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Asymmetric Cell Divisions: Zygotes of Fucoid Algae as a Model System

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Abstract Asymmetric cell divisions are commonly used across diverse phyla to generate different kinds of cells during development. Although asymmetric divisions play important roles during development in plants, algae, fungi, and animals, emerging data indicate that there is some variability amongst the mechanisms that are at play in these different organisms. Zygotes of fucoid algae have long served as models for understanding early developmental processes including cell polarization and asymmetric cell division. In addition, brown algae are phylogenetically distant from other organisms, including plant models, a feature that makes them interesting from a comparative perspective (Andersen 2004; Peters et al. 2004). This monograph focuses on advances made toward understanding how asymmetric divisions are regulated in fucoid algae and, where appropriate, comparisons are made to higher plant zygotes.

1 Introduction

How does a single cell, the zygote, give rise to a complex organism with many different cell and tissue types? The answer to this question lies in the ability of cells in a growing embryo to acquire separate identities, a feat that is often accomplished by asymmetric cell divisions. By definition, asymmetric cell divisions produce nonidentical daughter cells and can thereby initiate the process of cell differentiation. Asymmetric cell divisions are known to play important roles in development across diverse plant and algal phyla. Examples include the first cell division in many zygotes (Brownlee 2004; Gallagher and Smith 1997; Okamoto et al. 2005; Zernicka-Goetz 2004), as well as the production of gonidial and somatic cells in *Volvox carteri* (Kirk 2004; Schmitt 2003), reproductive initial cells from caulonema filaments in moss (Cove et al. 2006; Schumaker and Dietrich 1998), rhizoids from prothalli cells in ferns (Murata and Sugai 2000), stomata on the epidermal surfaces of leaves (Lucas et al. 2006; Nadeau and Sack 2002, 2003), and microspores during pollen development (Park et al. 2004; Twell et al. 1998). Because of the importance of asymmetric divisions in development, the mechanisms that regulate the pro-

cess are under investigation in several model organisms. In this monograph, we focus on advances made toward understanding how asymmetric divisions are regulated in zygotes of fucoid brown algae.

1.1 Asymmetric Divisions and Cell Fate Decisions

Generally, there are three ways by which the products of an asymmetric division acquire separate identities (Fig. 1):

1. Developmental determinants can be differentially partitioned between cells during division. In this case, each cell inherits a different set of cytoplasmic instructions that lead it down a unique developmental pathway. Because cell fate is controlled by determinants located within the cytoplasm, this type of development is often referred to as intrinsic or cell-autonomous (Fig. 1a). Both the first division of the *Caenorhabditis elegans* zygote and the divisions of neuroblasts in *Drosophila melanogaster* embryos represent examples of asymmetric divisions in which intrinsic factors control daughter cell fates (Betschinger and Knoblich 2004; Cowan and Hyman 2004).
2. In some cases, the cytokinetic plane is positioned such that the daughter cells are placed in different locations within the developing organism. Each cell then receives a unique set of positional cues from neighboring cells or the environment that dictate its fate (Fig. 1b). Since cell identities are determined by signals received from external sources, this type of development is known as extrinsic or non-cell-autonomous. In the *Arabidopsis thaliana* root, for example, the decision to become either an endodermal or a cortical cell depends on an asymmetric cell division that places daughter cells in different cell files in the root. Signals from neighboring cells then direct the daughters down different developmental pathways (Heidstra et al. 2004; Scheres et al. 2002).
3. An asymmetric division can produce daughters of different sizes and/or shapes, and these morphological differences determine the developmental pathway that each cell will follow (Fig. 1c). In *V. carteri*, asymmetric divisions generate small and large daughter cell pairs, and the size of the cell then activates either a somatic or a germline developmental program (Cheng et al. 2005; Kirk et al. 1993; Schmitt 2003).

Asymmetric divisions are commonly regulated in a three-step process. In the first step, cells polarize (Fig. 2). Sometimes there are obvious cytological or morphological changes associated with cell polarization while in other cases the polarity is more subtle, and may be manifested simply by the fact that the ends of the cell lie in different positions in the developing organism. After cell polarization, the mitotic apparatus (step 2) and the site of cytokinesis (step 3) must be positioned appropriately with respect to the axis defined by

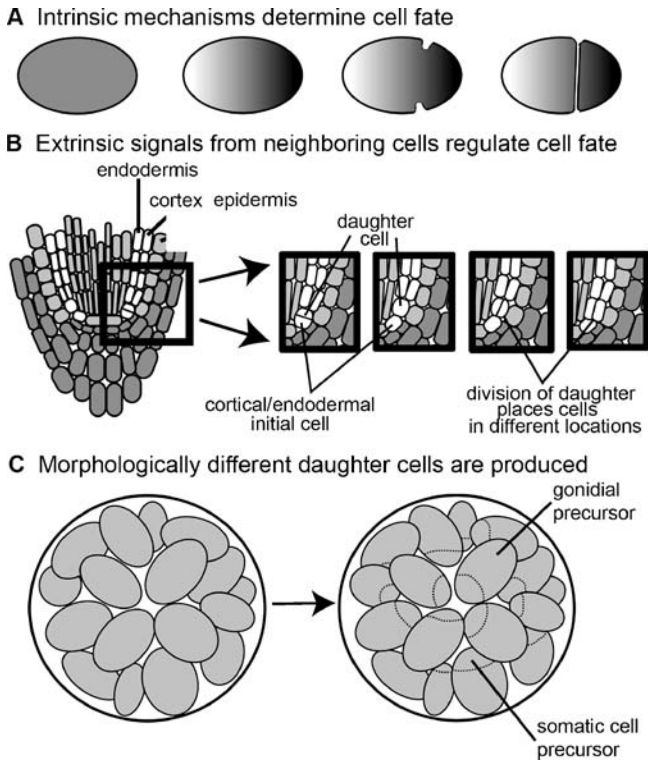

Fig. 1 Mechanisms by which asymmetric cell divisions generate diverse cell types during development. **a** In *C. elegans* zygotes, developmental determinants (*gray shading*) are segregated to one end of the zygote. When cytokinesis occurs, the daughter cells each inherit cytoplasm that is qualitatively different. **b** In *A. thaliana* roots, cortical and endodermal cells are produced through cell divisions that ultimately place cells in different files within the developing root. A cortical/endodermal initial cell divides to produce a daughter cell. When the daughter divides, the cell plate is laid down parallel with the longitudinal axis of the root, placing the two new cells in different cell files. Signals from neighboring cells then direct the adoption of either a cortical or an endodermal fate (redrawn from Scheres et al. 2002). **c** Asymmetric cell divisions in *V. carteri* embryos generate larger cells that will become reproductive gonidia and smaller, somatic cell precursors (Green and Kirk 1981). *Dashed lines* indicate sites of cytokinesis

the polarity of the cell. Alignment of the mitotic apparatus with the cellular axis ensures that each daughter will inherit both the appropriate cellular domains and a full chromosomal complement after division. During cytokinesis, the cell plate is positioned to bisect both the mitotic apparatus and the cellular axis correctly. Although cells usually polarize first, the order in which the latter two steps occur can vary. In zygotes of fucoid algae, for example, the mitotic apparatus is positioned first and then the site of cytokinesis is specified by the position of the mitotic apparatus (Fig. 2a; Bisgrove et al. 2003).

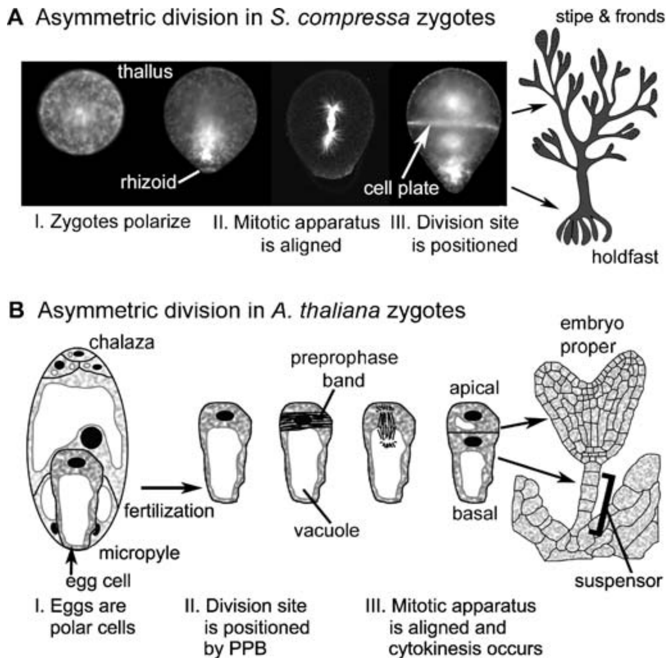

Fig. 2 Asymmetric cell divisions are commonly regulated in three steps. **a** *Silvetia compressa* eggs are spherical in shape with no obvious asymmetries, and polarization (I) is first manifested morphologically several hours after fertilization when increased secretion on one hemisphere produces a bulge, the rhizoid. The opposite end of the zygote is termed the thallus, and the axis defined by the two poles is the rhizoid/thallus axis. Next, the mitotic apparatus aligns parallel with the rhizoid/thallus axis (II). Finally, cytokinesis occurs and the cell plate is positioned perpendicular to the rhizoid/thallus axis (III). The three zygotes shown in the panels corresponding to I and III were stained with fluorescein diacetate which labels the cell plate, perinuclear regions, and cytoplasm. The zygote in II is in metaphase and was labeled with anti-alpha tubulin antibodies (image kindly provided by Nick T. Peters). **b** Asymmetric divisions in *A. thaliana* zygotes are also regulated in a three-step process, but the order in which the steps occur is different than it is in fucoid algae. In plants, polarity is acquired by the egg cell during development of the embryo sac (I). After fertilization, a preprophase band of microtubules marks the position of the first zygotic division (II) and then the mitotic apparatus is positioned with respect to both the cellular axis and the predetermined division site (Webb and Gunning 1991). Drawn using Drews and Yadegari (2002), Mayer et al. (1993), and Webb and Gunning (1991) as guides

In this case, proper placement of the spindle is required for correct specification of the cytokinetic site. Alternatively, the site of cytokinesis can be specified prior to mitosis in accordance with cues located in the cortex of the cell (Fig. 2b). Because the site of cytokinesis is determined before mitosis, this mechanism does not require precise positioning of the spindle. Instead, the mitotic apparatus needs only to align well enough to ensure that each daugh-

ter cell inherits a nucleus after telophase. This method is commonly employed by plant somatic cells, including zygotes. In these cells, a preprophase band of microtubules transiently forms in the cell cortex and marks the upcoming division site (Brown and Lemmon 2001; Marcus et al. 2005; Webb and Gunning 1991).

2 Zygotes of Fucoid Algae as a Model System

Zygotes of fucoid algae have, for many years, been a fruitful system in which to study the mechanisms by which cells acquire polarity and regulate asymmetric cell divisions, mainly because they are easy to manipulate and analyze in the laboratory (for recent reviews, see Brownlee 2004; Katsaros et al. 2006). Fucoid algae are marine brown algae, belonging to the Phaeophyceae class of stramenopiles (Andersen 2004). In nature they grow attached to rocks in the intertidal zone where they reproduce by releasing large, spherical eggs and biflagellated, motile sperm into the surrounding seawater. Gamete release can be induced from reproductive fronds in the lab and thousands of synchronously developing zygotes are easy to obtain for experimental analyses. The zygotes are relatively large, up to 100 μm in diameter, a size that renders them amenable to micromanipulation and analyses that require spatial measurements of subcellular features. Soon after fertilization zygotes settle onto the substratum, a rock in the intertidal zone, or a coverslip in the lab, and a sticky adhesive is secreted that firmly anchors them in place. Eggs are spherically shaped cells with no detectable asymmetries. However, within the first few hours following fertilization there are extensive cytoplasmic and morphological changes that result in asymmetric cells with rhizoid and thallus poles (Fig. 2a). To establish polarity, zygotes sense a wide array of environmental cues, although light is probably the dominant signal in nature. Zygotes developing in unidirectional light form rhizoids on their shaded hemispheres. An early sign of polarity is the preferential localization of secretion to the rhizoid pole, and increased secretion at this pole eventually produces a bulge, the tip-growing rhizoid (Fig. 2). When the first division occurs, about 24 h after fertilization (AF), it is oriented transverse to the rhizoid/thallus axis and bisects the zygote into two morphologically distinct cells with different developmental fates. The thallus cell gives rise to most of the photosynthetic and reproductive organs of the mature alga, while the rhizoid cell eventually becomes the holdfast that anchors the alga to its rock on the beach.

The first zygotic division in higher plants is also an asymmetric one that produces two morphologically distinct daughter cells with different developmental fates (Fig. 2b). The smaller apical cell is cytoplasmically dense and its progeny give rise to most of the developing embryo, while the larger, vacuolate basal cell divides only a few more times to form a single file of cells. The

uppermost cell in this file becomes part of the root meristem and the remaining cells form the suspensor, a structure that attaches the embryo to the ovule (Laux et al. 2004; Souter and Lindsey 2000; Torres-Ruiz 2004). Although the developmental pattern that is set up by the first zygotic cell division is similar in plants and fucoid algae, there are key mechanistic differences between the two. In many plants, for example, polarity arises in the egg prior to fertilization rather than in the zygote. Plant eggs and zygotes are also buried within the ovule where their development can be influenced by surrounding maternal tissues. Zygotes of fucoid algae, on the other hand, are free-living and they develop in response to vectorial information in the environment such as sunlight from above (Brownlee 2004). Because plant eggs and zygotes are relatively inaccessible, approaches that involve manipulating individual cells are difficult. Instead, molecular/genetic analyses of mutants are being used to address questions of cell polarity and asymmetric divisions. This research is yielding interesting data, but our understanding of how asymmetric divisions are regulated in plant zygotes is still rudimentary. In contrast, the free-living zygotes of fucoid algae are easy to access and are amenable to physical manipulations. Over the years research on fucoid algae has provided a wealth of mechanistic data and, although many questions still remain, we are beginning to understand how asymmetric cell divisions are regulated in these zygotes.

3 Polarization and Germination in Zygotes

Fucoid zygotes have long served as a paradigm for investigating the mechanisms by which polarity is established following fertilization. In 1920, Hurd reported that monochromatic blue light polarizes zygotes (Hurd 1920), and since that time many other vectorial cues, including electrical, ionic, and osmotic gradients, have been shown to induce a growth axis (for a review, see Jaffe 1969). These diverse stimuli likely activate distinct signal transduction pathways that converge at a common response, formation of a growth axis (Kropf et al. 1999). The presumed goal is to maximize the chance that the rhizoid will grow into a crevice on the rocky surface and thereby permanently anchor the developing embryo in the turbulent intertidal environment.

But when is polarity first set up? Is the fertilized egg apolar until it senses its environment? Recent work has shown that in fact polarity is first set up at fertilization (Hable and Kropf 2000). Sperm entry induces a rhizoid pole to form at that site and a branching actin network rapidly assembles in the cell cortex there (Fig. 3a). A zygote has a greater density than seawater and settles rapidly onto the rocky substratum with its sperm-induced rhizoid pole randomly oriented with respect to the surface. Over the next 2 h the sperm

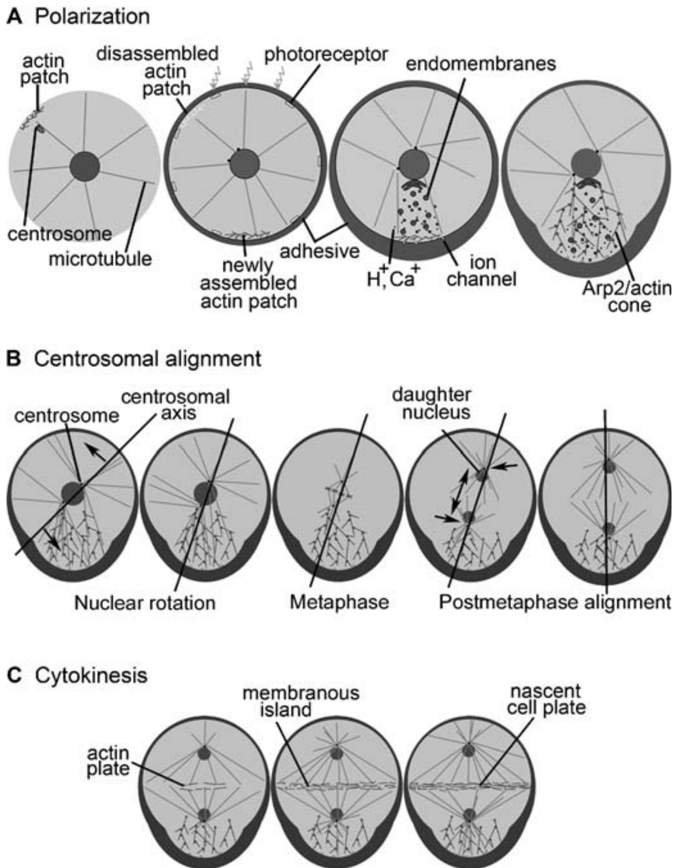

Fig. 3 Mechanism of asymmetric cell division in zygotes of fucoid algae. **a** Fertilization induces formation of a cortical actin patch that marks the rhizoid pole of a default axis. Photopolarization causes disassembly of the sperm-induced patch and assembly of a new patch at the shaded pole. Endomembrane cycling then becomes focused to the rhizoid pole as the nascent axis is amplified, and cytosolic ion gradients are generated. At germination, the actin array is remodeled into a cone nucleated by the Arp2/3 complex. During early development the paternally inherited centrosomes migrate to opposite sides of the nuclear envelope and acquire microtubule nucleation activity, but microtubules play only an indirect role in polarization. See text for details. **b** Centrosomal alignment begins with a premitotic rotation of the nucleus that partially aligns the centrosomal axis (defined by a line drawn through the two centrosomes) with the rhizoid/thallus axis. When the metaphase spindle forms it is partially aligned with the rhizoid/thallus axis. Postmetaphase alignment brings the telophase nuclei into almost perfect register with the rhizoid/thallus axis. *Arrows* indicate directions of nuclear movements. **c** Cytokinesis is positioned between the two daughter nuclei. A plate of actin assembles in the midzone between the nuclei, then membranous islands are deposited in the cytokinetic plane. The islands consolidate and cell plate materials are deposited in the division plane. All of these structures mature centrifugally, beginning in the middle of the zygote and progressing outward to the cell cortex

pronucleus migrates to the egg pronucleus utilizing microtubules (Swope and Kropf 1993), and the zygote secretes a cell wall (Quatrano 1982) and an adhesive that attaches it firmly to the rock (Hable and Kropf 1998). Once attached, the young zygote monitors its environment for positional information. Perceived environmental cues are integrated and used to specify a new growth axis that is appropriate for the environmental context. Under normal growth conditions the sperm-induced axis is usually overridden by environmental cues, and it can therefore be considered a default axis to be used only if the zygote fails to perceive positional information.

Unidirectional light is probably the most relevant vector in the intertidal environment, and is easy to apply in a laboratory setting. Photopolarization induces a new rhizoid pole on the shaded hemisphere (Fig. 3a), toward the rocky substratum. Although zygotes can perceive different light qualities, blue light is most effective. The photoreceptor is thought to reside at or near the plasma membrane (Jaffe 1958), and may be a rhodopsin-like protein (Gualtieri and Robinson 2002; Robinson et al. 1998). How light perception on one hemisphere of the zygote is transduced into a rhizoid pole on the opposite hemisphere is not well understood, but may involve formation of cGMP gradients resulting from differential photoreceptor activation (Robinson and Miller 1997) and/or activation of a plasma membrane redox chain on the shaded hemisphere (Berger and Brownlee 1994). Pharmacological studies indicate that photopolarization also requires signaling through a tyrosine kinase-like protein (Corellou et al. 2000a). At the downstream end, signal transduction results in depolymerization of the cortical actin at the sperm-entry site and polymerization of a new branching actin network nucleated by the Arp2/3 complex at the new rhizoid pole (Alessa and Kropf 1999; Hable et al. 2003; Hable and Kropf 2005). Thus, cortical actin localization is a faithful marker of the existing developmental axis.

Beginning about 4 h AF, the existing axis becomes steadily reinforced, or amplified. The essence of axis amplification is targeting of the endomembrane system and generation of cytosolic ion gradients (Fig. 3a). Both endocytotic and exocytotic limbs of membrane cycling are dispersed throughout the cytoplasm in young zygotes, but gradually become focused to the rhizoid pole (Hadley et al. 2006). This results in preferential secretion of adhesive at the rhizoid and may also establish a cortical domain with unique molecules in the rhizoid membrane and/or cell wall (Belanger and Quatrano 2000b; Fowler and Quatrano 1997). Simultaneously, cytosolic gradients of H^+ and Ca^{2+} are generated with highest activity at the rhizoid pole (Berger and Brownlee 1993; Kropf et al. 1995; Pu and Robinson 2003). Cytosolic H^+ and Ca^{2+} gradients and endomembrane cycling may comprise a positive feedback loop in which local elevation of H^+ and Ca^{2+} activity stimulate secretion and insertion of ion transporters at the rhizoid pole, thereby strengthening the ion gradients and promoting further secretion. However, it should be noted that to date there is no direct evidence for transporter accumulation at the rhi-

zoid pole. Surprisingly, the axis remains labile throughout the amplification period; when the direction of the light vector is changed actin, endomembranes, and ion gradients reposition to the new rhizoid pole.

Just prior to germination, the developmental axis becomes fixed in space and insensitive to subsequent environmental cues. Axis fixation is thought to involve formation of axis-stabilizing complexes at the rhizoid pole comprised of transmembrane bridges from the cortical actin to sulfated polysaccharides in the cell wall (Fowler and Quatrano 1997). Total mRNA accumulates at the thallus pole during axis fixation (Bouget et al. 1996), and some localized mRNAs may serve as developmental determinants that are asymmetrically partitioned when the zygote divides.

Rhizoid outgrowth denotes germination and is driven by an increase in targeted secretion. The branching Arp2/actin network expands dramatically at germination forming a continuum that extends from the rhizoid face of the nuclear envelope to the cortical domain in the rhizoid tip (Fig. 3a; Hable and Kropf 2005). The very apex is relatively devoid of cytoskeleton and is filled with secretory vesicles, as has been observed in other tip growing cells including pollen tubes (Lovy-Wheeler et al. 2005). Germinated zygotes exhibit negative phototropism, which is preceded by a shift in the actin array and the vesicle accumulation zone toward the shaded side of rhizoid where new growth becomes focused (Hable and Kropf 2005). These and other findings (Brawley and Quatrano 1979) suggest that the extensive actin array transports secretory vesicles from Golgi to the apical growth site. Microtubules are not required for polarization or germination, but may help organize the actin/endomembrane system. Microtubule depolymerization or stabilization results in a more dispersed endomembrane system (Hadley et al. 2006) and fat rhizoids (Kropf et al. 1990).

4
Microtubules and Asymmetric Cell Division

Although microtubules are not required for polarization or germination, they are essential for cell division. They are the major structural component of the mitotic spindle, and their organization within the cell determines both the position of the mitotic apparatus and the placement of the cell plate during division. How, then, are microtubules organized in developing zygotes? Like animals, fucoid algae have discrete microtubule organizing centers called centrosomes that regulate the distribution and organization of microtubules in the cell (Fig. 3b; Bisgrove et al. 1997; Motomura and Nagasato 2004; Nagasato et al. 1999). Hence, the location of the centrosomes during cell division determines both the position of the mitotic apparatus and the subsequent site of cell plate deposition. Because of their importance, the centrosomes have been monitored in zygotes during polarization and cell division.

4.1 Microtubule Organization During Polarization

Unfertilized eggs do not have centrosomes and microtubules emanate from the nucleus in an array that is evenly dispersed around the nuclear periphery (Bisgrove et al. 1997; Motomura 1994; Nagasato et al. 1999). The centriolar components of the centrosomes are acquired from the flagellar basal bodies of the sperm at fertilization (Fig. 3a). Since sperm are biflagellated, the egg receives two centrioles; they migrate with the sperm pronucleus through the cytoplasm and are deposited on the nuclear envelope at karyogamy (Bisgrove et al. 1997; Motomura 1994; Motomura and Nagasato 2004; Nagasato et al. 1999; Nagasato 2005; Swope and Kropf 1993). As development proceeds, the centrosomes slowly separate from each other by migrating around the nucleus until they reach positions on opposite sides of the nuclear envelope. At the same time, there is a gradual reorganization of the microtubules into an array in which microtubules emanate mainly from the two perinuclear centrosomes outward into the cortex of the cell. These steps occur over several hours and are not completed until shortly before zygotes enter mitosis, about 16 h AF. Although centrosomal separation does occur concurrently with polarization of the zygote, the two processes appear to proceed independently of each other since treatments that inhibit polarization or tip growth do not affect centrosomal separation and vice versa (Bisgrove and Kropf 1998).

Just prior to mitosis, the centrosomes come to rest on opposite sides of the nucleus and microtubules extend from them out into the cortex of the cell. The rhizoid appears to provide a favorable environment for microtubules, since they are more abundant in this part of the zygote. In addition to the microtubules that emanate from the centrosomes, recent studies in living zygotes microinjected with fluorescently labeled tubulin have revealed a cortical array that is not seen in fixed preparations (Corellou et al. 2005). In young zygotes the cortical microtubules are randomly arranged and distributed evenly around the cell. However, as zygotes develop, the cortical microtubules localize preferentially to the presumptive rhizoid where they become denser as zygotes germinate and the rhizoid elongates. Although the function of these cortical microtubules is unknown, it has been postulated that they might be involved in shaping the rhizoid as it grows. The microtubules appear to originate in the cell cortex where they form an array that is not contiguous with the centrosomes. It is, therefore, unlikely that the cortical microtubules are involved in positioning the mitotic apparatus or the division site (Corellou et al. 2005). Nonetheless, the abundance of both centrosomal and cortical microtubules suggests that the rhizoid provides an environment conducive to microtubule assembly and/or stabilization.

4.2
Positioning the Mitotic Apparatus

When zygotes enter mitosis the centrosomes form the poles of the metaphase spindle, and their position determines the placement of the spindle. Initially, the centrosomal axis, defined by a line drawn through the two centrosomes, is not well aligned with the rhizoid/thallus growth axis (Fig. 3b). However, before zygotes enter mitosis there is a nuclear rotation that partially aligns the centrosomal axis with the growth axis and results in crudely aligned metaphase spindles (Allen and Kropf 1992; Bisgrove and Kropf 1998, 2001; Corellou et al. 2000b). Alignment of the centrosomes continues as zygotes progress through mitosis, and by the end of telophase the centrosomal axis is parallel with the growth axis (Bisgrove and Kropf 2001).

Treating zygotes with a battery of inhibitors at different times during centrosomal alignment disrupts the premetaphase rotation of the nucleus but does not affect alignment during telophase, suggesting that the pre- and postmetaphase alignments are mechanistically different. The existing evidence supports a model in which premetaphase nuclear rotation is effected by microtubules that extend from the centrosomes out toward the cortex of the zygote (Allen and Kropf 1992; Bisgrove and Kropf 1998). These microtubules are most likely dynamic, growing out from the centrosomes and disassembling back toward them. Microtubules that reach the cell cortex appear to be captured in the actin-containing bridges that link the plasma membrane to the cell wall, since treatments that affect actin or the cell wall also disrupt nuclear rotation (Alessa and Kropf 1999; Bisgrove and Kropf 2001; Henry et al. 1996). Actin–cell wall bridges are concentrated in the rhizoid apex (Henry et al. 1996) and so microtubules are preferentially captured there. Motors located either at the centrosome or the cortex are postulated to exert a pulling force on the captured microtubules. By chance, one centrosome usually resides closer to the rhizoid apex; this centrosome will have more microtubules in contact with the cortex and will be pulled toward the rhizoid apex. The other centrosome will move toward the thallus pole, resulting in a rotation that partially aligns the centrosomal axis. A similar microtubule-based "search and capture" mechanism is thought to align the mitotic apparatus in budding yeast and animal cells (reviewed by McCarthy and Goldstein 2006). When the metaphase spindle forms, it is crudely aligned with the rhizoid/thallus axis. Spindle formation requires the activities of Kinesin-5 motors to maintain spindle bipolarity. In addition, Kinesin-5 motors also appear to be involved in maintaining the integrity of spindle poles in fucoid algae, an activity that has not yet been reported for these motors in other cell types (Peters and Kropf 2006).

As zygotes exit metaphase, the centrosomal axis continues to align, albeit by a mechanism that appears to be different from the nuclear rotation that occurs before mitosis. Although this phase of alignment is not well under-

stood, it is temporally associated with an elongation of the mitotic apparatus that occurs during anaphase and telophase (Fig. 3b). One possibility is that microtubule-based centering mechanisms acting on the centrosomes during spindle elongation could contribute to this phase of alignment (Bisgrove and Kropf 2001). Centrosomal centering involves interactions of microtubule ends with stationary objects such as the periphery of the cell. Polymerizing microtubules that impact the cell boundary can exert a force that pushes the centrosome toward the middle of the cell or, alternatively, cytoplasmic motors acting on shortening microtubules can pull the centrosome toward the cortex (Howard 2006). In theory, in a cell that is longer than it is wide, centrosomal centering forces could align the anaphase/telophase mitotic apparatus if the centrosomes move as a unit. Similar microtubule-based forces appear to be involved in centering the nucleus in fission yeast cells (for example see Daga et al. 2006).

4.3 Cytokinesis

By the end of telophase, the centrosomal axis is aligned parallel with the rhizoid/thallus axis. Microtubules radiating from the centrosomes on the daughter nuclei meet and interdigitate in the midzone of the remnant spindle. The zone of microtubule overlap extends outward to the cell cortex and predicts the position of the future division site (Bisgrove et al. 2003). During cytokinesis, a plate of actin first appears in the zone where microtubules meet, and then membrane is deposited in islands throughout the cytokinetic plane (Fig. 3c). The membranous islands fuse into a continuous compartment into which cell wall materials are deposited. All of these structures mature in a centrifugal fashion, from the center of the cell outward (Belanger and Quatrano 2000a; Bisgrove and Kropf 2004). Similar cytoskeletal arrays have been observed in other brown algal cells during cytokinesis (Karyophyllis et al. 2000; Katsaros et al. 1983, 2006; Katsaros and Galatis 1992; Nagasato and Motomura 2002a,b; Varvarigos et al. 2005). Plant cells also divide centrifugally, but they utilize a unique microtubule-based structure, the phragmoplast, during cytokinesis (see Jurgens 2005 for a recent review).

How is the division site chosen? In general, there are two ways by which cells determine a site for cytokinesis:

1. In metazoan, protist, and some plant cells the position of the mitotic apparatus during metaphase/anaphase or telophase determines the site of cytokinesis. In animal cells cytokinesis occurs by furrowing, and spindle microtubules appear to deliver signals to the cell cortex that determine the site of furrow formation (reviewed by Wadsworth 2005). Similarly, during cellularization in endosperm and female gametophytes, radial microtubules define cellular spaces around nuclei and cell plate deposition

occurs at the boundaries (Brown and Lemmon 2001; Otegui and Staehelin 2000; Pickett-Heaps et al. 1999).
2. Alternatively, in somatic plant cells, fission yeast, and budding yeast, cell polarity specifies the site of cytokinesis in accordance with localized cortical cues. In these cells the site of cytokinesis is determined before mitosis rather than by the mitotic apparatus during or after the nuclear division (Arkowitz 2001; Hoshino et al. 2003; Marcus et al. 2005; Wasteneys 2002; Wu et al. 2003).

In fucoid algae, the position of the two daughter nuclei at the end of telophase determines the division site. This conclusion is based on experiments in which the colinearity between the telophase nuclei and the rhizoid/thallus axis was uncoupled. Cytokinesis always occurred between telophase nuclei rather than perpendicular to the rhizoid/thallus axis, indicating that it is nuclear position and not cell polarity that defines the site of cytokinesis (Bisgrove et al. 2003). At the time of cytokinesis, microtubules radiating from the centrosomes define domains around the nuclei. Cytokinesis occurs in the zone of microtubule overlap between telophase nuclei, in a manner similar to the cellularization that occurs in endosperm and female gametophytes.

5
Zygotic Cell Division and Cell Fate Decisions

Is proper placement of the zygotic division developmentally important in fucoid algae? If so, why? Generally, there are three ways by which asymmetric cell divisions influence cell fate decisions: via the intrinsic, extrinsic, and morphological pathways discussed above. In fucoid algae, there is evidence indicating that all three pathways may be operational. Zygotic polarity develops in response to positional cues from the environment (extrinsic signals). Sperm entry and environmental vectors, light or ion gradients for instance, determine where the rhizoid will form. When the zygote divides, rhizoid and thallus cells of different shapes are produced, and there is evidence to support the idea that these morphological differences are developmentally important. Pulse-treating zygotes with pharmacological agents that perturb either the cytoskeleton or secretion disrupts placement of the division and can affect subsequent embryogenesis. In particular, severely misaligned divisions in which the cell plate bisects the rhizoid tip disrupt the ability of embryos to elongate their rhizoids normally. Rhizoid extension is either blocked or two rhizoids are initiated, depending on the pharmacological agent used (Bisgrove and Kropf 1998; Shaw and Quatrano 1996). Finally, there is also evidence that suggests developmental determinants may be asymmetrically partitioned between rhizoid and thallus cells when the zygote divides (intrinsic signals). Poly(A)+ RNA is preferentially segregated to the thallus in

germinated zygotes and two-celled embryos, and this asymmetric distribution of mRNA could play a role in determining cell fates (Bouget et al. 1995). Also, in an elegant set of laser microsurgery experiments, Berger et al. (1994) found that thallus cells quickly redifferentiated into rhizoid cells once they contacted residual cell wall from an ablated rhizoid, suggesting that developmental determinants might be localized in the rhizoid cell wall. Curiously, Bisgrove and Kropf (1998) found that moderate misalignments of the zygotic division had little effect on subsequent development. This observation suggests that if the division does segregate determinants, they are either tightly localized to the apical wall or daughter cell fates do not depend on precisely partitioning them.

In plants, assessing how the first zygotic division influences development is difficult because the relevant cells are buried deep within the maternal tissues of the ovule. Nonetheless, there is reason to believe that intrinsic, extrinsic, and morphological pathways may also have roles in plant zygotes and young embryos. Plant cells commonly make cell fate decisions in response to positional information (extrinsic cues), and genetic studies indicate that gametophytic and sporophytic tissues surrounding the zygote contribute to its development (reviewed by Laux et al. 2004). In addition, analyses of expression patterns have identified transcripts that are expressed in the zygote and differentially localized to either the apical or the basal cell of the two-celled embryo, suggesting that the first zygotic division differentially partitions determinants (Haecker et al. 2004; Laux et al. 2004; Lukowitz et al. 2004; Okamoto et al. 2005; Weterings et al. 2001). Finally, embryos of mutants with mispositioned division planes, such as *fass* and *gnom*, have morphological defects, suggesting that division plane alignment is important for morphogenesis (Busch et al. 1996; Geldner et al. 2003; Mayer et al. 1993; McClinton and Sung 1997; Shevell et al. 1994; Torres-Ruiz and Jurgens 1994).

6
Conclusions and Future Directions

Analyses conducted over the last several years have provided us with a basic understanding of how asymmetric cell divisions are regulated in zygotes of fucoid algae. The emerging evidence indicates that there are mechanistic differences between asymmetric divisions in brown algal and plant zygotes, a fact that is not surprising given the large phylogenetic distances that separate the two groups. In plants, the availability of genomic resources and molecular/genetic techniques are facilitating the identification of molecules that may play roles in asymmetric divisions and cell fate decisions. The lack of these resources for any species in the phaeophyte lineage has been perhaps the largest technical hurdle hindering molecular analyses in the brown algae. Recently, a project to sequence the genome of the marine brown alga *Ectocarpus*

siliculosis was initiated by the French sequencing center GENOSCOPE (Peters et al. 2004). This project will move brown algae forward into the molecular/genomics era and enhance the feasibility of comparative analyses between phaeophytes and other eukaryotic lineages at the molecular level.

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