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BROWN ALGAE AS A MODEL FOR PLANT ORGANOGENESIS

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Abstract

Brown algae are an extremely interesting, but surprisingly poorly explored, group of organisms. They are one of only five eukaryotic lineages to have independently evolved complex multicellularity, which they express through a wide variety of morphologies ranging from uniseriate branched filaments to complex parenchymatous thalli with multiple cell types. Despite their very distinct evolutionary history, brown algae and land plants share a striking amount of developmental features. This has led to an interest in several aspects of brown algal development, including embryogenesis, polarity, cell cycle, asymmetric cell division and a putative role for plant hormone signalling. This review describes how investigations using brown algal models have helped to increase our understanding of the processes controlling early embryo development, in particular polarization, axis formation and asymmetric cell division. Additionally, the diversity of life cycles in the brown lineage and the emergence of *Ectocarpus* as a powerful model organism, are affording interesting insights on the molecular mechanisms underlying haploid-diploid life cycles. The use of these and other emerging brown algal models will undoubtedly add to our knowledge on the mechanisms that regulate development in multicellular photosynthetic organisms.

Key words

Brown algae, development, polarization, asymmetric cell division, auxin, hormone, life cycle, cell cycle, *Fucus*, *Ectocarpus*

1. Introducing brown algae

The brown algae are a group of photosynthetic organisms or 'plant systems' belonging to the Heterokonts (Figure 1) ***(1)***. Heterokonts are an extremely diverse kingdom that includes photosynthetic as well as non-photosynthetic protists classified in 17 classes, including diatoms, chrysophytes (golden algae) and xanthophytes (yellow-green algae), but also oomycetes or water molds, a group of pseudo-fungi many of which are notorious plant parasites such as *Phytophtora* and *Phytium* causing late blight of potato and seed rot, respectively ***(2, 3)***. Photosynthetic heterokonts have characteristic chlorophyll *c* – containing plastids surrounded by 4 membranes and thylakoids grouped in stacks of three ***(2)***. Chloroplast multi-gene trees established that heterokonts and other chlorophyll c – containing algae have acquired their plastids by a secondary endosymbiotic event involving a heterotrophic eukaryote and a red alga ***(4-8)***. The relationships among chlorophyll *c* – containing organisms and whether these acquired their secondary plastids independently or from a common ancestor remain uncertain ***(8-13)***.

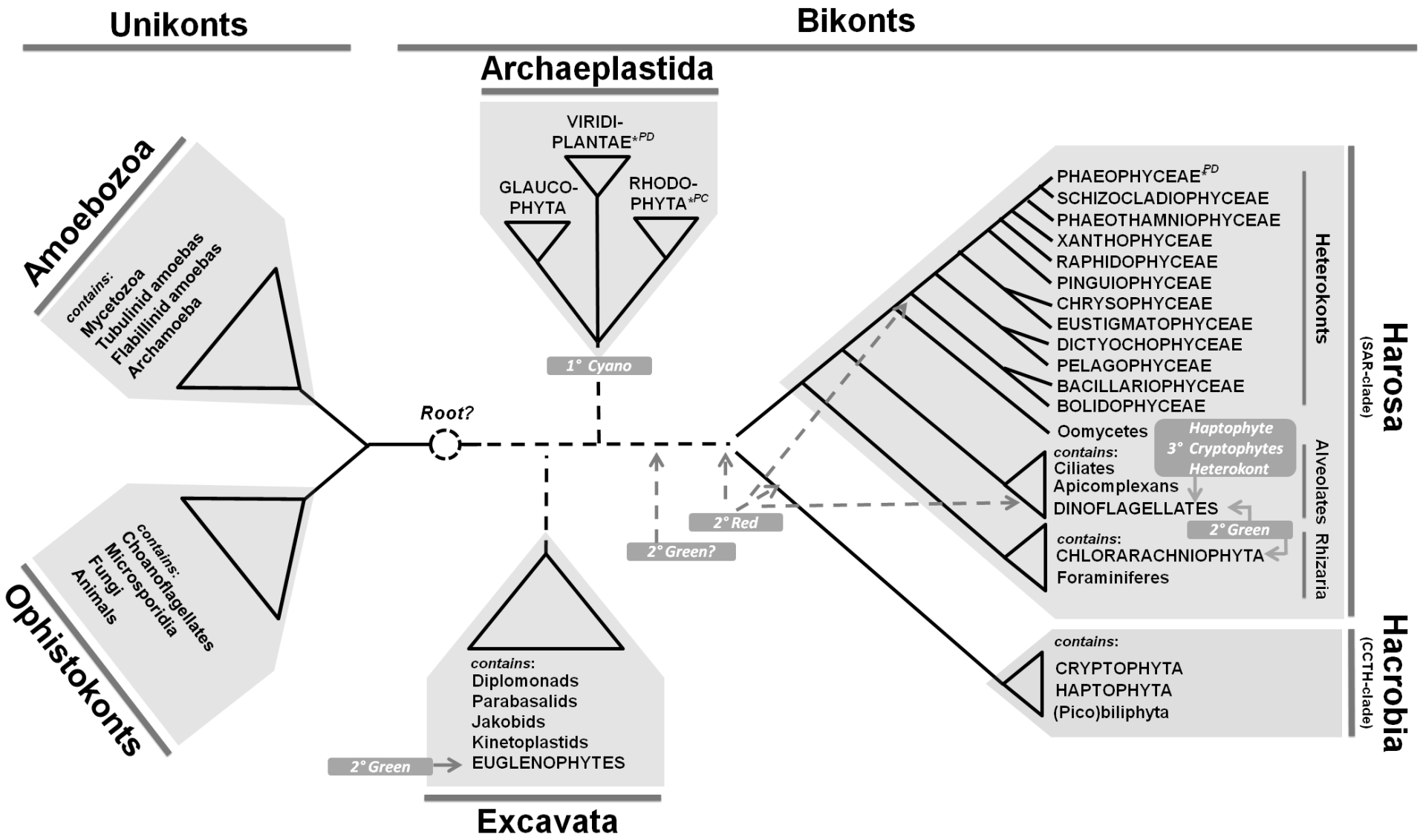

Figure 1. Relationships between major eukaryotic lineages (after (3, 15, 19, 20, 212-217)) indicating photosynthetic lineages and complex multicellularity. Endosymbiotic gene transfer is indicated with gray rectangles specifying the nature of the organelle donor. Dashed lines, and question marks refer to controversial phylogenetic relationships among the hypothesized "supergroups". Unresolved relationships are indicated as polytomies. Clades containing photosynthetic eukaryotes are marked with capitals. Parenchymatous photosynthetic lineages are marked by an asterisk, (1°) Primary endosymbiosis, (2°) secondary endosymbiosis, (3°) tertiary endosymbiosis, (PD) multicellularity by means of plasmodesmata (Phaeophyceae & Viridiplantae), (PC) multicellularity by means of pit connections (Rhodophyta), endosymbiotic relationships are indicated by grey rectangles. Branch lengths are not proportional to time.

Interestingly, brown algae are one of only five groups of eukaryotes that acquired complex multicellularity, the others being red algae (Rhodophyta), green algae (Chloroplastida, including the land plants), animals (Metazoa) and fungi (Fungi). Being Heterokonts brown algae share a common ancestor with land plants well over 1,500 million years ago ***(14)***. A genomic repertoire distinct from land plants is the result of this long independent evolution, only occasionally disturbed by horizontal and endosymbiotic gene transfer ***(9, 15)***. Remarkably, and despite of their evolutionary distance, brown algae and land plants share many common developmental features: (i) Following mitosis, daughter cells remain attached to each other, producing a multicellular body plan often showing a considerable amount of cell differentiation. (ii) Cells remain embedded in a rigid extracellular matrix which contains cellulose fibrils. (iii) Individual cells maintain cytoplasmic continuity through the formation of plasmodesmata. (iv) In general, both land plants as well as brown algae use an open-growth strategy in which a potential infinite sequence of new organs is produced by meristematic cells. (v) Some lineages have evolved meristems that are able to divide in three dimensions with daughter cells establishing also secondary cytoplasmic continuity with their direct neighbours (parenchymatous tissues). (vi) Multicellular development relays on positional control and clear cell lineages are absent. (vii) Unlike animals, totipotency or the ability to dedifferentiate upon isolation is frequent. In contrast, other cell biological features such as the absence of a preprophase band, the presence of centrioles ***(16)***, production of eicosanoid oxylipins ***(17)*** set them clearly apart from land plants and are more reminiscent of fungal and animal cells.

Brown algae contain about 1800 species belonging to 285 genera, all of which are multicellular ***(18)*** (Figure 2). Phaeophyceae are a sister group of the Schizocladiophyceae ***(19)***. The latter is a monotypic lineage that shares many characteristics with brown algae, but differs by the absence of plasmodesmata connecting neighbouring cells ***(19)***. The latter is considered significant with regard to the evolution of complex multicellularity. Intercellular connections, enabling transport of photosynthates and signaling molecules, are crucial in the development of a multicellular, morphologically differentiated thallus. The most recent calibrated phylogenies indicate that the

Schizocladiophyceae and Phaeophyceae diverged in the lower Jurassic, ca. 190 mya ***(20)***. Subsequent diversification of the Phaeophyceae in the Cretaceous resulted in a bewildering diversity of thallus morphologies, life cycles and sexual strategies ***(21-23)***.

The size of the thallus varies dramatically among species. The sporophytes of kelp species, large brown algae belonging to the order Laminariales, may attain sizes of up to 60 m and grow as fast as half a meter per day ***(1, 23)***. They are able to translocate photosynthates, nitrogen and phosphorous across all parts of the plant using differentiated cells, trumpet hyphae, resembling sieve tubes in land plants ***(24, 25)***. Other brown algae are small epi- or endophytes that are rarely to be noticed with the naked eye ***(26, 27)***. Thallus complexity varies from uniseriate, branched filaments with diffuse growth over filamentous thalli with localized meristematic zones or filaments uniting to form a pseudoparenchyme, to complex thalli whereby a single or a group of meristematic cells divides in three dimensions and the produced daughter cells establish also secondary symplastic continuity with their direct neighbours to produce parenchymatous tissue ***(28)***.

In this review we will focus on how brown algal models can be attractive and powerful systems that will offer a wider phylogenetic perspective of the underlying processes controlling development. The similarities in development between brown algae and land plants and the advantages of brown algae in terms of manipulation for a number of experimental protocols have led researchers to use fucoids, such as *Fucus* and *Silvetia*, as general models for a number of mechanisms such as embryogenesis, polarity and asymmetric cell division ***(29-33)***. Additionally, the diversity of life cycles among brown alga, and the particularly complex haploid-diploid life cycle of *Ectocarpus* has drawn the interest of evolutionary ecologists as well as functional biologists ***(21, 34, 35)***.

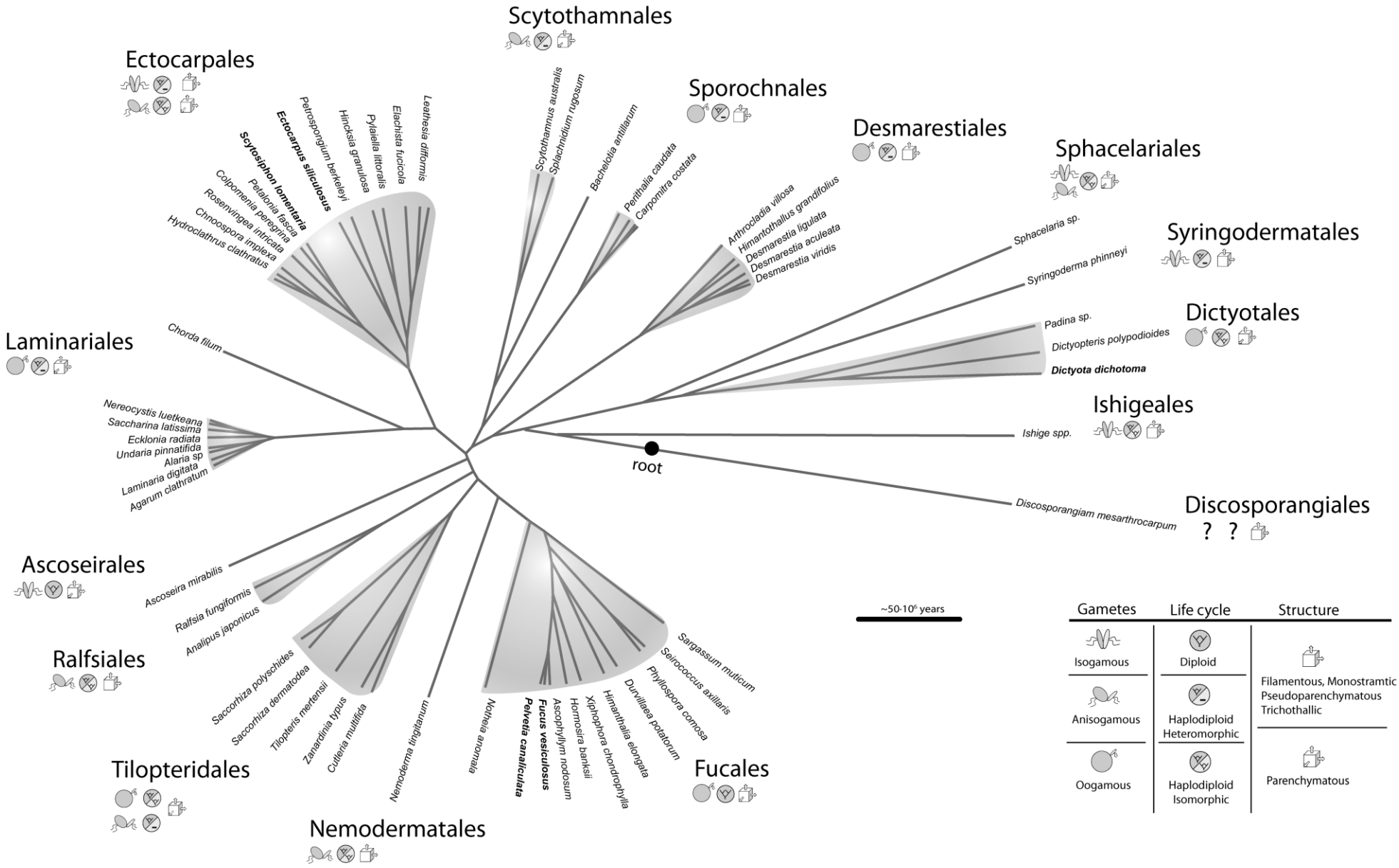

Figure 2. Schematic representation of brown algal diversity based on phylogenies by Kawai et al. (43) and Silberfeld et al. (18). Branch lengths are roughly proportional to time based on a time-calibrated phylogeny by Silberfeld et al. (18). Gamete dimorphism, type of life history and thallus structure are indicated for the most important brown algal orders. Definitions of thallus structure follow Niklas (28, 200) other characters states are gleaned from Silberfeld et al. (18).

In recent years, following the selection of *Ectocarpus* as a model for the brown algae ***(31)***, a considerable effort was invested in the development of genomic and genetic tools for this organism. The most important of these was the assembly and analysis of the complete 214 Mbp genome sequence ***(36)***, together with the development of a range of molecular tools including whole genome tiling arrays, deep sequencing of small RNAs, mutant screens ***(31)***, microarray analysis of gene expression ***(37)***, a sequence-tagged genetic map ***(38)***, stramenopile-adapted bioinformatic tools ***(39)***, proteomics ***(40)*** and metabolomic methodologies. Finally, we also address the question of whether plant hormones, such as auxin, are involved in the growth and development of brown algae. The latter question has been raised and addressed several times in the past ***(41)***, but despite numerous reports indicating the presence of plant hormones in brown algae the effects on development remain controversial.

2. Life cycle and development

Sexual life cycles in the eukaryotes involve a cyclic alternation between diploid and haploid generations with meiosis mediating the transition from the diploid to the haploid state and cell fusion (syngamy) reconstituting a diploid genome. Brown algae are highly interesting models for studies on the life cycles not only because of their evolutionary distance from other eukaryotic lineages but also because they exhibit a broad variety of life cycles, ranging from isomorphic haploid-diploid life cycles, in which both gametophyte and sporophyte generations exhibit multicellular development, to diploid life cycles, where only the diploid generation of the life cycle is multicellular (reviewed in ***(34)***).

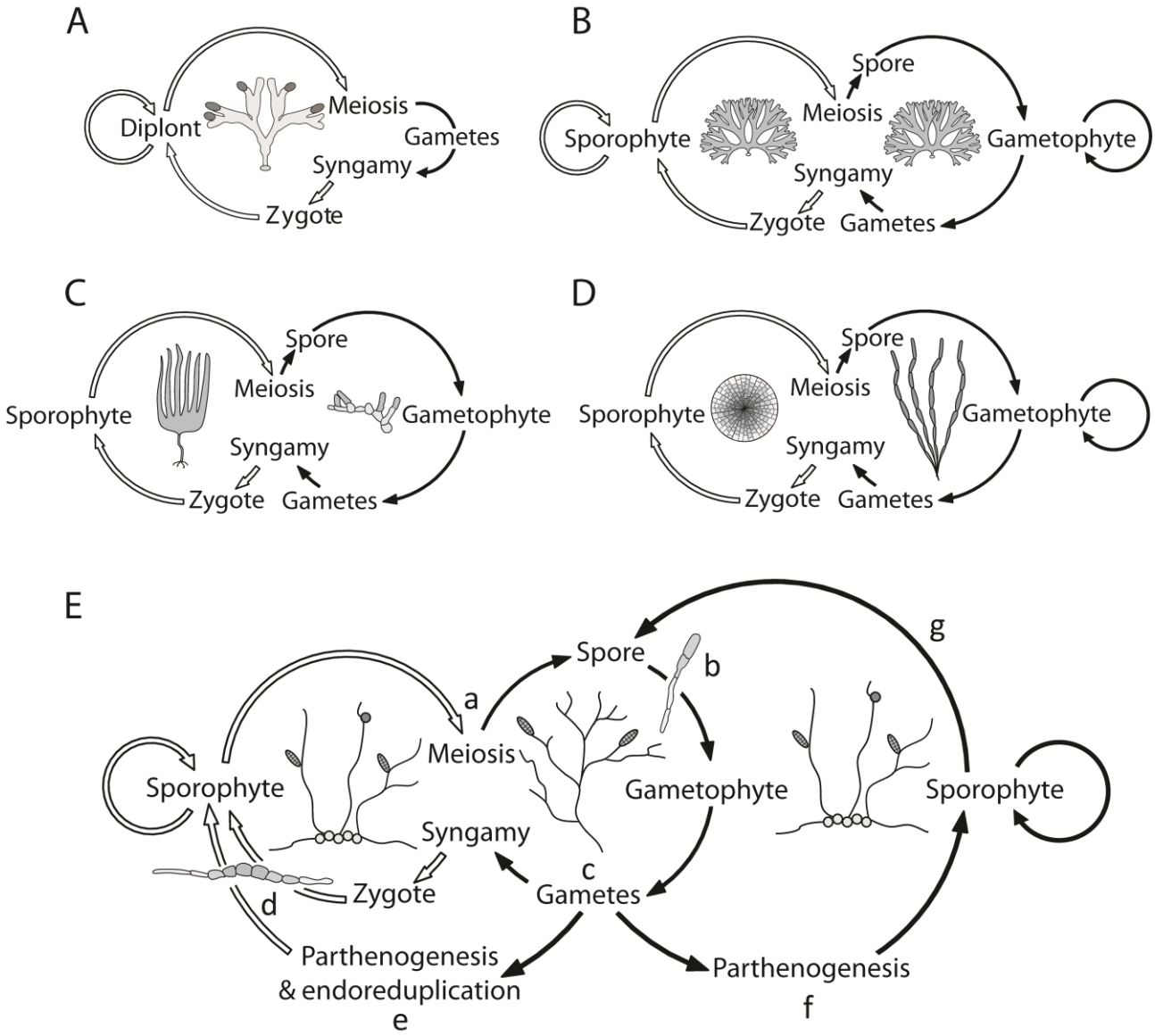

Figure 3. Schematic representation of brown algal life cycles. Diploid and haploid stages are marked by white and black arrows, respectively. (A) Fucales are characterized by a diplontic life cycle. Meiosis in the reproductive tissue is immediately followed by gametogenesis and syngamy producing a diploid zygote (218). (B) (C) (D) Most brown algae exhibit a diplohaplontic life cycle where a haploid gametophyte alternates with a diploid sporophyte. Here following meiosis the resulting spore develops into a multicellular organism. Both haploid and diploid phases may be of identical morphology (isomorphic) (B, Dictyota dichotoma) (219), or one of both phases may develop differently (heteromorphic) with either the gametophyte (C, Laminaria digitata) (220) or the sporophyte (D, Scytosiphon lomentaria) (221) being microscopic. (E) Simplified diplohaplontic life cycle of Ectocarpus siliculosus (31, 45). Meiosis (a) takes place in the sporophyte (diploid) to produce haploid spores. First cell division in germinating spores is asymmetric (b) and they grow into multicellular gametophytes. Gametophytes produce morphologically identical but physiologically differentiated male and female gametes (c), which fuse to form a zygote. After a symmetric first cell division (d) the zygote grows into a diploid sporophyte. Alternatively, gametes that do not meet a partner of opposite sex grow parthenogenically into diploid parthenosporophytes by means of parthenogenesis combined with endoreduplication (e) or in a haploid parthenosporophyte (f). The latter produce meiospores via a nonreductive apomeiotic event (g).

Loops connecting a generation with itself denote asexual reproduction mediated by vegetative reproduction (e.g. fragmentation, propagule formation) (A, B) (219, 222), the formation of asexual spores (mitospores) (B, E) (45, 223) or parthenogenetic development of unfertilized gametes (D) (221).

The ancestral brown algal sexual life cycle most likely involved alternation between two isomorphic generations: the diploid sporophyte and the haploid gametophyte ***(42, 43)***. However, many deviations from this situation are found in extant brown algae, which have independently evolved a remarkably fluid range of life cycles, usually involving morphological differentiation and reduction of one of the two generations (Figure 3). For instance, in the kelps the gametophyte generation is reduced but still develops independently of the sporophyte. In the Fucoids, the gametophyte generation has been completely lost, producing a diploid life cycle. Variations in life cycle structure can even be seen within a single order, as demonstrated by the Ectocarpales in which some families have life cycles with isomorphic generations (the Acinetosporaceae) and other families have strongly heteromorphic generations, with either the gametophyte (Chordariaceae, Adenocystaceae) or the sporophyte (Scytosiphonaceae) generation being microscopic ***(44)***.

Gametes of brown algae may be of equal size (isogamous), unequal size (anisogamous) or differentiated into female eggs and male sperm cells. Isogamy most likely presents the ancestral condition ***(42)*** and several lineages have evolved independently towards anisogamous and oogamous sexual strategies. Interestingly, the life history of the model brown alga *Ectocarpus* involves alternation between two morphologically distinct, sporophyte and gametophyte generations, both free living and macroscopic (Figure 2). In addition to this sexual cycle, an asexual cycle presents evidence of the extraordinary reproductive and developmental plasticity of *Ectocarpus*. Gametes that do not meet a partner of the opposite sex can still germinate parthenogenetically to produce haploid parthenosporophytes, a proportion of which will endoreduplicate to produce homozygous diploid parthenosporophytes ***(45)***. Of particular significance is the fact that haploid or endoreduplicated parthenosporophytes and diploid heterozygous sporophytes (produced from a zygote) are morphologically indistinguishable, and no

obvious correlation exists between ploidy level and cell volume. This is in contrast to what is typically described for most land plants where the level of ploidy is closely associated with the size of cells ***(46)***.

Gametophyte and sporophyte generations in *Ectocarpus* develop independently of the parent organism from a single progenitor cell that is released into the surrounding sea water. Remarkably, the morphological dissimilarity between generations is already discernible at the first cell division, which is asymmetrical in the gametophyte (just as in fucoids, producing a rhizoid cell and a "thallus" cell) as opposed to symmetrical in the sporophyte ***(31)***. It would be tempting to speculate that the pattern of this first cell division is linked to the developmental fate as a sporophyte or as a gametophyte. Mutant studies suggest that this may not be the case: the sporophyte generation of the *Ectocarpus* life cycle mutant *immediate upright* exhibits an asymmetrical first cell division (typical of a gametophyte) yet still develops into a fully functional sporophyte ***(31)***.

What are the biological functions of the two heteromorphic generations in haploid–diploid life cycles? Possible advantages of possessing distinct gametophyte and sporophyte developmental patterns include allowing the two generations to exploit alternative ecological niches or to avoid biotic aggressors due to differential susceptibility. Interestingly, the degree of morphological dissimilarity between the generations is highly variable between ecotypes, although a relationship between the degree of dissimilarity and the homogeneity of the environment, in terms of niche or pathogen prevalence, remains to be demonstrated.

How are the sporophyte and gametophyte developmental programs coordinated with the alternation between meiosis and syngamy? Molecular approaches using established model systems with haploid-diploid life cycles such as *Arabidopsis* would be expected to shed light on this matter. From a practical point of view, however, identification of genes that synchronize sporophyte/gametophyte development with life cycle progression can be hampered by the fact that mutations in such genes would most probably induce gametophyte or embryo lethality. In theory, though, a mutation in a gene that regulates the transition between the two generations could also lead to the development of

the “wrong” generation (for example, production of a gametophyte where a sporophyte would be expected). Redirection from a gametophyte to a sporophyte developmental program occurs during androgenesis and gynogenesis in land plants but the molecular mechanisms that regulate these processes have not yet been accessed genetically. *Ectocarpus*, therefore, represents a promising system to search for mutations that affect the switch between the sporophyte and gametophyte generations.

In the brown algae, the relationship between ploidy and the alternation of generations during the life cycle is not absolute, and the “classic” idea that gametophytes are haploid and sporophytes are diploid is not strictly applicable to these organisms. In *Ectocarpus*, both gametophytes and sporophytes can be haploid or diploid, showing that nuclear ploidy and life cycle generation are uncoupled ***(47)***. Therefore, the “choice” to deploy the gametophyte or the sporophyte developmental program is not determined by the ploidy of the initial cell but rather is under genetic control. Two single-locus mutations supporting this interpretation have recently been isolated in *Ectocarpus*. These mutations cause partial and complete conversion, respectively, of the sporophyte into a gametophyte developmental program ***(31, 35***). The identification of these two master regulatory genes responsible for the switch between life cycle generations will surely add to our knowledge on how development is controlled at the level of the whole organism.

3. Cell polarization and asymmetric cell division

Asymmetric cell division (ACD) is a nearly universal key mechanism that generates cellular diversity in complex multicellular systems. Because of its importance, the mechanisms involved in ACD have been investigated in a wide range of model systems. Cell polarity, the asymmetrical distribution of organelles, is a fundamental prerequisite for ACD, and it enables tip growth ***(48)***, lobe formation ***(49)***, polar diffuse growth ***(50)***, localized secretion ***(51)***, cell motility ***(52)*** and polar

transport of morphogens such as auxins ***(53)***.

Zygotes of brown algae have served as practical model systems for cell polarization and ACD, a cell division which produces two or more daughter cells with different cell fates, since the late 19th century (e.g. ***(54, 55)***). In particular, zygotes of *Fucus* and *Silvetia* (formerly called *Pelvetia*) have proved to be tractable cell biological models with important advantages over angiosperm systems. Studies on the establishment of polarity in angiosperms are technically challenging because asymmetrically dividing cells are often embedded in the mother tissue or surrounding cells (e.g. the zygote and lateral root initiation respectively) and polarity is often predetermined (e.g. pollen tubes). Fucoid eggs, conversely, are released in the surrounding seawater as radial symmetric spheres. Polarity is established after fertilization and can be manipulated by external cues. Development of embryos is highly synchronous, facilitating pharmacological treatments, cell cycle and biochemical studies ***(56)***. These characteristics have enabled researchers to manipulate the polarization axis according to the needs of the experimental set-up. In addition, zygotes of oogamous brown algae are relatively large (50-100 µm) making it relatively easy to visualize the internal cytological organization and making them amenable to micromanipulation (micro-injection, patch-clamping). The released eggs provide a "natural" system to study synchronous cell wall differentiation, in contrast to protoplast in land plants which are produced using potentially deleterious agents such as lytic enzymes. Finally, *in vitro* cultured zygotes maintain their polarity, while most of the cells from multicellular plants dedifferentiate and lose their original polarization axis upon isolation and *in vitro* culture ***(57)***.

The historic use of fucoid reproduction and early development as a model system has provided a number of crucial findings. In fucoid zygotes it has been clear for decades that ACD is dependent on both internal and external factors ***(58)***. Fucoid zygotes, being able to sense a whole range of environmental gradients and polarize accordingly, have put the concept of multiple polarization signals at the forefront ***(59)***. The widespread and fundamental role of free cytoplasmic calcium

(Ca^{2+}cyt) gradients ***(60, 61)***, the cytoskeleton ***(32, 62)***, and their interplay in the establishment of cell polarization, tip growth and fate determination in plant systems resulted to a considerable extent from insights gained on fucoid zygotes. The same applies to the importance of the plasma membrane ***(63)*** and targeted secretion to the cell wall ***(64, 65)***. Additionally the fucoid zygotes were the first plant systems to show the importance of increases in free Ca^{2+}cyt and membrane depolarization in egg activation (reviewed in ***(66, 67)***).

ACDs are traditionally divided in two categories each using a different mechanism ***(68, 69)***. The first mechanism involves the asymmetrical distribution of fate determinants according to a polarization axis established prior or during cell division. Subsequently, orientation of the mitotic spindle in parallel with the polarization axis ensures that one daughter cell will inherit most of the determinants. Because the cell fate is controlled by cell fate determinants within the cytoplasm, this mechanism is called 'intrinsic' or 'cell-autonomous'. The second mechanism, in contrast, involves a cell producing two initially identical daughter cells, which differentiate via positional signalling. Because cell fate in the latter scenario is controlled by external sources, the mechanism is termed 'extrinsic' or 'non-cell-autonomous'.

The polarization mechanism and ACD in the fucoid zygote has shown that both intrinsic and extrinsic cues can operate simultaneously ***(58)*** (Figure 4). After fertilization, if there are no detectable environmental cues, the sperm entry site will determine the axis of polarity ***(70, 71)***. However, the zygote remains responsive to an impressive range of environmental cues (blue light, gravity, ionic, electrical and osmotic gradients, presence of neighbouring zygotes, etc) until the polarization axis is fixed about 15-18 hours after fertilization ***(72-77)***. The cell will preferentially polarize according to these environmental cues and consequently its mRNA ***(78, 79)***, endomembrane system ***(80)***, sulfated fucans in the cell wall ***(81)***, dihydropyridine receptors (presumably calcium channels) ***(81, 82)*** and vitronectine ***(83)*** will become asymmetrically distributed. These asymmetries are established before cell division and therefore represent intrinsic

control over ACD which produces two daughter cells with a different fate (one rhizoid cell and one thallus cell).

Nowadays it is clear that every ACD depends on both intrinsic and extrinsic factors ***(84)***. In spite of the fact that this categorical framework has been fruitful in the research of both internal and external factors, it stretches the truth in the sense that the framework concentrates on the state of the daughter cells as its starting point rather than approaching ACD as a developmental process ***(85)***. Therefore it neglects the fact that the asymmetrical state of the *Fucus* thallus and rhizoid cell is dependent on external information in the first place. Similarly it has been shown that the orientation of the theoretically extrinsic ACD of *Drosophila* male germline stem cells depends on the intrinsic asymmetrical retention of the mother centrosome at the stem cell niche and migration of the daughter centrosome towards the opposite cell pole ***(86)***.

The dependence of the ACD on cues as light, gravity, presence of neighbouring tissue and the sperm entry site shows the fucoid zygotes are receptive to multiple polarization cues rather than only one. ACDs in plant systems are also receptive to multiple cues, but being in a multicellular context these signals are rather ligand-receptor mediated signals ***(87)*** or cell-to-cell movement transcription factors and miRNAs instead of physical factors as light and gravity like in fucoids (see ***(59)*** for a review).

The mechanisms underlying the perception of the environmental signals in fucoids remain obscure, although a rhodopsin-like protein ***(88)***, cGMP ***(89)*** and redox activity at the plasma membrane ***(90)*** have been proposed to be involved in perception of the light direction (see ***(91, 92)*** for a review). A speculative model involving calcium channel untethering at the lit side and tethering at the dark side in response to local cGMP concentration has also been proposed ***(92)***.

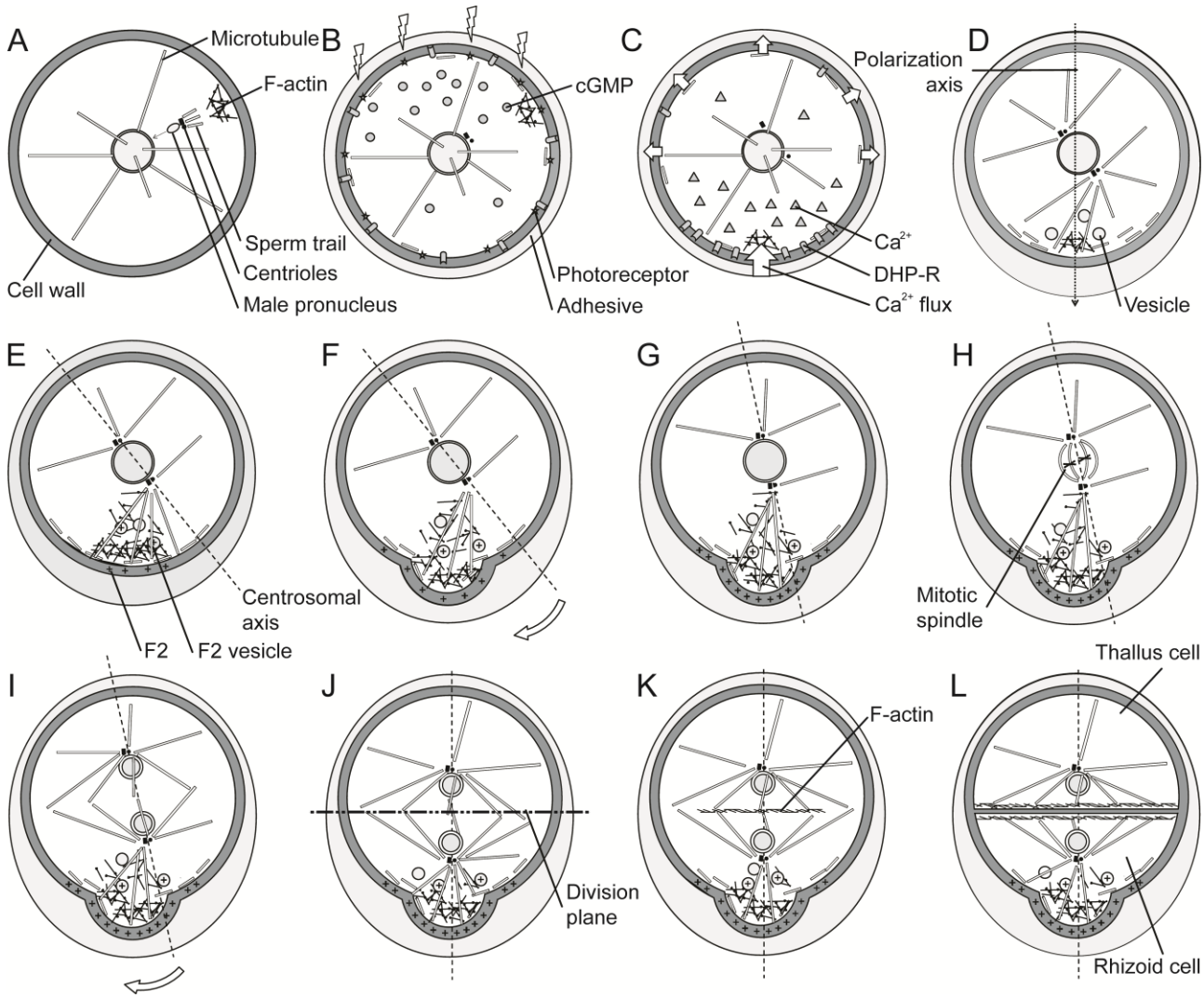

Figure 4. Schematic overview of a working model of photopolarizaton and ACD in fucoid zygotes. (A) Following fertilization the sperm pronucleus migrates towards the egg pronucleus. At the site of sperm entry, an F-actin/Arp2/3 patch is established and marks the default rhizoid pole. The microtubular cytoskeleton is mainly nucleated at the level of the nuclear enveloppe. (B) A couple of hours after fertilization the zygote secretes a uniform adhesive and attaches to the substrate. The cell becomes sensitive to the direction of the light. Rhodopsin-like receptors perceive the direction of the incoming light and establish a cGMP gradient decreasing from the thallus towards the rhizoid pole. In the meantime karyogamy has taken place and the centriole pair is deposited near the nuclear enveloppe. (C) The centrioles migrate towards two opposite poles independently of the future polarization axis (dotted arrow). A new F-actin patch is established at the shaded side of the zygote. Putative calcium channels (DHP-R, dihidropyridine receptors) accumulate progressively at the rhizoid pole. A calcium gradient is established decreasing from the future rhizoid pole towards the thallus pole. (D) Concurrently, the endomembrane system becomes asymmetrically distributed at the rhizoid pole and a polar secondary adhesive is deposited in addition to the uniform primary one. Also the cortical and cytoplasmic microtubular cytoskeleton become polarly distributed at the rhizoid pole early in the polarization process. The centrioles have arrived at opposite poles and the two centrosomes have become the most prominent nucleating centers of the cell. (E) During the polarization process the F-actin patch expands to form a cone extending from the cortex at the rhizoid pole towards the nucleus. Targetted secretion towards the rhizoid pole establishes the ASC ('axis stabilizing complex'). Among others a highly sulfated fucan (F2) is secreted at the rhizoid

pole. (F) Following F2 localization tip growth starts. Microtubules that get stabilized at the level of the ASC result in an asymmetry in the microtubular distribution which is thought to generate the force for the nuclear rotation which aligns the centrosomal axis with the polarization axis. (G) Prior to the metaphase the centrosomal axis is partially aligned. (H) The mitotic spindle is established in a partially aligned orientation. (I) After metaphase the alignment is completed. (J) Out of the two centrosomes microtubules radiate and delineate the plane of cell division at the zone where they interdigitate. (K) F-actin is deposited at this plane and the cell plate is deposited in a centrifugal way. (L) Similar to land plants, the ACD of the zygote establishes the apical-basal pattern with a large thallus cell and a smaller rhizoid cell.

3.1 The role of Ca^{2+}

Calcium appears to be an important player in cell polarity signalling during e.g. pollen growth, polarized cell expansion of pavement cells and trichome development in *A. thaliana* ***(57)***. The involvement of Ca^{2+}cyt in asymmetric cell division of angiosperms, however, has not yet been unequivocally established ***(84)***, and this is mostly due to difficulties in cell manipulation. The pioneering work of L. Jaffe and colleagues ***(93-95)*** showed that fucoid zygotes establish endogenous ionic currents consisting of mainly Ca^{2+}, K^{+} and Cl^{-} soon after fertilization. This research drew interest towards the important role for Ca^{2+}cyt in polarization and early development ***(61, 96)***.

The first evidence for a Ca^{2+} influx at the future rhizoid pole and eflux at the thallus pole resulting in a high Ca^{2+}cyt at the differentiating rhizoid pole came from the use of $^{45}Ca^{2+}$ isotopes as early as 1975 ***(97)***. Later, it was shown that rhizoids preferentially germinate towards the side exposed to higher concentrations of Ca^{2+} ionophores ***(98)***. The use of calcium sensitive micro-electrodes, calcium reporter Quin-2 ***(60)*** and Fura-2 ***(99)*** established directly a high Ca^{2+}cyt at the rhizoid tip. Just as in other tip growing systems, the high Ca^{2+}cyt in polarizing and tip growing zygotes is regulated by reactive oxygen production (ROS), illustrating the commonality of the ROS-Ca^{2+}cyt motif in development despite phylogenetic distances ***(29, 30)***.

The role of calcium influx during polarization of *Fucus* zygotes has been studied using different

calcium buffers ***(100)***, calcium reporters ***(101)***, patch and current clamp techniques ***(102, 103)*** and ion substitutions or additions to the medium ***(104, 105)***. By quantitative micro-injection of Calcium Crimson dextran, high concentrations of Ca^{2+}cyt were detected already within one hour after exposure of the cells to unilateral light ***(101)***. These elevations are F-actin dependent ***(106)***. Pharmacological data and antibody micro-injections suggest a role for the conserved calmodulin protein downstream of Ca^{2+}cyt in the polarization process of fucoids ***(101, 105)***. Further information on the role of calcium and calmodulin in fucoid polarization can be found in several excellent reviews ***(92, 107, 108)***.

Upon selection of the polarization axis based on external factors the axis is still labile and can be changed for example if the current axis is not parallel to the current light cue ***(109)***, indicating that polarity is acquired in distinct phases ***(59)***. Later, the axis is amplified by a positive feedback loop involving local increases of Ca^{2+}cyt and secretion at the rhizoid pole (see ***(110)*** for a review). It is still unclear how this positive feedback loop is achieved. It has been suggested that Ca^{2+}cyt induces local secretion of Ca^{2+} channels at the rhizoid pole ***(91)***. This hypothesis, however, contradicts the observation that inhibiting secretion does not affect redistribution of dihydropiridine receptors (putative Ca^{2+}-channels) ***(111)***. An alternative hypothesis involves Ca^{2+} elevations which induces cell wall softening secretion and consequently allows turgor pressure to locally expand the cell wall and the plasma membrane while activating stretch-sensitive calcium channels (see ***(96)*** for a review). Today, fucoid zygotes remain a valuable system for Ca^{2+} research ***(96)*** and serve in the development of new technical advances such as biolistic delivery of Ca^{2+} dyes into plant cells ***(112)***.

3.2 The role of the cytoskeleton

A lot of effort has been invested in determining the role of the cytoskeleton in polarity acquisition and ACD in *Fucus* zygotes (see ***(113)*** for a review). F-actin, nucleated by the Arp 2/3 complex ***(109)***, localizes at the future rhizoid pole within minutes after axis selection ***(110)***. The actin patch then increases and expands to form a cone extending from the cortex at the rhizoid pole towards the

nucleus ***(114)***. A similar importance of F-actin for the acquisition of polarity is corroborated by studies on protoplast regeneration in *Macrocystis pyrifera* ***(115)*** and on the ACD during branch initiation of *Sphacelaria rigidula* ***(116)***. Interestingly, asymmetric cell division of stomatal subsidiary mother cell in *Zea mays* also involves an F-actin patch extending towards the nucleus to localize to the pole close to the guard mother cell ***(117)***. F-actin plays an important role during polar growth of e.g. the *A. thaliana* root hair ***(118)***, *Nicotiana tabacum* pollen tubes ***(48)***, *A. thaliana* pavement cells ***(119)*** and *A. thaliana* trichomes ***(120)***. In these systems, F-actin is highly dynamic, oscillating between a polymerized and depolymerized state. These dynamics are regulated by effectors of ROP1, a Rho-like GTPase ***(119, 121, 122)***. These small GTPases act as molecular switches in many processes, both in animals, plants and yeast ***(123, 124)***. In *Fucus distichus* two small GTPases, FdRac1 and FdRab8, have been detected using degenerated PCR. Sequence analysis indicates that these proteins are members of respectively the Rho-family and the Rab-family ***(125)***. FdRac1 has been shown to accumulate at the rhizoid pole of polarized zygotes ***(125)*** and the Rac1 inhibitor NSC23766 has been shown to inhibit endomembrane polarization and adhesive polarization ***(126)***. The finding that Rac1 has a function in ACD in a heterokont alga supports the hypothesis that the role of Rho GTPases in cell polarity is a common feature in eukaryotes ***(125)***.

In land plants the orientation of cellulose microfibril deposition in the cell wall is controlled by the cortical microtubular cytoskeleton ***(127)***. The orientation of these microfibrils determines the direction of polar cell expansion ***(127, 128)***. Brown algae also contain cellulose and hexameric cellulose synthase complexes ***(129-131)***, however cortical F-actin determines the orientation of the cellulose microfibrils ***(132-134)***. Cortical microtubules in fucoid zygotes have been detected using immunofluorescence ***(135)***, and micoinjection of fluorescent tubulin monomers ***(136)***. It has been proposed that they have a role in tip growth ***(136)***. Additionally, the cytoplasmic microtubular skeleton has been shown to polarize and co-localize with the endoplasmatic reticulum and to direct exocytosis towards the rhizoid pole ***(137)***. The finding that microtubules are important for

exocytosis and tip growth, just as in land plants ***(138)***, is corroborated by studies on the tip growing apical meristem cells of *Sphacelaria*. The polarization of the microtubular skeleton in fucoid zygotes is regulated by phospholipase D, which demonstrates the conservation of phospholipase D signalling in microtubule regulation in eukaryotes ***(139)*** and illustrates the similarities with polarity establishment during tip growth in land plants (see ***(33)*** for a recent review).

3.3 Division plane determination and cytokinesis

In contrast to animals cells and brown algae, land plants do not have centrioles in their microtubule organizing complex ***(140)***. In animal cells and budding yeast it is the position of the centrosomal axis that determines the orientation of the division plane. The spindle is reoriented by a 'search and capture' mechanism that aligns the centrosomal axis with the polarization axis and off-center placement of the nucleus can be achieved by stronger pulling forces at the pole of the smaller cell ***(141, 142)***. It is still unclear how the plane of cytokinesis is determined in angiosperm cells. In some land plant cells the future cell plate is positioned by the pre-prophase band (PPB), a dynamic microtubular band beneath the plasma membrane that predicts the position of the plasma membrane (see ***(143)*** for a review). The land plant PPB and the animal spindle have opposite relations and therefore it has been argued that the 'plant polarity proteins' that are asymmetrically distributed towards one pole of the polarization axis are not able to coordinate the division plane orientation during asymmetric cell division like they do in animals and yeast. Consequently plant division mechanisms are regarded as intrinsically different ***(85)***.

In brown algae, correct positioning of the plane of cytokinesis is of fundamental importance, as misaligned asymmetrical division in fucoid zygotes disrupts normal development ***(81)***. In contrast to land plants, the position of the cell plate is determined in a way similar to furrow plane determination in animals and budding yeast. Similarly to animals, in brown algae centrioles serve as the most important nucleation sites for microtubuli. One important difference, however, is that the centrioles duplicate and migrate to opposite poles soon after the last cytokinesis and not during S-

phase of the cell cycle. Therefore, almost all vegetative cells have two pairs of centrioles instead of only one. Using polyspermic zygotes of *Scytosiphon* and zygotes of *Fucus* it has been shown that the position of the cytokinetic plane is determined by the two centrosomes out of which microtubules radiate and delineate the plane of cell division at the zone where they interdigitate ***(144, 145)***. During the first cell cycle of fucoid zygotes, the centrosomal axis is rotated until it is parallel with the polarization axis using a mechanism similar to the 'search and capture' mechanism of microtubuli in animals and budding yeasts (see ***(32)*** for a review). A similar reorientation of the centrosomal axis parallel to the new polarization axis has also been observed during branch formation in *Ectocarpus siliculosus, Sphacelaria rigidula* and *Macrocystis pyrifera* ***(116, 146)***. In highly vacuolated *Macrocystis pyrifera* protoplasts and vegetative cells, however, the cytokinetic mechanism has been modified ***(147)*** in a way more reminiscent to the one in land plants ***(148)***.

The finding that fucoid zygotes use a division mechanism similar to ophistokonts suggests that the direct specification of the plane perpendicular to the polarization axis is not inherently coupled to plant developmental biology. In land plant cells with a PPB it remains completely unknown how the PPB is positioned ***(149)***. In other land plants cells like pollen ***(150)*** and *Physcomitrella patens* protonemata ***(151)*** no PPBs are present, still the cell plate is positioned according to the polarization axis of the cell. This indicates that an additional mechanism should be involved in division plane determination in land plants ***(59)***. Interestingly it has been recently suggested that the division mechanism in land plants and animals does not differ that substantially as previously thought. During cell division of *A. thaliana* GFP-tagged plus-end binding EB1a and EB1b were reported to form polar caps during prophase and to penetrate inside the nuclear envelope ***(152-154)***. The polar caps were suggested to take up a role similar to centrioles in ophistokont and heterokonts. During tobacco pollen development, which lacks PPBs, also a microtubular cap structure was reported during the ACD and the EB1 gene product has been suggested to interact with TOBACCO MICROTUBULE BUNDLING POLYPEPTIDE OF 200 kDa (TMBP200), a MAP215/DIS1-family member which was shown to be essential for asymmetric cell division ***(150)***.

Brown algal cytokinesis usually occurs by centrifugal outgrowth of a partition membrane which gets progressively filled with cell wall material delivered by the Golgi bodies and transported by microtubules, as in land plants. However representatives of the Dictyotales-Sphacelariales-Syringodermatales clade such as *Dictyota* and *Halopteris* (no clear pattern) and *Sphacelaria* (centripetal) ***(155)*** present an exception to this pattern. There are also additional differences compared to land plants like the involvement of ER derived flat cisternae ***(156)*** that are supported by a peculiar actin structure at the future division plane ***(133, 157)*** and probably serve as the main membrane source for the partition membrane. Golgi derived vesicles in contrast rather serve as the main source of cell wall material (recently reviewed in ***(155, 158)***).

3.4 The axis stabilizing complex

Fucoid zygotes have been instrumental in appreciating the importance of the plasma membrane, cortical sites, cell wall and targeted secretion for patterning and morphogenesis in plant systems (reviewed in ***(63, 65, 159)***). In earlier work it has been established that the period during which the zygotes can reorient their polarization axis is prolonged as long as the cell wall is repeatedly removed ***(160)***. Similarly when the cell wall of a rhizoid cell is removed, the cell is able to regenerate but loses its polarity ***(161)***. Sulfated F2 fucans and at least one binding protein is preferentially secreted at the rhizoid pole with the same kinetics as the final alignment ***(63, 111)***. Also tip growing meristem cells of *Sphacelaria* lose their polarity following cell wall removal and divide symmetrically ***(162)***. Together with the idea that F-actin localizes to the rhizoid pole, this led to the hypothesis that transmembrane interactions between the cytoskeleton and the cell wall at the rhizoid pole (collectively termed the 'axis stabilizing complex' or 'ASC') are involved in polar growth. This positional information at the ASC may also play a role in the rotation of the centrosomal axis that aligns it with the polarization axis, because inhibiting polarized secretion to the cell wall with brefeldine A inhibits not only tip growth (as expected) but also alignment of the centrosomal axis with the polarization axis ***(81)***. Inhibiting polarized secretion, however, does not

inhibit polar localization of dihydropyridine receptors and F-actin. Next to a role in cell plate determination, polar growth and fixation of the polarization axis, the ASC also plays a role in cell fate specification. Laser-ablation of one cell of two-celled zygotes established that the remnants of cell walls are able to induce a thallus or a rhizoid cell fate in the daughter cell of the regenerating remaining cell that comes into contact with this cell wall ***(64)***. The finding that intrinsic factors are not necessarily transcription factors, mRNA's, membrane proteins and kinases but might be secreted cell wall components is intriguing and calls for additional research ***(163)***.

Also for land plants the role of targeted secretion of fate determinants towards cortical sites is relevant (reviewed in ***(65)***). In addition the recently discovered BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) in *Arabidopsis* meristemoid mother cell ACD ***(164)*** and the LRR-RLK PANGLOSS1 (PAN1) in maize subsidiary mother cell ACD ***(165)***, whose localization overlaps with the F-actin patch, also have polarized distribution patterns at a cortical domain and are reported to control ACD. Polar localization of PIN proteins is dependent on the actin cytoskeleton and on localized secretion towards cortical sites ***(53, 166)***. The apical-basal pattern of zygotes of tobacco that develop *in vitro* is dependent on the presence of the original cell wall, and even cell walls that hang loosely around the zygote are enough to signal the apical-basal axis ***(167)***. Interestingly arabinogalactan proteins were reported to be asymmetrically distributed in the tobacco zygote cell wall before ACD and pharmacological interference with these arabinogalactan proteins increases the frequency of symmetrical cell divisions ***(168)***.

3.5 Cell cycle and development

Zygotes of brown algae serve as excellent model systems for cell cycle research as they are naturally synchronized by fertilization ***(56)***. For example the inhibition of CDK proteins through a tyrosine phosphorylation at the S/M DNA replication checkpoint was first demonstrated in fucoid zygotes ***(56)*** and later confirmed in *A. thaliana* ***(169)***. Because fucoid zygotes polarize and divide asymmetrically free from the surrounding tissue, they are particularly interesting models for the

study of the interactions between cell cycle progression and the early patterning in a unicellular context because the first cell cycle of the zygote is concomitant with cell polarization. Cell cycle alterations during late embryogenesis and organogenesis of animals and adult development of land plants seem to have little effect on patterning. In contrast, cell cycle alternation during early embryogenesis in animals and budding in the unicellular yeast seems to result in severe effects on patterning because of a tight coordination between cell cycle and polarization. Indeed, the G1/S checkpoint in fucoid zygotes simultaneously controls S-phase entry and polarization, confirming that this trend also extends to plant systems. Coordination of cell cycle and polarization in *Fucus* resembles the cell cycle of the unicellular budding yeast in its presence of a DNA replication checkpoint a G1/S checkpoint which coordinates cell cycle and polarization ***(170)***, but it differs in the lack of a morphogenesis checkpoint ***(171)***.

Fucoid zygotes are excellent systems to test whether there are differences in cell cycle regulation between early embryo's and somatic cells in plant systems. The cell cycle in early embryos of animals differs substantially from cell cycle regulation in somatic cells. S-phases and M-phases alternate in quick succession without G1 and G2 phases which is regulated by periodic synthesis and degradation of cyclin B which activates the CDK CDC2 ***(172, 173)***. In somatic cells during organogenesis or upon terminal differentiation, however, the cell cycle appears to be more tightly controlled by CDKs then in the early embryos (see ***(174)*** for a review). In *Fucus* zygotes however the cell cycle is also tightly regulated and parallels a somatic cell cycle ***(170)***. This confirms the general idea that embryogenesis in plant systems in contrast to animals is not a distinct and independent stage in a plants life history, illustrating how brown algae can serve as an phylogenetic independent source of information for understanding plant systems.

4. Hormonal and positional control

4.1 A long-standing controversy

In animal systems cell fate decisions during embryogenesis is based on interactions between the cells or parts of the embryo ('regulative development') or by predetermined segregation of the intrinsic determinants specifying cell lineages ('mosaic development'). Plant systems typically develop in a more indeterminate way in which the plant lays out only the basic body plan during embryogenesis and adapts its adult shape and physiology to the environment by responding to external stimuli. Consequently positional information rather than cell lineages play the most important role in cell fate decisions of plant systems ***(175)***. Positional information is often controlled by gradients of diffusible factors which are interpreted by the target tissue ***(176)***. Some phytohormones such as auxin take up the role of such a diffusible factor comparable to animal morphogens or play an important role in growth regulation ***(177)***.

From regeneration experiments after ablation or wounding using *Sphacelaria* ***(178)*** or *Dictyota* meristems ***(179)*** and *Fucus* embryos ***(180)*** it is clear that brown algal development is under positional control. Whether the classical phytohormones of land plants play a role in organogenesis of brown algae is a long-standing but intriguing question. Many old and new reports provide arguments for a function for auxins, cytokinins, abscisic acid and gibberellins in wide range of brown algal developmental processes such as tropisms, polarity acquisition, gametogenesis, apical dominance and branching (reviewed in ***(181-187)***). Most attention has been drawn towards IAA (indole-3-acetic acid), an auxin, and consequently we will focus on this phytohormone

Despite the many reports of a role for auxins in brown algal development their conclusiveness remains highly debated. A lot of the older reports involve bioassays in which purified algal extracts are applied to *Avena* coleoptiles and the degree of curvature is tested. Despite being indications for the presence of phytohormones in these extracts, positive reactions may result from aspecific

activity rather than presence of auxins ***(188)***. More recent evidence involves the identification of the phytohormones in the extracts using GC-MS or HPLC. In fruiting tips of *F. distichus* concentrations up to 10 ng/g fresh weight have been recently reported, which are in the same order of magnitude as in land plants ***(189, 190)***. However, the mere detection of phytohormones does not provide a conclusive argument for its regulative potential on brown algal organogenesis. For example auxin has been detected (using GC-MS) also in unikonts, including human cerebrospinal fluid, at concentrations up to 15 ng/ml ***(191)***. Therefore these compounds may just be ordinary intermediates or breakdown products of the tryptophane metabolism. In addition, most if not all of the studies used field collected material or non-axenic cultures, which raises the question whether the detected phytohormones are actually produced by brown algae and not by epiphytic bacteria ***(188)***. Axenic cultures are difficult to obtain and often seem to be non-viable. Axenic cultures have been obtained from *Fucus spiralis* and *Ascophyllum nodosum*, but the thalli had an aberrant morphology and limited growth relative to the non-axenic controls ***(192, 193)***. Interestingly in *Ascophyllum* the aberrant phenotype could be (at least partially) rescued by respectively applying phenylacetic acid (PAA) and p-hydroxy-phenylacetic acid (p-OHPAA) and combinations of indole-3-acetic acid (IAA), 6-(γ,γ-dimethyl-allylamino)-purine (2IP) and zeatin at concentrations ranging around 10^{-7} M ***(193)***. PAA and IAA are next to indole-3-butyric acid (IBA) and 4-chloroindole-3-acetic acid (4-Cl-IAA) one of four endogenous auxins of higher plants ***(194)***. Zeatin and 2PI are cytokinins. These observations suggest that part of the detected auxins might be of bacterial origin. Regardless of their origin, however, these findings do illustrate the regulatory effect of phytohormones on brown algal organogenesis.

Many studies examined the effect of adding exogenous phytohormones and auxin transport inhibitors on the phenotype of brown algae. Care needs to be taken interpreting data obtained from application of transport inhibitors, since the effects may be totally unrelated to a putative auxin function in brown algae. For example the inhibitory effects of triiodobenzoic acid (TIBA) and genistein on polarization of fucoid zygotes can be understood in the context of respectively a

breakdown product of TIBA affecting sulfhydryl-groups of the sulfated fucans that fix the polarization axis (see above) ***(195)*** or dependence of the polarization process on a protein tyrosine kinase in *Fucus* zygotes ***(196)***. Secondly, the overall evidence is often conflicting and equivocal ***(188)***. Sometimes positive reports of effects are reported to be not reproducible (see e.g. ***(197)***).

Despite these objections, a number of recent studies are providing additional evidence for the role of auxins. For example radioactive [^{3}H]IAA pulse-chase experiments provided a strong argument for the effect of NPA and TIBA on auxin transport in *F. distichus* zygotes ***(189)***. Moreover, the auxin transport and F-actin cytoskeleton were shown to interact during photopolarization of *F. distichus* embryos ***(77)***. Immunolocalisation of IAA showed IAA also to be present inside *E. siliculosus* cells and putative homologs for enzymes of the tryptamine (TAM) and indole-3-acetaldoxime (IAOx) Trp-dependent IAA biosynthesis pathways were identified in the *E. siliculosus* genome. An auxin responsive gene, *EsGRP1*, was identified using micro-array analysis, which showed increased expression levels in mutants affected in their apical dominance ***(198)***.

Tracing the auxin action in brown algae may provide valuable answers to evolutionary developmental questions in plant systems. For example, it has been argued that tracing the evolutionary patterns of auxin action can more easily uncover how regulation of IAA action relates to organogenesis as more basal plants show a simpler regulation than land plants ***(199, 200)***. The brown algal clade might provide an independent test case for the hypothesis that simple body plans like the filamentous *Ectocarpus* or the parenchymatous *Dictyota* or *Fucus* are characterized by homeostasis established by regulation of IAA biosynthesis and degradation rather than by conjugation ***(190, 198, 199)***.

4.2 Auxin function as a homoplasious or homologous character?

Various explanations may be applied to account for the putative function of auxin as a phytohormone in brown algae. *In silico* analyses of whole genome sequences and EST libraries

demonstrated that the canonical auxin response system and auxin transport mechanism of land plants evolved gradually in the streptophyte lineage ***(201)***. Homologues of key enzymes (e.g. ARF's, TIR1, PIN like proteins) were not found in the genomes of unicellular green algae. An AUX–IAA-ARF-mediated signalling system is absent from other unicellular photosynthetic algal lineages and from *Ectocarpus* ***(41, 198)***. These results, therefore, do not support a common origin of an AUX–IAA-ARF-mediated signalling mechanism.

Alternatively, several physico-chemical characteristics render IAA an ideal signalling molecule and therefore make convergent evolution a plausible scenario. Its size leads to rapid diffusion in aqueous solutions. When protonated (pKa 4.75) IAA becomes lipophilic, readily diffuses through cell walls and most eukaryotes have the machinery to synthesize IAA ***(41)***. It is therefore possible that Phaeophyceae evolved convergently alternative auxin-dependent signalling and transport mechanisms. The absence of a common origin of an AUX–IAA-ARF-mediated signalling mechanism in *E. siliculosus* and unicellular Viridiplantae, however, does necessarily mean that auxin function in brown algae has no homologous origin (by either vertical or horizontal gene transfer) and that the auxin pathway had to evolve from scratch. Firstly, Cooke ***(199)*** proposed that IAA initially served as a growth-regulating pheromone in photosynthetic unicellular aquatic organisms. The possibility of alternative pathways for auxin signalling in unicellular green algae suggested by putative IBR5 or ABP1 homologues ***(201)*** and reports on IAA function in polarization (e.g. of *Fucus* zygotes), or cell division of unicellular organisms or rhizoid growth suggest that auxin function predates the advent of multicellularity ***(41, 201)***. Secondly, the only whole genome sequences available from respectively the red and the brown lineage are the ones of *Cyanidioschyzon merolae* (unicellular) and *E. siliculosus* (filamentous) which have both a relatively simple body plan and a relatively small genome ***(36, 202, 203)***. As complexity in green plants is thought to be linked to the number of auxin genes ***(204)***, this developmental simplification and genome reduction might have resulted in the secondary loss of auxin related genes, leaving only scarce traces of the common origin and subsequent independent divergence. Indeed some putative

homologs of the ABCB efflux IAA transporter family, in *E. siliculosus* ***(198)*** and single celled members of Chlorophyta ***(201)*** have been found. Also a putative homolog for BIG, a callosin-like protein needed for auxin mediated inhibition of PIN protein endocytosis ***(205)***, was found in *E. siliculosus.*

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5. Future perspectives

Over one century of research on algal development, mainly focussing on embryogenesis and patterning of fucoid zygotes, has strongly positioned brown algae as widely used model organisms to study polarization and asymmetrical cell division. In addition, the rich variation in life cycles and associated developmental control of the gametophyte and sporophyte generations make the Phaeophyceae an extremely interesting group of organism. Studies on the relations between life cycle and developmental patterns is spearheaded by *E. siliculosus*, which has the benefit of having its whole genome sequenced as well as being easily cultured. Classical genetics have been proven to be a valuable tool for the research in the mechanisms underlying *E. siliculosus* development and the control of its life cycle ***(31, 35, 198)***.

Importantly, in spite of the extensive knowledge of brown algal embryogenesis and development, tools enabling functional characterization (e.g. transformation, RNAi) have yet to be developed for brown algal model systems. Development of transformation protocols may highlight the need for additional species which in contrary to the fucoids can be cultured throughout their life cycle. Completion of the fucoid life cycle in laboratory conditions is extremely complicated ***(206)***, posing obvious problems for performing mutant analysis and stable transformations. From a comparison of several brown algae as candidates for genomic research ***(207)***, *Dictyota dichotoma* emerged as a valuable option which may be complementary to *Fucus*. Just like the latter, *Dictyota* has evolved parenchymatous growth (Figure 2), though the tissue of *Dictyota* remains thin enough to allow lab cultures and consequently has been cultured in the lab for decades ***(208, 209)***. The ability to culture

Dictyota throughout its life cycle enables crossing experiments, and potentially maintenance of isolated mutants or transformations for longer time periods. In addition, *Dictyota* is one of few oogamous brown algae where large eggs are suspended in the medium after egg release. Preliminary findings suggest that different but complementary insights on polarization and asymmetric cell division can be gained by using *Dictyota* (Bogaert & De Clerck, unpublished data). In addition to its tractability, the distinct phylogenetic position as a heterokont in the eukaryotic tree of life (Figure 1) and as a member of the early diverging SSDO group in the brown algal phylogeny ***(210)*** makes *Dictyota* an interesting physiological and phylogenomic data point for unravelling a plethora of evolutionary developmental questions, such as for example the evolutionary origin of auxin action in the regulation of the plant body plan.

The limited rather 'patchy' taxonomic sampling of plants systems concentrating almost exclusively on the green plant lineage (Viridiplantae) is hampering comparative genomics and phylogenomics ***(15, 211)***. Sequencing projects of multicellular 'brown' and 'red' plant systems as well as the further development of tools enabling functional characterization of genes will undoubtedly provide important insights on the development of complex multicellular organisms outside of the well-trodden domain of 'higher plants' and offer a more inclusive view on plant morphogenesis.

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