inherited from an ancestral red algal symbiont but genes coding for D-mannitol metabolism arose via HGT from a species of Actinobacteria, and those responsible for sulfated-fucans typical of brown algal cell walls may have come from the eukaryotic host involved in the original primary endosymbiotic event that separated plants from animals (Cock *et al.* 2010a).

Furthermore, sexual reproduction and Darwinian theories on natural selection are not the only mechanism of generating genetic variation (reviewed in Monro and Poore 2009a). Modular organisms lack germ-soma segregation, and do not undergo meiosis but instead pass on genetic information via mitosis (somatic embryogenesis) to form ramets. Asexually produced *Asparagopsis armata*, for example, has a high level of phenotypic plasticity, illustrating that sexual reproduction is not a pre-requisite for morphological variability and, consequently, adaptation. The costs and benefits of sexual recombination versus clonal transmission of genetic material are discussed by Monro and Poore (2009a). Epigenetic phenomena, which affect gene transcription via changes in histone proteins and DNA methylation, but do not change the DNA sequence, are also likely to be important in generating genetic variation (Maumus *et al.* 2011).

The question of whether closely related seaweeds can hybridize has also benefited from molecular biological techniques (see Essay 1, Fig. 1). Before ~2000, laboratory crosses were used to demonstrate putative hybridization (see Table 1 in Kamiya and West 2010; Table 2 in Bartsch *et al.* 2008), but the application of molecular tools has allowed such studies to be extended to assess the level of hybridization in field populations. One of the most studied genera is *Fucus*. Species within this genus are closely related and have radiated relatively recently (Serrão *et al.* 1999a). Their thalli display substantial morphological variation and putative hybrids have been described for over 100 years (Coyer *et al.* 2002, 2006b). Coyer *et al.* (2002) verified in Eastern Norway the existence of hybrids between native *Fucus serratus* and introduced *Fucus evanescens*. Introgression (backcrossing) and paternal leakage are other determinants of *Fucus* population genetics. For example, during its northward post-glacial range extension, estuarine *Fucus ceranoides* carried the introgressed cytoplasm of *Fucus vesiculosus* with it, the first marine example of “alien cytoplasm surfing the wave of range expansion” (Neiva *et al.* 2010). Paternal leakage, whereby paternal mtDNA is not degraded but instead transmitted into the F1 generation (heteroplasmy), from *F. serratus* to *F. evanescens*

has been detected in their hybrid zone in Denmark (Hoarau *et al.* 2009). Hybridization can also occur between *F. vesiculosus* and *F. spiralis*, but this is considered a relatively rare event because they have different mating strategies: *F. vesiculosus* is dioecious and out-crossing while *F. spiralis* is predominantly a selfing hermaphrodite (Coleman and Brawley 2005; Engel *et al.* 2005; Perrin *et al.* 2007; Billard *et al.* 2010). The result is that *F. vesiculosus* has greater within-population genetic diversity, but *F. spiralis* has greater between-population diversity, because mutations are retained within the selfing population.

Self-fertilization has also been detected in the Laminariales. For *Macrocystis pyrifera* in California, selfing is thought to negatively affect local populations by increasing inbreeding depression (Raimondi *et al.* 2004). On the other hand, Barner *et al.* (2010) detected no negative effects of selfing for *Postelsia palmeformis*, and suggest that selfing is an important mechanism enhancing fertilization success in the extremely wave-exposed habitats in which it thrives.

Nuclear genome size varies widely among algae, and if the red, green, and brown lineages of seaweeds are taken together, there is a 1300-fold variation (Gregory 2005; Kapraun 2005). The size of nuclear genomes of plants and animals is typically reported as a C-value (pg) which refers to the amount of nuclear DNA in a haploid nucleus (e.g. sperm for animals). However, if diploid cells are studied (e.g. blood of animals) then the value reported is 2C. For an accurate assessment of the C-value, the ploidy level of the plant/algae is needed. For a polyploid, DNA content may be reported as 4C, 8C or 16C. Also, the C-value does not necessarily relate to the number of chromosomes. For terrestrial plants and seaweed, because cells are continually dividing, and neighboring cells may be at a different stage of cell division, it can be difficult to know precisely the ploidy level (Gregory 2005). Kapraun (2005) conducted the first wide-scale analysis of 2C nuclear DNA content in seaweeds. He found a minimum 2C nuclear genome size of 0.2 pg for all groups, with the Chlorophyta having the greatest range of up to 6.1 pg, the Rhodophyta up to 2.8 pg, and the Phaeophyceae 1.8 pg. This study raised some interesting questions: within the brown seaweeds, for

example, orders with oogamous reproduction tended to have more nDNA than isogamous or anisogamous modes. There was also a trend of cold-water Fucales (*Ascophyllum* and *Fucus*) having larger nuclear genomes than warm-water genera (*Sargassum* and *Turbinaria*), a finding that was supported by the nuclear DNA content of 19 Fucalean seaweeds from Spain, although whether a larger genome size causes enhanced cold tolerance requires testing (Garreta *et al.* 2010).

In addition to nuclear, plastid, and mitochondrial genomes, some seaweeds contain plasmids – small loops of DNA. Plasmids have been identified in red seaweeds including *Gracilaria* spp., *Gracilaropsis* spp., and *Porphyra pulchra* (e.g. Goff and Coleman 1990; Moon and Goff 1997) and the coenocytic green seaweeds *Ernadesmis verticillata* and *Valonia ventricosa* (previously *Ventricaria ventricosa*) (La Clair II and Wang 2000). Plasmid size ranges from 1.6–8 kbp, compared with ~110–190 kbp for plastid genomes in a range of red algae (Goff and Coleman 1988). Their functions in seaweeds are still unclear (Moon and Goff 1997). The plasmid complement appears to be a stable species character, not the result of infection (e.g. by viruses or parasites) (Goff and Coleman 1990).

1.4.3 Nucleocytoplasmic interactions

Eukaryotes have three main compartments for gene expression: the nucleus/cytosol, with the perforated nuclear membrane partially separating them, the plastids and mitochondria (Nott *et al.* 2006; sec. 1.3.2). Nucleocytoplasmic interactions include the full range of interactions between these compartments. In a giant, uninucleate cell like *Acetabularia*, with thousands of plastids and mitochondria, the organellar DNAs are significant components (Mandoli 1998a). *Acetabularia* has a very high cpDNA content compared to other algae and plants; much RNA synthesis takes place in the plastids but the extent to which cpDNA may be involved in cell morphogenesis, and the interactions between plastid and nuclear genomes, has yet to be resolved (Mandoli 1998a). Also, despite its large cpDNA size, the DNA is repeated and so the actual number of *Acetabularia* genes may be fairly

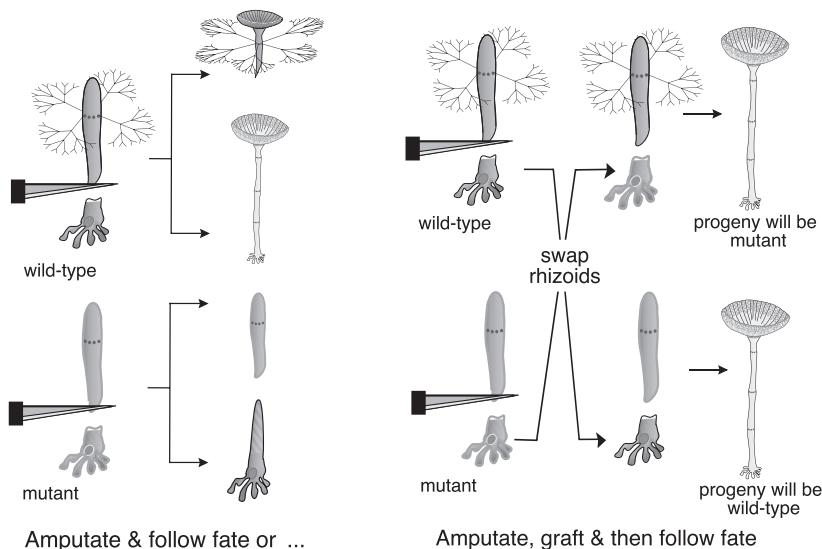


Figure 1.22 Responses of *Acetabularia acetabulum* to amputation and grafting. The vertically aligned cartoons are to scale with each other, but horizontally aligned cartoons are not necessarily at the same scale. (Left) Typical fates of the enucleate apex and the nucleate rhizoid to amputation are shown for the wild type and for a Class I type defect which is arrested in development, e.g. *kurkku* (Mandoli and Hunt 1996). (Right) The fates of graft chimeras of a reciprocal graft in which rhizoids of a wild-type and mutant are exchanged. Progeny of these graft chimeras are shown. The depiction may also be valid for some other species and some combinations of interspecific graft chimeras. (From Mandoli 1998a, reproduced with permission.)

similar to other seaweeds (Simpson and Stern 2002). *Acetabularia* has been a model system for studying nucleocytoplasmic interactions since Hämmерling's classic studies in the 1930s. The main advantage of *Acetabularia* is that interspecific grafts can be made: both nuclei and cytoplasm can be transferred between species (Fig. 1.22; reviewed by Menzel 1994; Mandoli 1998a,b; Mine *et al.* 2008).

Hämmérling concluded, long before messenger RNA (mRNA) was known, that "morphogenetic substances" were released from the nucleus into the cytoplasm, where they could be stored for some time, but were gradually used up. *Acetabularia* cells can still form a cap if, after reaching about one-third of their final length, the nucleus is removed. There are apico-basal and baso-apical gradients of morphogenetic substances. That these substances come from the nucleus has been shown by transplanting nuclei into opposite ends of enucleated cells (Fig. 1.22). The type of cap formed by an enucleated stalk is characteristic of the species, but if

a nucleus from another species or mutant is inserted, either as an isolated nucleus or by grafting on a basal fragment (Fig. 1.22 – right-hand panel), the caps formed are first intermediate and then have the characteristics of the nucleus donor species. This, and the fact that enucleated cells can form a cap only once, provides evidence that the morphogenetic substances are used up in cap formation.

Hämmérling's morphogenetic substances are now known to be mRNA, and mRNAs specific to morphological and reproductive developmental processes in *Acetabularia acetabulum* have been identified. Homeobox genes are the "master control genes" of eukaryotes with important roles in morphological development (Buchanan *et al.* 2000). For example, homeobox gene *Aaknox1* is evenly expressed along the stalk of mature, vegetative *A. acetabulum* but at the onset of reproduction mRNA it is localized at the base, close to the primary nucleus, indicating post-transcriptional control of gene expression (Serikawa and Mandoli 1999). Mine *et al.*

(2005) found two mRNAs (as polyadenylated RNA) for *A. peniculus*. “Poly(A)⁺ RNA striations”, derived from the primary nucleus, are distributed evenly within the stalk cytoplasm and associated with actin bundles and filaments. These mRNA striations are thought to be a transport form of mRNA and are involved in vegetative developmental processes such as stalk elongation and whorl development because they disappear during the early stages of cyst formation. The second type of mRNA, the “perinuclear poly(A)⁺ RNA mass”, creates a mass around each secondary nucleus and is closely associated with ribosomes, indicating active translation of these genes during cyst formation. This mRNA is also associated with microtubules, rather than actin filaments. Indeed, there are multiple mRNA gradients along the stalk of mature, vegetative *Acetabularia acetabulum* and despite being a unicellular algae there is considerable regional differentiation (Serikawa *et al.* 2001; Vogel *et al.* 2002).

This completes our review of seaweed cells and thalli. In Chapter 2, we focus on seaweed life cycles, reproduction, and morphogenesis, and how these processes are affected by both intrinsic and extrinsic factors.

1.5 Synopsis

Benthic ocean vegetation is dominated by macroscopic multicellular and unicellular members of the red, green, and brown seaweeds. The term “seaweeds” represents an ecological grouping of disparate taxa, which are related by the endosymbiotic events that

gave rise to plastids. Seaweeds have a microscopic phase to their life history, as gametes, spores, or zygotes, and for some as a free-living alternate life-history stage. The most common forms of seaweed construction are filamentous and pseudoparenchymatous, with parenchymatous construction being prevalent only in the Phaeophyceae. Seaweeds may be unitary or clonal, and some clonal species coalescence to form chimeras. Modularity is common in all the seaweed phyla. Seaweed cells differ from the cells of “higher” plants in general by their broader range of metabolic functions. Some special features of seaweed cells include vesicles that may store bioactive secondary metabolites, a high proportion and diversity of cell wall matrix polysaccharides, a wide variety of plastid structures and pigmentation, the different arrangement of flagella in motile cells, and the details of cell division. The unique characteristics of some seaweeds has led to their use as model organisms, e.g. *Acetabularia* for nucleocytoplasmic interactions and *Fucus* for cell polarization. Classical seaweed genetics, especially using color mutants in some red algae, have shown Mendelian and non-Mendelian inheritance. For asexually reproducing seaweed populations, genetic variation of clones arises via somatic embryogenesis. The application of molecular biology techniques has had a profound impact on phylogenetics, biogeography, and population biology. *Ectocarpus siliculosus* was the first seaweed to have its genome fully sequenced, and applications of other “omic” techniques are providing novel insights into seaweed physiological ecology.