

***Fucus* Embryogenesis: A Model to Study the Establishment of Polarity**

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INTRODUCTION

It has been known for more than 100 years that when spherical zygotes of the brown alga *Fucus* are subjected to a gradient of light, the plane of the first cell division is always perpendicular to the light axis. As illustrated in Figure 1, the resulting cell plate divides the zygote into two unequal cells; the smaller rhizoid cell emerges from the shaded portion of the gradient, whereas the larger thallus cell is on the lighted side (Figures 1A to 1C). Each cell has a different organellar composition. Golgi and mitochondria are enriched in the rhizoid cell, whereas the thallus cell contains most of the chloroplasts (Brawley et al., 1977). Each cell of the two-celled embryo also has a different developmental fate. For the most part, cells derived from the initial rhizoid cell form the holdfast portion of the mature plant, whereas descendants of the initial thallus cell form the embryo proper and fronds (Figures 1D and 1E). Although the developmental fate of an isolated thallus or rhizoid cell has not been determined directly, and the exact cell lineage from each cell of the two-celled embryo has not been mapped precisely, it is clear that the polar axis imposed on the zygote by an external gradient establishes the polarity and division plane of the two-celled embryo, results in the differentiation of the first two cells of the embryo, and sets the developmental axis for the whole organism (for reviews, see Quatrano, 1978, 1990; Harold, 1990; Jaffe, 1990; Kropf, 1992).

The basic mechanisms involved in the generation of cell asymmetry in the zygote and the directed transport of specific cytoplasmic components to the resulting daughter cells are developmental phenomena of critical importance. These polar processes are common not only to algae but also to many different organisms, from flowering plants, yeasts, and nematodes to insects, amphibians, and mammals. In flowering plants, for example, polar asymmetric divisions play critical roles in the first zygote division (Mayer et al., 1993) as well as in the formation of pollen grains, guard cells, and epidermal hairs (Wardlaw, 1968). However, in most systems, how a cell transports cytoplasmic material to a specific site defined by an extracellular gradient and orients the division plane

relative to this gradient are difficult to study and manipulate experimentally.

Because of the ease in manipulating the *Fucus* embryo, zygotes and young embryos of the Fucales have been used as a model to study these critical developmental processes (Quatrano, 1990; Kropf, 1992). Eggs of the Fucales are large (75 to 100 μm in diameter), apolar, and released, along with sperm, from mature plants under laboratory conditions (Quatrano, 1980). Fertilization occurs in a defined sea water medium, with the resulting diploid zygote attaching to the substratum within a few hours. This attachment to a substratum allows for easy manipulation of solutions and for orientation of populations of zygotes to various external gradients (Robinson and Jaffe, 1975).

After fertilization, polar development occurs synchronously for several cell divisions in the absence of surrounding cells and in a pattern similar to the early development of flowering plant embryos. The *Fucus* embryo diagrammed in Figure 1D is morphologically quite similar to the octant stage (stage 6) of the *Arabidopsis* embryo (Mayer et al., 1993). The polar axis of the zygote is labile for 10 hr following fertilization and can be oriented and reoriented by a number of gradients (Jaffe, 1968) before it becomes set (Quatrano, 1973). The most simple to manipulate gradient is unilateral white light. There is no apparent spatial patterning in the egg or early zygote, as determined by serial sectioning and analysis by electron microscopy (Brawley et al., 1976), and "polar determinants" are not localized in the egg and rotated by extracellular vectors such as light (Jaffe, 1958). Instead, the polar axis in *Fucus* appears to arise epigenetically and can be easily and predictably manipulated. Although a genetic approach is not yet available for analyzing these events in *Fucus*, general principles associated with polar development have been identified from cellular and biochemical experimentation.

Our goal in this review is to describe the mechanisms by which a polar axis is imposed on a cell, as deduced from experimentation with zygotes of the Fucales during the last several decades. These findings will be compared with the results of experiments with other polar systems in a search for general mechanisms involved in polarity and to suggest future directions for the analysis of polarity in *Fucus*.

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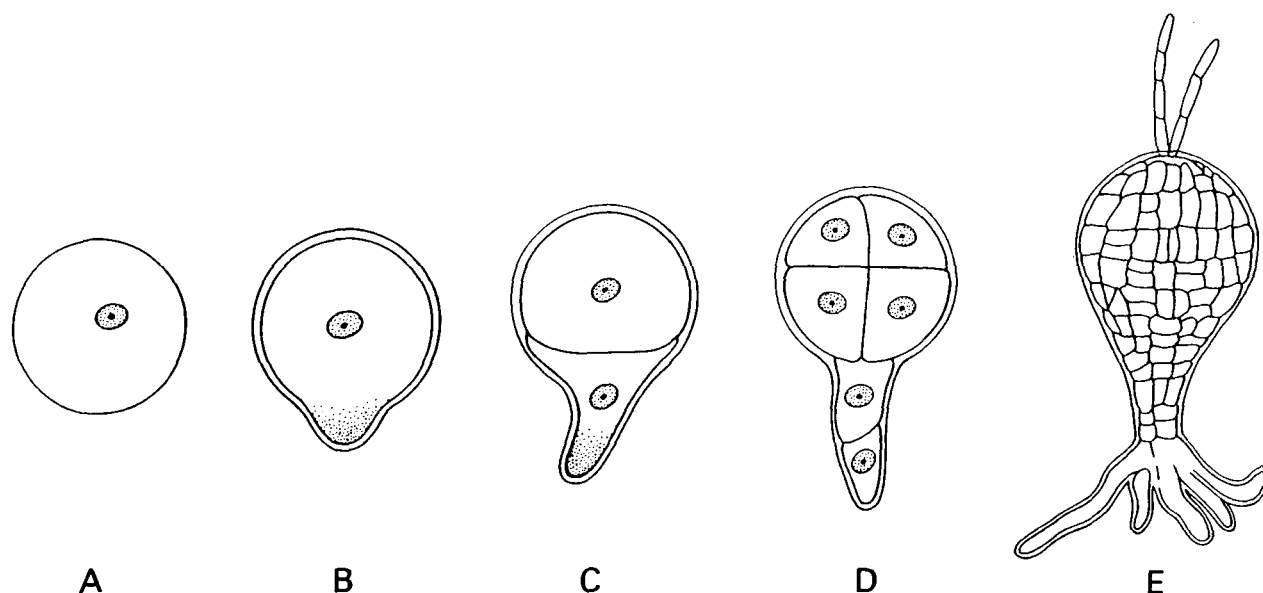


Figure 1. Schematic Diagram Depicting the Early Stages of *Fucus* Development.

(A) After fertilization, the zygote is initially apolar. Within the next 4 to 10 hr, the zygote forms an axis of polarity in response to environmental stimuli such as light.

(B) This axis becomes fixed 10 to 12 hr after fertilization, followed by polar growth to produce the rhizoid. Secretory vesicles and other cytoplasmic particles (stipules) are localized to the region of the growing rhizoid tip.

(C) The first cell division occurs ~24 hr after fertilization, with the new cell plate oriented perpendicular to the long axis of the rhizoid. The daughter cells of this division differ in size, shape, and cytoplasmic content, with the smaller rhizoid cell containing the growing tip.

(D) The next cell division is parallel to the first division in the rhizoid cell, followed by a cell division perpendicular to the first division in the thallus cell.

(E) Further cell divisions give rise to a plantlet with a globular thallus and multiple branching rhizoids.

COMPONENTS OF THE POLAR AXIS

Polarization of the developmental axis in *Fucus* can be separated into three basic components: formation of the polar axis by an external gradient (4 to 10 hr after fertilization), stabilization or fixation of the axis (10 to 14 hr), and morphological expression of the polarity (14 to 18 hr), as visualized by the directed transport of vesicles to the site of polar outgrowth, the rhizoid (Quatrano, 1990; Kropf, 1992). All of these processes occur before and are independent of the first cell division (18 to 24 hr) (Quatrano, 1973). The rotation of the microtubule organizing centers to orient the plane of the first division so that it is perpendicular to the rhizoid axis has been reviewed recently (Kropf, 1992) and will not be discussed in this article.

Expression of Polarity in *Fucus*

Cytoskeleton and the Cell Wall

The structure of the emerging rhizoid tip resembles that of other tip-growing cells from higher plants, such as pollen tubes and epidermal hairs, as well as fungal hyphae (Harold, 1990). The

cytoplasm at the tip of the elongating rhizoid is rich in Golgi vesicles, which deposit their contents (i.e., cell wall material) into the newly forming cell wall by exocytosis (Brawley et al., 1976). The resulting rhizoid cell wall is structurally and biochemically different from the thallus cell wall (Brawley and Quatrano, 1979). For example, a unique sulfated fucan polysaccharide (F2) (Quatrano et al., 1979) and a vitronectin-like protein (VnF) (Wagner et al., 1992) are localized only in the cell wall of the elongating tip. Both F2 and VnF are believed to be packaged in Golgi-derived vesicles (F granules), and these F granules are randomly distributed in the early zygote. However, pulse-chase experiments using $^{35}\text{SO}_4$ indicate that the F granules are directionally transported to the site of rhizoid outgrowth after the axis is irreversibly determined (Brawley and Quatrano, 1979; Quatrano et al., 1985).

The targeted transport of F granules to the site of tip growth requires actin microfilaments (Brawley and Quatrano, 1979). The tip of the elongating rhizoid displays a highly concentrated network of actin microfilaments (Kropf et al., 1989), as do other tip-growing systems (Heath, 1990). In the presence of drugs that disrupt microfilament assembly (i.e., cytochalasin B [CB]), polar elongation of the rhizoid ceases, but cell division is unaffected. A spherical two-celled embryo results. Although enzymatic sulfation of F2 occurs normally in CB-treated

embryos, the F granules are not transported to a localized site and remain associated with the perinuclear region of the zygote or daughter cell nuclei (Brawley and Quatrano, 1979). The actin network localized at the rhizoid tip is believed to serve as tracks to direct or guide the F granules to the site of cell wall elongation (Quatrano et al., 1979, 1985).

Localized accumulation of F granules also requires the enzymatic sulfation of F2 (Quatrano and Crayton, 1973). When sulfation of F2 is prevented by growth in artificial sea water lacking inorganic sulfate, tip growth of the rhizoid and cell division are unaffected. A morphologically normal two-celled embryo is formed (Crayton et al., 1974). However, when the rhizoid is examined by immunocytochemistry with antibodies to human Vn antibodies (Wagner et al., 1992) or by staining with a lectin probe for the unsulfated fucan (Hogsett and Quatrano, 1978), F2 and VnF are not observed in the cell wall of the rhizoid tip. Rather, both F2 and VnF remain in F granules within the zygote cytoplasm. Interestingly, although a normal rhizoid is formed without these components in the rhizoid cell wall, two-celled embryos lacking F2 and VnF in the tip of the rhizoid do not attach to the substratum (Crayton et al., 1974; Wagner et al., 1992). Similarly, if young zygotes are incubated continually with anti-Vn antibodies, the embryos that form are free floating and do not adhere to the substrate (Wagner et al., 1992). Two-celled embryos that remain unattached continue to divide but ultimately form a multicellular, globular embryo that lacks (or has a reduced) polarity. These results suggest that secretion of sulfated F2 and VnF into the growing rhizoid cell wall is required for adhesion of the embryo to a substratum. Adhesion is not required for the initial formation of a polar two-cell embryo but does have an impact on subsequent maintenance of a polar embryonic structure.

Sulfated polysaccharides have not been reported in flowering plants, although specific anionic polysaccharides in the cell walls may perform similar functions. Vn-like proteins have been detected in suspension cells of carrot (Wagner and Matthyse, 1992) and tobacco (Zhu et al., 1993) and observed in the stylar tissue of lily and broad bean (Sanders et al., 1991). What role, if any, cell wall macromolecules such as F2 and VnF play in polarity expression and function in other tip-growing filaments or in the early reproductive development of flowering plants remains to be determined.

Plasma Membrane, Ca^{2+} , and the Intracellular Current

A transcellular current is associated with rhizoid tip growth in *Fucus* embryos and with tip growth in many other systems (Harold, 1990). Exocytosis of localized F granules at the tip of the elongating rhizoid not only establishes an asymmetric distribution of F2 and VnF in the cell wall of the zygote but also results in the localized insertion of F granule membranes into the plasma membrane of the elongating tip. The membranes of F granules could potentially carry ion channels. Although the transport properties of these new "membrane patches" have not been determined, they may maintain and/or

amplify the transcellular current (Brawley and Robinson, 1985). The transcellular current flows inward at the rhizoid tip and outward at the opposite (thallus) pole and is carried in part by Ca^{2+} . Circulation of Ca^{2+} through the cell or a local accumulation at the rhizoid tip might lead to a cytoplasmic Ca^{2+} gradient, highest at the rhizoid tip (Jaffe, 1990; Kuhnreiter and Jaffe, 1990). Use of ion-selective microelectrodes has demonstrated a localization of free Ca^{2+} at the rhizoid tip, with the cytoplasmic concentration being 10 times greater in the tip than in the subtip region (Brownlee and Wood, 1986; Brownlee and Pulsford, 1988). Localization of membrane-bound Ca^{2+} has been detected just beneath the plasma membrane of the elongating rhizoid tip by the fluorescence probe chlorotetracycline (Kropf and Quatrano, 1987).

The conclusion that localized Ca^{2+} plays an important role in polarity expression is further substantiated by data that demonstrate that if internal Ca^{2+} gradients are made uniform by cytoplasmic injection of Ca^{2+} buffers, rhizoid formation is prevented (Speksnijder et al., 1989). Furthermore, if zygotes are treated with EGTA, which chelates extracellular Ca^{2+} , or with the Ca^{2+} channel blockers lanthanum, D-600, or verapamil, both membrane-bound and free Ca^{2+} localizations are eliminated (Kropf and Quatrano, 1987; Brownlee, 1989, 1990) and rhizoid outgrowth is prevented. The localization of unique plasma membrane components and Ca^{2+} in the cytoplasm at the rhizoid tip thus appear to be essential for maintaining tip growth as well as the transcellular ionic current. These general conclusions appear to apply to other tip-growing cells as well (Reiss and Herth, 1979).

The localization of F2 and VnF in the cell wall and Ca^{2+} and actin in the cytoplasm are clearly required for maintaining polar tip growth and adhesion. They also serve as useful markers for the expression of cellular asymmetries related to polar function such as cell elongation and adhesion. However, it is not as clear what role they play in the establishment of the polar axis. When are the first signs of these asymmetries detected in the zygote (axis formation), and, once established, how are these asymmetries maintained (axis fixation)? Can one assign a role for these molecules in polar axis formation or fixation?

Axis Formation

An axis can be imposed on a population of zygotes by a 1-hr oriented light pulse given between 4 and 10 hr after fertilization. This results in rhizoid formation on the shaded side of the light gradient 14 to 18 hr after fertilization. If the orienting light pulse is followed by growth in uniform light or darkness, the rhizoids still form on the shaded side of the light gradient. Hence, the zygote has a "memory" of several hours as to the direction of the light gradient (Quatrano, 1973). The responsible photoreceptor is in or near the plasma membrane, absorbs blue light, and is clearly not phytochrome (Kropf, 1992). Very little else is known about the perception of the light signal or the mechanisms involved in transducing this signal to the

cytoplasm. Rather, most of the research on axis formation so far has centered on the changes that occur at the presumptive site of rhizoid formation following the orienting light pulse.

Jaffe and colleagues (Robinson and Jaffe, 1975; Nuccitelli and Jaffe, 1976; Jaffe, 1990) have elegantly demonstrated that an electrical current flows through the zygote during the formation of the axis. The current flows into the presumptive rhizoid pole and out of the presumptive thallus pole. Hence, the inward current precedes and accurately predicts the fixed site of future rhizoid outgrowth. This is the first sign of asymmetry that can be detected in a zygote grown in unilateral light. Jaffe and colleagues proposed that this current results from the accumulation of membrane current leaks at the presumptive rhizoid site, either by directed recruitment or localized activation of ion channels. Also associated with the site of inward current is a small amount of localized vesicle exocytosis known as "cortical clearing." Cortical clearing should not be confused with the relatively massive F granule exocytosis that occurs later during rhizoid tip growth.

Even though an asymmetric current is set up during axis formation, it is important to stress that the inward-directed current and cortical clearing can be changed during axis formation by imposing a light gradient from a different direction (Nuccitelli, 1978). Hence, during this time of a labile polarity, a transcellular current and cortical clearing predict the site of polar growth. It has been presumed that the transcellular current detected during axis formation is mechanistically related to the transcellular current involved in rhizoid tip growth, but there is very little data available on the ions and ion channels involved at these different stages to test this hypothesis. Moreover, the debate still continues as to whether the inward current detected during axis formation is required for subsequent events. The experimental data have been discussed at great lengths in several recent reviews, but not enough is known at this time to alter the conclusion of Kropf (1992) that "the chain of causality in this web is obscure."

Beyond the association of the inward current and cortical clearing with the future site of polar growth, polar axis formation does require that K^+ or a related ion be present in the medium. Axis formation is not dependent on any one specific ion, but it has been shown that K^+ salts alone (10 mM KCl, with sucrose for an osmoticum) can support axis formation (Hurst and Kropf, 1991). It is not known whether this requirement for K^+ is directly related to axis formation or is indirectly related through some essential housekeeping function such as maintenance of turgor pressure. As pointed out by Kropf (1992), more information is needed on this ionic requirement, including whether zygotes generate a transcellular current in minimal medium containing K^+ .

In addition to K^+ or a related ion, polar axis formation requires intact actin microfilaments. If microfilaments are disassembled by CB treatment during the orienting light pulse and are then allowed to reassemble in the absence of CB and the zygotes are then grown in uniform light or darkness, rhizoids that form are oriented randomly with respect to the light pulse (Quatrano, 1973). It appears, therefore, that the CB pulse

interferes with the perception or transduction of the light vector into oriented polar elongation. However, even though CB has this profound effect on the orientation of the axis, there is no detectable change in the distribution of actin in the zygote at this time (Kropf et al., 1989). Perhaps in untreated cells, links between the actin microfilaments and membrane proteins are severed on the lighted side of the gradient (Brawley and Robinson, 1985), allowing those proteins to distribute freely to plasma membrane sites on the dark side, where they are stabilized subsequently. In CB-treated cells, microfilaments are disassembled, uniformly severing links to the plasma membranes around the zygote. Upon random reestablishment of those links in the absence of an orienting light, rhizoids that form are not oriented with respect to the first light pulse.

The best model for axis formation now appears to be that a gradient of light results in the asymmetric distribution of proteins in the plasma membrane. The mechanism by which this occurs is not clear, but it may involve recruiting ion channels within the plasma membrane to the shaded side of the light gradient. The requirement for actin to form an axis suggests that a microfilament-dependent differential linking of membrane proteins on the light (unstable) and dark (stable) sides may be a mechanism worth testing. Finally, polarized secretion (cortical clearing) at the presumptive rhizoid pole might be used to incorporate ion channels preferentially into this area of the plasma membrane to generate a current. In any case, these initial changes are reversible with light from a different direction and hence do not represent the fixation of the polar axis. How might these asymmetries be maintained (axis fixation)?

Axis Fixation

The primary aim of much research has been to determine the cytological and biochemical bases of the processes involved in the stabilization or fixation of the initial asymmetries generated by a light gradient. An integral component of this work has been the use of a polarization assay. This assay is based on the observation that just before rhizoid emergence, unidirectional light cannot reverse a previously established axis—i.e., the polar axis is fixed. However, if any treatment interfered with the fixation process, light from a direction different from any previous gradient could reorient the polar axis (Quatrano, 1973). A diagram outlining this assay is presented in Figure 2. Inhibitors of specific processes or structures are added to a zygote population during the time of axis fixation (10 to 14 hr after fertilization) but after an orienting pulse of light (LIGHT I; Figure 2). After a period of hours, the inhibitors are removed and the population is subjected to unidirectional light from a different direction (LIGHT II; Figure 2). The direction of rhizoid outgrowth relative to LIGHT I or II is then recorded several hours later. If the fixation process is unaffected by the inhibitors, LIGHT I would be the orienting light and LIGHT II would have no orienting effect. If the inhibitors interfered reversibly with the fixation process, however, LIGHT II would be orienting.

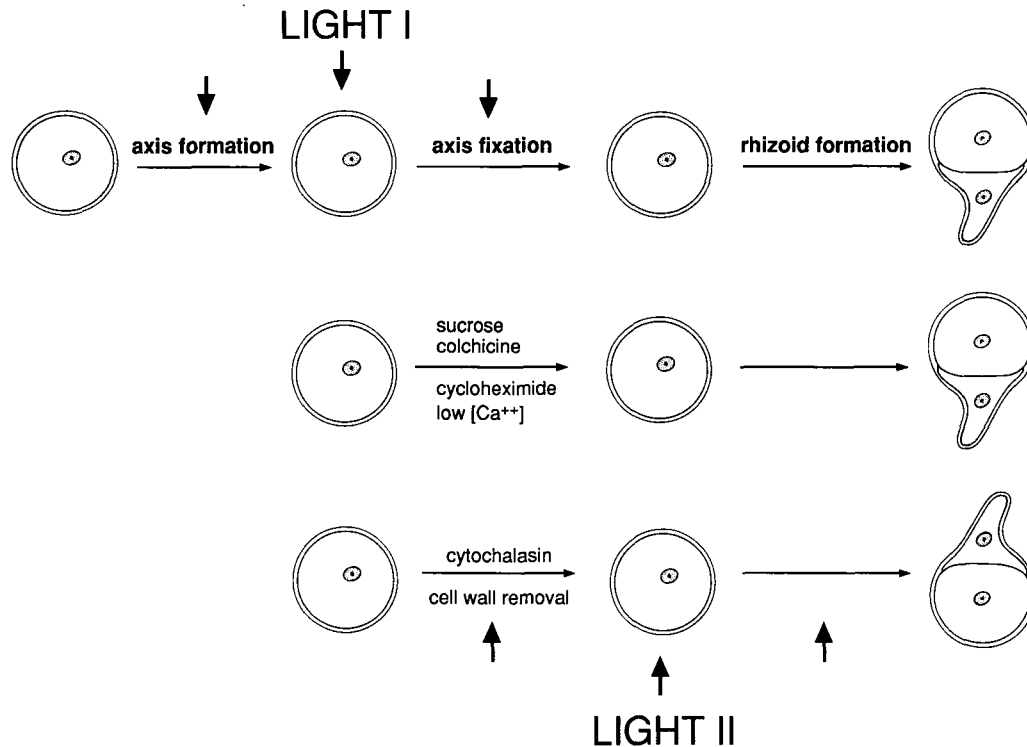


Figure 2. Schematic Diagram of the Polarization Assay and Some Experimental Results.

A synchronous population of zygotes is subjected to an unilateral light gradient (LIGHT I) during the first 12 hr after fertilization, when axis formation and fixation occur. Once the axis is fixed, it cannot be realigned by exposure to a new unilateral light gradient (LIGHT II). The rhizoid emerges from the shaded region of the zygote. To test if axis fixation can be inhibited, zygotes in LIGHT I are subjected to a treatment during the time of axis fixation. After the untreated controls have fixed their axis, the treated zygotes are switched to the LIGHT II gradient and placed in normal conditions. A few hours later, the zygotes are scored for the orientation of their growing rhizoids. Sucrose, colchicine, cycloheximide, or the reduction of Ca^{2+} levels in the medium do not prevent axis fixation. Cytochalasins, which disrupt the actin cytoskeleton, or removal of the cell wall by hydrolytic enzymes prevent axis fixation.

Using this assay, it has been found that if zygotes are incubated in the presence of a sea water medium containing low Ca^{2+} (Kropf and Quatrano, 1987) or microtubule or protein synthesis inhibitors or that is hypertonic, axis fixation is not affected (Quatrano, 1973). However, if during axis fixation, zygotes are incubated with CB (Quatrano, 1973), or if the cell wall is removed enzymatically (Kropf et al., 1988), LIGHT II is able to reorient the final axis. Hence, we have concluded that both microfilaments and a cell wall are required for a previously formed axis to become stabilized (Quatrano, 1990). In the absence of these structural components, polarity remains labile, and the axis is not fixed.

If microfilaments are required for axis fixation, one might expect to observe a spatial localization or temporal appearance of filamentous actin (F-actin) that would correlate with its role in the fixation process. Three isoforms of actin are present throughout early development of the zygote, but no increases in their levels or rates of synthesis are detected at the time of axis fixation or rhizoid outgrowth (Kropf et al., 1989). Fluorescently labeled (rhodamine) phalloidin, which binds to

F-actin, shows that F-actin is distributed symmetrically in the cortical region of zygotes prior to the time of polar axis fixation. However, spherical zygotes with a fixed polar axis show a localized accumulation of F-actin at the presumptive site of rhizoid formation (Kropf et al., 1989). A similar localization of F-actin has been recorded at the tip of two-celled or older embryos (Brawley and Robinson, 1985), as discussed earlier. Furthermore, in a population of zygotes, the initial signs of F-actin localization coincide with the acquisition of a fixed polar axis (10 to 14 hr) and not with initiation of polar elongation of the rhizoid, which occurs later (14 to 18 hr) (Kropf et al., 1989). Hence, the redistribution of F-actin to the site of rhizoid formation is temporally correlated with the fixation process and spatially related to the site of polar elongation. This localization of actin is the first stable asymmetry observed in the zygote. The mechanism(s) by which actin becomes redistributed are not known but could include the differential depolymerization of actin at the thallus pole, contraction of the cortical actin network, or a myosin-based movement of actin to the rhizoid pole (Harold, 1990; Kropf, 1992).

In addition to the requirement for an F-actin network, an intact cell wall must be present to detect a fixed axis by the polarization assay (Kropf et al., 1988). By removing the cell wall during axis fixation (Kropf et al., 1988) and varying the timing of the orienting light pulse(s) (Figure 2), it was determined that (1) wall removal and regeneration prior to an orienting light pulse do not interfere with subsequent axis formation; (2) an orienting light pulse (LIGHT I) given to intact zygotes followed by wall removal and regeneration does not interfere with rhizoids emerging from the shaded side of LIGHT I; (3) if an orienting light pulse (LIGHT I) is given to protoplasts, zygotes with a regenerated wall form rhizoids from the shaded side of LIGHT I; and (4) if zygote protoplasts are given two orienting light pulses from different directions (LIGHT I followed by LIGHT II), rhizoids form from the shaded side of LIGHT II. Hence, the perception and memory of a light gradient occurs in protoplasts, but protoplasts always have a labile polarity. That is, unless a cell wall is present, zygotes cannot fix or stabilize the polar axis.

The additional requirement for an intact cell wall to fix the polar axis raised the possibility that the cell wall provides an external matrix to which the required cytoplasmic structural component, i.e., actin microfilaments, is attached. We have proposed that axis fixation involves linking cytoskeletal filaments and wall fibrils at the future rhizoid site through integral membrane proteins (Kropf et al., 1988). Such a complex that structurally links the cytoplasm with the cell wall could serve to (1) immobilize asymmetries in the plasma membrane of the presumptive rhizoid tip that are generated by the orienting light gradient, (2) provide cytoplasmic "tracks" for the directional transport of F granules to the site of polar growth, and (3) anchor the developing embryo to the substratum by adhesive macromolecules (VnF and F2) localized in the cell wall of the elongating tip. The structural complex comprising this postulated transmembrane bridge is referred to as the axis stabilizing complex (ASC) (Quatrano, 1990).

The ASC has many similarities to elaborations of the plasma membrane at sites where animal cells adhere to an underlying substratum. These sites, known as focal adhesions, represent transmembrane connections between the cytoskeleton on the cytoplasmic face and adhesive glycoproteins in the extracellular matrix (ECM) (BurrIDGE et al., 1988). The composition of these focal adhesions and some of the interactions of the various components have been characterized. For example, the adhesive glycoproteins fibronectin (Fn) or Vn and anionic polysaccharides such as heparin comprise the ECM components on the extracellular face. The highly conserved transmembrane proteins, the integrins, link the ECM to the cytoplasm. The extracellular domain of integrin serves as the receptor for Fn or Vn through an RGD tripeptide, whereas its cytoplasmic domain supplies attachment sites for actin-associated cytoplasmic proteins (e.g., α -actinin, talin, vinculin) that in turn connect to the actin cytoskeleton (Luna and Hitt, 1992). In the proposed ASC of *Fucus*, VnF and F2 are localized in the cell wall only in the region of the ASC and could be analogous to animal Vn or Fn and the sulfated glycoprotein heparin, respectively. Actin has been localized to the cytoplasmic face

of the proposed *Fucus* ASC, and the presence of integrins and vinculin in *Fucus* extracts has been reported (Quatrano et al., 1991).

As discussed above, asymmetrically localized ion channels in this anchored domain of the ASC could generate an inward current involved in subsequent rhizoid elongation (Taylor et al., 1992). High concentrations of Ca^{2+} in this region of the ASC would enhance vesicle fusion and the possible deposition of more ion channels, thereby amplifying the electrical and structural polarity expressed in the elongating rhizoid tip. In our working model, shown in Figure 3, these unique plasma membrane transport components are postulated to be anchored in the plasma membrane between actin (cytoplasmic) and F2/VnF (extracellular) and to be unable to diffuse freely. The membrane proteins localized in this ASC domain have not been directly identified, but their postulated stabilization in the ASC corresponds to axis fixation.

FUTURE PROSPECTS FOR THE ANALYSIS OF POLARITY IN *FUCUS*

Cellular and Biochemical Approaches

The working model described in Figure 3 highlights two localized components that are essential for the stabilization of the polar axis: the actin cytoskeleton and the cell wall. The ASC at the presumptive rhizoid pole, composed of the localized actin network and the cell wall components VnF/F2, encloses a region of the plasma membrane containing a unique set of ion transporters. As described so far, this ASC is similar to focal adhesions in mammalian cells (BurrIDGE et al., 1988; Quatrano, 1990). Compared to focal adhesions, however, we know very little about the overall composition of the ASC. Screening of whole cell extracts, plasma membrane fractions, or fractions enriched in rhizoid cell components with antibodies to known components of focal adhesions or proteins postulated to be part of the *Fucus* ASC (e.g., actin binding proteins and ion transporters), should prove helpful in characterizing the localized proteins of the ASC.

A less biased approach would be to isolate rhizoid tip extracts after chemical cross-linking of embryos and to immunoprecipitate from these extracts actin or VnF, the two known components of the ASC. Complexes precipitated by antibodies to either or both proteins should contain additional proteins that are part of the ASC and are linked to actin or VnF in vivo. Monoclonal antibodies generated against components of these immunoprecipitated complexes could then be used to immunolocalize the proteins and screen *Fucus* zygote expression libraries. Antibodies that recognize localized components of the ASC or specific gene products from *Fucus* that can be shown to be involved in axis fixation would represent ideal probes to assess whether similar proteins are found in flowering plants. If so, the distribution of these gene products in cells undergoing asymmetric cell divisions (e.g., zygotes and early embryos, guard cells, epidermal hairs, pollen grains) may demonstrate

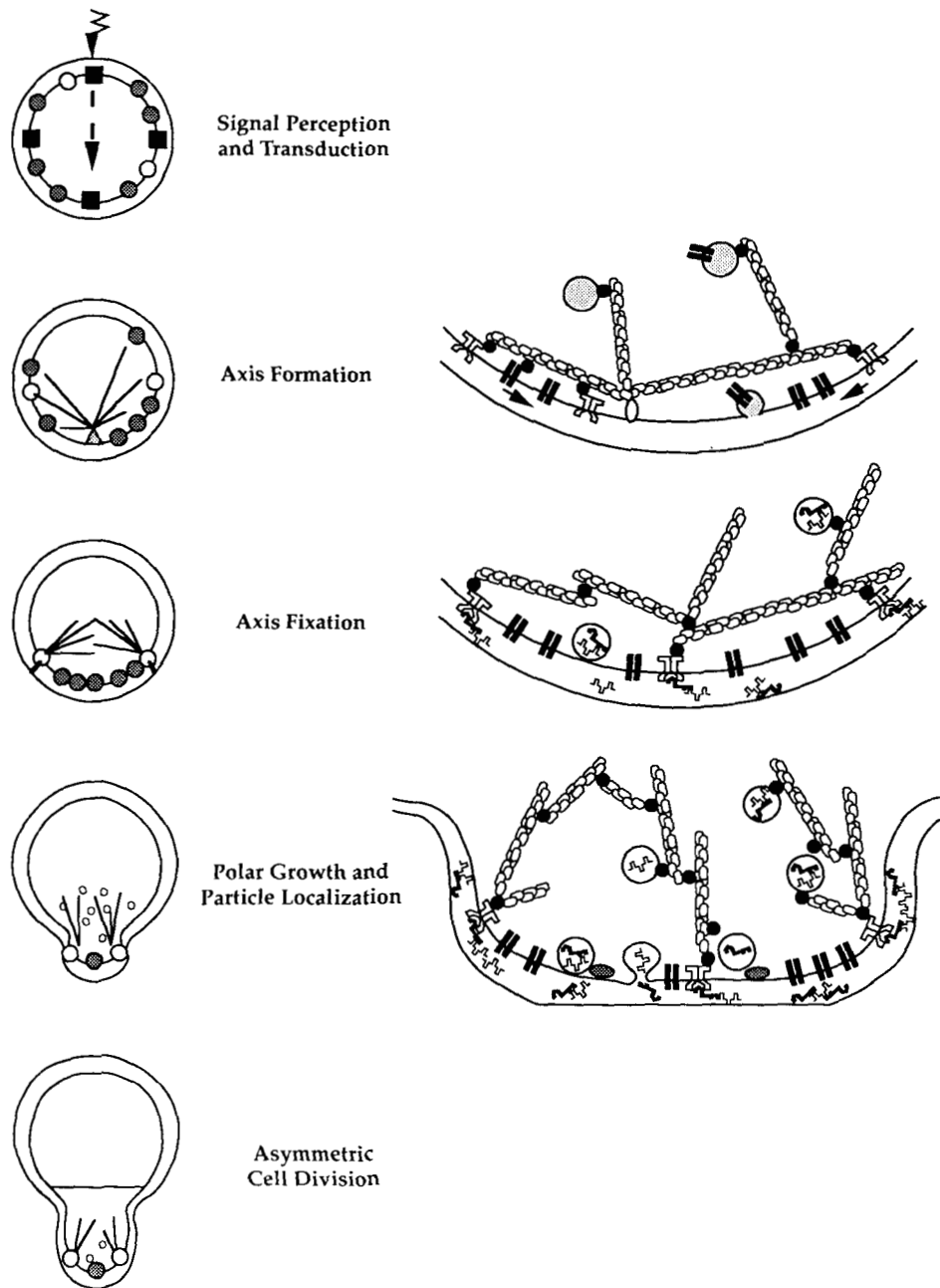


Figure 3. Schematic Diagram of a Working Model for Polarity Establishment and Polar Growth in *Fucus*.

The five figures on the left give a broad view of the process. Soon after fertilization, signal perception and transduction take place. This probably involves integral membrane receptors (■) for environmental signals such as light (⚡). After perception of a signal, information is transduced into the cytoplasm (→), and the zygote forms a polar axis. During axis formation, the future site of rhizoid tip growth is determined (Δ), the actin cytoskeleton (↔) reorganized, and membrane components such as integrins (○) and ion transporters (●) recruited and/or selectively retained at that site. During axis fixation, the connections between the actin cytoskeleton and membrane proteins such as integrins are stabilized, as are connections between these membrane proteins and components of the cell wall (||). Polarized growth occurs with the selective transport and fusion of secretory vesicles (○) at the rhizoid tip. The asymmetric first cell division produces two cells with different development fates.

The figures on the right give an expanded view of the molecular events occurring at the site of rhizoid tip growth during axis formation, axis fixation, and polar growth. The components shown in these figures include actin microfilaments (↔), protein(s) involved in determining the site of the future rhizoid tip (○), various actin binding or actin-associated proteins (●), integrins (⌘), ion transporters (■), secretory vesicles containing cell wall material (●), secretory vesicles containing rhizoid-specific cell wall components (○) (such as a vitronectin-like protein [↔]) and the F2 sulfated fucan glycoprotein [↔], and proteins related to SEC4 involved in vesicle fusion with the plasma membrane (■).

possible structural and functional homologies of gene products between *Fucus* and flowering plants.

Because genetic analysis is not yet possible in *Fucus*, the role of gene products in axis fixation must be tested directly. Microinjection of plasmids has proven to be an effective approach to study the regulation of spatial and temporal regulation of gene expression in sea urchin (Coffman and Davidson, 1992), and microinjection of antibodies into sea urchin eggs has been shown to bind and inactivate proteins *in vivo* (Dinsmore and Sloboda, 1989). The ability to microinject *Fucus* zygotes (Spektnijder et al., 1989) suggests the possibility of injecting specific antibodies into *Fucus* zygotes to interfere with the appropriate process. For example, monoclonal antibodies that recognize actin or the highly conserved terminal 37-amino acid cytoplasmic fragment of β -integrin (Marcantonio and Hynes, 1988) should block the actin network from binding integrins that are postulated to be the transmembrane link between the cell wall components and actin in the ASC. After injection of either of these antibodies into the *Fucus* zygote, we can ask a variety of questions. Is the polar axis prevented from becoming fixed after injection of either antibody? Can a polar axis form in the presence of these antibodies? Will injection of fluorescently labeled actin, Vn, or any other protein component of the ASC into early zygotes allow us to follow the *in vivo* localization of these introduced proteins into the ASC?

Comparative Genetic Approaches

The temporal and spatial processes of polar axis formation, fixation, and expression in *Fucus* are similar to the stages of bud site selection, polarity establishment, and bud growth during the asymmetric division of the budding yeast *Saccharomyces cerevisiae* (Chant and Pringle, 1991). For example, polar axis establishment in *S. cerevisiae* occurs well before, and can be experimentally separated from, the actual process of bud growth. Further, actin microfilaments, and not microtubules, play a central role in budding (Adams and Pringle, 1984). The powerful genetic tools in *S. cerevisiae* have been used to identify genes whose products are directly involved in each of the three steps of budding: bud site selection, polarity establishment, and bud growth. Genes encoding many of the components required for these steps are currently under study at the molecular level.

Are the morphological similarities of the asymmetric growth and division and experimental results of these two systems close enough to expect conservation of gene function? For example, can one identify gene products involved in *Fucus* by homology to *S. cerevisiae* genes? Can *Fucus* gene products function in *S. cerevisiae* (i.e., can they complement *S. cerevisiae* mutants)? If so, are the homologous gene products in *Fucus* temporally and spatially related to the fixation of the polar axis and/or rhizoid outgrowth? We are now evaluating the merit of this comparative approach by determining whether any of the gene products known to function in polarity establishment in *S. cerevisiae* are present in *Fucus*. Further descriptions of some

of the characterized *S. cerevisiae* genes may help to illustrate the utility of this approach.

Axis Formation and Bud Site Selection

In *S. cerevisiae*, a number of genes have been identified that are involved in bud site selection. Mutations in these genes (*BUD1-5*) specifically alter the wild-type pattern of bud site selection without altering subsequent steps in budding (Chant and Herskowitz, 1991; Chant et al., 1991). One of these genes, *BUD1*, codes for a ras-like GTP binding protein. The *BUD* gene products are probably localized or activated at the site of subsequent bud outgrowth (Chant and Pringle, 1991). Another protein that accumulates at the future bud site is *CDC42p*, another ras-like GTP binding protein whose activity might be linked to the activity of *BUD1p* through the action of *CDC24p* (Bender and Pringle, 1989; Johnson and Pringle, 1990). Site-directed mutagenesis has revealed that the *BUD1* and *CDC42* gene products require GTPase activity and membrane localization for their proper function (Ruggieri et al., 1991; Ziman et al., 1991). The mechanisms by which the other *BUD* gene products and *CDC24p* are involved in bud site selection are still not clear but may involve regulation of the activities of *BUD1p* and *CDC42p* through modulation of GDP/GTP exchange and activation of GTPase activity (Chant and Pringle, 1991).

The site of localized growth in *Fucus* is not predetermined as it is in *S. cerevisiae*. The ability to experimentally manipulate the site of targeting for subsequent rhizoid outgrowth in *Fucus* makes it possible to observe the molecular changes associated with the formation of the targeting site. Are proteins similar to the *BUD* gene products found in *Fucus*, and are they localized at the presumptive rhizoid site? Preliminary results indicate that ras-like genes can be detected in *Fucus*. These genes are currently being characterized, and their protein products are being localized by immunological methods (B. Goodner, R. Moore, and R.S. Quatrano, unpublished data).

Axis Fixation and Polarity Establishment

We know that the actin cytoskeleton is localized at the future rhizoid outgrowth site at the time of axis fixation in *Fucus*. In *S. cerevisiae*, the actin cytoskeleton is also localized to the future budding site and is essential for polarity establishment. Microtubules are not required for polarity establishment in either system and are only recruited to the budding site in *S. cerevisiae* later, in preparation for nuclear migration and cell division (Adams and Pringle, 1984; Kilmartin and Adams, 1984). In *S. cerevisiae* and in mammalian cells, a group of ras-like proteins (*CDC42p*, *rac*, *rho*) influence the organization of the actin cytoskeleton by as-yet-uncharacterized mechanisms (Chardin et al., 1989; Hall, 1992). Moreover, in *S. cerevisiae*, other proteins have been identified that are required for polarity establishment and may interact with *CDC42p*. One of these proteins, *BEM1p*, contains two SH3 (src homology domain 3) domains and has been shown genetically to interact with

CDC24p and BUD5p (Bender and Pringle, 1991; Chant et al., 1991; Chenevert et al., 1992). Proteins containing SH3 domains have been implicated in connecting signal transduction events with alterations in the actin cytoskeleton (Mayer and Baltimore, 1993). In addition, a set of novel cytoplasmic filaments known as neck filaments are assembled at the future bud site in *S. cerevisiae* (Byers, 1981). The proteins that make up these filaments, CDC3p, CDC10p, CDC11p, and CDC12p, lie immediately beneath the plasma membrane at the bud site (Haarer and Pringle, 1987; Ford and Pringle, 1991; Kim et al., 1991). This ring of neck filaments, along with the actin patches, appears to arrive at the future bud site minutes before the start of bud growth. These proteins may interact structurally with the cell polarity machinery in *S. cerevisiae* to delimit the area used for budding.

We are currently determining whether similar proteins are present in *Fucus* and, if so, whether their localization suggests a similar role in the polarized outgrowth of the rhizoid. We have preliminary immunological evidence that there are proteins homologous to neck filament proteins in *Fucus*, and we are attempting to clone the genes and localize the proteins (B. Goodner, R. Moore, and R.S. Quatrano, unpublished data).

Polar Growth, Particle Localization, and Bud Growth

Just as the transport of new cell wall components such as F2 and VnF in F granules to the site of rhizoid outgrowth requires an actin-based network in *Fucus*, the transport of intracellular vesicles occurs along actin microfilaments in *S. cerevisiae* (Nelson, 1992). What are the molecular motors and gene products involved with vesicle targeting in *Fucus*? Can directed vesicle transport to the site of bud growth in *S. cerevisiae* help in our understanding of vesicle transport in *Fucus*? In *S. cerevisiae*, a novel myosin gene (*MYO2*) has been implicated in the targeting of vesicles to the bud site. Mutations in this gene do not affect bulk flow of proteins to other areas of the cell surface, implying that vesicles associated with the MYO2p protein are specifically directed to the bud site (Johnston et al., 1991). Additional proteins, such as SEC4p, YPT1p, and the rab proteins, are also involved in regulating vesicle transport and exocytosis in *S. cerevisiae* and animal systems (Balch, 1990). Are similar proteins present in *Fucus*? Again, detection of these proteins in fractions enriched in F granules or at the presumptive site of rhizoid outgrowth using immunolocalization techniques would suggest a similar functional role in the asymmetrical distribution of cytoplasmic vesicles.

Applications to Polarity In Flowering Plants

The use of a system as a "model" implies that the system has some traits that are unique and of a special nature to enhance our ability to understand a general process. Additionally, the role of a particular gene elucidated in the model system can be tested directly in another system. It is now possible for us to begin to determine whether the mechanisms and

macromolecules implicated in polarity establishment and polar growth in *Fucus* are also involved in asymmetrical cell processes in flowering plants. Some of these asymmetric processes occur during the earliest stages of embryogenesis (Mayer et al., 1993) and others occur later in development in pollen tubes and root hairs (Heath, 1990; Schiefelbein et al., 1993). In these cases, as in *Fucus*, the actin cytoskeleton has been found to be localized to the region of polar growth and has been implicated in vesicle transport to the growing tip. Ca²⁺ fluxes are also localized to the growing tips of pollen tubes and root hairs. These systems and the study of early development should continue to provide new information using powerful genetic and biochemical tools. For example, the recently described Arabidopsis body pattern mutants *gnom*, *gurke*, *fackel*, and *monopteros* suggest that a genetic approach might lead to a further understanding of asymmetric cell divisions in the early development of flowering plants (Mayer et al., 1991, 1993). When genes such as these are eventually cloned and characterized, it will be interesting to look for analogies, if not homologies, with *Fucus*.

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