Genetic and developmental mechanisms of cellular differentiation in algae

Susana M Coelho

Max Planck Institute for Developmental Biology, Max-Planck-Ring 5, 72076 Tübingen, Germany

ORCiD: 0000-0002-9171-2550

coelho@sb-roscoff.fr

Mark Cock

Sorbonne Université, UPMC Univ Paris 06, CNRS, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, CS 90074, F-29688, Roscoff, France

ORCiD: 0000-0002-2650-0383

cock@sb-roscoff.fr

Abstract

Algae are photosynthetic eukaryotes whose taxonomic breadth covers a range of branches of the tree of life. Green, red and brown macroalgae have made the transition from simple multicellularity to complex multicellular body plans. This chapter will describe what is known about developmental events in each macroalgal lineage and discuss the developmental features of each group that are currently under investigation in relation to the evolution of multicellularity.

15.1 Introduction

The term ‘algae’ is generally used to group together all photosynthetic eukaryotes with the exception of land plants. The algae are therefore a highly polyphyletic group that includes organisms from many of the major eukaryotic lineages (Figure 15.1). The broad phylogenetic distribution of these organisms is explained by the evolutionary history of their photosynthetic organelles (plastids). With only one known exception (Marin et al., 2005), these organelles are all thought to be derived from a cyanobacterium captured by an ancestor of the Archaeplastida (the lineage that includes green and red algae; Figure 15.1; Keeling, 2013). Following this primary endosymbiosis, plastids were subsequently acquired by organisms in other eukaryotic supergroups over the course of evolution via secondary endosymbiosis events involving capture of red or green algae and integration of their plastid systems into the machinery of the host cell (Keeling, 2013). It was these events, which presumably involved unicellular ancestors in each lineage, that led to the very broad distribution of algae across the eukaryotic supergroups. For many of the lineages, the extant species are still unicellular (glaucophytes, dinoflagellates, chlorarachnids, euglenids, cryptophytes, haptophytes). In other lineages different levels of multicellularity have evolved, ranging from the formation of simple colonial chains or clusters of cells (e.g. some diatom species) to the emergence of large, complex multicellular organisms with differentiated organs and multiple cell types (brown, red and green macroalgae; Figure 15.1).

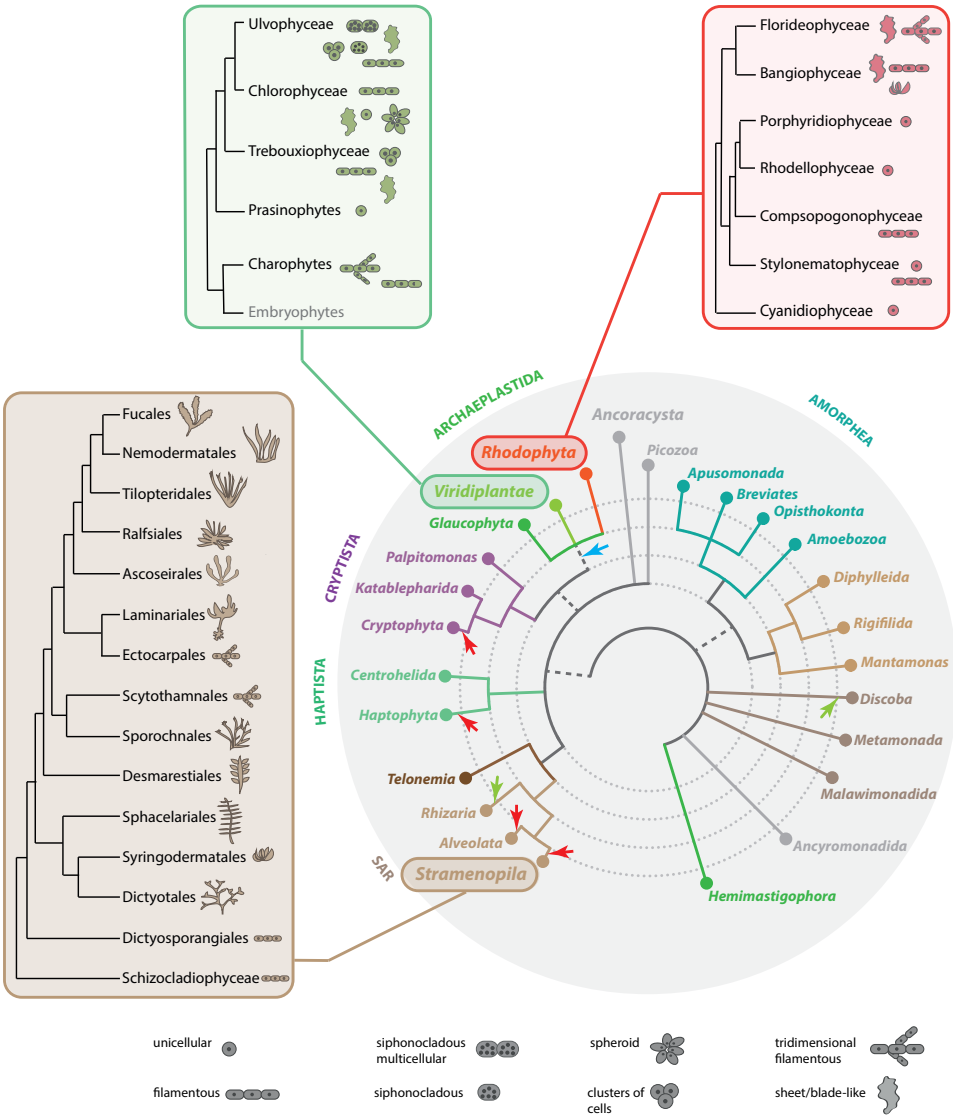

Figure 15.1. Schematic view of the eukaryotic tree of life and multicellular and morphological complexity in the brown, red and green algal lineages. The eukaryotic tree (adapted from (Burki et al., 2020) is based on a consensus of recent phylogenomic studies. Broken lines indicate uncertainty about the monophyly of certain groups. Coloured arrows indicate plastid endosymbiosis events (Bodył, 2018; Keeling, 2013). The blue arrow indicates the primary plastid endosymbiosis event that occurred in a common ancestor of glaucophytes, red (Rhodophyta) and green (Viridiplantae) algae. Red or green arrows indicate plastids acquired by endosymbiosis from algae of the red or green lineages, respectively. Note that these acquisitions could have been direct via a secondary endosymbiosis or indirect, involving two or more transfers of plastids between hosts (tertiary or quaternary endosymbioses). Note also that the presence of a coloured arrow does not necessarily indicate that all the members of the lineage corresponding to that branch of the tree acquired a plastid. The simplified trees for the three major algal lineages were based on Leliaert *et al*. (Leliaert et al., 2012) and De Clerck *et al*. (De Clerck et al., 2012) for the green algae, Qiu *et al*. (Qiu et al., 2016) for the red algae and Silberfeld *et al*. (Silberfeld et al., 2014), Kawai *et al*. (Kawai et al., 2015) and Bringloe *et al*. (Bringloe et al., 2020) for the brown algae. Only the brown algae, together with their sister clade, the Schizocladiophyceae, are shown for the Stramenopila.

This chapter will focus on the three macroalgal lineages, which are the algal groups that exhibit the highest level of multicellularity and are the only algal groups that are considered to have made the transition from simple multicellularity to complex multicellular body plans (along with the animals, land plants and fungi; Cock et al., 2010; Cock and Collén, 2015; Knoll, 2011). The chapter will describe what is known about developmental events in each lineage and will discuss the developmental features of each group that are currently under investigation in relation to the evolution of multicellularity.

15.2 Green algae

The green algae and the land plants compose the green lineage (Viridiplantae), one of the major groups of oxygenic photosynthetic eukaryotes. Current hypotheses propose early divergence of two discrete clades from an ancestral green flagellate organism. One clade comprises the chlorophytes. The other clade, the streptophytes, includes the charophyte green algae from which the land plants evolved (Leliaert et al 2012). Multicellular taxa have repeatedly evolved in the green algae, as well as macroscopically complex unicellular forms that show many of the features that are emblematic of multicellularity. We will focus here on two multicellular groups of chlorophytes, the volvocines and ulvophytes, whilst charophyte algae will be treated in another chapter (*ref. Bowman chapter*).

The chlorophytes diverged from the streptophytes approximately 900-1000 MYA (Bhattacharya et al., 2009; Hedges et al., 2004). Ancestral green algal species were probably small unicellular marine biflagellates (Leliaert et al., 2012) and this form is still prevalent among modern aquatic algae, such as the well-studied unicellular, biflagellate alga *Chlamydomonas reinhardtii*. Of the four classes of chlorophyte, three (Chlorophyceae, Trebouxiophyceae, and Ulvophyceae) include members that are multicellular during at least part of their life cycle. Transitions to multicellularity or macroscopic forms occurred several times independently (Lewis and McCourt, 2004; Mattox and Stewart, 1984; Chapter 9). These transitions gave rise to an exceptional range of morphological patterns, from relatively large algae (up to 1 m) with complex tissues and organs to several species that form small motile multicellular colonies (van den Hoek, Mann & Jahns, 1995; Lewis & McCourt, 2004) (Figure 15.1, Figure 15.2).

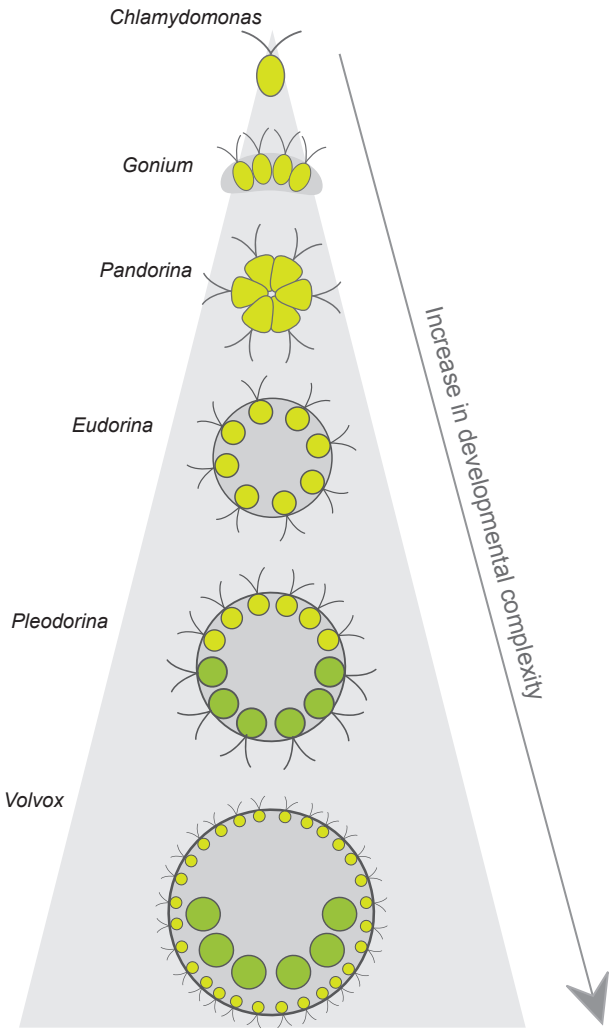

Figure 15.2. Schema indicating different degrees of developmental complexity in volvocine algae, from *C. reinhardtii* to *V. carteri*. Different shades of green represent different cell types.

In multicellular green algae, cells are connected by adhesives to maintain a coherent, physically attached body. Cells may also communicate through plasmodesmata, which ensure a supra-cellular organisation at the level of the whole organism (Raven, 1997). Some multicellular green algae, however, are merely unconnected groups of cells embedded in an extracellular matrix, and the form of the alga can be extremely variable, from simple unbranched filamentous forms to organisms with, for example, highly branched thalli or leaf-like blades. Note that complex and macroscopic unicellular organization is found in giant unicellular algae (e.g. *Acetabularia*) that possess a single nucleus (siphonous), but also in coenocytic algae where large single cells contain several nuclei within a common cytoplasm (reviewed in Leliaert et al., 2012). Although these organisms are not 'multicellular', they possess remarkable structures with specialised sub-domains. For example, *Caulerpa* has different 'tissues' that resemble roots, stems and leaves (Jacobs, 1970). Some green algae may also be both multicellular and coenocytic (siphonocladous) because they are composed of multiple cells, each of which contains multiple nuclei (e.g., *Cladophora*).

In the next sections, we will discuss both established and emerging model green algae and describe how these models are being used to explore the mechanisms underlying cell differentiation and the emergence of complex multicellular developmental patterns.

15.2.2 Volvocine algae (Chlorophyceae)

The volvocine algae, in the order Volvocales, are a monophyletic group of chlorophyceae algae that together form a fascinating study set for the emergence of multicellularity and evolution of developmental complexity. They include a series of species exhibiting increasing levels of cell-type specialisation and developmental complexity, from unicellular forms such as *Chlamydomonas*, through multicellular groups with undifferentiated cells (e.g. *Gonium* and *Eudorina*) to relatively complex multicellular individuals that may have one (e.g. *Pleodorina*) or two (*Volvox*) specialised cell types.

The Volvocales include experimentally tractable model-systems for which a range of molecular tools are available (Merchant et al. 2007; Prochnik et al. 2010; Umen and Olson 2012, Umen 2020). The genomes of three species that represent a broad range of developmental complexity —*Tetrabaena socialis*, *Gonium pectorale*, and *Volvox carteri*, together with the genome of a unicellular close relative, *C. reinhardtii*, have been sequenced and compared (Featherston et al., 2018; Hanschen et al., 2016; Merchant et al. 2007; Prochnik et al., 2010). Analysis of these genomes has revealed that, although the four species exhibit contrasting levels of morphological complexity, their genomes are relatively similar with the notable exception of the expansion of gene families involved in extracellular matrix (ECM) formation and cell-cycle regulation (Featherston et al., 2018; Hanschen et al., 2016; Prochnik et al., 2010). These comparative genomic analyses, together with classical genetic studies, have shed light on the mechanisms underlying the stepwise progression of increasing cell number, colony size and degree of cell-type specialization. Specifically for *V. carteri*, genetic screens have been a powerful tool in revealing the molecular pathways involved in developmental and morphological traits (e.g. Kirk, 1998, 2001).

The evolution of multicellularity in the Volvocales is thought to have involved a series of 12 specific innovations (Kirk, 2005; Umen, 2014). These innovations include, for example, genetic modulation of the number of cells per individual and modifications to the cell cycle program (Kirk, 1998, 2005; Umen and Olson, 2012). *C. reinhardtii* cells can divide as soon as they double in size, whereas cells in colonial species must grow to a specific size between reproductive cycles so that entire new colonies can be formed with the appropriate number of cells. In *C. reinhardtii*, the retinoblastoma (Rb) tumor suppressor pathway has been shown to control cell size and the number of cell divisions a cell undergoes before the daughter cells separate (Fang et al., 2006; Umen and Goodenough, 2001). It is thought that the Rb pathway may have been modified in colonial species to increase the minimum size threshold at which division can occur.

Another important feature of the evolution of multicellularity in this group is the transformation of the cell wall into an ECM and ECM expansion and complexification. For example, the walls of individual *Gonium* cells have an additional outer layer, compared to those of *C. reinhardtii*, that maintains colony cohesion (Nozaki, 1990). The more complex, spheroidal species *Pandorina*, *Eudorina*, *Pleodorina*, and *Volvox* also have expanded ECMs and additional modifications of this structure in these species include the formation of a colony boundary layer.

One of the best-studied innovations during the transition to multicellularity in the volvocines is the differentiation of germ and somatic cell lineages that is observed in the genera *Pleodorina* and *Volvox*. In *V. carteri*, germ-soma differentiation takes place after completion of embryonic cell division. Germ versus soma cell fate is established solely based on cell size

and not by inheritance of cytoplasmic cell fate factors (Kirk et al., 1993). The nature of the cell size signal is unknown but it may be linked to the Rb pathway, which has a role in cell size control in *C. reinhardtii* (Fang et al., 2006; see above). Somatic cell fate determination involves differential expression of a master regulatory gene (somatic regenerator, *RegA*), which is thought to encode a transcriptional repressor of genes for growth and reproduction. Mutation of this gene results in somatic cells regaining reproductive capability (Kirk et al., 1999; Stark et al., 2001). *RegA* expression is restricted to somatic cells and its mRNA is first detectable shortly after embryogenesis is completed (Kirk et al., 1999). RegA belongs to a family of VARL (Volvocine algae RegA-like) proteins with 14 members in *V. carteri* and 12 in *C. reinhardtii* (Duncan et al., 2007). The closest homologue of *RegA* in *C. reinhardtii* is *RLS1*, and its expression is induced under nutrient and light limitation and during stationary phase (Nedelcu, 2009; Nedelcu and Michod, 2006). Based on these observations, it has been hypothesised that the evolution of somatic cells in *V. carteri* involved the co-option of an ancestral environmentally-induced *RLS1*-like gene by switching its regulation from a temporal/environmental to a spatial/developmental context.

Recent comparative transcriptomic approaches indicated that *V. carteri* orthologs of genes that are diurnally controlled in *C. reinhardtii* exhibit a strongly partitioned pattern of expression, with expression of dark-phase genes overrepresented in somatic cells and light-phase genes overrepresented in the gonidial cells of the germ cell lineage (Matt and Umen, 2018). This observation further supports the idea that cell-type programs in *V. carteri* arose by co-option of temporally or environmentally controlled genes from a unicellular ancestor that came under cell-type control in the *V. carteri* lineage.

The idea that large-scale changes in gene expression underlie the transition to a multicellular life cycle is further supported by experimental evolution approaches. Herron and colleagues used experimentally evolved *C. reinhardtii* to explore the genetics underlying a *de novo* origin of multicellularity. They identified changes in gene structure and expression that distinguish an evolved ‘multicellular’ clone from its unicellular ancestor. Among these, changes to genes involved in cell cycle and reproductive processes were overrepresented, supporting previous indications from genetic screens (see above; Herron et al., 2018). Isolation of new regulatory loci using genetic screens and further investigation of *V. carteri* and *C. reinhardtii* mutants will help to clarify the origins of cell-type specification in this lineage and to further deepen our understanding of how multicellularity emerged.

15.2.2 Ulvophyceae

The Ulvophyceae, or green seaweeds, represent an independent emergence of a macroscopic plant-like vegetative body. Members of the class Ulvophyceae display a fascinating morphological and cytological diversity, including multicellular organisms such as the sea lettuce *Ulva* but also species with macroscopic siphonous coenocytes (Figure 15.1).

Despite the interest of this group of green algae with regard to the transition to multicellular development and the evolution of the cyto-morphological diversity, genomic resources for the Ulvophyceae have remained relatively scarce, except for a transcriptomic study of *U. linza* (Zhang et al., 2012), a description of the mating-type locus of *U. partita* (Yamazaki et al., 2017) and a study of the distribution of transcripts in the thallus of the siphonous green seaweed *Caulerpa* (Ranjan et al., 2015).

This situation is changing rapidly with the emergence of *Ulva* sp. as an exciting novel model organism for studies of green algal growth, development and morphogenesis as well as mutualistic interactions (Wichard et al., 2015). Several interesting aspects of *Ulva* biology,

including cell cycle, cytology, life-cycle transition, induction of spore and gamete formation, and bacterial-controlled morphogenesis, have been studied in detail (reviewed in Wichard et al., 2015). The *Ulva* thallus has a relatively simple multicellular organisation, with small uninucleate cells and a limited number of cell types. In natural environments, *Ulva* may exhibit two morphological patterns: either flattened blades that are two-cells thick or thalli that develop as tubes that are one-cell thick (Figure 15.3). These morphologies require the presence of symbiotic bacteria, without which only slow-growing, undifferentiated "callus-like colonies" develop (Spoerner et al., 2012; Stratmann et al., 1996). The growth, cell differentiation, and morphogenesis of *Ulva* depend on interactions with specific bacteria and the chemical mediators these bacteria produce, in particular the compound thalusin (Egan et al., 2013; Goecke et al., 2010; Wichard et al., 2015). The possibility of triggering morphogenesis by adding an engineered microbiome (Wichard et al., 2015) or a compound produced by these microbes is a major asset that may allow insights to be gained into molecular events during cellular differentiation. Therefore, *Ulva* is being increasingly used as a model organism for investigating morphogenesis (De Clerck et al., 2018; Wichard et al., 2015).

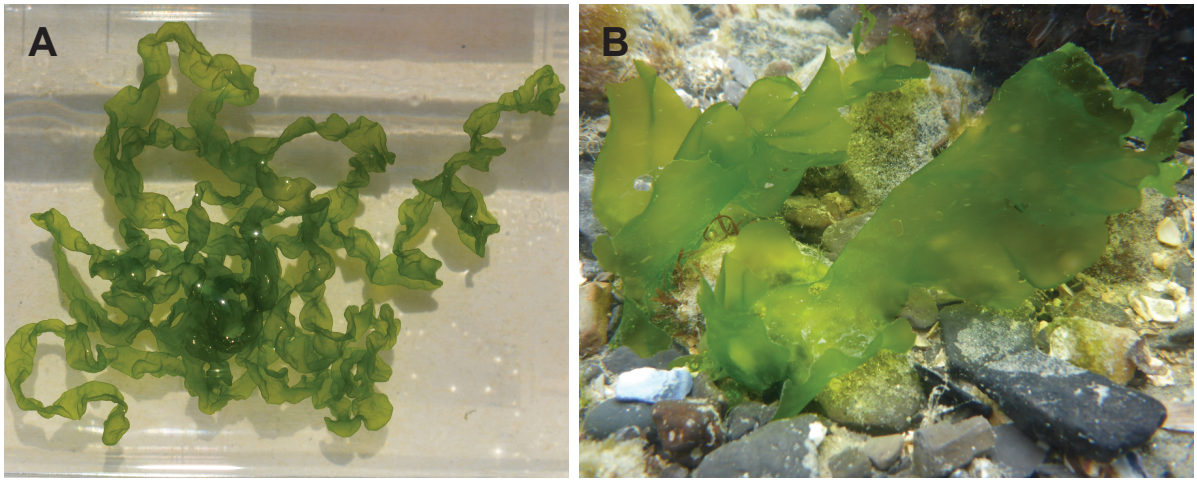

Figure 15.3. Alternative morphological patterns in *Ulva* spp.. Thalli either develop as tubes (A) or as flattened blades (B).

The first whole-genome sequence of an *Ulva* species, *U. mutabilis*, has recently opened new opportunities for the study of the emergence of multicellularity in the green lineage (De Clerck et al., 2018). Remarkably, analysis of the *U. mutabilis* genome has detected loss of genes that encode components of the retinoblastoma (RB)/E2F pathway and associated D-type cyclins. Comparative genomic studies of volvocine algae have revealed that the co-option of the RB cell-cycle pathway is key to the emergence of multicellularity in this lineage (see above). It appears therefore that the paths towards multicellularity in *U. mutabilis* and in volvocines progressed through different routes. Parallels can however still be drawn between ECM gene family expansions in the volvocine algae and the diversity of protein domains associated with the ECM in *U. mutabilis* relative to closely related unicellular taxa, especially given the proposed role of expanded volvocine ECM gene families in environmental signalling (De Clerck et al., 2018).

15.3 Red algae

The red algae (Rhodophyta) are an ecologically important group of organisms, dominating many coastal environments, and are also of economic interest as a source of food and industrial colloids. They are a sister group to the green lineage (Viridiplantae) and acquired their plastids via the same primary endosymbiosis (Figure 15.1; Keeling, 2013). From an evolutionary point of view, red algae are also important because they donated plastids to several other eukaryotic supergroups via secondary endosymbioses (Figure 15.1; (Keeling, 2013). Fossil evidence indicates that the red algae were the first eukaryotic lineage to evolve complex multicellularity, as early as 1600 Mya (Bengtson et al., 2017; Butterfield, 2000). Extant red algae exhibit a broad range of complexity, including species that are unicellular, colonial, grow as simple filaments or that form large, foliose thalli (Cock and Collén, 2015; Waaland, 1990). The latter can be either pseudoparenchymatous (made up of amalgamated filaments) or parenchymatous (true tissues formed by three-dimensional cell divisions). The larger multicellular red algae can possess several types of organ, including stems, holdfasts, bladders and bladelike fronds and can reach lengths of up to three metres. However, even the largest red algae exhibit significantly less morphological and cellular complexity than the most complex representatives of the brown algae.

In the 1970s and 1980s, genetic approaches were applied to the red algae with the objective, in part, of studying multicellular development. Red algal morphological mutants were isolated and analysed genetically, but these studies did not go as far as identifying the underlying genetic loci (van der Meer et al., 1990). Mutant analyses did however provide some insights into developmental processes at the cellular level. For example, in several members of the Bangiales, meiosis coincides with the first cell divisions of the gametophyte resulting in chimeric individuals descended from all four meiotic products. Using colour mutants, this phenomenon was exploited to study cell lineage patterns in the developing gametophyte (Mitman and Meer, 1994; Niwa, 2010).

Interest in red algal multicellular development has been revived recently as genome data has become available for this lineage. Complete genome sequences, with varying qualities of assembly, have currently been reported for 11 red algal species, including eight multicellular taxa (Table 15.1). Comparison of these assemblies indicates that multicellular species tend to have larger genomes than unicellular species (50-100 Mbp compared with 16-20 Mbp; Table 15.1), a trend that has been observed in other multicellular lineages. It has been proposed that expansion of the genomes of multicellular organisms occurs due to a weakening of purifying selection as a result of reduced effective population sizes (Lynch and Conery, 2003). Genome expansion in multicellular red algae appears to have occurred principally as a result of the proliferation of transposable elements (Table 15.1) rather than by polyploidisation (Collén et al., 2013; Lee et al., 2018). Collén *et al*. (2013) have proposed a general scenario for genome evolution in the red algae in which there was a marked reduction in genome size during the early evolution of the lineage, followed by genome expansion associated with the transition to multicellularity. The genomes of multicellular red algae have been analysed for features that may be related to the transition to multicellularity, such as gene family expansions and the composition of signalling gene families including transcription factors (Brawley et al., 2017; Collén et al., 2013). Interestingly, it has been suggested that the constricted repertoire of cytoskeleton genes in red algae may have prevented the lineage from evolving highly complex multicellular body plans because of limitations on cell size and complexity (Brawley et al., 2017). Genomic data, together with transcriptomics, is also being used to identify potential key developmental genes. For example, a recent study showed that a three amino acid loop extension homeodomain transcription factor (TALE HD TF) was upregulated in the

conchosporangium of *Pyropia yezoensis*, suggesting a possible role in the regulation of life-cycle-related developmental processes (Mikami et al., 2019).

From an evolutionary point of view, it would clearly be of interest to have a deeper understanding of multicellular development in the red algae. Genome sequencing has provided the necessary gene catalogues on which to build such analyses but genetic tools are also required to directly investigate gene function. Another key factor is likely to be the selection of a model species and focusing of effort across the red algal community on that species. *Pyropia yezoensis* (then *Porphyra yezoensis*) was proposed as a model several years ago (Waaland et al., 2004) and this species remains a strong candidate, but other species should also be considered.

Table 15.1. Red algal genome assemblies.

Species	Multicellular or unicellular	Genome size (Mbp)	Repeat sequences	Reference
Chondrus crispus	Multicellular	104.8	59%	(Collén et al., 2013)
Calliarthron tuberculosum	Multicellular	(51.1)*	nd	(Chan et al., 2011)
Gracilariopsis chorda	Multicellular	92.1	61%	(Lee et al., 2018)
Gracilariopsis lemaneiformis	Multicellular	81.2	55%	(Zhou et al., 2013)
Gracilaria changii	Multicellular	(35.8)*	nd	(Ho et al., 2018)
Porphyra umbilicalis	Multicellular	87.7	43%	(Brawley et al., 2017)
Pyropia yezoensis	Multicellular	(43.0)*	nd	(Nakamura et al., 2013)
Pyropia yezoensis	Multicellular	108	48%	(Wang et al., 2020)
Pyropia haitanensis	Multicellular	53.3	24%	(Cao et al., 2020)
Cyanidioschyzon merolae	Unicellular	16.5	20%	(Matsuzaki et al., 2004)
Galdieria sulphuraria	Unicellular	13.7	16%	(Schönknecht et al., 2013)
Porphyridium purpureum	Unicellular	19.7	4%	(Bhattacharya et al., 2013)

**Draft genomes and therefore probably incomplete assemblies. nd, not determined.*

15.4 Brown algae

The brown algae (Phaeophyceae) are part of the stramenopile (or heterokont) supergroup (Figure 15.1), which also includes diatoms and oomycetes. Brown seaweeds have therefore had a very different evolutionary history to the green and red algae, having diverged from the Archaeplastida lineage (i.e. Glaucophyta, Viridiplantae and Rhodophyta) at the time of the eukaryotic crown radiation. The photosynthetic stramenopiles (ochrophytes) acquired their plastid via a secondary endosymbiosis involving a red alga (Figure 15.1; Keeling, 2013).

All brown algae are multicellular but they exhibit a broad range of complexities, from simple filamentous species to large complex organisms with distinct, parenchymatous tissues and organs (Charrier et al., 2012). Kelps of the order Laminariales, for example, rival land plants in their complexity, with well-defined organs such as the holdfast, which anchors the alga to its substratum, the stipe, which serves a similar function to a land plant stem, and the frond, which represents the main photosynthetic surface, equivalent to land plant leaves. Each of these structures is composed of several cell types, including epidermal structures, cortical tissues and other specialised cells. Of particular interest are the trumpet hyphal cells, which represent a primitive vascular system. *Macrocystis pyrifera* (giant kelp) is often cited as an example of the level of multicellular complexity that has been attained by the brown algae. This kelp can grow up to lengths of 50 metres and therefore represents one of the largest organisms on the planet.

Stramenopiles other than the brown algae are all unicellular or filamentous organisms, including the stramenopile taxa closest to the brown algae, the filamentous alga *Schizocladia*. Moreover, the thalli of the most basal group within the brown algae, the Discosporangiales, also consist of uniseriate filaments (chains of single cells) whereas the most complex brown algae tend to belong to recently evolved taxa such as the Laminariales or the Fucales. These observations suggest a gradual acquisition of multicellular complexity during the evolution of the brown algae. However, while this conclusion is probably correct overall, a closer look at the phylogeny indicates a slightly more complex story because, for example, some recently evolved groups such as the Ectocarpales exhibit quite simple filamentous morphologies and some basal groups, such as the Dictyotales, have more complex features. These observations suggest that there has either been independent, parallel evolution of complex features in different brown algal groups or that orders such as the Ectocarpales have experienced some loss of complexity over evolutionary time.

The following sections will discuss how model organisms have been used and are being used to investigate developmental processes in the brown algae.

15.4.1 *Fucus* and *Dictyota*

Fucoids represent the group of brown algae that has attained a high level of multicellular complexity in terms of number of cell types and developmental complexity of tissues and organs (Figure 15.1). They are also the only brown algal group that has evolved diploid life cycles, where the gametophyte stage is inexistent, their life cycle resembling that of an animal (Heesch et al., 2019) (Figure 15.4).

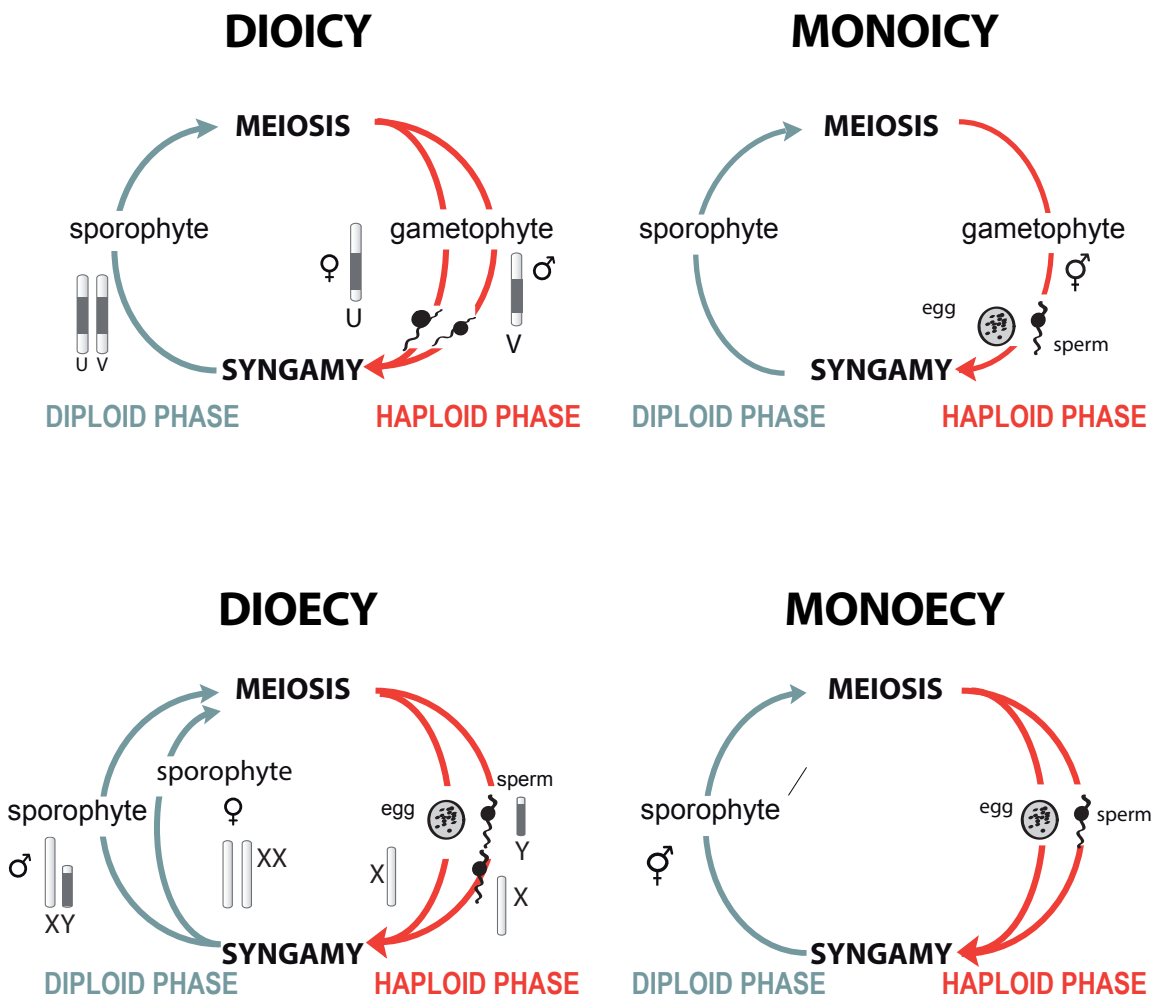

Figure 15.4. Schematic representation of the different types of sexual systems in the brown algae. The diversity of sexual systems include haploid or diploid sex determination, each associated with either co-sexuality (monoicy, monoecy) or separate sexes (dioicy, dioecy). Sex chromosomes are represented in the scheme as grey bars with the non-recombining region highlighted in dark grey. Note that some species have a large sexual dimorphism in terms of gamete size (oogamy, e.g. *Fucus* spp.) or a subtle difference in terms of male versus female gamete size (near-isogamy, e.g. *Ectocarpus*).

Brown algal zygotes and embryos have served as a system to explore early developmental events, such as polarity and asymmetric cell division because fertilisation is external and embryos develop free from maternal tissue. This feature allows access to the cellular events underlying polarisation, the first cell division and cell fate determination. Accordingly, *Fucus* zygotes and embryos have been employed to study polarity and asymmetry during early embryo development (reviewed in Brownlee et al., 2001). The initial asymmetric cell division produces two cells - a rhizoid and a thallus cell - with distinct morphologies and fates. The rhizoid cell generates the holdfast which will attach the alga to substrates, whereas the thallus cell will give rise to the stipe and the rest of the algal body (fronds, air bladders, and reproductive structures in *Fucus*). Fucoid eggs possess no intrinsic polarity and no cell wall. After fertilization, zygotes synthesize a cell wall within minutes and become polarized in response to external vectors, most frequently unilateral light. Photopolarisation and germination are followed by the asymmetric division of the zygote, with subsequent divisions occurring in a highly ordered, spatial, and temporal pattern (Brownlee and Bouget, 1998). The

cell wall was identified as a source of position-dependent information required for polarisation and fate determination in the zygote and 2-celled embryo (Berger et al., 1994). Regeneration is regulated in a position-dependent manner and is strongly influenced by intercellular communication, likely involving transport or diffusion of inhibitory signals which appear to be essential for regulation of cell fate decisions (Bouget et al., 1998). Apoplastic diffusible gradients of unknown nature were proposed to be involved in pattern formation in the multicellular embryo (Bouget et al., 1998).

While a large amount of work has been done in the cell biology mechanisms underlying asymmetrical cell division, we still know very little about the molecular mechanisms that drive cell fate determination in fucoid algae, mostly because there are very few molecular tools developed for this group of organisms. Injection of double-stranded RNA has been shown to provide a potential means to knockdown gene expression by RNA interference but no stable transformation system has been described yet. Classical genetic approaches are not feasible due to the slow growth rate and long life cycle.

A more recent model for cell fate determination and evolution of multicellularity is the brown alga *Dictyota dichotoma* (Bogaert et al., 2020; Coelho and Cock, 2020). In contrast to the situation for *Fucus*, the life cycle of *D. dichotoma* can be completed under laboratory conditions, opening the possibility of applying classical genetic approaches (Bogaert et al., 2016). As in other brown algae, the establishment of the apical-basal multicellular pattern is achieved through an initial asymmetric cell division (Bogaert et al., 2017; De Smet and Beeckman, 2011; Peters et al., 2008) and recent studies have indicated a potential role for auxin in patterning multicellular development in *D. dichotoma* (Bogaert et al., 2019).

15.4.2 Ectocarpus

Both *Fucus* and *D. dichotoma* have provided important insights into the cellular events during early embryogenesis but neither model is currently adapted to the analysis of the molecular events underlying developmental processes. This situation may change for *D. dichotoma* in the future as the life cycle of this species can be completed in the laboratory (see above), but this is not the case for *Fucus*. Currently, the most powerful model system for exploring developmental processes in the brown algae at the molecular level is the filamentous alga *Ectocarpus* (Coelho and Cock, 2020; Coelho et al., 2020). As a filamentous alga, this species does not exhibit the same level of developmental complexity as the kelps, for example, but the Ectocarpales are a sister order to the Laminariales (kelps) and the genetic systems that control developmental processes are expected to be conserved between the two taxa.

Ectocarpus was proposed as a general model system several years ago (Peters et al., 2004) based on its potential for genetic and genomic analyses. *Ectocarpus* cultures can be easily maintained in the laboratory and earlier work, principally by Dieter Müller and colleagues at Konstanz University in Germany, had shown that genetic approaches could be successfully applied to this organism (e.g. Müller, 1964; Müller and Eichenberger, 1997). Since 2004, a broad range of tools has been developed for *Ectocarpus* (Coelho and Cock, 2020; Coelho et al., 2020) including notably a high quality genome assembly (Cock et al., 2010; Cormier et al., 2017). These tools are currently being employed to investigate the genetic basis of developmental processes with the objective of comparing developmental programs in brown algae with those of other eukaryotic lineages that have evolved complex multicellularity.

Analysis of the *Ectocarpus* genome sequence, which was the first macroalgal genome to be described (Cock et al., 2010), allowed the identification of a number of genomic features that may be related to the evolution of multicellularity in the brown algal lineage. These included

structural features, such as the presence of a considerable amount of repeated sequence and large numbers of introns, but also the identification of genes that may be important for the regulation of developmental processes, such as transcription factors, ion channels and microRNAs (Cock et al., 2010; Cock and Collén, 2015; Tarver et al., 2015). Interestingly, genome analysis revealed that the brown algae independently evolved membrane-localised receptor kinases, a feature they share with two other complex multicellular lineages: animals and land plants (Cock et al., 2010). An additional four complete brown algal genome sequences have been reported since the publication of the *Ectocarpus* genome, allowing these analyses to be extended to other brown algal species (Table 15.2).

Table 15.2. Brown algal genome assemblies.

Species	Multicellular or unicellular	Genome size (Mbp)	Repeat sequences	Reference
Ectocarpus sp.	Multicellular	214	22.7%	(Cormier et al., 2017)
Cladosiphon okamuranus	Multicellular	140	4.1%	(Nishitsuji et al., 2016)
Nemacystus decipiens	Multicellular	154	8.8%	(Nishitsuji et al., 2019)
Saccharina japonica	Multicellular	537	40%	(Ye et al., 2015)
Undaria pinnatifida	Multicellular	511	34.2%	(Shan et al., 2020)

Whilst comparative genomics can provide important information about genes potentially linked to the transition to multicellularity, this approach has the weakness that it relies on predicting gene function based on sequence homology and, therefore, that it only allows the detection of features that are shared with the reference organisms that are used for the comparisons. A major recent advance in the brown algal field has been the development of forward genetic approaches, including positional cloning and cloning-by-sequencing, for *Ectocarpus* (Godfroy et al., 2017; Macaisne et al., 2017), which allow the identification of genes that play important roles in developmental processes based solely on mutant phenotypes.

The first brown algal developmental gene to be identified by a genetic approach was *IMMEDIATE UPRIGHT* (*IMM*; Macaisne et al., 2017). This gene is particularly interesting because it is a member of a large gene family that is completely absent from animal and land plant genomes and therefore corresponds to the type of gene that would not be identified by comparative genomic approaches. Mutation of *IMM* leads to a change in the morphology of the basal system of the sporophyte generation. *Ectocarpus* has a haploid diploid life cycle that involves alternation between a diploid sporophyte generation and haploid, male and female (dioicous) gametophytes (Figure 15.4). Both generations are multicellular, filamentous organisms and are of similar size but with distinct morphologies. In *imm* mutants, the normally extensive basal system of the sporophyte is replaced by a structure that resembles the gametophyte basal system, i.e. a small rhizoid-like structure. The IMM protein does not contain any known protein domains but includes a motif that resembles an atypical zinc

finger, suggesting a possible role in signalling. Mutations in a second gene, *DISTAG (DIS)*, also affect the development of the basal system, but in this mutant both the sporophyte and the gametophyte generation are affected and both completely lose their basal systems (Godfroy *et al.*, 2017). *DIS* encodes TBCCd1, a protein that has been implicated in cytoskeleton function and cellular architecture in diverse eukaryotic species (Andre et al., 2013; Feldman and Marshall, 2009; Goncalves et al., 2010). Interestingly, the existence of the sporophyte and gametophyte generations means that *Ectocarpus* has had to evolve developmental programs to construct two different multicellular body plans. Based on the phenotypes of the *imm* and *dis* mutants, this process appears to have involved both sharing of developmental components by the two generations (such as the *DIS* gene) and evolution of developmental components specific to each generation (such as the *IMM* gene).

Land plants also have haploid-diploid life cycles, and the deployment of the sporophyte generation has been shown to be under the control of a pair of TALE HD TFs (Horst et al., 2016; Sakakibara et al., 2013), orthologues of which also control the deployment of the diploid phase of the life cycle in unicellular green algae (Lee et al., 2008). Remarkably, deployment of the sporophyte developmental program in *Ectocarpus* has also been shown to be under the control of a pair of TALE HD TFs (called OUROBOROS and SAMSARA; Arun et al., 2019). Based on these observations, together with studies implicating HD TFs in life cycle regulation in other eukaryotic lineages (Hedgethorne et al., 2017; Nasmyth and Shore, 1987), we have suggested that these proteins are all derived from an ancient life cycle regulatory system that has been independently exapted to act as a developmental regulator in at least two complex multicellular lineages, the land plants and the brown algae. This is a compelling example of a phenomenon referred to as latent homology (Merényi et al., 2020; Nagy et al., 2014) in which an evolutionary process, in this case the emergence of complex multicellularity, is constrained by the shared, ancestral genetic tool kit, leading to convergent evolution of similar regulatory systems.

Although only a limited number of brown algal developmental genes have been characterised so far, these analyses have already provided several interesting insights into the evolution of developmental processes and multicellularity, including the identification of evolutionary novelties, convergent evolution constrained by an ancestral tool kit and differences between the developmental programs of body plans deployed at different generations of the life cycle. Ongoing efforts to use forward genetic approaches, coupled with complementary methodologies such as transcriptomics and network analysis, to identify additional key regulatory genes are expected to significantly expand our knowledge of brown algal regulatory gene networks in the coming years. Additional approaches of interest include recent studies aimed at characterising chromatin modifications in brown algae (Bourdareau et al., 2020; Fan et al., 2020; Gueno et al., 2020), as epigenetic processes presumably play important roles in establishing and maintaining the differentiated cell states that underlie multicellular development.

15.5 Conclusion

The algae played a prominent role in the emergence of multicellularity over the course of evolution. Three of the five transitions to complex multicellularity involved algal lineages (the red, green and brown algae) and multiple additional algal taxa underwent transitions from unicellularity to simple forms of multicellularity. They also include the first eukaryotic lineage to undergo the transition to complex multicellularity, the red algae. As a consequence, algae potentially represent highly interesting model systems to understand many aspects of the evolution of multicellularity, including the two key transitions from a unicellular state to

multicellularity and from simple to complex multicellularity. However, with the exception of long-standing model systems within the Volvocales (Matt and Umen, 2016), algae have remained a relatively underexploited resource, mainly due to a dearth of genomic information and a lack of effective genetic tools to explore gene function. This situation is changing as an increasing number of whole genome sequences are being made available for a broad range of algal species and with the emergence of model species associated with powerful tools for forward and reverse genetic approaches (Brodie et al., 2017; Coelho and Cock, 2020). This process is expected to accelerate in the coming years with the completion of large-scale genome sequencing projects and adaptation of new tools such as CRISPR-Cas9 to algal systems (Nymark et al., 2016; Shin et al., 2016). With these advances, we expect algae to continue to make important contributions to multicellularity research.

Acknowledgements

We thank Luna Van der Loos and Xiaojie Liu for Ulva spp. photos.

S.M.C. is supported by the ERC (grant 638240) and MC by the ANR (ANR-19-CE20-0028-01).

References

Andre J, Harrison S, Towers K, Qi X, Vaughan S, McKean PG, Ginger ML. 2013. The tubulin cofactor C family member TBCCD1 orchestrates cytoskeletal filament formation. *J Cell Sci* **126**:5350–5356. doi:10.1242/jcs.136515

Arun A, Coelho SM, Peters AF, Bourdareau S, Pérès L, Scornet D, Strittmatter M, Lipinska AP, Yao H, Godfroy O, Montecinos GJ, Avia K, Macaisne N, Troadec C, Bendahmane A, Cock JM. 2019. Convergent recruitment of TALE homeodomain life cycle regulators to direct sporophyte development in land plants and brown algae. *eLife* **8**:e43101. doi:10.7554/eLife.43101

Bengtson S, Sallstedt T, Belivanova V, Whitehouse M. 2017. Three-dimensional preservation of cellular and subcellular structures suggests 1.6 billion-year-old crown-group red algae. *PLoS Biol* **15**:e2000735. doi:10.1371/journal.pbio.2000735

Berger F, Taylor A, Brownlee C. 1994. Cell Fate Determination by the Cell Wall in Early *Fucus* Development. *Science* **263**:1421–1423.

Bhattacharya D, Price DC, Chan CX, Qiu H, Rose N, Ball S, Weber APM, Arias MC, Henrissat B, Coutinho PM, Krishnan A, Zäuner S, Morath S, Hilliou F, Egizi A, Perrineau M-M, Yoon HS. 2013. Genome of the red alga *Porphyridium purpureum*. *Nat Commun* **4**:1941. doi:10.1038/ncomms2931

Bhattacharya D, Yoon H, Hedges S, Hackett J. 2009. Eukaryotes In: Hedges S, Kumar S, editors. The Timetree of Life. New York: Oxford University Press. pp. 116–120.

Bodył A. 2018. Did some red alga-derived plastids evolve via kleptoplastidy? A hypothesis. *Biol Rev* **93**:201–222. doi:10.1111/brv.12340

Bogaert KA, Beeckman T, De Clerck O. 2017. Two-step cell polarization in algal zygotes. *Nat Plants* **3**:16221. doi:10.1038/nplants.2016.221

Bogaert KA, Blommaert L, Ljung K, Beeckman T, De Clerck O. 2019. Auxin Function in the Brown Alga *Dictyota dichotoma*. *Plant Physiol* **179**:280–299. doi:10.1104/pp.18.01041

Bogaert KA, Delva S, De Clerck O. 2020. Concise review of the genus *Dictyota* J.V. Lamouroux. *J Appl Phycol* **32**:1521–1543. doi:10.1007/s10811-020-02121-4

Bouget FY, Berger F, Brownlee C. 1998. Position dependent control of cell fate in the *Fucus* embryo: role of intercellular communication. *Development* **125**:1999–2008.

Bourdareau S, Tirichine L, Lombard B, Loew D, Scornet D, Wu Y, Coelho SM, Cock JM. 2020. Histone modifications during the life cycle of the brown alga *Ectocarpus*. *Genome Biol* **in press**.

Brawley SH, Blouin NA, Ficko-Blean E, Wheeler GL, Lohr M, Goodson HV, Jenkins JW, Blaby-Haas CE, Helliwell KE, Chan CX, Marriage TN, Bhattacharya D, Klein AS, Badis Y, Brodie J, Cao Y, Collén J, Dittami SM, Gachon CMM, Green BR, Karpowicz SJ, Kim JW, Kudahl UJ, Lin S, Michel G, Mittag M, Olson BJSC, Pangilinan JL, Peng Y, Qiu H, Shu S, Singer JT, Smith AG, Sprecher BN, Wagner V, Wang W, Wang Z-Y, Yan J, Yarish C, Zäuner-Riek S, Zhuang Y, Zou Y, Lindquist EA, Grimwood J, Barry KW, Rokhsar DS, Schmutz J, Stiller JW, Grossman AR, Prochnik SE. 2017. Insights into the red algae and eukaryotic evolution from the genome of *Porphyra umbilicalis* (Bangiophyceae, Rhodophyta). *Proc Natl Acad Sci* **114**:E6361–E6370. doi:10.1073/pnas.1703088114

Bringloe TT, Starko S, Wade RM, Vieira C, Kawai H, Clerck OD, Cock JM, Coelho SM, Destombe C, Valero M, Neiva J, Pearson GA, Faugeron S, Serrão EA, Verbruggen H. 2020. Phylogeny and Evolution of the Brown Algae. *Crit Rev Plant Sci* **39**:281–321. doi:10.1080/07352689.2020.1787679

Brodie J, Chan CX, De Clerck O, Cock JM, Coelho SM, Gachon C, Grossman AR, Mock T, Raven JA, Smith AG, Yoon HS, Bhattacharya D. 2017. The Algal Revolution. *Trends Plant Sci* **22**:726–738. doi:10.1016/j.tplants.2017.05.005

Brownlee C, Bouget FY. 1998. Polarity determination in *Fucus*: from zygote to multicellular embryo. *Semin Cell Dev Biol* **9**:179–85. doi:10.1006/scdb.1997.0212

Brownlee C, Bouget FY, Corellou F. 2001. Choosing sides: establishment of polarity in zygotes of fucoid algae. *Semin Cell Dev Biol* **12**:345–51. doi:10.1006/scdb.2001.0262

Burki F, Roger AJ, Brown MW, Simpson AGB. 2020. The New Tree of Eukaryotes. *Trends Ecol Evol* **35**:43–55. doi:10.1016/j.tree.2019.08.008

Butterfield NJ. 2000. *Bangiomorpha pubescens* n. gen., n. sp.: Implications for the evolution of sex, multicellularity, and the Mesoproterozoic/Neoproterozoic radiation of eukaryotes. *Paleobiol* **26**:386–404.

Cao M, Xu K, Yu X, Bi G, Liu Y, Kong F, Sun P, Tang X, Du G, Ge Y, Wang D, Mao Y. 2020. A chromosome-level genome assembly of *Pyropia haitanensis* (Bangiales, Rhodophyta). *Mol Ecol Resour* **20**:216–227. doi:10.1111/1755-0998.13102

Chan CX, Yang EC, Banerjee T, Yoon HS, Martone PT, Estevez JM, Bhattacharya D. 2011. Red and green algal monophyly and extensive gene sharing found in a rich repertoire of red algal genes. *Curr Biol* **21**:328–33. doi:10.1016/j.cub.2011.01.037

Charrier B, Le Bail A, de Reviers B. 2012. Plant Proteus: brown algal morphological plasticity and underlying developmental mechanisms. *Trends Plant Sci* **17**:468–77. doi:10.1016/j.tplants.2012.03.003

Cock JM, Collén J. 2015. Independent emergence of complex multicellularity in the brown and red algae In: Ruiz-Trillo I, Nedelcu AM, editors. Evolutionary Transitions to Multicellular Life, Advances in Marine Genomics. Springer Verlag. pp. 335–361.

Cock JM, Sterck L, Rouzé P, Scornet D, Allen AE, Amoutzias G, Anthouard V, Artiguenave F, Aury J, Badger J, Beszteri B, Billiau K, Bonnet E, Bothwell J, Bowler C, Boyen C, Brownlee C, Carrano C, Charrier B, Cho G, Coelho S, Collén J, Corre E, Da Silva C, Delage L, Delaroque N, Dittami S, Doulbeau S, Elias M, Farnham G, Gachon C, Gschloessl B, Heesch S, Jabbari K, Jubin C, Kawai H, Kimura K, Kloareg B, Küpper F, Lang D, Le Bail A, Leblanc C, Lerouge P, Lohr M, Lopez P, Martens C, Maumus F, Michel G, Miranda-Saavedra D, Morales J, Moreau H, Motomura T, Nagasato C, Napoli C, Nelson D, Nyvall-Collén P, Peters A, Pommier C, Potin P, Poulain J, Quesneville H, Read B, Rensing S, Ritter A, Rousvoal S, Samanta M, Samson G, Schroeder D, Ségurens B, Strittmatter M, Tonon T, Tregear J, Valentin K, von Dassow P, Yamagishi T, Van de Peer Y, Wincker P. 2010. The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature* **465**:617–621. doi:10.1038/nature09016

Coelho S, Cock J. 2020. Brown algal model organisms. *Ann Rev Genet* **54**:71–92.

Coelho SM, Peters AF, Müller D, Cock JM. 2020. *Ectocarpus*: an evo-devo model for the brown algae. *EvoDevo* **11**:19. doi:10.1186/s13227-020-00164-9

Collén J, Porcel B, Carré W, Ball SG, Chaparro C, Tonon T, Barbeyron T, Michel G, Noel B, Valentin K, Elias M, Artiguenave F, Arun A, Aury JM, Barbosa-Neto JF, Bothwell JH, Bouget FY, Brillet L, Cabello-Hurtado F, Capella-Gutiérrez S, Charrier B, Cladière L, Cock JM, Coelho SM, Colleoni C, Czjzek M, Da Silva C, Delage L, Denoeud F, Deschamps P, Dittami SM, Gabaldón T, Gachon CM, Groisillier A, Hervé C, Jabbari K, Katinka M, Kloareg B, Kowalczyk N, Labadie K, Leblanc C, Lopez PJ, McLachlan DH, Meslet-Cladiere L, Moustafa A, Nehr Z, Nyvall Collén P, Panaud O, Partensky F, Poulain J, Rensing SA, Rousvoal S, Samson G, Symeonidi A, Weissenbach J, Zambounis A, Wincker P, Boyen C. 2013. Genome structure and metabolic features in the red seaweed *Chondrus crispus* shed light on evolution of the Archaeplastida. *Proc Natl Acad Sci U A* **110**:5247–52. doi:10.1073/pnas.1221259110

Cormier A, Avia K, Sterck L, Derrien T, Wucher V, Andres G, Monsoor M, Godfroy O, Lipinska A, Perrineau M-M, Van De Peer Y, Hitte C, Corre E, Coelho SM, Cock JM. 2017. Re-annotation, improved large-scale assembly and establishment of a catalogue of noncoding loci for the genome of the model brown alga *Ectocarpus*. *New Phytol* **214**:219–232. doi:10.1111/nph.14321

De Clerck O, Bogaert KA, Leliaert F. 2012. Chapter Two - Diversity and Evolution of Algae: Primary Endosymbiosis In: Piganeau G, editor. Advances in Botanical Research, Genomic Insights into the Biology of Algae. Academic Press. pp. 55–86. doi:10.1016/B978-0-12-391499-6.00002-5

De Clerck O, Kao S-M, Bogaert KA, Blomme J, Foflonker F, Kwantes M, Vancaester E, Vanderstraeten L, Aydogdu E, Boesger J, Califano G, Charrier B, Clewes R, Del Cortona A, D'Hondt S, Fernandez-Pozo N, Gachon CM, Hanikenne M, Lattermann L, Leliaert F, Liu X, Maggs CA, Popper ZA, Raven JA, Van Bel M, Wilhelmsson PKI, Bhattacharya D, Coates JC, Rensing SA, Van Der Straeten D, Vardi A, Sterck L, Vandepoele K, Van de Peer Y, Wichard T, Bothwell JH. 2018. Insights into the Evolution of Multicellularity from the Sea Lettuce Genome. *Curr Biol CB* **28**:2921-2933.e5. doi:10.1016/j.cub.2018.08.015

De Smet I, Beeckman T. 2011. Asymmetric cell division in land plants and algae: the driving force for differentiation. *Nat Rev Mol Cell Biol* **12**:177–88. doi:10.1038/nrm3064

Duncan L, Nishii I, Harryman A, Buckley S, Howard A, Friedman NR, Miller SM. 2007. The VARL gene family and the evolutionary origins of the master cell-type regulatory gene, regA, in *Volvox carteri*. *J Mol Evol* **65**:1–11. doi:10.1007/s00239-006-0225-5

Egan S, Harder T, Burke C, Steinberg P, Kjelleberg S, Thomas T. 2013. The seaweed holobiont: understanding seaweed-bacteria interactions. *FEMS Microbiol Rev* **37**:462–476. doi:10.1111/1574-6976.12011

Fan X, Han W, Teng L, Jiang P, Zhang X, Xu D, Li C, Pellegrini M, Wu C, Wang Y, Kaczurowski MJS, Lin X, Tirichine L, Mock T, Ye N. 2020. Single-base methylome profiling of the giant kelp *Saccharina japonica* reveals significant differences in DNA methylation to microalgae and plants. *New Phytol* **225**:234–249. doi:10.1111/nph.16125

Fang S-C, de los Reyes C, Umen JG. 2006. Cell size checkpoint control by the retinoblastoma tumor suppressor pathway. *PLoS Genet* **2**:e167. doi:10.1371/journal.pgen.0020167

Featherston J, Arakaki Y, Hanschen ER, Ferris PJ, Michod RE, Olson BJSC, Nozaki H, Durand PM. 2018. The 4-Celled *Tetrabaena socialis* Nuclear Genome Reveals the Essential Components for Genetic Control of Cell Number at the Origin of Multicellularity in the Volvocine Lineage. *Mol Biol Evol* **35**:855–870. doi:10.1093/molbev/msx332

Feldman JL, Marshall WF. 2009. ASQ2 encodes a TBCC-like protein required for mother-daughter centriole linkage and mitotic spindle orientation. *Curr Biol* **19**:1238–1243. doi:10.1016/j.cub.2009.05.071

Godfroy O, Uji T, Nagasato C, Lipinska AP, Scornet D, Peters AF, Avia K, Colin S, Mignerot L, Motomura T, Cock JM, Coelho SM. 2017. DISTAG/TBCCd1 Is Required for Basal Cell Fate Determination in *Ectocarpus*. *Plant Cell* **29**:3102–3122. doi:10.1105/tpc.17.00440

Goecke F, Labes A, Wiese J, Imhoff JF. 2010. Chemical interactions between marine macroalgae and bacteria. *Mar Ecol Prog Ser* **409**:267–299. doi:10.3354/meps08607

Goncalves J, Nolasco S, Nascimento R, Lopez Fanarraga M, Zabala JC, Soares H. 2010. TBCCD1, a new centrosomal protein, is required for centrosome and Golgi apparatus positioning. *EMBO Rep* **11**:194–200. doi:10.1038/embor.2010.5

Gueno J, Bourdareau S, Cossard G, Godfroy O, Lipinska A, Tirichine L, Cock JM, Coelho SM. 2020. Chromatin dynamics associated with sexual differentiation in a UV sex determination system. *bioRxiv* 2020.10.29.359190. doi:10.1101/2020.10.29.359190

Hanschen ER, Marriage TN, Ferris PJ, Hamaji T, Toyoda A, Fujiyama A, Neme R, Noguchi H, Minakuchi Y, Suzuki M, Kawai-Toyooka H, Smith DR, Sparks H, Anderson J, Bakarić R, Luria V, Karger A, Kirschner MW, Durand PM, Michod RE, Nozaki H, Olson BJSC. 2016. The *Gonium pectorale* genome demonstrates co-option of cell cycle regulation during the evolution of multicellularity. *Nat Commun* **7**:11370. doi:10.1038/ncomms11370

Hedges SB, Blair JE, Venturi ML, Shoe JL. 2004. A molecular timescale of eukaryote evolution and the rise of complex multicellular life. *BMC Evol Biol* **4**:2. doi:10.1186/1471-2148-4-2

Hedgethorne K, Eustermann S, Yang J-C, Ogden TEH, Neuhaus D, Bloomfield G. 2017. Homeodomain-like DNA binding proteins control the haploid-to-diploid transition in *Dictyostelium*. *Sci Adv* **3**:e1602937. doi:10.1126/sciadv.1602937

Heesch S, Serrano-Serrano M, Luthringer R, Peters AF, Destombe C, Cock JM, Valero M, Roze D, Salamin N, Coelho S. 2019. Evolution of life cycles and reproductive traits: insights from the brown algae. *bioRxiv* 530477. doi:10.1101/530477

Herron MD, Ratcliff WC, Boswell J, Rosenzweig F. 2018. Genetics of a *de novo* origin of undifferentiated multicellularity. *R Soc Open Sci* **5**:180912. doi:10.1098/rsos.180912

Ho C-L, Lee W-K, Lim E-L. 2018. Unraveling the nuclear and chloroplast genomes of an agar producing red macroalga, *Gracilaria changii* (Rhodophyta, Gracilariales). *Genomics* **110**:124–133. doi:10.1016/j.ygeno.2017.09.003

Horst NA, Katz A, Pereman I, Decker EL, Ohad N, Reski R. 2016. A single homeobox gene triggers phase transition, embryogenesis and asexual reproduction. *Nat Plants* **2**:15209. doi:10.1038/nplants.2015.209

Jacobs WP. 1970. Development and regeneration of the algal giant coenocyte *Caulerpa*. *N Acad Sci Ann*.

Kawai H, Hanyuda T, Draisma SGA, Wilce RT, Andersen RA. 2015. Molecular phylogeny of two unusual brown algae, *Phaeostrophion irregulare* and *Platysiphon glacialis*, proposal of the Stschapoviales ord. nov. and Platysiphonaceae fam. nov., and a re-examination of divergence times for brown algal orders. *J Phycol* **51**:918–928. doi:10.1111/jpy.12332

Keeling PJ. 2013. The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. *Annu Rev Plant Biol* **64**:583–607. doi:10.1146/annurev-arplant-050312-120144

Kirk D. 1998. Volvox: Molecular-Genetic Origins of Multicellularity and Cellular Differentiation. Cambridge, UK: Cambridge University Press.

Kirk DL. 2005. A twelve-step program for evolving multicellularity and a division of labor. *BioEssays News Rev Mol Cell Dev Biol* **27**:299–310. doi:10.1002/bies.20197

Kirk DL. 2001. Germ-soma differentiation in volvox. *Dev Biol* **238**:213–223. doi:10.1006/dbio.2001.0402

Kirk MM, Ransick A, McRae SE, Kirk DL. 1993. The relationship between cell size and cell fate in *Volvox carteri*. *J Cell Biol* **123**:191–208. doi:10.1083/jcb.123.1.191

Kirk MM, Stark K, Miller SM, Müller W, Taillon BE, Gruber H, Schmitt R, Kirk DL. 1999. *regA*, a *Volvox* gene that plays a central role in germ-soma differentiation, encodes a novel regulatory protein. *Dev Camb Engl* **126**:639–647.

Knoll AH. 2011. The Multiple Origins of Complex Multicellularity. *Annu Rev Earth Planet Sci* **39**:217–239.

Lee J, Yang EC, Graf L, Yang JH, Qiu H, Zelzion U, Chan CX, Stephens TG, Weber APM, Boo GH, Boo SM, Kim KM, Shin Y, Jung M, Lee SJ, Yim H-S, Lee J-H, Bhattacharya D, Yoon HS. 2018. Analysis of the Draft Genome of the Red Seaweed *Gracilariopsis chorda* Provides Insights into Genome Size Evolution in Rhodophyta. *Mol Biol Evol* **35**:1869–1886. doi:10.1093/molbev/msy081

Lee JH, Lin H, Joo S, Goodenough U. 2008. Early sexual origins of homeoprotein heterodimerization and evolution of the plant KNOX/BELL family. *Cell* **133**:829–840. doi:10.1016/j.cell.2008.04.028

Leliaert F, Smith DR, Moreau H, Herron MD, Verbruggen H, Delwiche CF, De Clerck O. 2012. Phylogeny and Molecular Evolution of the Green Algae. *Crit Rev Plant Sci* **31**:1–46. doi:10.1080/07352689.2011.615705

Lewis LA, McCourt RM. 2004. Green algae and the origin of land plants. *Am J Bot* **91**:1535–1556. doi:10.3732/ajb.91.10.1535

Lynch M, Conery JS. 2003. The origins of genome complexity. *Science* **302**:1401–4. doi:10.1126/science.1089370

Macaisne N, Liu F, Scornet D, Peters AF, Lipinska A, Perrineau M-M, Henry A, Strittmatter M, Coelho SM, Cock JM. 2017. The *Ectocarpus IMMEDIATE UPRIGHT* gene encodes a member of a novel family of cysteine-rich proteins with an unusual distribution across the eukaryotes. *Development* **144**:409–418. doi:10.1242/dev.141523

Marin B, Nowack EC, Melkonian M. 2005. A plastid in the making: evidence for a second primary endosymbiosis. *Protist* **156**:425–32. doi:10.1016/j.protis.2005.09.001

Matsuzaki M, Misumi O, Shin-I T, Maruyama S, Takahara M, Miyagishima SY, Mori T, Nishida K, Yagisawa F, Yoshida Y, Nishimura Y, Nakao S, Kobayashi T, Momoyama Y, Higashiyama T, Minoda A, Sano M, Nomoto H, Oishi K, Hayashi H, Ohta F, Nishizaka S, Haga S, Miura S, Morishita T, Kabeya Y, Terasawa K, Suzuki Y, Ishii Y, Asakawa S, Takano H, Ohta N, Kuroiwa H, Tanaka K, Shimizu N, Sugano S, Sato N, Nozaki H, Ogasawara N, Kohara Y, Kuroiwa T. 2004. Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. *Nature* **428**:653–7. doi:10.1038/nature02398

Matt G, Umen J. 2016. *Volvox*: A simple algal model for embryogenesis, morphogenesis and cellular differentiation. *Dev Biol*. doi:10.1016/j.ydbio.2016.07.014

Matt GY, Umen JG. 2018. Cell-Type Transcriptomes of the Multicellular Green Alga *Volvox carteri* Yield Insights into the Evolutionary Origins of Germ and Somatic Differentiation Programs. *G3 Bethesda Md* **8**:531–550. doi:10.1534/g3.117.300253

Mattox K, Stewart K. 1984. Classification of the green algae: a concept based on comparative cytology In: Irvine D, John D, editors. The Systematics of Green Algae. London: Academic Press. pp. 29–72.

Merényi Z, Prasanna AN, Wang Z, Kovács K, Hegedüs B, Bálint B, Papp B, Townsend JP, Nagy LG. 2020. Unmatched Level of Molecular Convergence among Deeply Divergent Complex Multicellular Fungi. *Mol Biol Evol* **37**:2228–2240. doi:10.1093/molbev/msaa077

Mikami K, Li C, Irie R, Hama Y. 2019. A unique life cycle transition in the red seaweed *Pyropia yezoensis* depends on apospory. *Commun Biol* **2**:299. doi:10.1038/s42003-019-0549-5

Mitman GG, Meer JP van der. 1994. Meiosis, Blade Development, and Sex Determination in *Porphyra Purpurea* (rhodophyta)1. *J Phycol* **30**:147–159. doi:10.1111/j.0022-3646.1994.00147.x

Müller DG. 1964. Life-cycle of *Ectocarpus siliculosus* from Naples, Italy. *Nature* **26**:1402.

Müller DG, Eichenberger W. 1997. Mendelian genetics in brown algae: inheritance of a lipid defect mutation and sex alleles in *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae). *Phycologia* **36**:79–81.

Nagy LG, Ohm RA, Kovács GM, Floudas D, Riley R, Gácser A, Sipiczki M, Davis JM, Doty SL, de Hoog GS, Lang BF, Spatafora JW, Martin FM, Grigoriev IV, Hibbett DS. 2014. Latent homology and convergent regulatory evolution underlies the repeated emergence of yeasts. *Nat Commun* **5**:4471. doi:10.1038/ncomms5471

Nakamura Y, Sasaki N, Kobayashi M, Ojima N, Yasuike M, Shigenobu Y, Satomi M, Fukuma Y, Shiwaku K, Tsujimoto A, Kobayashi T, Nakayama I, Ito F, Nakajima K, Sano M, Wada T, Kuhara S, Inouye K, Gojobori T, Ikeo K. 2013. The First Symbiont-Free Genome Sequence of Marine Red Alga, Susabi-nori (*Pyropia yezoensis*). *PLoS One* **8**:e57122. doi:10.1371/journal.pone.0057122

Nasmyth K, Shore D. 1987. Transcriptional regulation in the yeast life cycle. *Science* **237**:1162–1170.

Nedelcu AM. 2009. Environmentally induced responses co-opted for reproductive altruism. *Biol Lett* **5**:805–808. doi:10.1098/rsbl.2009.0334

Nedelcu AM, Michod RE. 2006. The evolutionary origin of an altruistic gene. *Mol Biol Evol* **23**:1460–1464. doi:10.1093/molbev/msl016

Nishitsuji K, Arimoto A, Higa Y, Mekaru M, Kawamitsu M, Satoh N, Shoguchi E. 2019. Draft genome of the brown alga, *Nemacystus decipiens*, Onna-1 strain: Fusion of genes involved in the sulfated fucan biosynthesis pathway. *Sci Rep* **9**:4607. doi:10.1038/s41598-019-40955-2

Nishitsuji K, Arimoto A, Iwai K, Sudo Y, Hisata K, Fujie M, Arakaki N, Kushiro T, Konishi T, Shinzato C, Satoh N, Shoguchi E. 2016. A draft genome of the brown alga, *Cladosiphon okamuranus*, S-strain: a platform for future studies of "mozuku" biology. *DNA Res* **23**:561–570. doi:10.1093/dnares/dsw039

Niwa K. 2010. Genetic analysis of artificial green and red mutants of *Porphyra yezoensis* Ueda (Bangiales, Rhodophyta). *Aquaculture* **308**:6–12. doi:10.1016/j.aquaculture.2010.08.007

Nozaki H. 1990. Ultrastructure of the extracellular matrix of *Gonium* (Volvocales, Chlorophyta). *Phycologia* **29**:1–8. doi:10.2216/i0031-8884-29-1-1.1

Nymark M, Sharma AK, Sparstad T, Bones AM, Winge P. 2016. A CRISPR/Cas9 system adapted for gene editing in marine algae. *Sci Rep* **6**:24951. doi:10.1038/srep24951

Peters AF, Marie D, Scornet D, Kloareg B, Cock JM. 2004. Proposal of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) as a model organism for brown algal genetics and genomics. *J Phycol* **40**:1079–1088.

Peters AF, Scornet D, Ratin M, Charrier B, Monnier A, Merrien Y, Corre E, Coelho SM, Cock JM. 2008. Life-cycle-generation-specific developmental processes are modified in the *immediate upright* mutant of the brown alga *Ectocarpus siliculosus*. *Development* **135**:1503–1512. doi:10.1242/dev.016303

Prochnik SE, Umen J, Nedelcu AM, Hallmann A, Miller SM, Nishii I, Ferris P, Kuo A, Mitros T, Fritz-Laylin LK, Hellsten U, Chapman J, Simakov O, Rensing SA, Terry A, Pangilinan J, Kapitonov V, Jurka J, Salamov A, Shapiro H, Schmutz J, Grimwood J, Lindquist E, Lucas S, Grigoriev IV, Schmitt R, Kirk D, Rokhsar DS. 2010. Genomic analysis of organismal complexity in the multicellular green alga *Volvox carteri*. *Science* **329**:223–226. doi:10.1126/science.1188800

Qiu H, Yoon HS, Bhattacharya D. 2016. Red Algal Phylogenomics Provides a Robust Framework for Inferring Evolution of Key Metabolic Pathways. *PLoS Curr* **8**. doi:10.1371/currents.tol.7b037376e6d84a1be34af756a4d90846

Ranjan A, Townsley BT, Ichihashi Y, Sinha NR, Chitwood DH. 2015. An intracellular transcriptomic atlas of the giant coenocyte *Caulerpa taxifolia*. *PLoS Genet* **11**:e1004900. doi:10.1371/journal.pgen.1004900

Raven JA. 1997. Miniview: Multiple origins of plasmodesmata. *Eur J Phycol* **32**:95–101. doi:10.1080/09670269710001737009

Sakakibara K, Ando S, Yip HK, Tamada Y, Hiwatashi Y, Murata T, Deguchi H, Hasebe M, Bowman JL. 2013. KNOX2 genes regulate the haploid-to-diploid morphological transition in land plants. *Science* **339**:1067–1070. doi:10.1126/science.1230082

Schönknecht G, Chen WH, Ternes CM, Barbier GG, Shrestha RP, Stanke M, Bräutigam A, Baker BJ, Banfield JF, Garavito RM, Carr K, Wilkerson C, Rensing SA, Gagneul D, Dickenson NE, Oesterhelt C, Lercher MJ, Weber AP. 2013. Gene transfer from bacteria and archaea facilitated evolution of an extremophilic eukaryote. *Science* **339**:1207–10. doi:10.1126/science.1231707

Shan T, Yuan J, Su L, Li J, Leng X, Zhang Y, Gao H, Pang S. 2020. First Genome of the Brown Alga *Undaria pinnatifida*: Chromosome-Level Assembly Using PacBio and Hi-C Technologies. *Front Genet* **11**:140. doi:10.3389/fgene.2020.00140

Shin S-E, Lim J-M, Koh HG, Kim EK, Kang NK, Jeon S, Kwon S, Shin W-S, Lee B, Hwangbo K, Kim J, Ye SH, Yun J-Y, Seo H, Oh H-M, Kim K-J, Kim J-S, Jeong W-J, Chang YK, Jeong B-R. 2016. CRISPR/Cas9-induced knockout and knock-in mutations in *Chlamydomonas reinhardtii*. *Sci Rep* **6**:27810. doi:10.1038/srep27810

Silberfeld T, Rousseau F, de Reviers B. 2014. An Updated Classification of Brown Algae (Ochrophyta, ‚Phaeophyceae). *Cryptogam Algol* **35**:117–156.

Spoerner M, Wichard T, Bachhuber T, Stratmann J, Oertel W. 2012. Growth and Thallus Morphogenesis of *Ulva mutabilis* (Chlorophyta) Depends on A Combination of Two Bacterial Species Excreting Regulatory Factors. *J Phycol* **48**:1433–1447. doi:10.1111/j.1529-8817.2012.01231.x

Stark K, Kirk DL, Schmitt R. 2001. Two enhancers and one silencer located in the introns of regA control somatic cell differentiation in *Volvox carteri*. *Genes Dev* **15**:1449–1460. doi:10.1101/gad.195101

Stratmann J, Paputsoglu G, Oertel W. 1996. Differentiation of *Ulva Mutabilis* (chlorophyta) Gametangia and Gamete Release Are Controlled by Extracellular Inhibitors1. *J Phycol* **32**:1009–1021. doi:10.1111/j.0022-3646.1996.01009.x

Tarver JE, Cormier A, Pinzón N, Taylor RS, Carré W, Strittmatter M, Seitz H, Coelho SM, Cock JM. 2015. microRNAs and the evolution of complex multicellularity: identification of a large, diverse complement of microRNAs in the brown alga *Ectocarpus*. *Nucl Acids Res* **43**:6384–6398.

Umen JG. 2014. Green algae and the origins of multicellularity in the plant kingdom. *Cold Spring Harb Perspect Biol* **6**:a016170. doi:10.1101/cshperspect.a016170

Umen JG, Goodenough UW. 2001. Control of cell division by a retinoblastoma protein homolog in *Chlamydomonas*. *Genes Dev* **15**:1652–1661. doi:10.1101/gad.892101

Umen JG, Olson BJSC. 2012. Chapter Six - Genomics of Volvocine Algae In: Piganeau G, editor. Advances in Botanical Research, Genomic Insights into the Biology of Algae. Academic Press. pp. 185–243. doi:10.1016/B978-0-12-391499-6.00006-2

van der Meer JP, Cole K, Sheath R. 1990. GeneticsBiology of the Red Algae. Cambridge University Press. pp. 103–121.

Waaland JR, Stiller JW, Cheney DP. 2004. Macroalgal Candidates for Genomics. *J Phycol* **40**:26–33. doi:10.1111/j.0022-3646.2003.03-148.x

Waaland SD. 1990. Development In: Cole K, Sheath R, editors. Biology of the Red Algae. Cambridge University Press. pp. 259–273.

Wang D, Yu X, Xu K, Bi G, Cao M, Zelzion E, Fu C, Sun P, Liu Y, Kong F, Du G, Tang X, Yang R, Wang J, Tang L, Wang L, Zhao Y, Ge Y, Zhuang Y, Mo Z, Chen Y, Gao T, Guan X, Chen R, Qu W, Sun B, Bhattacharya D, Mao Y. 2020. *Pyropia yezoensis* genome reveals diverse mechanisms of carbon acquisition in the intertidal environment. *Nat Commun* **11**:4028. doi:10.1038/s41467-020-17689-1

Wichard T, Charrier B, Mineur F, Bothwell JH, Clerck OD, Coates JC. 2015. The green seaweed *Ulva*: a model system to study morphogenesis. *Front Plant Sci* **6**:72. doi:10.3389/fpls.2015.00072

Yamazaki T, Ichihara K, Suzuki R, Oshima K, Miyamura S, Kuwano K, Toyoda A, Suzuki Y, Sugano S, Hattori M, Kawano S. 2017. Genomic structure and evolution of the mating type locus in the green seaweed *Ulva partita*. *Sci Rep* **7**:11679. doi:10.1038/s41598-017-11677-0

Ye N, Zhang X, Miao M, Fan X, Zheng Y, Xu D, Wang J, Zhou L, Wang D, Gao Y, Wang Y, Shi W, Ji P, Li D, Guan Z, Shao C, Zhuang Z, Gao Z, Qi J, Zhao F. 2015. *Saccharina* genomes provide novel insight into kelp biology. *Nat Commun* **6**:6986. doi:10.1038/ncomms7986

Zhang X, Ye N, Liang C, Mou S, Fan X, Xu J, Xu D, Zhuang Z. 2012. De novo sequencing and analysis of the *Ulva linza* transcriptome to discover putative mechanisms associated with its successful colonization of coastal ecosystems. *BMC Genomics* **13**:565. doi:10.1186/1471-2164-13-565

Zhou W, Hu Y, Sui Z, Fu F, Wang J, Chang L, Guo W, Li B. 2013. Genome survey sequencing and genetic background characterization of *Gracilariopsis lemaneiformis* (Rhodophyta) based on next-generation sequencing. *PloS One* **8**:e69909. doi:10.1371/journal.pone.0069909