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Green Algal Models for Multicellularity

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Keywords

Caulerpa, cellular differentiation, *Chara*, evolution, *Ulva*, *Volvox*

Abstract

The repeated evolution of multicellularity across the tree of life has profoundly affected the ecology and evolution of nearly all life on Earth. Many of these origins were in different groups of photosynthetic eukaryotes, or algae. Here, we review the evolution and genetics of multicellularity in several groups of green algae, which include the closest relatives of land plants. These include millimeter-scale, motile spheroids of up to 50,000 cells in the volvocine algae; decimeter-scale seaweeds in the genus *Ulva* (sea lettuce); and very plantlike, meter-scale freshwater algae in the genus *Chara* (stoneworts). We also describe algae in the genus *Caulerpa*, which are giant, multinucleate, morphologically complex single cells. In each case, we review the life cycle, phylogeny, and genetics of traits relevant to the evolution of multicellularity, and genetic and genomic resources available for the group in question. Finally, we suggest routes toward developing these groups as model organisms for the evolution of multicellularity.

Major transitions:

pivotal events in evolution that resulted in shifts in the units of reproduction and/or the way information is transmitted

Cellular

differentiation: the ability of genetically identical cells within a multicellular organism to differ in morphology, physiology, gene expression, and function, considered by many authors to be a requirement for true multicellularity

Plasmodesmata:

cytoplasmic channels between cells that allow transport and communication among cells

INTRODUCTION

Most life on Earth is unicellular, and this has probably always been so. Nevertheless, numerous unicellular species have evolved multicellular bodies (or thalli, coenobia, grexes, fruiting bodies, etc.), beginning at least 2.5 billion years ago (134). The evolution of multicellularity was a key innovation that led to massive radiations in several groups, such as plants, animals, fungi, and several algal clades. The ecological and evolutionary consequences of multicellularity can hardly be overstated. Many of Earth's biomes are defined by the dominant multicellular species, for example, tropical, temperate, and boreal forests; kelp forests; and coral reefs. Each of these, of course, has strongly influenced the evolution of numerous species adapted to living among the trees, kelps, and corals, which in turn has affected the predators, prey, competitors, and mutualists of those species. It has even been suggested that the evolution of multicellularity in cyanobacteria may have enhanced the Great Oxidation Event 2–2.4 billion years ago, ultimately affecting the ecology and evolution of nearly everything on Earth (134).

Aside from its effects on the biotic and abiotic environments to which organisms adapt, the evolution of multicellularity also affects subsequent evolutionary dynamics by changing what an organism is. Each time a population transitions from unicellular to multicellular life, a change occurs in the very units of evolution, from cells to multicellular organisms. For this reason, the evolution of multicellularity is often grouped with other evolutionary changes that altered the units of evolution, for example, the transitions from unitary (prokaryotic) to compound (eukaryotic) cells and from solitary insects to caste-differentiated societies or superorganisms. Because of an influential book by John Maynard Smith and Eörs Szathmáry (106), these and related events are called major transitions in evolution.

As with the other major transitions, there are important gaps in our understanding of the evolution of multicellularity. Unlike most others, though, for the evolution of multicellularity we have a decent sample size on which to test our hypotheses. The most frequently cited estimate of the number of independently evolving lineages that have evolved multicellularity is at least 25 (60). In fact, as we show here, the actual number is likely to be much larger than 25.

Although it is diverse, multicellular life does share some commonalities, referred to as dynamic patterning modules by Niklas and colleagues (11, 113). To be multicellular requires some form of cell–cell adhesion, most often mediated by the cell wall or extracellular matrix in green algae. Although aggregative multicellularity exists elsewhere (e.g., in cellular slime molds), in green algae and most other taxa, multicellular individuals are built from mitotic descendants of a single cell (either a zygote or a vegetative cell). Polarized and localized growth and cell division are also frequently found in multicellular taxa, as well as different degrees of cellular differentiation and specialization, often initiated with asymmetric cell division. As organismal size increases, additional structural and long-distance communication requirements may arise that can be met in various ways, such as cytoplasmic streaming and polarized transport through intercellular connections (e.g., plasmodesmata) or through chemical/hormonal signals. A newly emerging consideration whose prevalence remains unknown is the interaction of evolving multicellular individuals with not only their abiotic environment but also microbiota that can make essential contributions to the form and function of a multicellular individual.

Open questions about the evolution of multicellularity include everything from its philosophical implications to evolutionary processes and dynamics to specific phenotypic and genotypic changes. How, for example, does the unit of evolution shift from the single cell to the group of cells that make up an organism? What selective pressures drove the transition to multicellularity, or need the processes underlying this change have been adaptive at all? What is the relative importance of the particular selective pressures versus the nature of the unicells in determining whether and how multicellularity evolves? How do trait heritability and fitness shift from the unicellular

to the multicellular unit? What is the relative importance of novel traits versus the co-option of existing traits, and what role did phenotypic plasticity play in determining this? What specific genetic changes were involved?

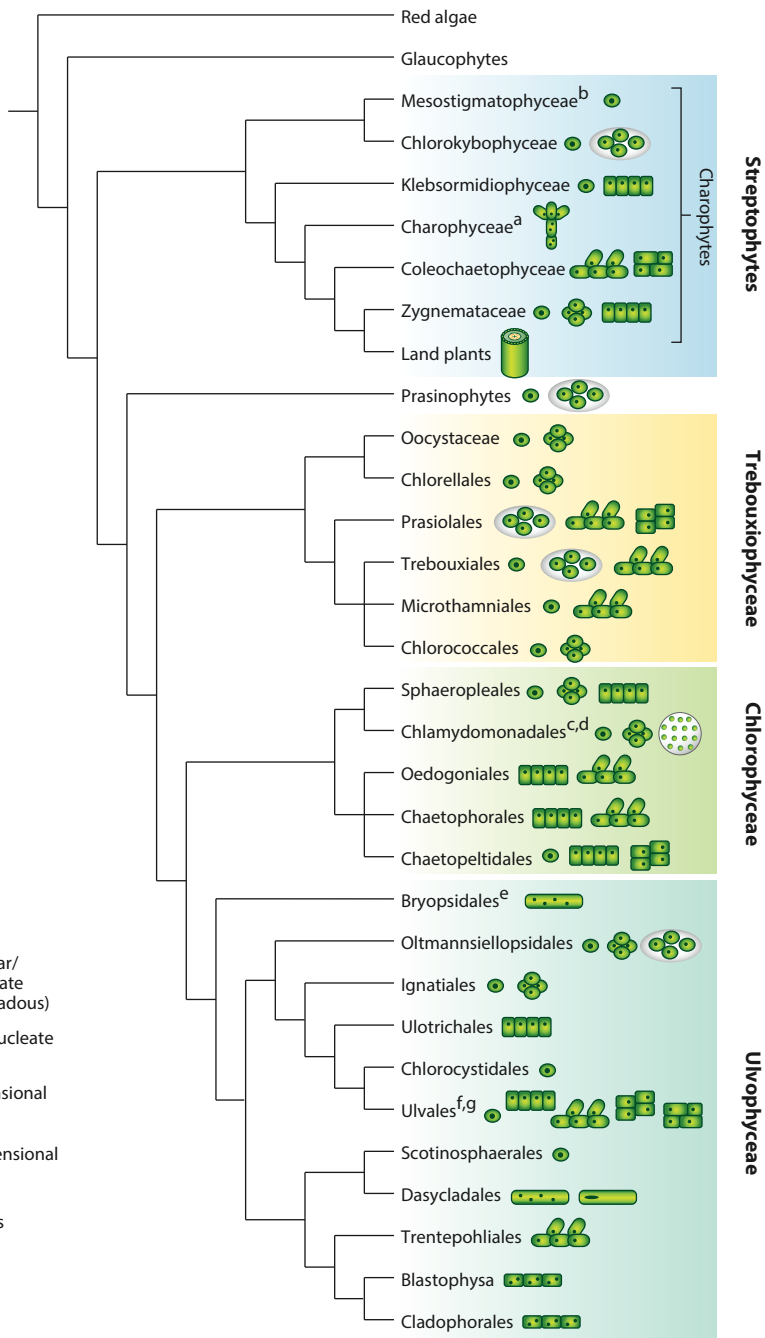
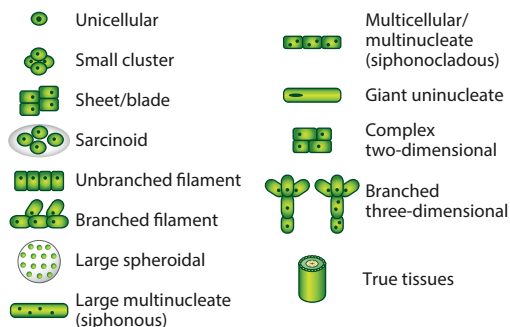
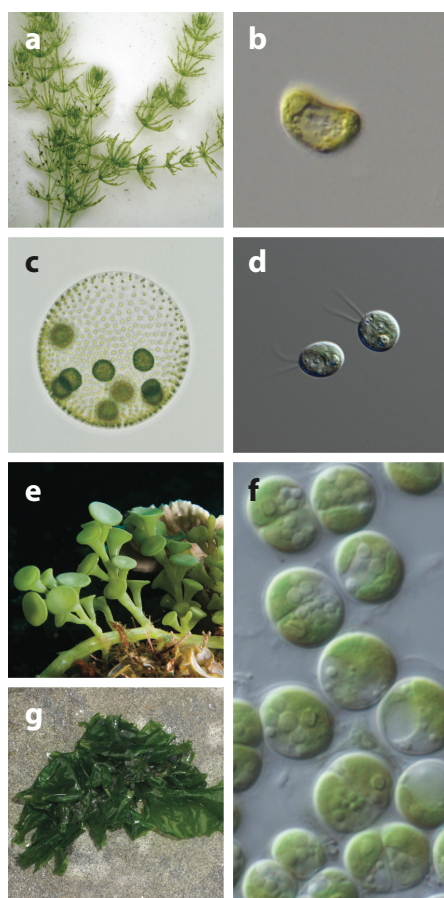
We consider some of these questions, mostly those later in the list, in the context of the green algae. Green algae are a diverse and ecologically important group of eukaryotes that include both unicellular and a wide variety of multicellular forms (**Figure 1**). Along with the red and glaucophyte algae and land plants, green algae are archaeplastidans, that is, they are direct descendants of a eukaryotic ancestor that engulfed (or otherwise came to contain) a cyanobacterium that evolved into the original chloroplast. This event is known as the primary endosymbiosis, in contrast to secondary and higher-order endosymbioses in which a eukaryotic alga was engulfed by another eukaryote and evolved into a chloroplast. The green algae are paraphyletic with respect to land plants, which together make up the Viridiplantae (**Figure 1**). Recent estimates of the number of species of green algae range from ~11,000 to ~22,000 (61, 62).

The green algae are an ideal group for exploring the evolution of multicellularity simply because they have done it so many times. The green algal crown lineages of chlorophytes, ulvophytes, and trebouxiophytes all include multicellular species descended from different unicellular ancestors (**Figure 1**), but that is only the tip of the iceberg. The number of suspected transitions to multicellular life in the Chlorophyceae alone inspired David Kirk (90, p. 22) to call them “master colony-formers,” and the other two classes likely include multiple independent origins of multicellularity as well. In fact, Grosberg & Strathmann’s (60, p. 622) estimate of “at least 25” independent origins of multicellularity might very well apply to the green algae alone.

Although multiple independent origins of multicellularity were already suspected from morphological and ultrastructural comparisons, the extent of such convergence only became clear from the increasing availability of taxon-rich phylogenetic reconstructions based on nucleic acid and amino acid sequence data. Since the late twentieth century, the rapidly decreasing cost of sequencing has facilitated the construction of increasingly larger phylogenies, phylogenies based on multiple unlinked genes, and, most recently, phylogenies based on very large numbers of genes, an approach often referred to as phylogenomics or phylotranscriptomics. The increased phylogenetic resolution allowed by such studies has often revealed complicated pictures of the evolution of multicellularity that were previously obscured by restricted taxonomic sampling (41, 95, 96, 112, 133).

Reduced sequencing costs have also brought us to the genomic era, in which large numbers of whole-genome sequences are available for most large taxonomic groups, including the green algae. A recent review identified 132 green algal genome assemblies; given the accelerating pace of sequencing, this number is sure to be outdated by the time this manuscript is published (71). Unfortunately, Hanschen & Starkenburg (71) also found that as the pace of sequencing new genomes has increased, their quality has declined. Furthermore, the taxonomic distribution of these assemblies is far from representative, with the Chlorophyceae, Trebouxiophyceae, and Mamiellophyceae overrepresented relative to the number of species in each of these classes and the Ulvophyceae and Charophyceae severely underrepresented (71).

Genomics and molecular phylogenetics have opened up new opportunities for investigating multicellularity in different algal groups. As phylogenies based on molecular sequence data became the norm in the late twentieth century, it became clear in many cases that taxonomies based on morphological characters were misled by frequent convergence. Some taxa were already suspected to be para- or polyphyletic based on detailed studies of cell morphology and ultrastructure, but as in many other taxa, molecular phylogenetics of algae revealed taxonomic problems at an unsuspected scale. However, where taxonomists see problems, evolutionary biologists see sample size. Convergent evolution provides statistical power to comparative analyses, allowing correlations to be measured between, for example, traits and environments or traits and other traits.



(Caption appears on following page)

Figure 1 (Figure appears on preceding page)

Approximate phylogeny of the Archaeplastida with representative growth forms. Phylogeny based on data from References 34, 41, 96, 135. Representative multicellular/siphonous algae discussed in the main text are pictured to the left of representatives of their closest unicellular relatives: (a) *Chara braunii*, adapted from Show_ryu, CC BY-SA 3.0 (<https://creativecommons.org/licenses/by-sa/3.0>), via Wikimedia Commons; (b) *Mesostigma viride* strain SAG 50–1, adapted from Tatyana Darienko, CC BY-SA 4.0 (<https://creativecommons.org/licenses/by-sa/4.0>); (c) *Volvox aureus*, adapted from Matthew D. Herron; (d) *Chlamydomonas reinhardtii*, adapted with permission from Deborah Shelton; (e) *Caulerpa racemosa* adapted from Nick Hobgood, CC BY-SA 3.0 (<https://creativecommons.org/licenses/by-sa/3.0>), via Wikimedia Commons; (f) *Desmochloris mollenbaueri* strain CCAP 6006, photo kindly provided by Tatyana Darienko and Thomas Pröschold; and (g) *Ulva* sp., adapted from Matthew D. Herron.

The available genome assemblies include several colonial and multicellular species (for the purposes of this paragraph, we are taking a broad view of coloniality, including some species that are described as unicellular or colonial). Within the Chlorophyceae, these include five species of Volvocaceae (order Chlamydomonadales), a similar number of Scenedesmaceae, two species of Selenastraceae, and one of Mychonastaceae (order Sphaeropleales). Three colonial species of trebouxiphytes are represented, two from the Chlorellaceae (order Chlorellales) and one from the Botryococcaceae (order Trebouxiales), as well as three species of ulvophytes, two of which are *Ulva* species (Ulvaceae, Ulvales) and one of which is a *Caulerpa* species (Bryopsidales). Four species of colonial/multicellular streptophyte algae are represented, from the classes Charophyceae (*Chara braunii*), Chlorokybophyceae (*Chlorokybus atmophyticus*), Klebsormidiophyceae (*Klebsormidium nitens*), and Zygnematophyceae (*Mesotaenium endlicherianum*) (71) (**Figure 1**).

The combination of genomics and molecular phylogenetics can be a powerful tool for understanding the evolution of multicellularity. When the genomes of a multicellular organism and its unicellular relatives are compared, we know that the differences between them must have evolved since they diverged from a common ancestor. Differences between the unicellular and multicellular genomes, especially when they can be polarized by sampling multiple taxa, are candidates for the genetic basis of multicellularity. When gene functions are known, this information can be used to narrow the focus to genes with plausible roles in multicellular development.

While genome sequences are necessary for understanding how multicellularity and other traits evolved, they are often remarkably uninformative about how complex traits such as multicellularity originated and were manifested. It is increasingly appreciated that many of the items in the genetic tool kits that were important for multicellularity were already present in simple unicellular ancestors (124, 128, 129, 131). Understanding the origins and evolution of multicellularity and other complex traits, therefore, requires a deeper analysis of how those tool kits were modified and redeployed in the descendant species. Much of the research into the origins of multicellularity focuses on a few well-developed model species as well as on animals and land plants due to their importance to humans. Our goal here is to highlight a few examples of how green algae offer additional underexploited opportunities for exploring the origins of complex multicellular or macroscopic body plans in systems that are increasingly tractable for experimental manipulation. The four genera highlighted here—*Volvox* and volvocine green algae, *Ulva*, *Caulerpa*, and *Chara*—all evolved their complex traits and body plans independently. Although these species represent just a small fraction of the diversity in the green algal universe, they each have the potential to contribute insights into important general questions about the evolution of organismal complexity and multicellularity, and the latter three deserve additional attention as they are relatively underdeveloped as models.

THE VOLVOCINE ALGAE

The volvocine green algae are a group of motile unicellular and colonial species within the Chlorophyceae. Their life cycle is haplontic, with a dormant, desiccation-resistant zygote or spore as the

Volvocaceae: the family that includes the genera *Colemanosphaera*, *Eudorina*, *Pandorina*, *Platydorina*, *Pleodorina*, *Volvox*, *Volvulina*, and *Yamagishiella*

Haplontic: a life cycle in which only the haploid phase includes a multicellular stage

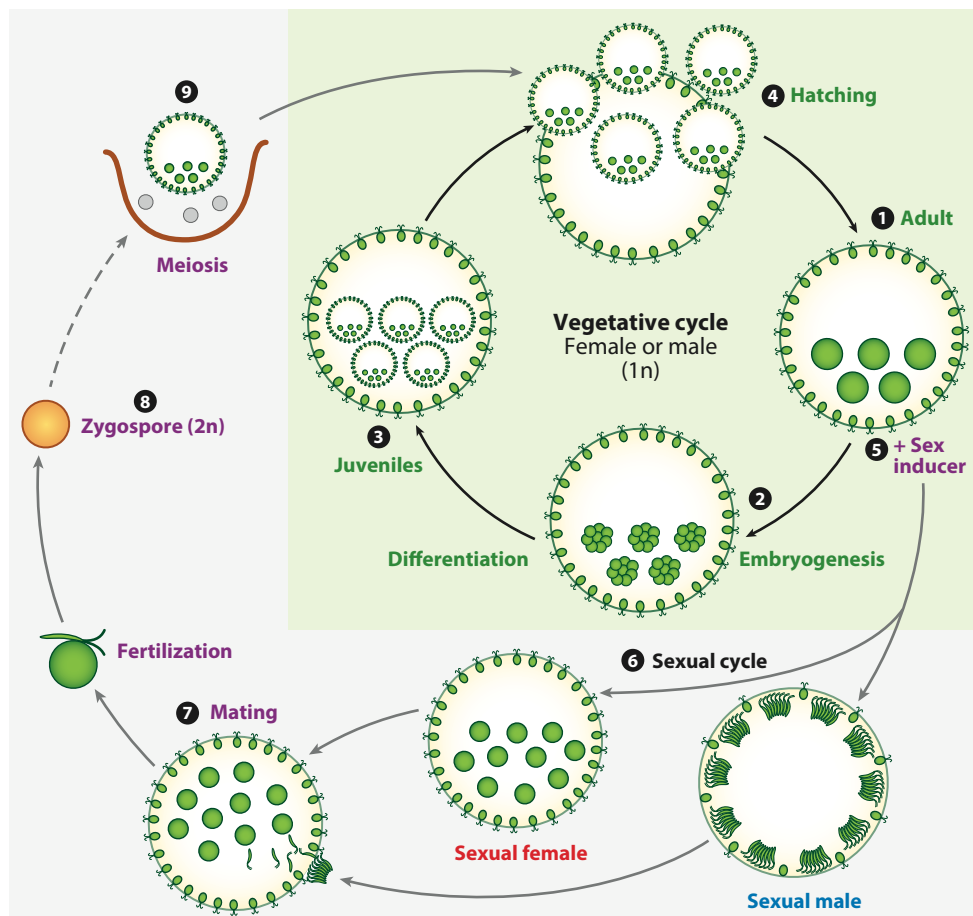


Figure 2

Volvox life cycle and development. Vegetative reproduction (*light green region*) occurs in the haploid ($1n$) phase and can be synchronized with a 16 h:8 h light:dark diurnal regime where one full reproductive cycle is completed every 48 h. Vegetative development starts with mature precleavage adults (1) and proceeds clockwise through embryogenesis (2), the cytodifferentiation of germ cells (gonidia) and somatic cells to form juveniles (3), the hatching of juveniles (4), and finally maturation to complete the cycle as the next generation of adults. After hatching, the parental somatic cells of the previous generation are discarded and undergo senescence and cell death. Sexual development (*light gray region*) is triggered by exposure to sex inducer (5) and leads to gonidia undergoing modified embryogenesis and development (not shown) into adult sexual egg-bearing females or adult sexual sperm-packet-bearing males (6). Sperm packets are released and swim to females where mating takes place with internal fertilization (7), resulting in the formation of diploid ($2n$) zygospores (8). Meiosis (*dashed gray line*) occurs upon germination and produces three polar bodies and one haploid progeny that re-enters the vegetative life cycle (9). Figure adapted from Reference 150.

Tetrabaenaceae: the family that includes the genera *Basichlamys* and *Tetrabaena*

Goniaceae: the family that includes the genera *Astrephomene* and *Gonium*

only diploid stage (Figure 2). Reproduction is normally asexual, with entry into the sexual phase triggered by various environmental stressors, such as heat shock or low nitrogen.

Volvocine is a term of convenience rather than a formal taxon, but it generally refers to three families of colonial algae—Tetrabaenaceae, Goniaceae, and Volvocaceae—and their close unicellular relatives in the genera *Chlamydomonas* and *Vitreochlamys* (Figure 3). Just which unicellular relatives are included in this group differs somewhat among authors. The Tetrabaenaceae (118) and Goniaceae (123) include small colonies of 4 and up to 64 cells, respectively. The Volvocaceae

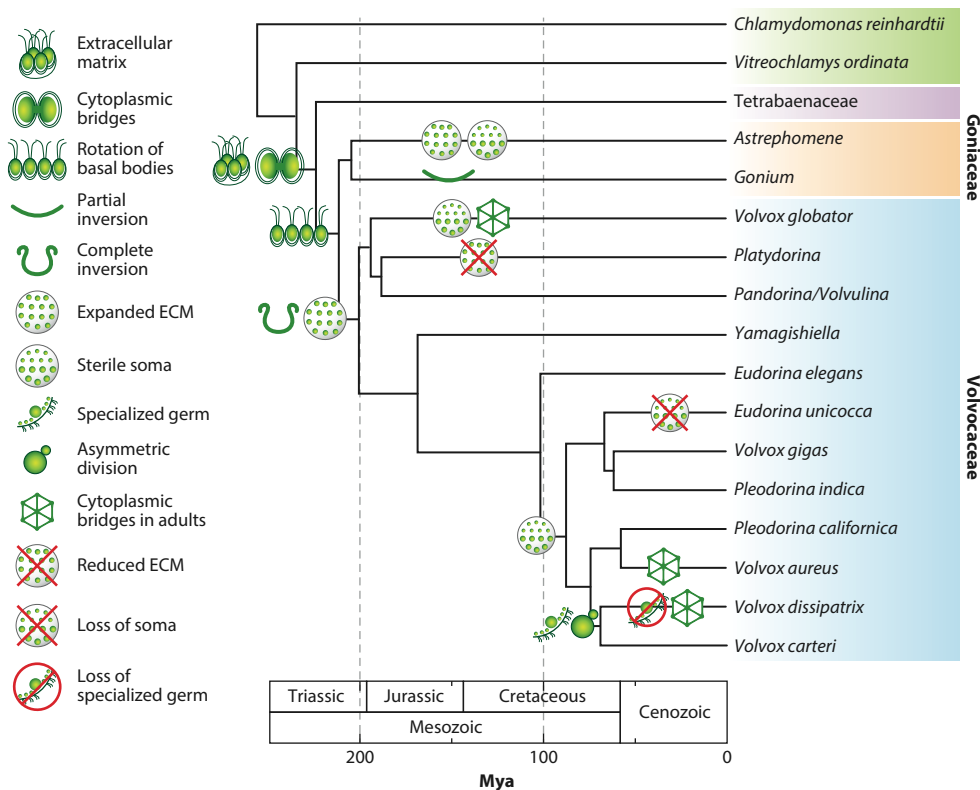


Figure 3

Approximate phylogeny (chronogram) of the volvocine algae. Phylogeny based on Reference 75. Developmental changes are those identified by Kirk (91). Extracellular matrix and cytoplasmic bridges are present in all three families of colonial/multicellular volvocine algae (Tetrabaenaceae, Goniaceae, Volvocaceae). Rotation of the basal bodies is present in Goniaceae and Volvocaceae. *Gonium* undergoes partial inversion; complete inversion is present in all members of the Volvocaceae. Expanded volume of extracellular matrix evolved independently in *Astrephomene* and in the Volvocaceae and has been lost in *Platydorina* and some members of the *Pandorina/Volvulina* clade. Sterile soma evolved independently in *Astrephomene* and at least two lineages of Volvocaceae and has been lost in *Platydorina* and some species of *Eudorina*. Specialized germ cells and asymmetric cell division evolved in the *Volvox carteri* lineage and were lost in *Volvox dissipatrix*. Retention of cytoplasmic bridges in adults evolved independently in three lineages of *Volvox*. Note that several nominal taxa are polyphyletic, including the genera *Pandorina*, *Volvulina*, *Eudorina*, *Pleodorina*, and *Volvox*, as well as some nominal species. Abbreviations: ECM, extracellular matrix; Mya, million years ago.

includes eight genera of spheroidal colonies ranging from 8 to ~50,000 cells, all of which undergo complete inversion during embryogenesis (29).

This diversity of forms makes the volvocine algae an outstanding model system for understanding the origins of multicellularity. Living volvocine species comprise a near continuum of sizes and degrees of complexity, including unicellular forms, simple colonies of 4–32 undifferentiated cells, and larger colonies with rudimentary cellular differentiation. Comparative studies have reconstructed the evolutionary history of traits relevant to the evolution of multicellularity, and most inferred ancestral states have analogs among extant species (68, 69, 74–76, 91).

Several volvocine genomes have been sequenced, starting with *Chlamydomonas reinhardtii* in 2007 (109) and some unicellular relatives of *Chlamydomonas* more recently (31). All three

Inversion: the process by which spheroidal colonies such as *Volvox* turn inside-out during development, beginning with the flagella inside and ending with the flagella outside

colonial families are now represented, including *Tetrabaena socialis* (49), *Gonium pectorale* (70), and several members of the Volvocaceae: *Volvox carteri* (124), *Yamagishiella unicocca*, and *Eudorina* sp. (65). Genome sizes are similar, ranging from 111 Mb in *C. reinhardtii* to 149 Mb in *G. pectorale* (49). There is no obvious relationship between genome size and organismal size or complexity; for example, the *V. carteri* genome (138 Mb) is similar in size to that of the 4-celled *T. socialis* (136 Mb) and smaller than that of the 8- to 16-celled *G. pectorale* (149 Mb), and it has fewer protein-coding genes than either (49).

Comparisons among sequenced genomes showed that the evolution of colony formation as such did not involve large-scale changes in the protein-coding capacity of the genome. However, some gene families did originate or expand around the time of this innovation, and among those are gene families involved in DNA repair, protein kinase activity, the extracellular matrix, and cell adhesion (49).

Changes in cell cycle regulation may have played a role in the early evolution of volvocine multicellularity, as such changes would be necessary for the multiple fission cell cycle to adapt to increasing cell numbers per colony and to more stereotyped cell division numbers compared with unicellular species where division numbers can range widely from one to five depending on growth conditions (91). The cyclin-D1-encoding gene has a single copy in *C. reinhardtii*, three in *T. socialis*, and four in *G. pectorale* and *V. carteri*, and a downstream target of D-type cyclin-dependent kinases, the volvocine retinoblastoma-related protein, experienced accelerated evolution in the colonial taxa relative to *C. reinhardtii* (49). Expression of the *G. pectorale* version of retinoblastoma in retinoblastoma-deficient *C. reinhardtii* was sufficient to cause a quasi-colonial phenotype, though the cause of this phenotype and how it related to changes in the cell cycle (if any) remain to be determined (70).

Another early step in the evolution of multicellularity was the formation of physical connections among cells, for example, by cytoplasmic bridges resulting from incomplete cytokinesis and transformation of cell wall components into a colonial boundary (91). The cytoplasmic bridges play a critical role in cell-cell adhesion early in development, but in all but a few species, they are absent in adult colonies (91). Their role in development involves a morphogenetic process known as inversion, with partial inversion (convex-to-concave shape change) occurring during *Gonium* development and complete inversion (inside-out colony reconfiguration) occurring in all Volvocaceae (52, 53, 64, 101, 139).

Many of the changes that distinguish the multicellular volvocines from their unicellular relatives were established early in their evolution, by about 200 Mya (75). Out of the 12 developmental changes identified by Kirk (91) that led from a unicellular ancestor to multicellular *V. carteri*, 8 occurred by shortly after the divergence among the three multicellular families (76).

A crucial subsequent event in the evolution of multicellular complexity is the differentiation of previously uniform cells into multiple functional types. The last 4 of Kirk's (91) 12 steps, those that took place after the divergence among the three multicellular families, all relate to cellular differentiation. The unicellular ancestor of the volvocine algae is presumed to have had, like all unicellular organisms, generalist cells capable of performing all necessary functions. This situation persists in the small multicellular taxa, such as *Tetrabaena*, *Gonium*, and *Pandorina*, and in their extant unicellular relatives. On at least three separate occasions, a subset of cells has differentiated into sterile somatic cells specialized in motility, leaving the remaining cells responsible for reproduction (76).

Experiments by Kirk and colleagues (138) showed that differential expression of the transcription factor *regA* (somatic regenerator) is necessary and sufficient to cause somatic cell differentiation in *V. carteri*. In *regA*⁻ mutants, somatic cells dedifferentiate and adopt a reproductive fate (82). *RegA* is part of a gene cluster that also includes three *regA*-like paralogs (*rlsA*, *rlsB*, *rlsC*) whose

roles are not known (47). The *regA* cluster is found in other volvocine species that evolved somatic cells independently (67), but its role in those species is unknown. Interestingly, *regA* orthologs are also present in species without soma, suggesting that *regA* in these species plays roles unrelated to somatic cell differentiation (58). However, at least in one lineage (*Astrephomene*), somatic cells are predicted to have evolved in the absence of *regA* orthologs, indicating that other genetic mechanisms for somatic differentiation exist in the volvocine lineage (58).

Two separate lineages within the polyphyletic nominal genus *Volvox* have further evolved specialized reproductive cells (gonidia) that, in addition to being much larger than the cells in undifferentiated species, do not contribute to motility (74). In *V. carteri*, *Volvox obversus*, *Volvox africanus*, and *Volvox reticuliferus*, cell fate is determined early in development through a set of asymmetric divisions in which the smaller daughter cell continues to divide, eventually producing somatic cells, and the larger daughter cell stops dividing and differentiates into a vegetative reproductive cell (43, 44, 74, 92).

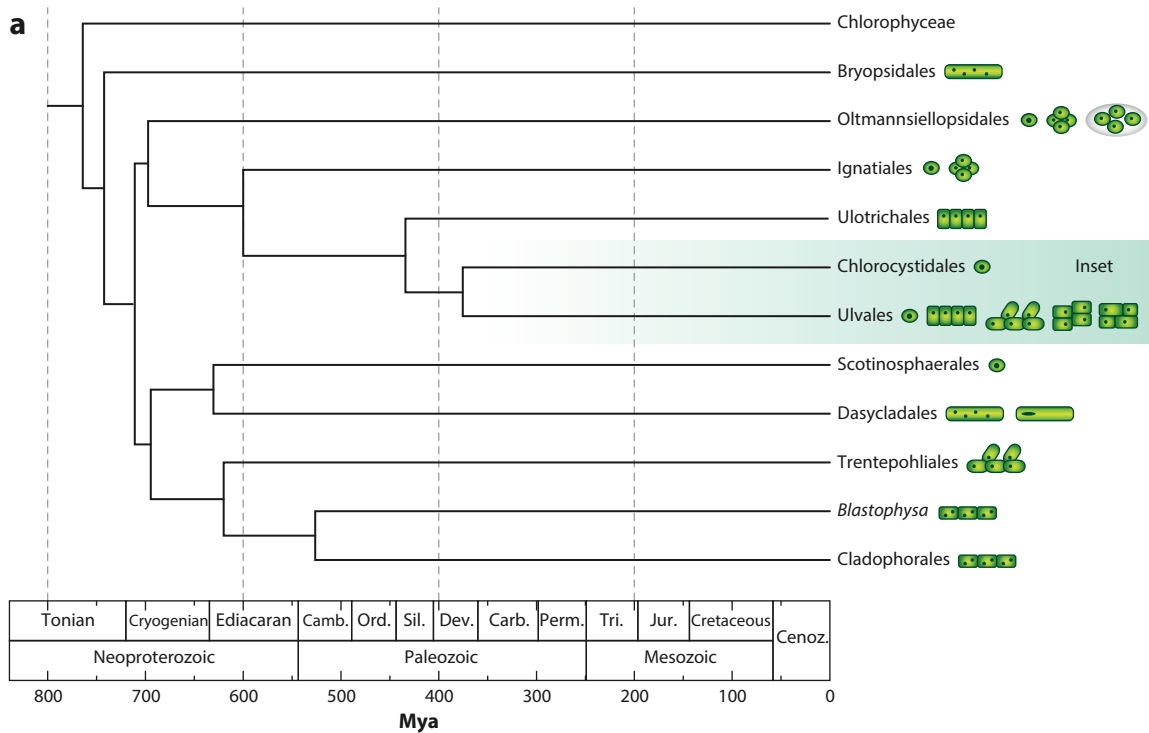
Although genes involved in several important developmental processes—somatic cell differentiation, asymmetric division, and inversion—have been characterized in the model organism within the colonial volvocine algae, *V. carteri* (104), very little is known about the genetics of these processes in other volvocine species. Still less is known about the genetic changes responsible for the early steps in the evolution of multicellularity in this group, for example, the incomplete cytokinesis that produces cytoplasmic bridges, the conversion of a cell wall into an extracellular matrix and colonial boundary, and the rotation of the basal bodies that aligns the flagella for colonial motility (91). Analyses of the origin and evolution of gene families in the available genome sequences have suggested some candidates. Of these, all but *regA* have orthologs or co-orthologs in *Chlamydomonas*. For example, changes to the retinoblastoma gene (*MAT3/RB*) and expansion of the D-type cyclin family have been suggested to have played a role in changes to the cell cycle related to the origin of multicellularity (70, 124). Defects in the *V. carteri glsA* gene cause defects in asymmetric cell division during embryogenesis. Interestingly, the predicted *glsA* gene product is a chromatin-associated protein conserved in *Chlamydomonas* and other eukaryotes (110). Likewise, the *invA* gene of *V. carteri* encodes a kinesin-related protein required for inversion, and this gene also has an ortholog in *Chlamydomonas* (115, 116). More generally, a transcriptome study of *V. carteri* cell types revealed that lineage-specific genes (those found in *Chlamydomonas* and multicellular volvocine algae, but not outside this clade) are enriched for cell-type-specific expression compared with all other genes (105). Thus, some of the roots for specialized processes found in *Volvox* first appeared in a unicellular ancestor where their functions remain unclear, but which could be further investigated in *Chlamydomonas* or other extant species that lack these innovations. Several gene families that may be associated with multicellularity and that expanded at its origins include those with inferred functions in cell adhesion, phosphorylation, and the extracellular matrix. *Chlamydomonas* is a well-established model with reverse genetic methods available for functional testing (132). Recent advances in stable nuclear transformation in several species of multicellular volvocine algae suggest that it may soon be possible to test the functions of these genes through reverse genetics (97–99, 122).

Soma: mortal body cells that do not produce offspring and die when the multicellular organism dies

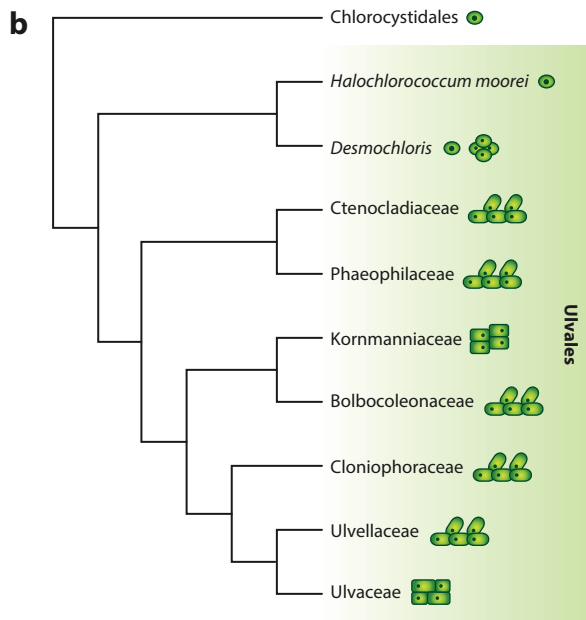
Gonidia: asexual reproductive cells that divide mitotically to produce multicellular offspring

ULVA: A MULTICELLULAR COMMUNITY ORGANIZER

The genus *Ulva* belongs to the order Ulvales, a clade of filamentous or blade-like marine algae (**Figure 4**). The closest unicellular relatives of the Ulvales are in the order Chlorocystidales (33, 34, 95, 135) (**Figure 4**). Chlorocystidales includes marine and terrestrial species with filamentous (*Ochlochaete*, *Ruthnielsenia*) and unicellular (*Chlorocystis*, *Desmochloris*, *Halochlorococcum*) morphologies (33, 120, 135); note that *Halochlorococcum* is polyphyletic, and not all described species belong



- Unicellular
- Small cluster
- Sheet/blade/tube
- Unbranched filament
- Branched filament
- Complex two-dimensional
- Large multinucleate (siphonous)
- Multicellular/multinucleate (siphonocladous)
- Giant uninucleate



(Caption appears on following page)

Figure 4 (Figure appears on preceding page)

Ulvophyceae. (a). Approximate phylogeny (chronogram) of the class Ulvophyceae with representative growth forms, adapted with permission from Reference 41 with additional information from References 34, 96, 135. (b) Approximate phylogeny (cladogram) of the order Ulvales with representative growth forms, based on data from References 34, 135. The closest unicellular relatives of the Ulvales are in the genera *Desmochloris* and *Halochlorococcum* (34). *Halochlorococcum* is polyphyletic, with *Halochlorococcum marinum* within the more distantly related Oltmannsiellopsidales (125). Abbreviations: Camb., Cambrian; Carb., Carboniferous; Cenoz., Cenozoic; Dev., Devonian; Jur., Jurassic; Mya, million years ago; Ord., Ordovician; Perm., Permian; Sil., Silurian; Tri., Triassic.

to Chlorocystidales (125). Divergences within the Ulvales extend to around 300 Mya, and their divergence from the order Ulotrichales to around 500 Mya (41). As with all molecular clock studies of ancient divergences, the confidence intervals associated with these inferences are large, so they should be treated as order-of-magnitude estimates. Nevertheless, they give some idea of the age of the origin of multicellularity within this group, which was likely prior to the earliest divergences within the Ulvales and, assuming that the most recent common ancestor of Ulvales and Chlorocystidales was unicellular, subsequent to their divergence. The genus *Ulva* has been merged with *Enteromorpha*, whose species have a branched tube-like morphology (72, 145). A partly blended tube-blade morphology is seen in some species of the *Ulva-Enteromorpha* clade, and experimental induction of both morphotypes within a clonal population (13) further supports the idea that the morphogenetic programs of the two genera are manifestations of developmental plasticity and possibly different degrees of canalization. Because *Ulva*-like species are better studied, they are the focus of this description.

Ulva has an isomorphic haplo-diplontic life cycle with diploid thalli of the sporophyte generation producing haploid unicellular parthenospores through meiosis (**Figure 5a**). The parthenospores then develop into haploid gametophytes that are similar in morphology to the sporophytes but are capable of mitotically producing unicellular isogametes of either *mt+* or *mt-* mating type. Additionally, unmated *mt+* gametes can produce either new haploid gametophytes or homozygous diploid parthenosporophytes (7, 80, 81, 155). Sequencing of *mt+* and *mt-* strains of *Ulva partita* revealed a complex pair of nonrecombining mating-type haplotypes controlling gamete differentiation, though the molecular details of mating-type determination remain to be worked out (161). The life cycle of *Ulva* from parthenospore or zygote to mature spore-forming adult takes about five weeks to complete in laboratory culture (137).

The body plan of *Ulva* is made up of three primary cell types positioned along an apical-basal axis: basal rhizoid cells, stem-forming stem cells, and apical blade cells, which proliferate the most actively and form the bulk of the thallus (**Figure 5b**). The lower portion of *Ulva* is composed of a hollow stem formed by stem cells from which filamentous rhizoid cells grow basally to produce a holdfast that can anchor the alga to a substrate. Loss of either apical or basal structures triggers stem cell proliferation and asymmetric division to reform the missing structures. Only stem cells can regenerate both of the other cell types (137). As the two-layered flat blade structures mature, they undergo differentiation starting from their apical margins, where haploid motile gametes or parthenospores are formed (155).

In plants, phytohormones control growth and morphogenesis, and in *Ulva*, there are analogous substances that regulate development. At least three types of endogenous inhibitory substances are produced by *Ulva* that control the timing and possibly the spatial distribution of reproductive development. These include two sporulation inhibitors (SIs)—a glycoprotein and a low-molecular-weight compound (142)—and an inhibitor of gamete release from the mother cells within thalli (157). Several developmental mutants have been isolated that disrupt normal morphology (15, 21, 50), including slender (*Sl*), which produces filamentous blades and a few basal rhizoid cells but lacks totipotent stem cells. The disrupted genes in *Sl* and other developmental mutants have yet to be identified.

Haplo-diplontic:
a life cycle in which both haploid and diploid phases include a multicellular stage

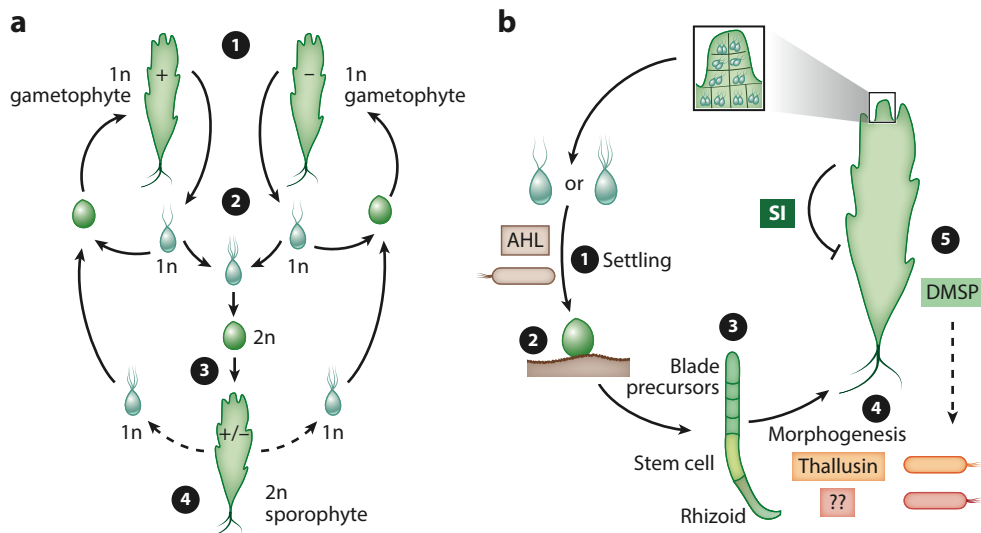


Figure 5

Life cycle and morphogenesis of *Ulva*. (a) Simplified diagram of the haplo-diplontic isomorphic life cycle of *Ulva*. The multicellular gametophytes (1) of either plus or minus mating type produce biflagellated gametes (2) that can mate to form a diploid zygote (3), which develops into a sporophyte (4), or they can settle and redevelop as gametophytes. The diploid sporophyte can produce quadriflagellated haploid meiotic zooids that settle and develop into gametophytes. (b) Microbial signaling and chemical communication in *Ulva* development. Simplified life cycle diagram showing stages where chemical and microbial interactions influence development. (1) Motile phase single-celled spores will settle to the seafloor under the influence of bacterially produced quorum-sensing acyl-homoserine lactones (AHLs) and (2) transition to the sessile multicellular phase. (3) Three main cell types (blade cells, stem cells, and rhizoids) proliferate to produce (4) the adult thallus, with morphogenesis under the control of bacterially produced thallusin and as-yet-unidentified signals. (5) Dimethylsulfoniopropionate (DMSP) and other metabolites produced by *Ulva* may supply organic nutrients to associated bacteria. Unidentified sporulation inhibitors (SIs) control the timing of sporulation that takes place on the distal portion of the thallus where either gametes or zoospores are produced.

The Role of the Microbiome

Macroalgae, including *Ulva*, have associated microbiomes that colonize surface tissue and may play roles in growth and morphogenesis (48, 55). *Ulva* is unable to develop normally when cultured axenically and instead adopts a callus-like morphology with undifferentiated cells when its microbiome is removed. Normal morphogenesis can be restored when *Ulva* is cocultured with one or more bacterial species that produce essential morphogens or developmental regulators. Research into the identity of morphogenesis-inducing bacteria and the morphogens produced by the bacteria has yielded important insights and helped frame a new set of questions. Bacterial associations with *Ulva* and relatives (e.g., *Enteromorpha*, *Monostroma*) have been studied for many years, resulting in the identification of specific bacterial taxa that associate with some species and of morphogenetic compounds produced by bacteria (155). It remains unclear how much taxonomic specificity is associated with the *Ulva* microbiome or whether morphogenesis-inducing functions are distributed across different bacterial groups (54, 130).

One interesting class of morphogenetic regulator is a quorum-sensing acyl-homoserine lactone (AHL) that enhances the substrate attachment of gametes or zooids (diploid spores) to promote the transition from the unicellular motile phase to the sessile multicellular phase of the life cycle (87, 144, 154) (Figure 5b). This finding is particularly interesting as it ties the communal

sensing behavior of prokaryotic species via quorum sensing to a major developmental transition in *Ulva* related to its decision to enter the multicellular stage of its life cycle (see the next section). The potential selective advantage for *Ulva* in settling in areas of high AHL concentration might be in finding bacteria that can promote its morphogenesis. It is unknown whether there is additional specificity regarding the class of AHLs recognized by *Ulva* or variability for this trait across different species.

A second bacterially produced sesquiterpenoid, thalussin, is a true morphogen for *Ulva* that promotes cell differentiation and organismal polarity, though it alone is not sufficient for the full morphogenesis of axenic *Ulva* cultures (**Figure 5b**). Thalussin was first discovered by researchers using bioassays for morphogenesis-promoting compounds secreted by bacteria in a relative of *Ulva*, *Monostroma oxyspermum*, where it promoted proliferation of the blade-like thallus (103). One of the morphogenesis-inducing bacteria for *Ulva mutabilis*, *Maribacter*, is a member of the Bacteroidetes family from which thalussin was identified (153). Indeed, thalussin was the molecule from *Maribacter* culture supernatants that was identified through fractionation, verified with synthetic analogs, and able to reproduce that effect of *Maribacter* coculturing on *Ulva*. The active form of thalussin was also a siderophore that enabled uptake of Fe(III) by *Ulva* (4). Notably, the effect of thalussin on *Monostroma* and *Ulva* was different (thallus proliferation versus polarity and cell differentiation, respectively), indicating that the effect of thalussin has evolved dynamically in the ulvophytes.

Besides thalussin, there is at least one other bacterial-produced morphogen for *Ulva* that can be provided by a much broader range of bacterial taxa and acts to stimulate thallus proliferation (153, 155) (**Figure 5b**). One species that can provide this as-yet-unidentified substance or substances is a *Roseovarius* strain that engages in a two-way communication with *Ulva*. The bacteria senses dimethylsulfoniopropionate (DMSP) produced by *Ulva*, which serves as a chemoattractant, and secretes a compound or compounds that promote thallus proliferation and morphogenesis in *Ulva*. These compounds in turn also stimulate *Ulva* to produce and secrete glycerol, which promotes bacterial growth (89). Coculturing of axenically derived *Ulva* callus with both *Maribacter* and *Roseovarius* leads to normal morphogenesis of *Ulva* in a simplified tripartite system (155). The identification of the second morphogenesis factor in this system would be another major step forward and help set the stage for dissecting the perception and signaling events that must lie downstream of the bacterial morphogens (130).

Microbiota and the Origins of Multicellularity

Ulva is an example of multicellularity being intertwined with prokaryote–eukaryote cross-kingdom interactions. Another example of such interactions comes from the choanoflagellate species *Salpingoeca rosetta*, which is part of the holozoan clade that includes metazoans and their closest unicellular and simple multicellular relatives (78). *S. rosetta* is facultatively multicellular, producing single cells, chains of cells, or rosettes (36). Interestingly, the propensity to form these different morphs is influenced by small molecules produced by a bacterial species isolated from its environment, *Algoriphagus machipongonensis* (159). The algal and choanoflagellate examples of bacterial-induced eukaryotic multicellular morphogenesis raise the interesting possibility that such cross-kingdom interactions were more widespread drivers of the origins of multicellularity than currently understood (160). While this is an appealing idea, it is difficult to untangle from what are likely long and complex histories of ecological interactions between prokaryotes and eukaryotes.

In the case of *Ulva*, the morphogenetic programming for normal development may have evolved independently of bacteria and then come to depend on bacterial associations secondarily.

A somewhat analogous situation is the widespread and frequent transition of algae from vitamin prototrophy to auxotrophy, where the auxotrophy is presumably complemented by bacterially produced vitamins in their environment or epibiome (32, 86). Further investigation into the role of bacterial morphogens in *Ulva* may help shed light on how these signals are integrated into developmental programs and whether in the past there might have been alternate routes for the development of *Ulva* without bacterial signals.

***Ulva mutabilis* Is a Model System for Algal Multicellularity and Dimorphic Life Cycles**

While research on *Ulva* is spread across multiple species, *U. mutabilis* [now also including conspecific *Ulva compressa* (140, 141)] is a promising candidate model organism for the genus *Ulva* (156) and related groups of ulvophytes. *U. mutabilis* has several key advantages, including a relatively high rate of spontaneous mutability to produce stable morphotypes such as Slender, a life cycle that can be easily controlled (146, 156), and a sequenced haploid genome (37) (**Table 1**). Interestingly, the genetic tool kit of *U. mutabilis* that was inferred from its genome was not especially enriched in gene families or functional categories that might be associated with multicellularity, such as transcription factors, and its overall gene content and genome size were on par with unicellular species of chlorophytes. This finding is similar to that for volvocine algae, where the *V. carteri* genome was not qualitatively very different from that of its unicellular relative, *C. reinhardtii* (124). Thus, the multicellularity genetic tool kit of *Ulva* may also be derived largely from one shared with its last unicellular ancestor.

Current understanding of ulvophycean relationships places members of the order Chlorocystidales as the closest unicellular relatives of *Ulva* (**Figure 4**). These unicellular relatives have a coccoid morphology, and most members have also undergone adaptation to terrestrial environments (33, 152). Although these relatives have been little studied to date, they are well positioned for use in comparative genomics and could potentially have members that are amenable to genetic or molecular genetic manipulation to understand gene function. By combining comparative genomics and developmental genetics, it may be possible to define the genes necessary for morphogenesis, including pathways that have coevolved with their prokaryotic symbionts, and even to compare homologous gene functions in a relatively close unicellular outgroup.

Additional genomic resources include mating locus (*MT*) haplotype sequences and transcriptome data for *U. partita* and *Ulva prolifera* (73, 83, 161). The promise of developmental genetics should also be enhanced by the relatively recent development of nuclear genome transformation using a method modified from yeast and a dominant selectable marker for zeocin resistance (119), as well as newly developed vectors (12). The transformation method not only enabled stable random integration and expression of transgenes but also yielded several classes of putative developmental mutants that may be tagged with the integrated transgene, thus facilitating identification of the disrupted loci in these strains. With these tools, it should be possible to gain new insights into the origins of developmental programming and its relationship to bacterial symbionts.

CAULERPA: COMPLEXITY WITHOUT CELLS?

Caulerpa is a green seaweed genus in the ulvophycean order Bryopsidales (**Figure 4**) whose individuals are multinucleate single cells that can grow to meters in length. *Caulerpa* can propagate vegetatively through fragmentation and regeneration and sexually through the production of small motile gametes (151) (**Figure 6**). Some species of *Caulerpa*, such as *Caulerpa taxifolia*, are invasive pests, while *Caulerpa lentillifera* (sea grapes) is grown as food and has potential medicinal properties (26, 163).

Table 1 Green algal comparative models for multicellularity and macroscopic complexity

Featured species	Order/group	Life cycle	Key derived features	Molecular genetics	Genome browser	Genome/genesc	Unicellular comparison	Relatives with genome sequences
<i>Volvox carter</i> (123)	Volvocine algae	Haplontic	Germ-soma differentiation, inversion, intercellular bridges, asymmetric cell division, expanded ECM, oogamy, internal fertilization	Forward genetics, developmental mutants, reverse genetics, gene editing, transgenics	https://phytozome.jgi.doe.gov/pz/portal.html#info?alias=Org_Vcarteri	131 Mbp/14,247 loci	<i>Chlamydomonas reinhardtii</i> (62)	<i>Chlamydomonas reinhardtii</i> (62), <i>Chlamydomonas incerta</i> (31), <i>Chlamydomonas schleiseri</i> (31), <i>Edaphobochlamys debaryana</i> (31), <i>Gonium pectoral</i> (70), <i>Tetrahena socialis</i> (49), <i>Yamagishiella utricocci</i> ^d (65), <i>Eudorina</i> sp. ^d (65)
<i>Ulva mutabilis</i> ^a (37), <i>Ulva pertusa</i> ^b (159)	Ulvales	Haplo-diplontic	Macroscopic growth, stem cells and asymmetric division, cell-type differentiation, chemical/hormonal signaling, microbiome interactions	Forward genetics, developmental mutants, transformation, transgenics	https://bioinformatics.psb.ugent.be/orcaae/overview/Ulvmu	99 Mbp/12,924 loci	Chlorocystidales, e.g., <i>Desmochloris</i> spp., <i>Halochlorococcum</i> spp.	NA
<i>Codium lenitillifera</i> ^a (5), <i>Codium tuxifolius</i> ^b (125)	Bryopsidales	Haplontic?	Macroscopic siphonous growth, pseudo-organ formation, cytoplasmic streaming/long-distance transport	NA	https://marinegenomics.oist.jp/umibudo/viewer?project_id=55	26 Mbp/9,311 loci	Chlorocystidales, e.g., <i>Desmochloris</i> spp., <i>Halochlorococcum</i> spp.	NA
<i>Chara braunii</i> (116)	Charales	Haplontic	Macroscopic growth, cellulose wall, plasmodesmata, apical polarized growth, three-dimensional patterning, cytoplasmic streaming/long-distance transport and communication, multiple cell types, asymmetric cell division, oogamy, specialized reproductive organs	NA	https://mycocosm.jgi.doe.gov/Chabral/Chabral1.home.html	1750 Mbp/23,546 loci	<i>Mesostigma viride</i> (100)	<i>Mesostigma viride</i> (100), <i>Klebsormidium flaccidum</i> (79), <i>Pontium marginaticum</i> (27), <i>Synedra muscicula</i> (85), <i>Mesotium endlicherianum</i> (85)

^aSpecies with full genome sequence.

²⁰Species with partial genome sequence and/or transcriptome data.

²Nuclear genome size/protein-coding loci.

^{1d}Full nuclear genome assemblies not published.

Abbreviations: ECM, extracellular matrix; NA, not available.

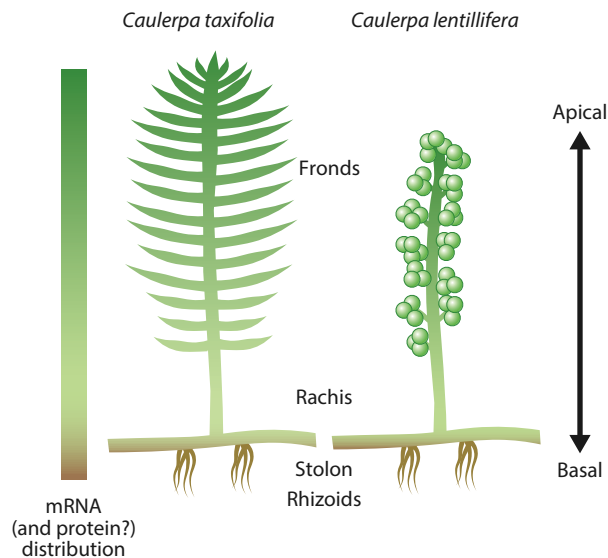


Figure 6

Morphology of giant multinucleate siphonous *Caulerpa* cells from two species, *Caulerpa taxifolia* and *Caulerpa lentillifera*. Anatomical features (pseudo-organs) and the apical-basal axis are labeled. Messenger RNA (mRNA) and, presumably, proteins from pseudo-organs show spatial differentiation (colored heat map) along the apical-basal axis. It is unknown to what extent this is due to the long-distance transport of mRNA and/or spatial differentiation of nuclei.

Caulerpa and other genera in the Bryopsidales challenge the notion that organismal complexity can only be achieved through multicellularity and cell-type differentiation (30, 114). Each giant single-celled *Caulerpa* individual is organized along an apical-basal axis with organ-like regions (pseudo-organs) growing from a long tube-shaped stolon that lies on the seabed. Fronds [or analogous structures, depending on the species (10, 163)] grow upwards from the stolon, while rhizoids (or holdfasts) grow downward into the benthic substratum. These pseudo-organs are structurally and functionally analogous to the tissues of a land plant: Rhizoids act as roots to anchor the *Caulerpa* individual to the seabed and are specialized for nutrient uptake (3, 28); stolons are similar to primary stems, providing structural connectivity between pseudo-organs and initiating new growth of fronds and rhizoids; fronds, like leaves, are structures where photosynthetic activity is presumably highest and are also organized along their own apical-basal axis where the apical tip is the site of growth, much like a shoot apical meristem (**Figure 6**) (25, 30, 84).

The striking convergence of form and function between land plant morphologies and those of *Caulerpa* and other algae in the family Bryopsidales was a key observation supporting Kaplan & Hagemann's (88) organismal theory of plant development. This organismal theory considers the roles of individual plant cells to be less important than the supracellular levels of organization mediated through symplastic intercellular connections and vasculature (30, 88).

In multicellular organisms, cell-type specialization into reproductive and somatic fates establishes a potential for genetic conflict that is typically mitigated by organismal clonality (i.e., all cells are genetically identical) established through a single-celled bottleneck during sexual or asexual reproduction (59). The multinucleate nature of *Caulerpa* cells creates a similar potential for conflict. Interestingly, propagation of *Caulerpa* can occur via vegetative fragmentation into portions containing only one pseudo-organ or a subset of pseudo-organs (22, 136). This observation implies that at least some nuclei in *Caulerpa* may be totipotent and raises additional questions about

Meristem:

tissue made up of undifferentiated cells capable of division and differentiation

whether there is any permanent nuclear specialization and how replicative competition between nuclei is managed.

Recent studies of the *Caulerpa* transcriptome lend molecular support to the idea that pseudo-organs are functionally specialized domains within each individual. Independently conducted studies of transcriptomes using RNA prepared from separate morphological domains of *C. taxifolia* (126) or *C. lentillifera* (6) both identified differential enrichment of transcripts encoding specific functional classes of proteins from each domain (**Figure 6**). Thus, even in the absence of individual cells, different regions within a single *Caulerpa* cell have the capacity for functional differentiation. The bases for spatially differential transcript accumulation remain to be determined for *Caulerpa*. Because *Caulerpa* cells are multinucleate, the spatial differentiation could be at the level of transcriptional programs of individual nuclei within pseudo-organs, and it could also be based on the long-distance transport of messenger RNAs (mRNAs) to specific subcellular locations. Additionally, spatial specialization could be mediated by the long-distance transport of proteins or other molecules within the cell. Cytoplasmic streaming is well documented in *Caulerpa* and may provide the mechanism for long-distance transport (35).

Given the size and complexity of *Caulerpa* cells, it might be expected that they possess large and complex genomes. On the contrary, the nuclear genome of *C. lentillifera* (the only currently sequenced species) was surprisingly modest in size (28.7 Mb), and its predicted protein-coding gene content (9,311 loci) was comparable with many unicellular species of green algae (5). Thus, as was also the case for *Ulva* and volvocine algae, the genetic tool kits for becoming more complex in size and in form—either through multicellularity or through cellular enlargement and subcompartmentalization—are not notably different from those of unicellular microalgae. To understand how *Caulerpa* and other coenocytic macroalgae achieve morphological complexity will require not just comparative genomics but also the advancement of genetic and molecular genetic tools for interrogating developmental mechanisms. While the production of small biflagellate gametes has been observed in different species of *Caulerpa*, ploidy levels and the nature of its sexual cycle remain unclear and may differ between species (163). The existence of highly distinct morphotypes at the species level (163) (**Figure 6**) suggests that heritable patterning modules exist and might be identified through forward genetics or even nuclear transplantation experiments analogous to those done to understand the nuclear control of morphogenesis in *Acetabularia* (66). It is also currently unclear which species of uninucleate unicellular microalgal ulvophytes might be the best proxy for an ancestral state that preceded the evolution of *Caulerpa* and other members of the Bryopsidales (**Figure 4**).

Microbiome Studies of *Caulerpa*

Caulerpa spp. possess distinct epiphytic and endophytic communities of bacteria that have mostly been characterized with respect to biogeography and as taxonomic markers. Functions in nutrient uptake, holobiont metabolism, and possibly chemical ecology have also been inferred (1, 2, 28, 111). It remains unknown whether, like *Ulva*, its microbial associates also shape *Caulerpa* development. It is also unknown whether axenic cultures of *Caulerpa* might be established for use in investigating this topic, but doing so would be beneficial.

THE CHAROPHYTE PARADOX

Charophytes are a grade of mostly freshwater green algae with a few terrestrial species. Like most chlorophytes, they have haplontic life cycles, and some (e.g., Klebsormidiophyceae) appear to have lost sexuality altogether. All but the order Mesostigmatophyceae have evolved some form of multicellularity, which, depending on the group, ranges from simple undifferentiated filaments or

clusters to plantlike forms with three-dimensional pseudoparenchymatous structures and multiple cell types. Charophyte algae are part of a larger taxonomic grouping, streptophytes, that also encompasses land plants (embryophytes), but charophyte algae are a paraphyletic grade (Figure 7). An interesting feature of charophytes as a whole is their relative paucity of species diversity compared with embryophytes and chlorophyte algae (61). Paradoxically, the closest charophyte relatives of embryophytes are members of the highly derived group Zygnematophyceae, which are unicellular or exhibit simple filamentous multicellularity without obvious cellular differentiation.

Recent research into charophyte algae has made use of genomic information as a guide to the early evolution of land plants, especially for understanding the genetic origins of key terrestrial adaptations such as abiotic stress protection (e.g., desiccation, high light, UV radiation) and hormonal signaling systems (16, 27, 38, 42, 79, 85, 94, 100, 117). Charophyte algae have also been championed as possible models for various aspects of land plant physiology and cell biology (46). Here, we consider charophyte algae from a different perspective, as potential models for the origins of streptophyte multicellularity (23). This topic is challenging given the age of the major charophyte orders (41) and the scarcity of well-developed model systems among charophytes. Thus, unlike the case for volvocine algae, where extant species with different degrees of size and cellular differentiation can serve as proxies or templates for understanding the progression from unicellular to more complex multicellular forms, the charophytes have no such subgroupings, with each order being fairly narrow in terms of the morphological diversity it contains. Nonetheless, it is worth considering the evolution of charophytean multicellularity and the potential for key members to serve as developmental genetic models, particularly in light of recently created genomic resources (Table 1) that now include sequences from several orders, including unicellular Mesostigmatophyceae (*Mesostigma viride*) (100), filamentous Klebsormidiophyceae (*Klebsormidium flaccidum*) (79), Zygnematophyceae (*Penium margaritaceum*, *Spirogloea muscicola*, *M. endlicherianum*) (27, 85), and Charophyceae (*C. braunii*) (117). Transcriptome data are also available for several more charophyte species that do not have genome sequences (95). Here we focus on multicellularity in the most morphologically complex genus, *Chara*.

Chara: Multicellular Atavism or Convergence?

The giant macroscopic internodal cells from members of the freshwater genus *Chara* have been of interest for many years as models for cytosolic transport and streaming and for electrophysiological studies (9, 18, 46). The multicellular body plans of *Chara* and other charalean algae are the most complex of all charophyte algae, and they exhibit some of the same architectural properties as land plants and vice versa (Figure 8). For example, the morphology of *Chara* bears a striking resemblance to the vegetative morphology of aquatic angiosperms (flowering plants) in the genus *Ceratophyllum*. Beyond sharing a similar body plan with land plants, the ontogenetic mechanisms that give rise to the body plans of *Chara* also bear similarities to those in land plants, including a mechanism involving indeterminate and determinate meristematic tip growth.

Prior to their phylogenetic repositioning (Figure 7), the Charales were considered the sister taxa to embryophytes, but now they have been conclusively displaced by two less complex orders, the Coleochaetophyceae and the Zygnematophyceae. Even after this displacement, it has been generally assumed that the common ancestor of these three charophytean orders and of embryophytes was similar in complexity to the extant Charales, but this assumption is without strong support and predicated on the parsimony-based concept that complexity is hard to gain and easy to lose. We believe this assumption needs revisiting, as the potential for convergent evolution of body plans in different algal taxa may be underestimated (88, 149). It remains an open question whether charalean algae are atavistic twins of a primitive embryophyte precursor lineage or the common ancestor of these two streptophyte orders was a much simpler organism whose charalean and

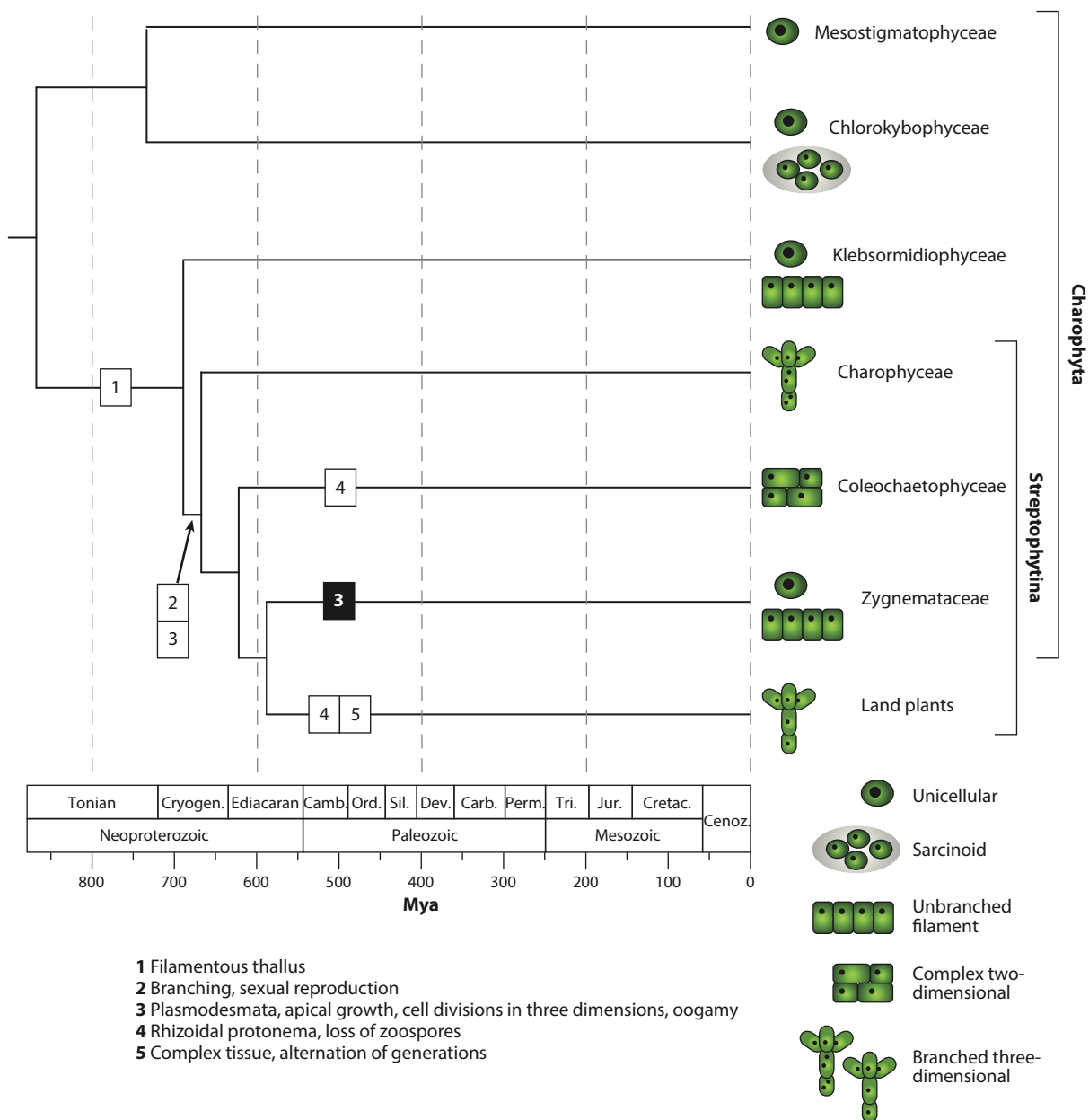


Figure 7

Approximate phylogeny (chronogram) of streptophytes with representative growth forms. Black numbers on white background indicate gain, and white on black indicate loss of (①) the filamentous thallus; (②) branching, sexual reproduction; (③) plasmodesmata, apical growth, cell divisions in three dimensions, and oogamy; (④) rhizoidal protonema and loss of zoospores; and (⑤) complex tissue and the alternation of generations. Abbreviations: Camb., Cambrian; Carb., Carboniferous; Cenoz., Cenozoic; Cretac., Cretaceous; Cryogen., Cryogenian; Dev., Devonian; Jur., Jurassic; Mya, million years ago; Ord., Ordovician; Perm., Permian; Sil., Silurian; Tri., Triassic. Adapted with permission from Reference 41 with additional information from References 107, 135.

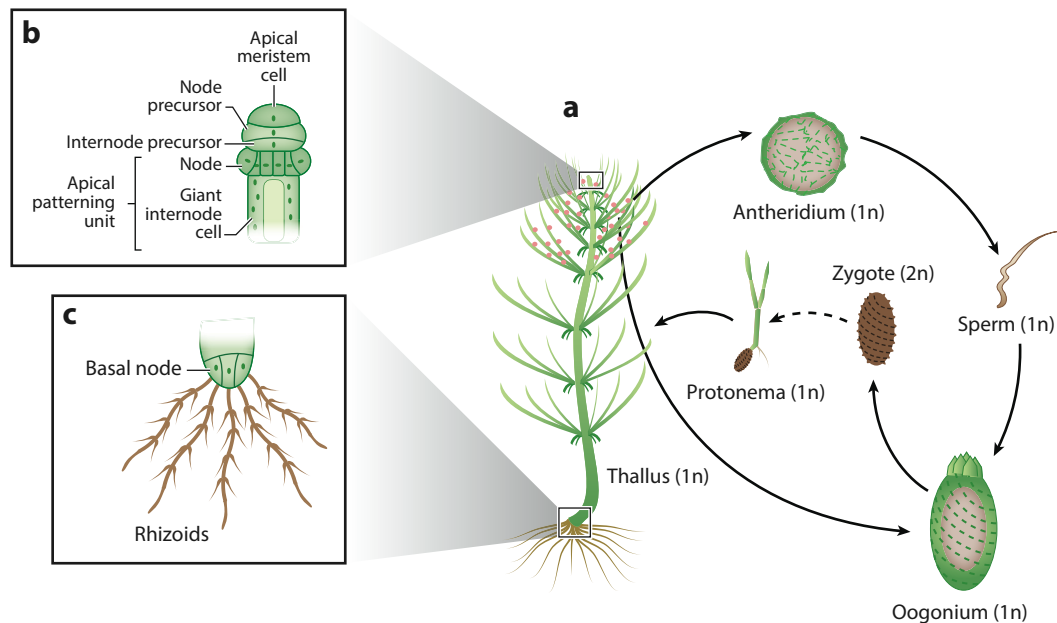


Figure 8

Life cycle of *Chara*. (a) The haploid thallus produces sperm in multicellular antheridia and eggs in oogonia. Gamete fusion results in a diploid zygote that undergoes meiosis (dashed line) before germinating to produce a rhizoid and protonema, which develop into a new mature thallus. (b) The apical meristem region of the thallus showing the apical to basal differentiation program. A single apical meristem cell divides transversely to produce daughters. The lower daughter divides again to produce a node precursor cell and an internode precursor cell. The internode precursor cell elongates without cytokinesis and becomes a giant multinucleated internode cell. The node precursor undergoes additional asymmetric divisions to produce different types of cells including lateral meristems and reproductive structures (see the section titled Multicellularity and Body Plan Patterning in *Chara* for details). (c) The basal nodes produce filamentous colorless rhizoids that can form thread-like branches at cell–cell boundaries. Adapted with permission from Reference 117.

embryophyte descendants convergently arrived at similar approaches for generating their body plans. Either way, we believe some of the most important and exciting discoveries about the streptophyte lineage will come from developing Charales and other charophyte orders as models for developmental, genetic, and comparative studies. Such an approach will ultimately lead to further insights into how complex multicellular body plans were generated among streptophytes, and whether the underlying mechanisms are truly homologous (23, 143).

Multicellularity and Body Plan Patterning in *Chara*

The capacity for polarized apical growth with a cellulosic wall, asymmetric cell division, and intercellular communication via plasmodesmata exists in *Chara* and was probably ancestral to the Zygnematophyceae, Coleochaetophyceae, and Charophyceae (ZCC) charophytes (149). These cellular features were the building blocks upon which a multicellular body plan evolved in *Chara*. The body plans and development of *Chara* and other charalean algae are described in excellent detail in other sources (51, 56) and are summarized here (Figure 8) (also see Reference 117 for informative color illustrations of the *C. braunii* body plan and life cycle). *Chara* plants display radial symmetry along an apical–basal axis. At the growing apex is an indeterminate meristematic cell that undergoes transverse divisions using a phragmoplast mechanism. The upper daughter cell of this pair undergoes further cell divisions to form a nodal structure, while the lower daughter

Phragmoplast: cell structure formed during cytokinesis that serves as a scaffold for cell wall formation

cell undergoes a massive expansion as it differentiates into a giant multinucleated internode cell that can grow to centimeter lengths. The lower cell divides again transversely to produce a pair of daughters. This general pattern of an apical stem cell producing alternating pairs of nodes and internodes forms the template for the upper (above ground) tissues of *Chara* (**Figure 8**). Similar to those in land plants, daughter cells remain connected by plasmodesmata after division. Nodal precursor cells undergo a further set of patterned divisions to form a radially symmetric nodal region that includes branchlet precursor cells; spike-shaped stipulode cells that resemble the stipules on the lateral organs of plants; and, in some species, cortical cells that surround and elongate with the internode cell to form a cortical layer. The branchlet precursor cells formed on the circumference of each node undergo the same type of apical tip growth and patterning as the apical meristem cell, but unlike the apical meristem, cell division of the branchlet meristem cells is determinate and will cease after several rounds of extension. However, dormant lateral branchlet meristem cells can be reactivated and form a new central axis if the central apical meristem is cut off—a phenomenon similar to apical dominance in embryophytes. *Chara* does not have the entire embryophyte auxin synthesis and signaling pathway that mediates apical dominance in plants (117), though auxin is known to undergo polar transport and modulates some physiological responses in Charales and other charophyte algae (14, 127, 149, 162). It remains unknown whether apical dominance in *Chara* is also controlled by some form of auxin signaling or a completely different long-range signal.

The basal region of a *Chara* plant is specialized with filaments formed from elongated root-like rhizoid cells emanating from the most basal nodes (**Figure 8**). Rhizoid cells are highly polarized (93) and nonphotosynthetic, but they do have colorless plastids and are specialized for nutrient uptake while also providing structural support and anchoring the above-ground portion of the plant. Rhizoid filaments are tip growing but can form lateral branches at the interface between postmitotic cells. Rhizoid cells are also capable of gravitropic responses mediated by statoliths whose positioning and sedimentation govern an intracellular signaling pathway for anisotropic growth (8).

Specialized ornate multicellular sexual reproductive structures called oogonia and antheridia produce female and male gametes in *Chara*, respectively (**Figure 8**). Oogonia and antheridia form at central and branchlet nodes and are easily recognizable by their shapes, the bright orange color of the antheridia, and the elongated helical pattern of tube cells surrounding each oogonium. The biflagellate sperm produced in antheridia swim to and enter the oogonia where fertilization takes place. The zygote forms a thick protective wall, while the oogonial tube cells surrounding the zygote can become calcified and form an additional protective layer. Meiosis occurs during germination where a single postmeiotic cell undergoes mitotic divisions and develops into a new plant (51).

Genomics and Potential for Development as Models for Streptophyte Multicellularity

C. braunii is currently the only charophycean species with a published nuclear genome sequence (117). Analyses of the *Chara* genome in the context of land plant evolution and terrestrialization are well covered topics (16, 27, 39, 85, 94, 100, 102). Of interest here is whether any aspects of multicellularity in *Chara* might be illuminated by genome or transcriptome data. Compared with distantly related charophytes, including unicellular *M. viride*, *C. braunii* has gained several hundred gene families (100, 117), though it is likely that most of these are related to innovations at the cellular level (e.g., phragmoplast, polyplastidy, plasmodesmata, cell wall) that helped shape the developmental landscape of multicellularity in *Chara* (11, 20, 24, 40, 45) and other streptophyte algae.

The capacity for asymmetric cell division and for daughter cells to differentiate and express complex cell-type gene networks typically depends on transcription factors, and *Chara* has many

Oogonia: specialized multicellular structures that produce ova

Antheridia: specialized multicellular structures that produce sperm cells

Monoicous: bearing sperm and eggs on the same gametophyte thallus

Dioicous: having separate male and female gametophyte thalli

of the same families as land plants as well as some of its own lineage-specific expansions (94, 117, 158). Transcriptomes of oogonia, antheridia, and zygotes of *C. braunii* confirmed expected patterns of expression for some genes (e.g., motility genes in antheridia), but much remains to be done for leveraging the genomics of *Chara* to understand its developmental mechanisms. Using charophyte genes to rescue the mutants of their homologs from genetically tractable embryophytes is a powerful method to help establish similarity in molecular function for charophyte genes (63, 77, 102) but is not sufficient to understand the developmental context in which these genes are utilized in their native charalean species. Being able to directly test gene function in *Chara*, especially for key developmental transcription factors, will be essential for understanding and comparing *Chara* multicellularity to embryophyte multicellularity.

Although *Chara* has a short life cycle with means of vegetative and sexual propagation, availability of both monoicous and dioicous species (108), and amenability to controlled crossing (57), developmental genetics has not been developed or exploited for *Chara* or any other charophycean species. With a sequenced genome, *C. braunii* would be a leading candidate, but sequencing additional genomes in this genus or other charophycean relatives should not be a major hurdle. Probably the most important consideration is amenability to mutagenesis and isolation of developmental mutants. Whether this has been attempted before is not known to us, but with their large genomes this would have been a daunting proposition before the current age of inexpensive genome sequencing. We argue that this group is ready for more serious consideration as a model for streptophyte multicellularity.

CONCLUSION AND PERSPECTIVES

Algae in general, and green algae in particular, seem to have a predisposition to evolve multicellular forms, some of which reach high levels of complexity. We have reviewed several existing and potential model organisms for the evolution of multicellularity, cellular differentiation, and multicellular development within the green algae. In each case, we have identified the closest known unicellular relatives of the multicellular species, a critical resource for the application of comparative methods. Recent technological advances, particularly cheap genome sequencing and methods for genomic manipulation, make the development of new model organisms easier than it has ever been.

If there is one universal principle that emerges from comparing the evolution of multicellularity across green algal taxa, it is that there are no universal principles. The diversity of ways in which green algae have made the transition from unicellular to multicellular life precludes generalizations, or at least generalizations that are free of exceptions. The sheer variety of life cycles, body plans, and lifestyles suggests that the particular path the transition to multicellularity or complex morphology takes is highly contingent on the nature of the unicellular ancestor, the ecological conditions that determine selective pressures, chance events, or some combination of the three. Whether the haploid stage, the diploid stage, or both develop multicellular structures; whether the resulting structures are motile or sessile, complex or simple; and even whether they are strictly multicellular at all (as opposed to giant multinucleate cells) vary without obvious pattern.

What, then, can we learn from the green algae that might generalize to the evolution of multicellularity more broadly? We can at least eliminate some proposed patterns or rules. For example, there is clearly no general requirement that the evolution of multicellularity be associated with a wholesale expansion or reorganization of the genome. *Volvox*, *Ulva*, and *Caulerpa* all have genomes similar in size and gene content to their closest unicellular (and uninucleate) relatives. This is an instance of the so-called C-value paradox, the observation that there is no

consistent relationship between genome size and the size and complexity of the organism (147). This is not to say that the evolution of multicellularity never involves a big increase in genome size, but there are clearly enough exceptions to dismiss such a relationship from consideration as a rule. All of this suggests an important role for co-option in the evolution of multicellularity, and in fact we have abundant evidence for particular co-opted functions (17, 121, 148). Moreover, it appears that lineage-specific genes (those genes found in the multicellular clade and its closest unicellular relatives but not elsewhere) may play disproportionately large roles in co-option and shaping the transition to multicellularity and cell-type specialization. This is evident in volvocine algae, where cell-type specialization is associated with expression of lineage-specific genes (105), and is likely to be a more general phenomenon. For example, what were once thought of as metazoan-specific genes and signaling pathways are now known to have their roots in holozoans (animals and their closest unicellular relatives, which include choanoflagellates and several other taxa) (19).

Understanding the genetic changes underlying the evolution of multicellularity more generally will require expanding both the depth and breadth of our search. Looking more deeply will mean experimentally investigating gene functions, and recent advances allowing precise genetic manipulation of nonmodel organisms are likely to facilitate this. Looking more broadly will require increasing the sample size of comparisons, that is, the number of sets of unicellular and multicellular relatives between which we can make comparisons. Although single-celled green algae seem superficially similar, there is immense diversity within this group, and it is clear from the examples we have presented here that the way multicellularity evolves is contingent on differences among them. With additional points of comparison, it may eventually be possible to understand what features of the unicellular ancestor affect the subsequent evolution of multicellularity and how they do so.

SUMMARY POINTS

1. Multicellularity has evolved many times independently, and a large proportion of those origins were within the green algae.
2. Green algae have evolved a diverse array of multicellular and giant unicellular forms that differ dramatically in their morphology, life cycles, and development.
3. Molecular phylogenetic studies show that convergent evolution of similar morphologies is rampant in several groups of green algae.
4. Genomic comparisons often reveal that the evolution of multicellularity does not require large-scale changes in gene content or genome size.
5. Many components of the molecular tool kits we associate with multicellularity were present in unicellular ancestors.
6. Multicellular development requires interactions with bacterial microbiomes in some red and green algae.
7. We identify several taxa spanning the major clades of green algae that are well positioned to serve as model organisms for the evolution of multicellularity and cellular differentiation.
8. Recent advances in DNA sequencing technology and newly developed techniques for genetic manipulation facilitate the development of new model organisms.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Aires T, Moalic Y, Serrao EA, Arnaud-Haond S. 2015. Hologenome theory supported by cooccurrence networks of species-specific bacterial communities in siphonous algae (*Caulerpa*). *FEMS Microbiol. Ecol.* 91(7):fiv067
2. Aires T, Serrão EA, Kendrick G, Duarte CM, Arnaud-Haond S. 2013. Invasion is a community affair: clandestine followers in the bacterial community associated to green algae, *Caulerpa racemosa*, track the invasion source. *PLOS ONE* 8(7):e68429
3. Alexandre A, Santos R. 2020. High nitrogen and phosphorous acquisition by belowground parts of *Caulerpa prolifera* (Chlorophyta) contribute to the species' rapid spread in Ria Formosa Lagoon, Southern Portugal. *J. Phycol.* 56(3):608–17
4. Alsufyani T, Califano G, Deicke M, Grueneberg J, Weiss A, et al. 2020. Macroalgal–bacterial interactions: identification and role of thallusin in morphogenesis of the seaweed *Ulva* (Chlorophyta). *J. Exp. Bot.* 71(11):3340–49
5. Arimoto A, Nishitsuji K, Higa Y, Arakaki N, Hisata K, et al. 2019. A siphonous macroalgal genome suggests convergent functions of homeobox genes in algae and land plants. *DNA Res.* 26(2):183–92
6. Arimoto A, Nishitsuji K, Narisoko H, Shoguchi E, Satoh N. 2019. Differential gene expression in fronds and stolons of the siphonous macroalga, *Caulerpa lentillifera*. *Dev. Growth Differ.* 61(9):475–84
7. Balar NB, Mantri VA. 2020. Insights into life cycle patterns, spore formation, induction of reproduction, biochemical and molecular aspects of sporulation in green algal genus *Ulva*: implications for commercial cultivation. *J. Appl. Phycol.* 32:473–84
8. Beilby MJ. 2019. *Chara braunii* genome: a new resource for plant electrophysiology. *Biophys. Rev.* 11(2):235–39
9. Beilby MJ, Casanova MT. 2014. *The Physiology of Characean Cells*. Berlin: Springer-Verlag
10. Belton GS, van Reine WFP, Huisman JM, Draisma SGA, Gurgel CFD. 2014. Resolving phenotypic plasticity and species designation in the morphologically challenging *Caulerpa racemosa*–*peltata* complex (Chlorophyta, Caulerpacae). *J. Phycol.* 50(1):32–54
11. Benítez M, Hernández-Hernández V, Newman SA, Niklas KJ. 2018. Dynamical patterning modules, biogeneric materials, and the evolution of multicellular plants. *Front. Plant Sci.* 9:871
12. Blomme J, Liu X, Jacobs TB, De Clerck O. 2021. A molecular toolkit for the green seaweed *Ulva mutabilis*. *Plant Physiol.* 186:1442–54
13. Bonneau ER. 1977. Polymorphic behavior of *Ulva lactuca* (Chlorophyta) in axenic culture. I. Occurrence of *Enteromorpha*-like plants in haploid clones. *J. Phycol.* 13(2):133–40
14. Boot KJM, Libbenga KR, Hille SC, Offringa R, van Duijn B. 2012. Polar auxin transport: an early invention. *J. Exp. Bot.* 63(11):4213–18
15. Børresen S, Fjeld A. 1977. Mutational instability in *Ulva mutabilis* and its relation to different stages of the life cycle. *Hereditas* 85(2):215–18
16. Bowles AMC, Bechtold U, Paps J. 2020. The origin of land plants is rooted in two bursts of genomic novelty. *Curr. Biol.* 30(3):530–536.e2
17. Bowman JL, Briginshaw LN, Florent SN. 2019. Evolution and co-option of developmental regulatory networks in early land plants. *Curr. Top Dev. Biol.* 131:35–53

18. Braun M, Foissner I, Löhning H, Schubert H, Thiel G. 2007. Characean algae: still a valid model system to examine fundamental principles in plants. *Prog. Bot.* 68:193–220
19. Brunet T, King N. 2017. The origin of animal multicellularity and cell differentiation. *Dev. Cell* 43:124–40
20. Brunkard JO, Zambryski PC. 2017. Plasmodesmata enable multicellularity: new insights into their evolution, biogenesis, and functions in development and immunity. *Curr. Opin. Plant Biol.* 35:76–83
21. Bryhni E. 1974. Genetic control of morphogenesis in the multicellular alga *Ulva mutabilis*: defect in cell wall production. *Dev. Biol.* 37(2):273–79
22. Bulleri F, Marzinelli EM, Voerman SE, Gribben PE. 2020. Propagule composition regulates the success of an invasive seaweed across a heterogeneous seascape. *J. Ecol.* 108(3):1061–73
23. Buschmann H. 2020. Into another dimension: how streptophyte algae gained morphological complexity. *J. Exp. Bot.* 71(11):3279–86
24. Buschmann H, Zachgo S. 2016. The evolution of cell division: from streptophyte algae to land plants. *Trends Plant Sci.* 21(10):872–83
25. Chen JCW, Jacobs WP. 1966. Quantitative study of development of the giant coenocyte, *Caulerpa prolifera*. *Am. J. Bot.* 53:413–23
26. Chen X, Sun Y, Liu H, Liu S, Qin Y, Li P. 2019. Advances in cultivation, wastewater treatment application, bioactive components of *Caulerpa lentillifera* and their biotechnological applications. *PeerJ* 7:e6118
27. Cheng S, Xian W, Fu Y, Marin B, Keller J, et al. 2019. Genomes of subaerial Zygnematophyceae provide insights into land plant evolution. *Cell* 179(5):1057–67.e14
28. Chisholm JRM, Dauga C, Ageron E, Grimont PAD, Jaubert JM. 1996. “Roots” in mixotrophic algae. *Nature* 381(6581):382
29. Coleman AW. 2012. A comparative analysis of the Volvocaceae (Chlorophyta). *J. Phycol.* 48(3):491–513
30. Coneva V, Chitwood DH. 2015. Plant architecture without multicellularity: quandaries over patterning and the soma-germline divide in siphonous algae. *Front. Plant Sci.* 6:287
31. Craig RJ, Hasan AR, Ness RW, Keightley PD. 2021. Comparative genomics of *Cblamydomonas*. *Plant Cell* 33:1016–41
32. Croft MT, Warren MJ, Smith AG. 2006. Algae need their vitamins. *Eukaryot. Cell* 5(8):1175–83
33. Darienko T, Friedl T, Pröschold T. 2009. *Desmochloris mollenbaueri*—a new terrestrial ulvophycean alga from south-west African soils. (Molecular phylogeny and systematics of terrestrial Ulvophyceae I.). *Algological Stud.* 129:25–40
34. Darienko T, Pröschold T. 2017. Toward a monograph of non-marine Ulvophyceae using an integrative approach. (Molecular phylogeny and systematics of terrestrial Ulvophyceae II.). *Phytotaxa* 324(1):1–41
35. Dawes CJ, Barilotti DC. 1969. Cytoplasmic organization and rhythmic streaming in growing blades of *Caulerpa prolifera*. *Am. J. Bot.* 56:8–15
36. Dayel MJ, Alegado RA, Fairclough SR, Levin TC, Nichols SA, et al. 2011. Cell differentiation and morphogenesis in the colony-forming choanoflagellate *Salpingoeca rosetta*. *Dev. Biol.* 357(1):73–82
37. De Clerck O, Kao S-M, Bogaert KA, Blomme J, Fofflonker F, et al. 2018. Insights into the evolution of multicellularity from the sea lettuce genome. *Curr. Biol.* 28(18):2921–33.e5
38. de Vries J, Archibald JM. 2018. Plant evolution: landmarks on the path to terrestrial life. *New Phytol.* 217(4):1428–34
39. de Vries J, Curtis BA, Gould SB, Archibald JM. 2018. Embryophyte stress signaling evolved in the algal progenitors of land plants. *PNAS* 115:E3471–80
40. de Vries J, Gould SB. 2018. The monoplastidic bottleneck in algae and plant evolution. *J. Cell Sci.* 131:jcs203414
41. Del Cortona A, Jackson CJ, Bucchini F, Van Bel M, D’hondt S, et al. 2020. Neoproterozoic origin and multiple transitions to macroscopic growth in green seaweeds. *PNAS* 117(5):2551–59
42. Delwiche CF, Cooper ED. 2015. The evolutionary origin of a terrestrial flora. *Curr. Biol.* 25(19):R899–910
43. Desnitskiy AG. 1995. A review on the evolution of development in *Volvox*—morphological and physiological aspects. *Eur. J. Protistol.* 31(3):241–47
44. Desnitskiy AG. 2016. Major ontogenetic transitions during *Volvox* (Chlorophyta) evolution: when and where might they have occurred? *Dev. Genes Evol.* 226:349–54

45. Domozych DS, Domozych CE. 2014. Multicellularity in green algae: upsizing in a walled complex. *Front. Plant Sci.* 5(204):649
46. Domozych DS, Popper ZA, Sørensen I. 2016. Charophytes: evolutionary giants and emerging model organisms. *Front. Plant Sci.* 7:1470
47. Duncan L, Nishii I, Harryman A, Buckley S, Howard A, et al. 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):1–11
48. Egan S, Harder T, Burke C, Steinberg P, Kjelleberg S, Thomas T. 2013. The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiol. Rev.* 37(3):462–76
49. Featherston J, Arakaki Y, Hanschen ER, Ferris PJ, Michod RE, et al. 2017. 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(4):855–70
50. Fjeld A. 1972. Genetic control of cellular differentiation in *Ulva mutabilis*. Gene effects in early development. *Dev. Biol.* 28(2):326–43
51. Fritsch FE. 1948. *The Structure and Reproduction of the Algae*, Vol. 1. Cambridge, UK: Cambridge Univ. Press
52. Fulton AB. 1978. Colonial development in *Pandorina morum*: II. Colony morphogenesis and formation of the extracellular matrix. *Dev. Biol.* 64(2):236–51
53. Gerisch G. 1959. Die Zelldifferenzierung bei *Pleodorina californica* Shaw und die Organisation der Phytomonadinekolonien. *Arch. Protistenkunde* 104:292–358
54. Ghaderiardakani F, Coates JC, Wichard T. 2017. Bacteria-induced morphogenesis of *Ulva intestinalis* and *Ulva mutabilis* (Chlorophyta): a contribution to the lottery theory. *FEMS Microbiol. Ecol.* 93(8):fix094
55. Goecke F, Labes A, Wiese J, Imhoff J. 2010. Chemical interactions between marine macroalgae and bacteria. *Mar. Ecol. Prog. Ser.* 409:267–99
56. Graham LE, Graham JM, Wilcox LW. 2009. *Algae*. San Francisco: Benjamin Cummings. 2nd ed.
57. Grant MC, Proctor VW. 1972. *Chara vulgaris* and *C. contraria*: patterns of reproductive isolation for two cosmopolitan species complexes. *Evolution* 26(2):267–81
58. Grochau-Wright ZI, Hanschen ER, Ferris PJ, Hamaji T, Nozaki H, et al. 2017. Genetic basis for soma is present in undifferentiated volvocine green algae. *J. Evol. Biol.* 30(6):1205–18
59. Grosberg RK, Strathmann RR. 1998. One cell, two cell, red cell, blue cell: the persistence of a unicellular stage in multicellular life histories. *Trends Ecol. Evol.* 13(3):112–16
60. Grosberg RK, Strathmann RR. 2007. The evolution of multicellularity: a minor major transition? *Annu. Rev. Ecol. Evol. Syst.* 38:621–54
61. Guiry MD. 2012. How many species of algae are there? *J. Phycol.* 48(5):1057–63
62. Guiry MD, Guiry GM. 2021. *AlgaeBase*. Natl. Univ. Irel., Galway, Irel. <https://www.algaebase.org>
63. Hackenberg D, Pandey S. 2014. Heterotrimeric G-proteins in green algae. An early innovation in the evolution of the plant lineage. *Plant Signal. Behav.* 9(4):e28457
64. Hallmann A. 2006. Morphogenesis in the family Volvocaceae: different tactics for turning an embryo right-side out. *Protist* 157(4):445–61
65. Hamaji T, Kawai-Toyooka H, Uchimura H, Suzuki M, Noguchi H, et al. 2018. Anisogamy evolved with a reduced sex-determining region in volvocine green algae. *Commun. Biol.* 1(1):17
66. Hämmerling J. 1953. Nucleo-cytoplasmic relationships in the development of *Acetabularia*. *Int. Rev. Cytol.* 2:475–98
67. Hanschen ER, Ferris PJ, Michod RE. 2014. Early evolution of the genetic basis for soma in the Volvocaceae. *Evolution* 68(7):2014–25
68. Hanschen ER, Herron MD, Wiens JJ, Nozaki H, Michod RE. 2018. Repeated evolution and reversibility of self-fertilization in the volvocine green algae. *Evolution* 72:386–98
69. Hanschen ER, Herron MD, Wiens JJ, Nozaki H, Michod RE. 2018. Multicellularity drives the evolution of sexual traits. *Am. Nat.* 192(3):E93–105
70. Hanschen ER, Marriage TN, Ferris PJ, Hamaji T, Toyoda A, et al. 2016. The *Gonium pectorale* genome demonstrates co-option of cell cycle regulation during the evolution of multicellularity. *Nat. Commun.* 7:11370

71. Hanschen ER, Starkenburg SR. 2020. The state of algal genome quality and diversity. *Algal Res.* 50:101968
72. Hayden HS, Blomster J, Maggs CA, Silva PC, Stanhope MJ, Waaland JR. 2012. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *Eur. J. Phycol.* 38(3):277–94
73. He Y, Ma Y, Du Y, Shen S. 2018. Differential gene expression for carotenoid biosynthesis in a green alga *Ulva prolifera* based on transcriptome analysis. *BMC Genom.* 19(1):916
74. Herron MD, Desnitskiy AG, Michod RE. 2010. Evolution of developmental programs in *Volvox* (Chlorophyta). *J. Phycol.* 46(2):316–24
75. Herron MD, Hackett JD, Aylward FO, Michod RE. 2009. Triassic origin and early radiation of multicellular volvocine algae. *PNAS* 106(9):3254–58
76. Herron MD, Michod RE. 2008. Evolution of complexity in the volvocine algae: transitions in individuality through Darwin's eye. *Evolution* 62(2):436–51
77. Higo A, Kawashima T, Borg M, Zhao M, López-Vidriero I, et al. 2018. Transcription factor DUO1 generated by neo-functionalization is associated with evolution of sperm differentiation in plants. *Nat. Commun.* 9(1):5283
78. Hoffmeyer TT, Burkhardt P. 2016. Choanoflagellate models—*Monosiga brevicollis* and *Salpingoeca rosetta*. *Curr. Opin. Genet. Dev.* 39:42–47
79. Hori K, Maruyama F, Fujisawa T, Togashi T, Yamamoto N, et al. 2014. *Klebsormidium flaccidum* genome reveals primary factors for plant terrestrial adaptation. *Nat. Commun.* 5:3978
80. Hoxmark RC. 1975. Experimental analysis on the life cycle of *Ulva mutabilis*. *Bot. Mar.* 18:123–29
81. Hoxmark RC, Nordby Ø. 1974. Haploid meiosis as a regular phenomenon in the life cycle of *Ulva mutabilis*. *Hereditas* 76(2):239–49
82. Huskey RJ, Griffin BE. 1979. Genetic control of somatic cell differentiation in *Volvox*: analysis of somatic regenerator mutants. *Dev. Biol.* 72(2):226–35
83. Ichihara K, Yamazaki T, Miyamura S, Hiraoka M, Kawano S. 2019. Asexual thalli originated from sporophytic thalli via apomeiosis in the green seaweed *Ulva*. *Sci. Rep.* 9(1):13523
84. Jacobs WP. 1970. Development and regeneration of the algal giant coenocyte *Caulerpa*. *Ann. N. Y. Acad. Sci.* 175(1):732–48
85. Jiao C, Sørensen I, Sun X, Sun H, Behar H, et al. 2020. The *Penium margaritaceum* genome: hallmarks of the origins of land plants. *Cell* 181(5):1097–111.e12
86. Johnson WM, Alexander H, Bier RL, Miller DR, Muscarella ME, et al. 2020. Auxotrophic interactions: a stabilizing attribute of aquatic microbial communities? *FEMS Microbiol. Ecol.* 96(11):fiae115
87. Joint I, Tait K, Callow ME, Callow JA, Milton D, et al. 2002. Cell-to-cell communication across the prokaryote-eukaryote boundary. *Science* 298(5596):1207
88. Kaplan DR, Hagemann W. 1991. The relationship of cell and organism in vascular plants. *BioScience* 41(10):693–703
89. Kessler RW, Weiss A, Kuegler S, Hermes C, Wichard T. 2017. Macroalgal–bacterial interactions: role of dimethylsulfoniopropionate in microbial gardening by *Ulva* (Chlorophyta). *Mol. Ecol.* 27(8):1808–19
90. Kirk DL. 1998. *Volvox*. Cambridge, UK: Cambridge Univ. Press
91. Kirk DL. 2005. A twelve-step program for evolving multicellularity and a division of labor. *BioEssays* 27(3):299–310
92. Kirk MM, Ransick A, McRae SE, Kirk DL. 1993. The relationship between cell size and cell fate in *Volvox carteri*. *J. Cell Biol.* 123(1):191–208
93. Kiss JZ, Staehelin LA. 1993. Structural polarity in the *Chara* rhizoid: a reevaluation. *Am. J. Bot.* 80(3):273–82
94. Lai X, Chahtane H, Martin-Arevalillo R, Zubieta C, Parcy F. 2020. Contrasted evolutionary trajectories of plant transcription factors. *Curr. Opin. Plant Biol.* 54:101–7
95. Leebens-Mack JH, Barker MS, Carpenter EJ, Deyholos MK, Gitzendanner MA, et al. 2019. One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* 574(7780):679–85
96. Lemieux C, Otis C, Turmel M. 2014. Chloroplast phylogenomic analysis resolves deep-level relationships within the green algal class Trebouxiophyceae. *BMC Evol. Biol.* 14(1):211
97. Lercche K, Hallmann A. 2009. Stable nuclear transformation of *Gonium pectorale*. *BMC Biotechnology* 9:64

98. Lerche K, Hallmann A. 2013. Stable nuclear transformation of *Eudorina elegans*. *BMC Biotechnology* 13(5):11
99. Lerche K, Hallmann A. 2014. Stable nuclear transformation of *Pandorina morum*. *BMC Biotechnology* 14:65
100. Liang Z, Geng Y, Ji C, Du H, Wong CE, et al. 2020. *Mesostigma viride* genome and transcriptome provide insights into the origin and evolution of Streptophyta. *Adv. Sci.* 7:1901850
101. Marchant HJ. 1977. Colony formation and inversion in the green alga *Eudorina elegans*. *Protoplasma* 93(2–3):325–39
102. Martin-Arevalillo R, Thévenon E, Jégu F, Vinos-Poyo T, Vernoux T, et al. 2019. Evolution of the Auxin Response Factors from charophyte ancestors. *PLOS Genet.* 15(9):e1008400
103. Matsuo Y, Imagawa H, Nishizawa M, Shizuri Y. 2005. Isolation of an algal morphogenesis inducer from a marine bacterium. *Science* 307(5715):1598
104. Matt G, Umen JG. 2016. *Volvox*: a simple algal model for embryogenesis, morphogenesis and cellular differentiation. *Dev. Biol.* 419(1):99–113
105. 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* 8(2):531–50
106. Maynard Smith J, Szathmáry E. 1995. *The Major Transitions in Evolution*. Oxford, UK: Oxford Univ. Press
107. McCourt RM, Delwiche CF, Karol KG. 2004. Charophyte algae and land plant origins. *Trends Ecol. Evol.* 19(12):661–66
108. McCracken MD, Proctor VW, Hotchkiss AT. 1966. Attempted hybridization between monoecious and dioecious clones of *Chara*. *Am. J. Bot.* 53(9):937–40
109. Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, et al. 2007. The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science* 318(5848):245–50
110. Miller SM, Kirk DL. 1999. *glsA*, a *Volvox* gene required for asymmetric division and germ cell specification, encodes a chaperone-like protein. *Development* 126(4):649–58
111. Morrissey KL, Çavaş L, Willems A, De Clerck O. 2019. Disentangling the influence of environment, host specificity and thallus differentiation on bacterial communities in siphonous green seaweeds. *Front. Microbiol.* 10:717
112. Nagy LG, Kovács GM, Krizsán K. 2018. Complex multicellularity in fungi: evolutionary convergence, single origin, or both? *Biol. Rev.* 93(4):1778–94
113. Niklas KJ. 2014. The evolutionary-developmental origins of multicellularity. *Am. J. Bot.* 101(1):6–25
114. Niklas KJ, Cobb ED, Crawford DR. 2013. The evo-devo of multinucleate cells, tissues, and organisms, and an alternative route to multicellularity. *Evol. Dev.* 15(6):466–74
115. Nishii I, Miller SM. 2010. *Volvox*: simple steps to developmental complexity? *Curr. Opin. Plant Biol.* 13(6):646–53
116. Nishii I, Ogiwara S, Kirk DL. 2003. A kinesin, InvA, plays an essential role in *Volvox* morphogenesis. *Cell* 113(6):743–53
117. Nishiyama T, Sakayama H, de Vries J, Buschmann H, Saint-Marcoux D, et al. 2018. The *Chara* genome: secondary complexity and implications for plant terrestrialization. *Cell* 174(2):448–64.e24
118. Nozaki H, Itoh M. 1994. Phylogenetic relationships within the colonial Volvocales (Chlorophyta) inferred from cladistic analysis based on morphological data. *J. Phycol.* 30(2):353–65
119. Oertel W, Wichard T, Weissgerber A. 2015. Transformation of *Ulva mutabilis* (Chlorophyta) by vector plasmids integrating into the genome. *J. Phycol.* 51(5):963–79
120. O’Kelly CJ, Wyso B, Bellows WK. 2004. Gene sequence diversity and the phylogenetic position of algae assigned to the genera *Phaeophila* and *Occhlobaete* (Ulvophyceae, Chlorophyta). *J. Phycol.* 40(4):789–99
121. Olson BJSC, Nedelcu AM. 2016. Co-option during the evolution of multicellular and developmental complexity in the volvocine green algae. *Curr. Opin. Genet. Dev.* 39:107–15
122. Ortega-Escalante JA, Jasper R, Miller SM. 2019. CRISPR/Cas9 mutagenesis in *Volvox carteri*. *Plant J.* 97(4):661–72
123. Pascher A. 1931. Systematische Übersicht über die mit Flagellaten in Zusammenhang stehenden Algenreihen und Versuch einer Einreihung dieser Algenstämme in die Stämme des Pflanzenreiches. *Beih. Bot. Centralbl.* 48(2):317–32

124. Prochnik SE, Umen J, Nedelcu AM, Hallmann A, Miller SM, et al. 2010. Genomic analysis of organismal complexity in the multicellular green alga *Volvox carteri*. *Science* 329(5988):223–26
125. Pröschold T, Surek B, Marin B, Melkonian M. 2002. Protist origin of the Ulvophyceae (Chlorophyta) revealed by SSU rDNA analyses of marine coccoid green algae. *J. Phycol.* 38(s1):30–31
126. Ranjan A, Townsley BT, Ichihashi Y, Sinha NR, Chitwood DH. 2015. An intracellular transcriptomic atlas of the giant coenocyte *Caulerpa taxifolia*. *PLOS Genet.* 11(1):e1004900
127. Raven JA. 2013. Polar auxin transport in relation to long-distance transport of nutrients in the Charales. *J. Exp. Bot.* 64(1):1–9
128. Richter DJ, King N. 2013. The genomic and cellular foundations of animal origins. *Annu. Rev. Genet.* 47:509–37
129. Rokas A. 2008. The origins of multicellularity and the early history of the genetic toolkit for animal development. *Annu. Rev. Genet.* 42:235–51
130. Roth-Schulze AJ, Pintado J, Zozaya-Valdés E, Cremades J, Ruiz P, et al. 2018. Functional biogeography and host specificity of bacterial communities associated with the Marine Green Alga *Ulva* spp. *Mol. Ecol.* 27(8):1952–65
131. Ruiz-Trillo I, Roger AJ, Burger G, Gray MW, Lang BF. 2008. A phylogenomic investigation into the origin of metazoa. *Mol. Biol. Evol.* 25(4):664–72
132. Salomé PA, Merchant SS. 2019. A series of fortunate events: introducing *Chlamydomonas* as a reference organism. *Plant Cell* 31(8):1682–707
133. Schaap P, Winckler T, Nelson M, Alvarez-Curto E, Elgie B, et al. 2006. Molecular phylogeny and evolution of morphology in the social amoebas. *Science* 314(5799):661–63
134. Schirrmeister BE, de Vos JM, Antonelli A, Bagheri HC. 2013. Evolution of multicellularity coincided with increased diversification of cyanobacteria and the Great Oxidation Event. *PNAS* 110:1791–96
135. Škaloud P, Rindi F, Boedeker C, Leliaert F. 2018. *Freshwater Flora of Central Europe*, Vol. 13: *Chlorophyta: Ulvophyceae*. Cham, Switz.: Springer Spektrum
136. Smith CM, Walters LJ. 1999. Fragmentation as a strategy for *Caulerpa* species: fates of fragments and implications for management of an invasive weed. *Mar. Ecol.* 20(3–4):307–19
137. 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(6):1433–47
138. 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.* 15:1449–60
139. Stein JR. 1965. On cytoplasmic strands in *Gonium pectorale* (Volvocales). *J. Phycol.* 1(1):1–5
140. Steinhagen S, Barco A, Wichard T, Weinberger F. 2018. Conspecificity of the model organism *Ulva mutabilis* and *Ulva compressa* (Ulvophyceae, Chlorophyta). *J. Phycol.* 55(1):25–36
141. Steinhagen S, Weinberger F, Karez R. 2019. Molecular analysis of *Ulva compressa* (Chlorophyta, Ulvales) reveals its morphological plasticity, distribution and potential invasiveness on German North Sea and Baltic Sea coasts. *Eur. J. Phycol.* 54(1):102–14
142. Stratmann J, Paputsoglu G, Oertel W. 1996. Differentiation of *Ulva mutabilis* (Chlorophyta) gametangia and gamete release are controlled by extracellular inhibitors. *J. Phycol.* 32(6):1009–21
143. Szövényi P, Waller M, Kirbis A. 2019. Evolution of the plant body plan. *Curr. Top. Dev. Biol.* 131:1–34
144. Tait K, Joint I, Daykin M, Milton DL, Williams P, Cámara M. 2005. Disruption of quorum sensing in seawater abolishes attraction of zoospores of the green alga *Ulva* to bacterial biofilms. *Environ. Microbiol.* 7(2):229–40
145. Tan IH, Blomster J, Hansen G, Leskinen E, Maggs CA, et al. 1999. Molecular phylogenetic evidence for a reversible morphogenetic switch controlling the gross morphology of two common genera of green seaweeds, *Ulva* and *Enteromorpha*. *Mol. Biol. Evol.* 16(8):1011–18
146. Thiadens AJH, Zeuthen E. 1966. Meiosis and sporulation induced in sporophytes of *Ulva mutabilis* (“slender”) with synchronous mitosis. *Planta* 72(1):60–65
147. Thomas CA Jr. 1971. The genetic organization of chromosomes. *Annu. Rev. Genet.* 5:237–56
148. True JR, Carroll SB. 2002. Gene co-option in physiological and morphological evolution. *Annu. Rev. Cell Dev. Biol.* 18:53–80

149. Umen JG. 2014. Green algae and the origins of multicellularity in the plant kingdom. *Cold Spring Harb. Perspect. Biol.* 6(11):a016170
150. Umen JG, Coelho S. 2019. Algal sex determination and the evolution of anisogamy. *Annu. Rev. Microbiol.* 73:267–91
151. Vroom PS, Smith CM. 2001. The challenge of siphonous green algae. *Am. Sci.* 89:525–31
152. Watanabe S, Kuroda N, Maiwa F. 2001. Phylogenetic status of *Helicodictyon planctonicum* and *Desmochloris halophila* gen. et comb. nov. and the definition of the class Ulvophyceae (Chlorophyta). *Phycologia* 40(5):421–34
153. Weiss A, Costa R, Wichard T. 2017. Morphogenesis of *Ulva mutabilis* (Chlorophyta) induced by *Maribacter* species (Bacteroidetes, Flavobacteriaceae). *Bot. Mar.* 60(2):197–206
154. Wheeler GL, Tait K, Taylor A, Brownlee C, Joint I. 2006. Acyl-homoserine lactones modulate the settlement rate of zoospores of the marine alga *Ulva intestinalis* via a novel chemokinetic mechanism. *Plant Cell Environ.* 29(4):608–18
155. Wichard T. 2015. Exploring bacteria-induced growth and morphogenesis in the green macroalga order Ulvales (Chlorophyta). *Front. Plant Sci.* 6:86
156. Wichard T, Charrier B, Mineur F, Bothwell JH, De Clerck O, Coates JC. 2015. The green seaweed *Ulva*: a model system to study morphogenesis. *Front. Plant Sci.* 6:72
157. Wichard T, Oertel W. 2010. Gametogenesis and gamete release of *Ulva mutabilis* and *Ulva lactuca* (Chlorophyta): regulatory effects and chemical characterization of the “swarming inhibitor.” *J. Phycol.* 46(2):248–59
158. Wilhelmsson PKI, Mühlich C, Ullrich KK, Rensing SA. 2017. Comprehensive genome-wide classification reveals that many plant-specific transcription factors evolved in streptophyte algae. *Genome Biol. Evol.* 9(12):3384–97
159. Woznica A, Cantley AM, Beemelmans C, Freinkman E, Clardy J, King N. 2016. Bacterial lipids activate, synergize, and inhibit a developmental switch in choanoflagellates. *PNAS* 113(28):7894–99
160. Woznica A, King N. 2018. Lessons from simple marine models on the bacterial regulation of eukaryotic development. *Curr. Opin. Microbiol.* 43:108–16
161. Yamazaki T, Ichihara K, Suzuki R, Oshima K, Miyamura S, et al. 2017. Genomic structure and evolution of the mating type locus in the green seaweed *Ulva partita*. *Sci. Rep.* 7(1):11679
162. Zhang S, de Boer AH, van Duijn B. 2016. Auxin effects on ion transport in *Chara corallina*. *J. Plant Physiol.* 193:37–44
163. Zubia M, Draisma SGA, Morrissey KL, Varela-Álvarez E, De Clerck O. 2020. Concise review of the genus *Caulerpa* J.V. Lamouroux. *J. Appl. Phycol.* 32(1):23–39



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Errata

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