Induction of Polarity in Fucoid Zygotes

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INTRODUCTION

Early events in the vegetative development of higher land plants are difficult to investigate because the young embryo is encased in the ovarian tissue of the previous gametophytic and sporophytic generations. Recently, genetic approaches have succeeded in identifying mutations that alter early development (Jürgens, 1995; see also Laux and Jürgens, 1997, in this issue), but cellular and physiological analyses of higher plant embryogenesis remain tedious.

Brown algae of the family Fucales (comprising the genera Fucus and Pelvetia) are, however, very tractable for investigating early plant embryogenesis, especially the establishment of developmental polarity. These marine organisms grow attached to rocks in the intertidal zone and are typically 0.25 to 0.5 m in height at maturity. Zygotes, eggs, and sperm are shed from fronds of mature plants in the laboratory and can easily be harvested in gram quantities, free of other tissues. Young zygotes attach firmly to the substrate, and populations of adhering zygotes transit synchronously through early development.

In the Fucales, mature eggs are radially symmetric, and cell polarization does not occur until after fertilization. The primary developmental axis is organized early in the first cell cycle and defines the growth axis of the young embryo. Unlike higher plants, in which positional cues are often conveyed by neighboring cells, fucoid zygotes develop autonomously and therefore rely on environmental signals for spatial information to orient their nascent axes. In the laboratory, an entire population can be induced to establish axes in parallel by the application of external vectors such as unidirectional light (Jaffe, 1968). A few hours after polarization, localized growth occurs at one pole of the nascent axis, as shown in Figure 1.

The first zygotic division is an invariant, asymmetric division, with the cell plate oriented transverse to the growth axis. The smaller rhizoid cell contains the growing apex and is the precursor to the holdfast of the alga; the larger thallus cell gives rise to the stipe and fronds of the mature plant (Figure 1). After the initial division of the zygote, the rhizoid cell elongates and repeatedly divides transverse to the growth axis, whereas the thallus cell undergoes proliferative divisions, each of which is transverse to the previous division.

Developing embryos of many land plants, including Arabidopsis, undergo a similar pattern of cell divisions (Figure 1; see also Laux and Jürgens, 1997, in this issue).

This review highlights recent progress in understanding the polarization of fucoid zygotes, with an emphasis on early events involved in polarity induction in Pelvetia. Readers are directed to a comprehensive review (Kropf, 1992) and to other more specialized reviews (Goodner and Quatrano, 1993; Kropf, 1994; Berger and Brownlee, 1995; Fowler and Quatrano, 1995; Quatrano and Shaw, 1997) for additional analyses and opinions.

POLARITY INDUCTION

The induction of polarity in fucoid zygotes is a continuum of overlapping events that occur during the first 10 hr after fertilization (AF). For convenience, I consider polarity induction to occur in two stages: axis selection, followed by axis amplification. Events occurring during these stages are considered below, and models describing polarity induction are presented later.

Axis Selection

Axis selection is the process by which the position of the developmental axis is specified. To select an axis, a zygote must perceive a signal, transduce that signal, and mark the developmental polarity.

Fucoid eggs have no demonstrable cellular asymmetries, and axis selection must therefore occur during or after fertilization. The egg pronucleus resides in the center of the egg cell, and microtubules nucleate uniformly from the surface of the pronucleus and radiate toward the cortex (Swope and Kropf, 1993). Short rods of F-actin are randomly oriented and uniformly distributed throughout the cortex (Kropf et al., 1992), and organelles are uniformly distributed throughout the cytoplasm (Brawley et al., 1976b). Glycoproteins are distributed in patches over the entire surface of the egg (Stafford et al., 1992).
derived organelles persist through much of the first cell cycle and have the potential to serve as positional markers. However, preliminary evidence indicates that the nucleus and perinuclear material, as marked by centrosomal position, do not align with the developmental axis until after growth begins (S.R. Bisgrove, unpublished results); therefore, they are unlikely to be involved in axis selection. Instead, polarity is likely to be perceived and marked in the plasma membrane and cell cortex (see below). Although the mechanisms by which sperm may mark the plasma membrane or cortex are unknown, Ca$^{2+}$ levels increase just beneath the plasma membrane at the sperm entry site (Roberts et al., 1994), and it is possible that, in turn, this local elevation in Ca$^{2+}$ activity triggers regional structural changes (see Trewavas and Malhó, 1997, in this issue).

**Environmental Cues**

The putative sperm-induced axis is weak and can be overridden by vectorial information in the environment (Jaffe, 1958; Robinson, 1996b). Approximately 3 hr AF, Pelvetia and Fucus zygotes secrete an adhesive symmetrically over the surface of the zygote and attach firmly to the substratum (Vreeland et al., 1993; Vreeland and Epstein, 1996). Once attached, zygotes begin to sense and respond to an impressive number of very different environmental cues. Indeed, the developmental axis, and, hence, the position of rhizoid outgrowth, can be controlled by the application of unidirectional light, gradients of ions or ionophores, gravity (G. Muday, personal communication), flow of seawater, temperature gradients, and many other factors (Jaffe, 1968). Plane polarized light induces the formation of two rhizoids (Jaffe, 1958), indicating that vectorial information does not simply result in rotation of the putative sperm-induced axis. Zygotes are sensitive to each gradient for a restricted period, and the windows of sensitivity overlap for many gradients (Bentrup, 1984).

The mechanisms by which environmental gradients are perceived and transduced are almost totally unknown in plants (see Trewavas and Malhó, 1997, in this issue). In fact, the diversity of stimuli that can elicit developmental axis selection in zygotes of the Fucales indicates that multiple, converging signal transduction pathways are involved. However, to date, only the photopolarization pathway has been investigated in detail. Unidirectional ultraviolet or blue light induces rhizoid growth from the shaded hemisphere of the zygote (Hurd, 1920; Robinson, 1996a), and the activation of cortical photoreceptors (Jaffe, 1958) preferentially stimulates plasma membrane redox chains at the presumptive rhizoid (i.e., shaded) pole (Berger and Brownlee, 1994). Photopolarization is inhibited by acidification or alkalization of the cytosol (Kropf et al., 1995) and by disruption of F-actin (Quatrano, 1973), but the roles of redox potential, cytosolic ions, and the cytoskeleton in signal transduction remain a mystery. Likewise, the mechanism by which the rhizoid pole is marked is unknown.

**The Role of Sperm Entry: An Open Question**

The role of sperm entry in inducing polarity in fucoid eggs is unclear (Roberts et al., 1993). Sperm entry is sufficient for polar development; external vectorial information (e.g., light) is not required for localized rhizoid outgrowth, oriented cell division, or subsequent development. These observations are consistent with sperm entry inducing an initial axis, and in a related brown alga, Cystosira, rhizoid growth occurs at the sperm entry site in the absence of vectorial cues (Knapp, 1931). However, the spatial relationship between sperm entry and rhizoid outgrowth has not been investigated in Pelvetia or Fucus, the organisms in which nearly all of the subsequent polarity studies have been conducted.

If sperm entry induces polarity, then the sperm entry site must somehow be marked in the egg cytoplasm. The sperm eyespot, mitochondria (Brawley et al., 1976a), and centrosomes (Motomura, 1994) migrate with the sperm pronucleus and are deposited on the zygotic nucleus at the site of pronuclear fusion, as shown in Figure 2A. These sperm-
Figure 2. Summary of Structural and Physiological Changes during the First Zygotic Cell Cycle.

(A) One hr AF. Centrosomes are inherited paternally, and organelles, microtubules, and F-actin are uniformly distributed in the zygotic cytoplasm.

(B) Four to 7 hr AF. Zygotes secrete local jelly, and electrical current flows through the cell. Inward current and jelly are positioned on the shaded side of the zygote. Centrosomes separate and begin to migrate along the nuclear envelope.

(C) Seven to 10 hr AF. F-actin in the cortex and dihydropyridine receptors in the plasma membrane accumulate at the presumptive rhizoid. Secretion becomes targeted to this site, depositing the sulfated fucan F2 in the wall and causing local cortical clearing. Gradients of H+ and Ca2+ are detectable.

(D) Ten to 13 hr AF. The rhizoid begins to elongate, centrosomes become the major site of microtubule nucleating activity, and microtubules extend preferentially into the rhizoid cortex.

(E) Thirteen to 16 hr AF. The nucleus rotates, bringing the centrosomes into axial alignment; the spindle forms.

(F) Twenty to 24 hr AF. At cytokinesis, the cross-wall forms transverse to the growth axis. F-actin and actin mRNA accumulate at the cross-wall. In the next division, only the rhizoid nucleus rotates, so spindles in the two-celled embryo are aligned transverse to one another.

DHP, dihydropyridine; MtOC, microtubule organizing center.
Axis Amplification

After axis selection, there is a prolonged period, lasting from ~4 to 10 hr AF, during which there is a gradual reorganization of the cytoplasm, plasma membrane, and cell wall in the young zygote. These rearrangements, which presumably amplify and strengthen the weak initial axis, overlap temporally and are discussed in their approximate chronological order.

Polar Jelly Deposition

One to 2 hr after Pelvetia zygotes adhere to a substratum and begin to sense environmental cues, they deposit a jelly material at the presumptive rhizoid pole of the nascent axis, as shown in Figure 3A (Schroter, 1978; Weisenseel, 1979). The jelly, which contains alginate, phenolic compounds, and sulfated fucans (Vreeland and Epstein, 1996), is deposited as an amorphous, translucent layer outside the cell wall. Fucus zygotes are slightly delayed compared with Pelvetia; uniform adhesive is secreted ~4 hr AF, and conspicuous polar jelly is deposited 6 to 8 hr AF (Vreeland et al., 1993). The rapidity with which jelly deposition occurs probably relates to function; the rhizoid-localized jelly strengthens the attachment of this region of the spherical zygote to the substratum, usually a rock in the intertidal zone (Vreeland and Epstein, 1996). Robust and rapid attachment helps prevent the cell from being washed out to sea in the next tidal cycle.

Jelly continues to be deposited over the surface of Fucus and Pelvetia embryos throughout early development, with greatest accumulations occurring toward the rhizoid pole (Schroter, 1978). However, jelly deposition is not required for early embryogenesis. Growth in seawater lacking sulfate prevents sulfation of fucans and thereby interferes with ionic interactions between polysaccharide molecules. The jelly layer is not deposited, yet zygotes germinate and divide normally (Schroter, 1978).

The jelly is likely deposited via vesicle secretion (Vreeland et al., 1993), but it is not yet clear whether uniformly distributed vesicles fuse with the plasma membrane locally or whether a subpopulation of secretory vesicles is preferentially localized at the presumptive rhizoid. Electron microscopy studies of Fucus zygotes provide no evidence for asymmetries in Golgi or vesicle distributions at this early stage of development (Quatrano, 1972; Brawley et al., 1976b). The early secretion of polar jelly in fucoid zygotes is surprising, because in most polarizing cells, secretion is a late event, occurring only after an axis has been amplified and reinforced (Drubin and Nelson, 1996).

Despite the lack of obvious structural polarity in the cell at the time of jelly secretion, there are local physiological changes in the cellular cortex that can be detected by plasmolysis or measurement of electrical current. When 5-hr-old photopolarized zygotes are placed in hypertonic seawater, plasmolysis occurs preferentially at the presumptive rhizoid pole (Reed and Whitaker, 1941). These changes in interactions between plasma membrane and cell wall at the presumptive rhizoid are detectable within 1 hr of photopolarization.

Ionic Currents

A transcellular ionic current (Figure 2B), which is generated at the plasma membrane, has been thoughtfully discussed in numerous articles and reviews (Jaffe et al., 1974; Jaffe and Nuccitelli, 1977; Robinson and McCaig, 1980) and is only discussed briefly here. In very young zygotes, unstable patches of inward and outward current (defined as the flow of positive charge) can be detected, but by 6 hr AF, an inward current stabilizes at the presumptive rhizoid pole (Nuccitelli, 1978). Treatment with cytochalasin D prevents localized current flux (Brawley and Robinson, 1985), indicating a dependence on cortical F-actin.
The current is thought to result in local differences in cytosolic ion concentrations at the presumptive rhizoid and thallus poles. Indeed, a fraction of the inward current is carried by \( \text{Ca}^{2+} \) at 5 to 6 hr AF (Robinson and Jaffe, 1975), yet cytosolic \( \text{Ca}^{2+} \) gradients have not been detected until 10 hr AF (Berger and Brownlee, 1993; Taylor et al., 1996). By contrast, a small but measurable cytosolic pH gradient (acidic at the presumptive rhizoid) has been detected at 5 hr AF, and the magnitude of the gradient increases throughout the period of amplification (Kropf et al., 1995). However, the difference in pH between rhizoid and thallus poles is <0.1 pH units and may not be intense enough to induce regional specialization.

**Late Events**

Several cellular rearrangements occur later in axis amplification, between 7 and 10 hr AF. Dihydropyridine receptors, which may be \( \text{Ca}^{2+} \) channels that carry part of the inward current, localize to the plasma membrane at the presumptive rhizoid soon after the stable current is first detectable (Shaw and Quatrano, 1996a). Shortly thereafter, F-actin (Brawley and Robinson, 1985; Kropf et al., 1989), cytosolic \( \text{Ca}^{2+} \) (Berger and Brownlee, 1993), and a clearing between plasma membrane and cell wall (cortical clearing; Peng and Jaffe, 1976; Nuccitelli, 1978) become localized to the cell cortex at the rhizoid pole, and a sulfated fucan (F2; Novotny and Forman, 1974) becomes localized to the rhizoid cell wall (Figure 2C). The precise temporal relationships between these localizations have not been investigated.

Both cortical clearing and polar plasmolysis may be manifestations of localized secretion, which locally disrupts interactions between the plasma membrane and the cell wall (Peng and Jaffe, 1976). Polar secretion also may function to deposit F2 into the wall at the rhizoid pole (Novotny and Forman, 1974). Because these localizations occur late in amplification, they may be important for subsequent events such as axis fixation or rhizoid growth (see below).

**Axis Realignement**

The developmental axis is labile and susceptible to realignment by applied vectors throughout the period of amplification (4 to 10 hr AF). Components of the axis become aligned with the most recently perceived vectorial cue, as demonstrated for polar jelly in Figure 3B. In addition to jelly (Schroter, 1978), dihydropyridine receptors (Shaw and Quatrano, 1996a), the ionic current, and cortical clearing (Nuccitelli, 1978) can all be repositioned. F2, however, is deposited after the axis can no longer be realigned (Fowler and Quatrano, 1995) and is therefore a marker for axis fixation (see below). Repositioning of F-actin, \( \text{Ca}^{2+} \), and H+ has not been investigated.

Traditionally, experiments designed to investigate polarity induction are conducted on well-attached, 6- to 8-hr-old zygotes that are then exposed to an environmental vector. Although these experiments are reported to investigate axis formation, it seems more likely that they address realignment of an axis selected before jelly secretion.

**Axis Fixation**

Just before germination, polar zygotes become insensitive to external cues, and the site of rhizoid outgrowth becomes fixed in space. Axis fixation occurs 10 to 12 hr AF and is marked by the polar secretion of F2 into the wall. For the first time in zygotic development, the secretory apparatus shows marked polarity; Golgi complexes accumulate in the perinuclear region on the rhizoid-facing side, and vesicles are transported preferentially toward the site of impending outgrowth (Brawley and Quatrano, 1979; Quatrano and Shaw, 1997). Cytochalasin treatment causes F2-containing vesicles (F granules) to accumulate near the Golgi complexes, indicating that vesicle transport is mediated by F-actin (Brawley and Quatrano, 1979). However, actin filaments in the region between the Golgi and the cortex have not been observed, perhaps because F-actin is difficult to preserve. Total mRNA and actin mRNA are excluded from the rhizoid and are abundant at the thallus pole at the time of axis fixation (Bouget et al., 1996), but the significance of these observations is unclear.

Agents that block axis fixation extend the period in which zygotes are sensitive to external stimuli; zygotes germinate in accordance with the most recently perceived light vector when the inhibitor is removed. Secretory inhibition (Shaw and Quatrano, 1996b), disruption of F-actin (Quatrano, 1973), and enzymatic removal of the cell wall (Kropf et al., 1988) all have been shown to prevent axis fixation. Incubation in hypertonic seawater of proper osmolarity also prevents axis fixation but not rhizoid outgrowth, demonstrating that the two phenomena can be uncoupled (Robinson, 1996b).

**Growth**

At germination, vesicle secretion intensifies, and a rhizoid, which elongates by tip growth, emerges from the chosen site (Figure 2D). The insertion of secretory vesicles deposits new membrane and wall components, including F2 (Brawley and Quatrano, 1979) and a vitronectin-like protein (Wagner et al., 1992), at the elongating tip. Some of the cellular components that are localized to the presumptive rhizoid during axis induction and fixation are important for tip elongation. This is the case for F-actin and cytosolic ions, which are thought to play fundamental roles in the elongation of most, if not all, tip growing cells (Steer and Steer, 1989; Heath, 1990; Jackson and Heath, 1993; Hepler, 1997).

The \( \text{Ca}^{2+} \) gradient in *Fucus* zygotes intensifies at germination and is maintained during rhizoid growth (Berger and Brownlee, 1993). This gradient likely results from flux through...
stretch-activated channels that are uniformly distributed but preferentially activated in the rhizoid apex (Taylor et al., 1996). In addition, the magnitude of the H+ gradient also increases at germination (Kropf et al., 1995). These ionic gradients appear to function in growth; when they are dissipated by treatment with H+ (Kropf et al., 1995) or Ca2+ (Speksnijder et al., 1989) buffers, tip elongation is inhibited. Presumably, the elevated ionic activities localize specific physiological processes to the tip.

Cortical F-actin also has an important role in rhizoid growth. F-actin is localized to the elongating tip, and treatment with cytochalasin results in the immediate cessation of growth (Kropf et al., 1989). However, the functions of F-actin during tip growth are not clear. Inhibitor studies suggest that vesicles are transported along F-actin (Brawley and Quatrano, 1979), but this has not been demonstrated directly. Cortical F-actin may also provide structural support to reinforce the apex against turgor pressure and/or may play a more direct role in generating force for rhizoid expansion (Harold et al., 1996).

Recently, cortical F-actin has been shown to be present at adhesion sites near the apex of growing Pelvetia rhizoids (Henry et al., 1996). These wall-membrane adhesions are visible during plasmolysis of germinated zygotes and are localized to the base of the apical dome, where they form a ring-like structure. They contain F-actin (Henry et al., 1996), dihydropyridine receptors (Shaw and Quatrano, 1996a), and Ca2+ (Kropf and Quatrano, 1987) on the cytoplasmic face and are thought to be manifestations of transmembrane complexes (see below). Although they do not appear to be needed for tip elongation, these adhesions may play a role in defining and maintaining the growth site (Kropf et al., 1993; Henry et al., 1996).

DIVISION PLANE ALIGNMENT

The first zygotic division is an invariant, asymmetric division with the wall oriented transverse to the growth axis. This orientation is determined by spindle alignment, which in turn is determined by the positioning of the centrosomes (Allen and Kropf, 1992). Two centrosomes are inherited paternally (Figure 2A) and separate to opposite sides of the zygotic nucleus before growth (Figure 2B; Motomura, 1994). Soon after germination, the nucleus and centrosomes rotate so that the axis defined by the centrosomes aligns with the growth axis, and the spindle subsequently forms in axial (longitudinal) alignment (Figure 2E; Kropf et al., 1990). Cytokinesis occurs centripetally by invagination and is supported by vesicle fusion (Brawley et al., 1977). The cleavage bisects the spindle, resulting in a transverse cross-wall (Figure 2F). Subsequent divisions in the rhizoid are also transverse (Figure 1D) and are thought to involve nuclear rotation (Allen and Kropf, 1992). Rotational alignment implies that information regarding the position of the rhizoid is communicated to the nucleus.

Division plane alignment can be perturbed by pulsed treatments with cytochalasins, brefedalin A, or microtubule depolymerizing agents (e.g., oryzalin or nocodazole; Allen and Kropf, 1992; Shaw and Quatrano, 1996b). These agents cause abnormal nuclear rotations that result in misaligned spindles and skewed division planes. Embryos with slightly misaligned division planes can develop normally, but more severe misalignment reduces growth rate. Embryos in which the division plane is displaced 90° and bisects the rhizoid tip often develop two rhizoids (Shaw and Quatrano, 1996b; Quatrano and Shaw, 1997), suggesting that rotational alignment may have evolved as a mechanism to prevent the formation of multiple rhizoids on a single embryo.

WORKING MODELS

The working models presented below are intended to provide a framework for interpreting the diverse findings discussed above and to help clarify the most important questions that need to be addressed.

Polarity Induction

A developmental axis is selected very early in fucoid zygotes, perhaps even at sperm entry. Investigation of the spatial relationships between the sperm entry site and the sites of jelly secretion and rhizoid outgrowth in the absence of external vectors will resolve the role of sperm entry in polarization. If sperm entry is found to induce polarity, the next challenge will be to identify the cortical markers of this site. F-actin is a likely candidate because localized cortical domains of F-actin mark important sites in other polar cells (Drubin and Nelson, 1996; White and Strome, 1996). Application of high-pressure freezing techniques will aid in preserving cortical F-actin structure, and microinjection of fluorescently tagged actin should provide insight into the dynamic properties of F-actin during fertilization.

The chain of causality during the early stages of polarity induction is not well understood. In published models, channel localization, transcellular current, and cytosolic Ca2+ accumulation are postulated to be causal to polar secretion (Jaffe et al., 1975; Brawley and Robinson, 1985). Alternatively, polar secretion may precede ionic localizations, as depicted in Figure 4A. In this version of polarity induction, axis selection is followed closely by vesicle secretion at the presumptive rhizoid, which inserts ion channels and/or cytoskeletal anchoring proteins into the presumptive rhizoid membrane (Drubin and Nelson, 1996). These membrane asymmetries then give rise to local current influx and Ca2+ localization. Identification of the spatial and temporal relationships among dihydropyridine receptor localization, jelly secretion, and cytosolic gradients by double-labeling studies will help distinguish between these two chronologies.
Polarity in Fucoid Zygotes

A cell wall protein

secretory vesicle

F-actin

ion channel

cytoskeletal anchoring protein

ion

integral membrane protein

jelly

Figure 4. Working Model.

(A) and (B) Axis amplification. (A) Polar secretion at the presumptive rhizoid pole of 4-hr zygotes establishes asymmetries in the plasma membrane by locally inserting membrane proteins, including ion channels and cytoskeletal anchoring proteins. (B) The weak asymmetry is amplified by continued local insertion of channels, recruitment of channels already in the plasma membrane, F-actin stabilization, and the establishment of locally elevated ionic activities at the presumptive rhizoid pole.

(C) Axis fixation. Transmembrane complexes (right side of drawing) containing F-actin, an integral membrane protein, and a secreted cell wall component fix the developmental axis and are involved in the rotational alignment of the nucleus. For simplicity, putative actin-binding proteins of the complex are not shown.

The initial asymmetry is hypothesized to be amplified and reinforced over the ensuing hours, as depicted in Figure 4B (Brawley and Robinson, 1985). Continued vesicle insertion adds new channels and amplifies the local increase in the activity of inward-flowing cations, particularly H\(^+\) and Ca\(^{2+}\). F-actin is stabilized locally by elevated H\(^+\) and Ca\(^{2+}\) and by interaction with cytoskeletal-anchoring proteins. This F-actin in turn anchors and stabilizes ion channels and other membrane proteins, which results in further accumulations at the presumptive rhizoid and reinforcement of the vesicle secretion site. Furthermore, F-actin may mediate the movement of additional plasma membrane components, such as dihydropyridine receptors, to the rhizoid site. This amplification loop increases asymmetries in Ca\(^{2+}\), H\(^+\), F-actin, and polar secretion.

This amplification model raises several important issues. First, is a Ca\(^{2+}\) gradient causal to the initial stages of axis induction? Previous experiments investigating the requirement for Ca\(^{2+}\) in early development produced contradictory and equivocal results (Kropf and Quatrano, 1987; Hurst and Kropf, 1991; Robinson, 1996b). Manipulating cytosolic Ca\(^{2+}\) in specific cellular domains may help resolve the issue. Cytosolic Ca\(^{2+}\) can be locally elevated by microinjection of caged Ca\(^{2+}\) followed by local Ca\(^{2+}\) release using laser irradiation. Putative interactions between cytosolic ionic activities and the cytoskeleton can be investigated by manipulating ionic activities and examining the effects on cytoskeletal assembly and stability. Finally, an investigation of secretory vesicle distribution at the time of jelly secretion should clarify whether vesicles are transported directionally at this early stage of development.

Axis Fixation and Rotational Alignment

Wall-membrane interactions mediated by transmembrane complexes are postulated to be important in axis fixation and rotational alignment of the nucleus. The essence of fixation is thought to be the formation of transmembrane complexes that link F-actin in the rhizoid cortex through the plasma membrane to the cell wall (Figure 4C). F-actin accumulates in the rhizoid by differential stabilization (see above), and plasma membrane and/or cell wall components are localized by the polar secretion of Golgi-derived material (Fowler and Quatrano, 1995; Shaw and Quatrano, 1996b). Although the components of these proposed complexes have not been identified, proteins that cross-react with antibodies raised against mammalian focal adhesion complex components, such as vinculin, \(\beta\)-1 integrin, and vitronectin (Burridge and Chrzanowska-Wodnicka, 1996), have been identified in Fucus (Quatrano et al., 1991; Wagner et al., 1992). However, the vitronectin-like protein and F2 are unlikely to be the cell wall components needed for axis fixation because fixation proceeds even when localization of these molecules is disrupted (Fowler and Quatrano, 1995). It is important to identify the true adhesion components, which may be different from molecules comprising focal adhesions in animal cells. Regardless of their composition, the formation of transmembrane complexes is thought to stabilize the rhizoid cortex and define a target site for vesicle fusion during ensuing growth.

However, available evidence indicates that there may not be a close association of plasma membrane and cell wall at the time of axis fixation. A clear zone in the cortex at the presumptive rhizoid pole is thought to be caused by displacement of the plasma membrane from the cell wall (Figure 2C; Peng and Jaffe, 1976). Moreover, wall-membrane adhesions visualized by plasmolysis are not localized at the presumptive rhizoid at the time of axis fixation (Henry et al., 1996).
Rotational alignment of the nucleus before mitosis is also thought to involve transmembrane complexes. At the time of rotational alignment, wall–membrane adhesions, presumably composed of transmembrane complexes, are localized at the elongating rhizoid tip (Henry et al., 1996), and treatments that disrupt adhesions, such as the application of cytochalasin D, cause improper rotation (Allen and Kropf, 1992). Treatment with brefeldin A also prevents nuclear rotation (Shaw and Quatrano, 1996b), perhaps by preventing deposition of the cell wall component of the transmembrane complexes (Quatrano and Shaw, 1997).

The current working model for rotational alignment postulates that microtubules anchored at the centrosomes grow into the rhizoid cortex and are captured there (Kropf, 1994). Although the nature of the microtubule-capturing site is unknown, it is postulated to contain the cortical F-actin associated with adhesion sites. Motors located at the centrosome or cortex exert force on the captured microtubules and pull the centrosome apically. Stochastically, one centrosome will be closer to the apex and is therefore likely to have more microtubules captured. This centrosome wins the tug-of-war and rotates apically (Figures 2D and 2E).

Similar mechanisms have been proposed to orient asymmetric divisions in Caenorhabditis elegans embryos (White and Strome, 1996) and in Saccharomyces cerevisiae (Palmer et al., 1992; Li et al., 1993; Muhua et al., 1994). The emerging picture is that a cortical patch of actin and capping protein serves as a nucleation site for a dynactin complex and dynein, which pull on the astral microtubules (Vallee et al., 1995). Whether the same cortical machinery operates in rotational alignment in fucoid zygotes remains to be determined.

FUTURE DIRECTIONS

Recent technological advances promise further rapid advances in studies of early development in fucoid algae. Reporter molecules can be introduced into the cytoplasm of living cells by microinjection, and redistributions can be followed during polarization (Hepler et al., 1993). Injection of multiple reporters will permit spatial and temporal relationships to be examined. Fluorescent reporters for ionic activity and for cytoskeleton are currently being injected into zygotes, and others will soon follow. Application of molecular tools has led to the cloning and spatial characterization of cytoskeletal sequences (Bouget et al., 1996; Coffman and Kropf, 1997), and microinjection of antisense RNA and antibodies will permit analyses of gene function.

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