MICROBIOLOGICAL REVIEWS, June 1992, p. 316–339
0146-0749/92/020316-24$02.00/0

Vol. 56, No. 2

Establishment and Expression of Cellular Polarity in Fucoid Zygotes

DARRYL L. KROPF

Department of Biology, University of Utah, Salt Lake City, Utah 84112

INTRODUCTION

In recent years, much of our knowledge of molecular and cellular events which give rise to pattern during embryogenesis in eukaryotes has come from investigations of animals (34, 93). A select group of organisms, most notably fruit flies, nematodes, sea urchins, and frogs, has been the subject of intensive investigation, and the rewards have been considerable. With the exception of yeasts, lower eukaryotes have received significantly less attention, yet the diversity and simplicity of developmental strategies within this group make them attractive organisms for illuminating fundamental aspects of morphogenesis and pattern formation. Fucoid algae provide a case in point. They have a long history as subjects for examination of the processes that control early embryogenesis. These investigations date from the middle of the 19th century, when developmental biologists first discovered the utility of gametes, zygotes, and embryos of fucoid algae as model organisms. The chronicle of research in this field can broadly be categorized into three areas: (i) gamete recognition and fertilization (36), (ii) wall biosynthesis (117), and (iii) acquisition of cellular polarity (111). This review will focus on the third aspect, establishment and expression of developmental polarity, and will consider fertilization and wall biosynthesis only as they relate to polarity. The approach is to focus entirely on the first cell cycle of the fertilized egg, approximately a 24-h period. A rhizoid/thallus axis is generated during the first half of the cell cycle, and in later stages this polarity is expressed as localized growth and oriented division. Most of the research has been conducted on two genera, *Fucus* and *Pelvetia*, and results indicate that they use very similar, if not identical, mechanisms to generate embryonic polarity. For this reason, investigations of *Fucus* and *Pelvetia* species will be considered interchangeably in this review.

Fucoid algae are common marine rockweeds in the intertidal zones of North America and Europe, and sexually mature fronds (receptacles) are easily collected at low tide. Receptacles can be stored for a few weeks at 4°C without loss of gamete viability. When exposed to light-dark regimes (*Pelvetia* spp.) or osmotic shock (*Fucus* spp.), receptacles release copious amounts of gametes. Both monoecious (*Fucus distichus* and *Pelvetia fastigiata*) and dioecious (*F. vesiculosis*, *F. serratus*, and *F. ceranoides*) species are common, so one can obtain large numbers of zygotes or unfertilized eggs, respectively. The young zygote displays no detectable asymmetry; it is a large, spherical, apolar cell. Yet, a few hours after fertilization it generates a developmental axis that marks rhizoid and thallus poles. Zygotes develop synchronously, and an entire population of cells can be induced to polarize simultaneously in a single direction. These features make the zygote of fucoid algae a paradigm for cell polarity (45).

Fertilization is oogamous, with large, sessile eggs and small, motile, biflagellated sperm. Sperm entry triggers an electrical fast block to polyspermy and wall biosynthesis (possibly a slow block) within minutes. The next few hours are critical for establishment of the young zygote. The cell must settle onto the rock substratum and attach so tenaciously that it cannot be dislodged by the pounding surf. Zygotes are dense and sink rapidly (1 cm/min) through seawater (63). The nascent cell wall is quite sticky and permits zygotes to adhere to nearly any substratum with which they come in contact. During this attachment period the cell remains spherical and does not increase in size (Fig. 1a). After attachment the zygote germinates and grows a rhizoid down onto the rock, anchoring the cell even further. To select the appropriate position for rhizoid initiation, a

Electronic mail address: Kropf@bioscience.utah.edu.

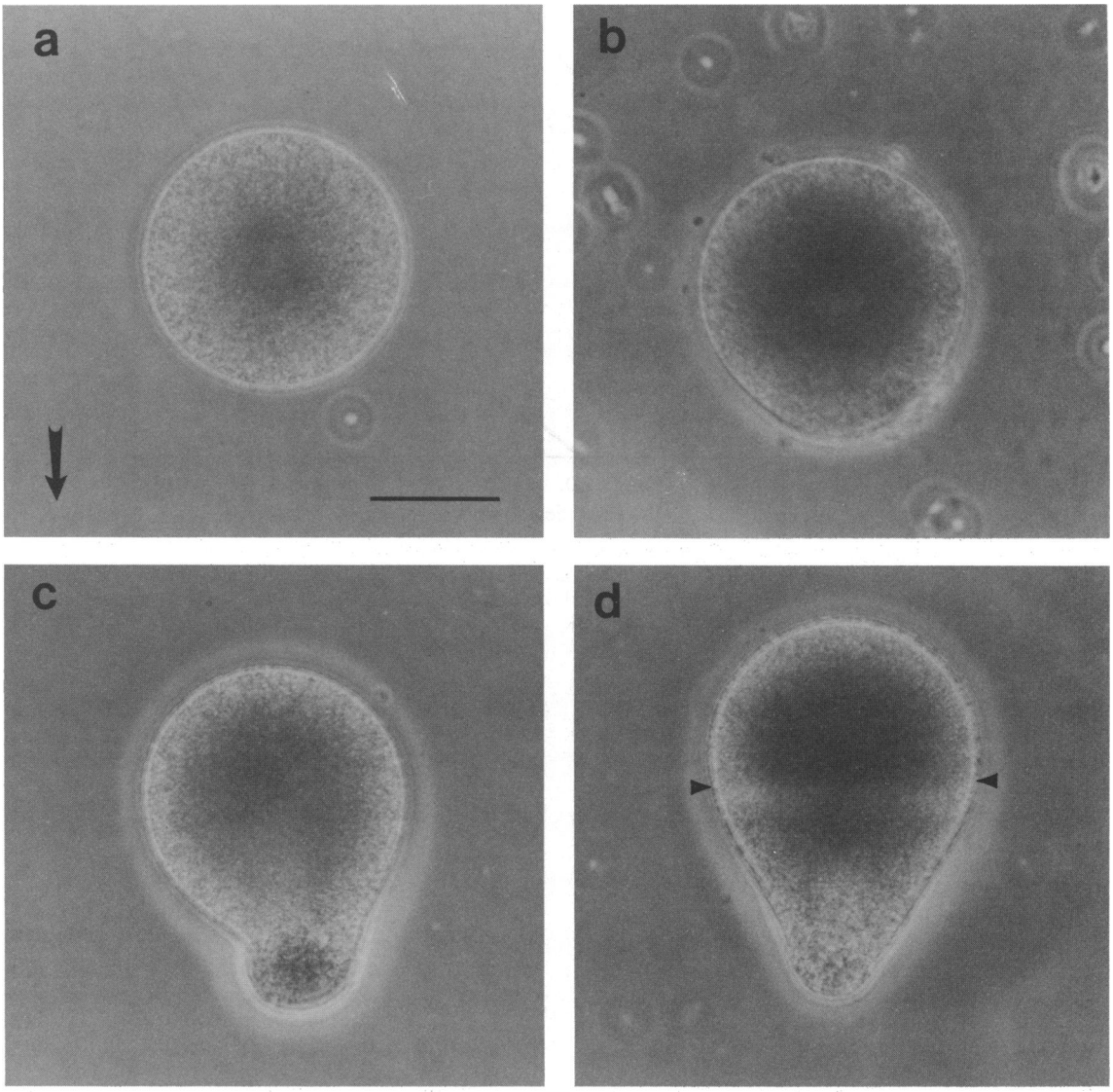

FIG. 1. Photomicrographs of *Pelvetia* zygotes. (a) Apolar zygote 3 h after fertilization. (b) Zygote just beginning to germinate at shaded pole 12 h after fertilization. Localized growth makes the zygote appear pear shaped. (c) Zygote (16 h old) in mitosis. The spindle is not visible through the opaque cytoplasm. The rhizoid is now well defined. (d) First cytokinesis is unequal, forming a larger thallus cell and a smaller rhizoid cell. Arrowheads mark the division plane. The arrow indicates the direction of unilateral light. Bar, 50 μm.

zygote monitors environmental conditions and establishes a primary developmental axis in accordance with vectorial cues. The axis is established by localization of cytoplasmic components such that the two ends of the zygote differ structurally and physiologically. In this way, the homogeneous egg cytoplasm is restructured into a polar cell.

Rhizoid growth constitutes the first obvious morphological expression of the inherent polarity. Growth is localized to one end of the developmental axis, creating a pear-shaped zygote (Fig. 1b). The rhizoid continues to elongate and is well defined morphologically by the time of first mitosis (Fig. 1c). Subsequently, an unequal division partitions the zygote into two very distinct parts; the rhizoid cell is smaller and highly polarized morphologically and contains the growing apex. By contrast, the larger thallus cell remains nearly spherical except for a flattened face at the cross wall (Fig. 1d). These two cells also have very distinct developmental fates: the rhizoid cell gives rise to the holdfast, whereas the thallus cell is the progenitor of stipe and frond tissues. The unique developmental lineages are determined by cytoplasmic determinants which are segregated unevenly to the two cells. Interaction of these localized determinants with the genetically identical nuclei presumably initiates different genetic programs in the rhizoid and thallus cells (111). To ensure proper partitioning of these determinants, it is essential that cytokinesis be invariant in its orientation. Indeed, the partition wall always forms perpendicular to the growth axis (Fig. 1d). Over the next days of development the rhizoid elongates by apical growth and the embryo is divided into increasingly smaller cells. Its morphology is strikingly similar to the globular stage of higher plant embryos (111). This developmental program is uniquely suited for investigations

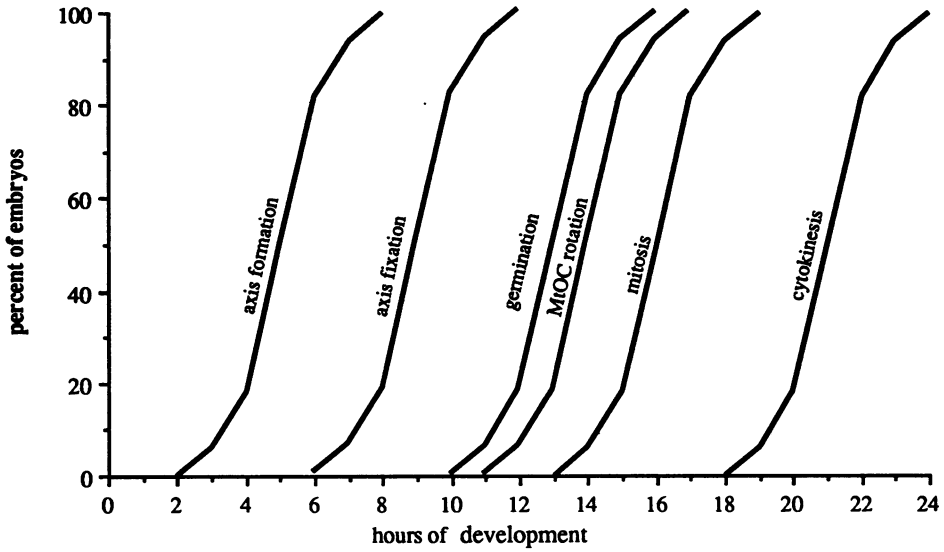

FIG. 2. Time course of progression through the developmental stages of the first cell cycle in *Pelvetia* species. In chronological order the stages are axis formation, axis fixation, germination, rotation of the axis defined by MtOCs, mitosis, and cytokinesis. A population of zygotes transits each of these stages in relative synchrony; even so, several of the stages overlap temporally. Time zero is defined as the time of fertilization, taken as 30 min after receptacles are placed in the dark.

of fertilization, polarization, and expression of polarity, questions which are intractable in other organisms, including most higher plants and animals.

Zygotes proceed through stages of the first cell cycle in relative synchrony (Fig. 2). The stages are defined so as to highlight the salient features of early development, but they are nonetheless an attempt to reduce a continuous developmental process into a series of successive events. Axis formation is the establishment of a labile polarity, usually in accordance with an external vector such as unilateral light. At axis fixation this polarity becomes irreversibly determined in space. Germination is simply the initiation of rhizoid outgrowth. Two microtubule-organizing centers (MtOCs) form in the perinuclear area soon after germination, and rotation of the axis defined by these MtOCs determines spindle orientation during mitosis. Cytokinesis, the final stage, does not follow mitosis closely in time but is instead delayed a few hours. Nearly all individuals in a population transit through a specific stage over a 4-h period (Fig. 2). Because there is considerable temporal overlap between some stages, it is often difficult to correlate the developmental stage with specific molecular, cellular, or physiological phenomena. For this reason, procedures have been developed to separate temporally overlapping stages. The intent of this review is to discuss our current understanding of each of these stages in the context of embryonic polarity.

Classical genetic approaches have not been developed for fucoid algae, owing to the relatively long life cycle and difficulties in growing zygotes into sexually mature plants in the laboratory. Techniques of molecular biology are only now being applied, and as yet little progress has been made. What is known concerning protein and RNA synthesis can be summarized in short order. Although most mRNAs are inherited maternally, zygotic transcription and translation are necessary for completion of the first cell cycle (76, 108). However, the requirements for transcription and translation markedly precede the dependent developmental processes. Completion of the first cell cycle depends on RNA synthesis only during the first 5 h postfertilization, and protein synthesis is needed only for the first 12 h (79, 108). Unfortunately, we have no idea of the identity of the specific molecules that must be synthesized. The array of proteins synthesized at each developmental stage has been analyzed by two-dimensional gel electrophoresis. Approximately 20% of the detectable proteins change quantitatively or qualitatively during the first cell cycle, and the pattern of protein synthesis at each stage is unique (79). However, morphogenesis and protein synthesis are not tightly coupled during early embryogenesis, and so treatments which affect morphogenesis do not necessarily alter patterns of protein synthesis, and vice versa. Taken together, these findings indicate that proteins relevant to early development are synthesized soon after fertilization and that a particular morphogenetic event is not dependent on concomitant protein synthesis. Therefore, establishment and expression of polarity do not appear to be closely governed by changes in gene expression, but instead arise in a more epigenetic manner. In fact, changes in gene expression alone cannot confer polarity upon a cell (45). In my opinion, the essence of polarity is the redistribution and localization of macromolecules, organelles, and metabolic processes within the cytoplasm, plasmalemma, and cell wall.

Considerable research has been aimed at identifying localized structures and processes during early development, and I will attempt to review the present status of this work, beginning with polarization and continuing through first cytokinesis. Where warranted, working models will be presented and critiqued in the hope of providing a framework to guide future research.

ESTABLISHMENT OF EMBRYONIC POLARITY

As Bünning stated nearly 40 years ago, "Ohne Polarität keine Differenzierung" (26); the establishment of cellular polarity is indispensable for development and differentiation. In general, mechanisms for establishing developmental cell polarity can be grouped into two broad categories, extrinsic and intrinsic. These two mechanisms are not mutually ex-

clusive, but instead operate coordinately. In multicellular tissues, polarity is often induced by external substances secreted from neighboring cells. A good example is the hormonal induction of mesoderm by transforming growth factor beta during vertebrate development (93). Polarity can also be established intrinsically by localization of cytoplasmic determinants within the egg. Subsequent inheritance of the local egg cytoplasm controls cell differentiation. Both extrinsic and intrinsic controls operate coordinately during early development of fucoid zygotes. Extrinsic factors are important in the initial polarization, but, later, intrinsic factors control specification of rhizoid and thallus cells in the two-celled embryo.

Unlike most higher animal and plant eggs, the intrinsic cytoplasmic determinants which specify cell fate are not prelocalized in the fucoid egg. Instead, the fucoid egg is an unpatterned, apolar (or weakly polar) cell which bears no obvious physiological, morphological, or biochemical localizations (61). Polarity arises epigenetically a few hours after fertilization, and cytoplasmic determinants are then redistributed in accordance with this nascent embryonic axis. This makes the young zygote an ideal organism for investigating the process of cytoplasmic localization and specification of cell lineages. It cannot be overstressed that although zygotic transcription is required for establishing polarity (79), it must be the localization of relevant gene products to opposite developmental poles that is fundamental in reorganizing a homogeneous fertilized egg into a polar cell bearing localized determinants.

Establishment of embryonic polarity in fucoid zygotes encompasses (i) selection of an axis, (ii) transduction and amplification of that positional information leading to localization of determinants within the cell (axis formation), and (iii) irreversible spatial fixation of the determinants (axis fixation).

Axis Selection

The first step in establishing polarity is the selection of an axis, and fucoid zygotes make this decision by sensing environmental gradients. The orientation of the developmental axis is sensitive to any number of applied gradients, as was first demonstrated by Rosenvinge in 1888, who used unilateral light as an orienting vector (128). Much of the research early in the 20th century carefully characterized responses to applied vectors. In most cases, the position and direction of rhizoid growth (tropistic responses), as well as differentiation of the two-celled embryo into thallus and rhizoid cells (morphogenetic responses), are influenced by applied gradients. Gradients, however, are not essential for morphogenesis. Zygotes incubated in the dark without vectorial cues germinate, divide, and develop normally, albeit somewhat slowly. In the absence of vectorial cues from the environment, the site of sperm entry is reported to mark the rhizoid end of the developmental axis in a related fucoid alga, *Cystoseira* (74). However, these experiments are now 60 years old and need to be repeated with *Fucus* and *Pelvetia* eggs. If the results are the same, these experiments indicate that cytoplasmic determinants are redistributed with respect to the site of sperm entry, predisposing the egg to germinate at that site. However, this weak axis is normally overwhelmed by environmental gradients experienced during the first hours after fertilization. One might expect that the environmental gradients act by rotating the weak preformed axis set up by sperm entry, but this is apparently not the case. In plane-polarized light, roughly half of the zygotes

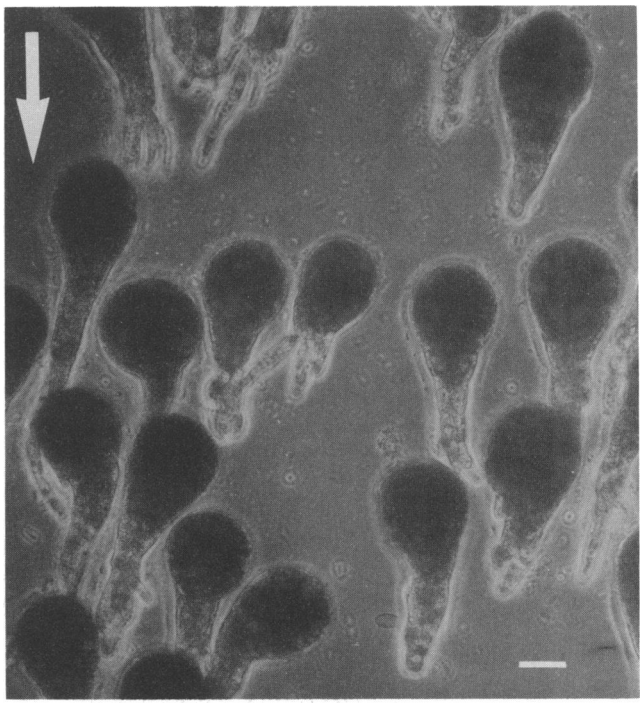

FIG. 3. Population of synchronously developing 5-day-old *Pelvetia* embryos oriented by bright unilateral light. Rhizoids grow away from the light source. The arrow indicates the direction of light. Bar, 50 μm.

form two rhizoids, oriented in accordance with the vibration of the electric vector (61). The induction of double rhizoids indicates that the developmental axis is selected de novo in response to vectorial stimuli.

The array of gradients that zygotes can perceive is impressive (for a detailed consideration, see references 6 and 63). In a natural setting (on rocks in the intertidal zone), zygotes experience numerous vectorial stimuli, any one of which is sufficient to determine polarity. In the laboratory, investigations of polarization are facilitated by the ease with which entire populations of zygotes can be oriented in unison. Prior to the polarization, fertilized eggs attach firmly to the substratum via the nascent cell wall and do not rotate even when the medium is exchanged. Because zygotes are well anchored, application of an appropriate gradient across the dish can induce parallel axes in all of the zygotes developing synchronously in a dish of seawater. The most commonly used vector is unidirectional light of high irradiance, which localizes rhizoid growth to the shaded portion of the zygote (53) (Fig. 3). In addition, physiologically relevant stimuli known to polarize fertilized eggs include a laminar flow of seawater (7), a heat gradient (87), osmotic and ionic (K^+ and H^+) gradients (8), and a nearby egg or piece of plant tissue (63). Many nonphysiological stimuli, including electric fields (89), are also effective. Interactions between the different vectors have been investigated by applying them sequentially. A second inducing gradient often overrides the first, and there appears to be a hierarchy of effectiveness (147). Very little is known concerning the mechanism(s) by which zygotes record gradients, so only two examples will be considered here, the positive group effect and photopolarization.

One of the strongest polarizing influences is the presence

of a neighbor. Fertilized eggs within a few cell diameters of one another grow rhizoids toward each other (positive group effect [151]). This observation led to speculation that the rhizoids secrete a growth-stimulating substance, termed rhizin (63). Because a negative group effect can be induced by changing the pH of the medium, the presence of a growth-inhibitory substance (antirhizin) was also postulated. Unfortunately, the chemical identity of these putative regulators has not been uncovered. Arguments in favor of auxin as rhizin (68) lost favor when it was shown that high levels of auxins added to the medium did not overcome the group effect (71). In recent years, no attempts have been made to identify rhizin, but it may prove to be CO_2 or metabolic acid (45). Young zygotes have recently been shown to secrete metabolic acid, which acidifies the medium near the wall (40) and may create a local pH gradient that orients close neighbors (8).

Most is known concerning axis induction by unilateral light. The photoreceptor is thought to be uniformly distributed about the cell cortex (or plasma membrane), with its main photon transition moment oriented parallel to the cell surface (61). In higher plants, two photoreceptor systems are involved in photomorphogenesis, and they are primarily distinguished by their action spectra. Plant responses governed by phytochrome are activated by red light and reversibly inactivated by far-red light. Another class of responses is generally activated by UV and blue light, but the action spectra vary and multiple pigments may be involved (52). Even so, the major blue-light receptor is cryptochrome. The nature of this pigment has not been unequivocally determined, and its mode of action is not known. Cryptochrome is most probably a flavoprotein with an action spectrum peak near 450 nm, but the possibility that it is a protein-bound carotenoid has not been ruled out. Photomorphogenesis in brown algae is, in most cases, a blue-light response acting through cryptochrome (33), and this appears to be true for photopolarization of fucoid algae (61). The action spectrum for photopolarization has two peaks; one is consistent with cryptochrome as the photoreceptor, and the other is in the UV, near 250 nm (4). Bentrup interpreted these data to indicate the existence of two photoreceptors: a low-intensity, probably carotenoid-containing receptor and a high-intensity, probably flavin-containing (cryptochrome) receptor (4). However, the findings are also consistent with a single pigment that must be photoactivated before functioning as a competent photoreceptor (22). More information is needed before this issue can be resolved (22). Unidirectional red light is ineffective in inducing an axis (53), so phytochrome does not participate in photopolarization.

Rhizoid growth is thought to be localized to the region where the least blue light is received and the photoreceptors are least activated (61, 147). The intensity of light received by photoreceptors at various positions around the spherical zygote is influenced mainly by two factors, screening and the lens effect (22). In an opaque cell such as the fucoid zygote, much of the light traversing the cytoplasm is absorbed or scattered by screening pigments (carotenoids, xanthophylls, and chlorophyll) such that photoreceptors on the shaded side are presumably least activated. If screening dominates, zygotes initiate rhizoids directly away from the light source. Superimposed on the screening effect is the lens effect. Since the refractive index of cytoplasm is higher than that of seawater, unilateral light will be refracted and focused on the shaded hemisphere, with the sides of the cell (equatorial zone) receiving the least light (see reference 22 for a recent review). When the lens effect dominates, rhizoids form in the equatorial zone. The position of rhizoid germination depends on the relative contribution of the lens effect and screening and on the nature of light applied (polarized versus unpolarized, irradiance, and light quality).

Despite the long history of characterizing responses to applied gradients, the mechanism by which zygotes record these stimuli is obscure. In no case is the signal perception pathway known, and, to make matters worse, there are almost certainly multiple pathways. Because so many diverse vectors can be detected, it seems unlikely that they all have the same primary effect on the cell. For example, imposed gradients of ions, ionophores, and voltage are thought to be perceived as local changes in membrane potential or intracellular ion concentration (66), whereas unilateral light causes differential activation of photoreceptors. If there is a unifying theme in signal perception, it is that all vectorial stimuli are recorded in the cortical cytoplasm (64), but even this is unproven.

It is assumed that, despite the wide variety in effective inducing gradients, the vectorial stimuli are all amplified and transduced into common cellular signals which lead to localization of determinants within the cytoplasm or membrane, or both. In this way, a small environmental perturbation would be transduced into a developmental axis. Like signal perception, the signal transduction pathway(s) is completely unknown. For photopolarization at least two plausible pathways can be conceived. First, light-induced activation of the photoreceptor may lead to electrical or ionic fluxes, or both, across the plasma membrane. Flavin-containing molecules are known to shuttle electrons by oxidation-reduction reactions (e.g., flavin adenine dinucleotide, electron-transferring flavoprotein), and there is some evidence that photoreceptor activation causes transmembrane ion fluxes via a plasma membrane electron transport chain containing cytochrome (119; see also reference 22). In stomatal guard cells, blue light absorption causes proton extrusion (3, 35), but in *Pelvetia* zygotes no changes in local external pH were found upon irradiating dark-grown zygotes (40). Alternatively, blue light may activate a phosphatidylinositol (PI) cascade, as recently proposed by Brownlee (22) (Fig. 4). In this pathway, light stimulation changes the conformation of the photoreceptor, permitting it to interact with a G protein. Activated G protein stimulates a phospholipase associated with the membrane, causing the hydrolysis of PI trisphosphate to inositol trisphosphate (IP_3) and diacylglycerol (DAG). Cytoplasmic IP_3 induces the release of Ca^{2+} from internal stores (mainly the endoplasmic reticulum). Local elevation of levels of cytosolic Ca^{2+} and DAG initiates regional differentiation. Protein phosphorylation via protein kinase C and other Ca^{2+}-dependent kinases is activated, as are other responses regulated by Ca^{2+}, DAG, or IP_3. The end result is localization of cytoplasmic determinants and cellular differentiation.

Signal transduction via the PI cycle has been extensively documented in animal cells (9), and recent evidence implicates this pathway in osmotically driven movements in plants. Closing of stomata and solar tracking by leaflets appear to be regulated by phosphoinositide cycles involving changes in IP_3 and intracellular Ca^{2+} levels (43, 94). Blue light also regulates these movements (90, 131), consistent with the hypothesis that the photoreceptor is coupled to the PI cycle. Other components of the pathway have been identified in higher plants (86), but, like many aspects of signal perception and transduction, they have not yet been investigated in fucoid zygotes.

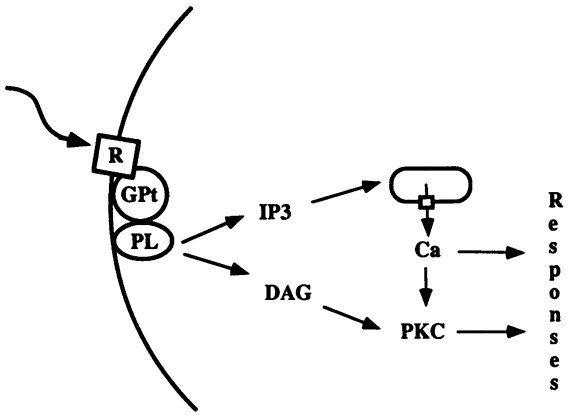

FIG. 4. Possible mechanism by which unilateral light is transduced into intracellular signals via a PI cycle. Light absorption by a membrane receptor (R) activates a G protein (GPt), which in turn interacts with and activates a phospholipase (PL) on the cytosolic face of the membrane. Phospholipid breakdown produces IP_3 and DAG, which function as intracellular messengers. IP_3 binds to internal membrane systems (endoplasmic reticulum, vacuole, etc.), causing release of stored Ca^{2+}, thereby amplifying the signal. DAG and Ca^{2+} activate protein kinase C (PKC), which, in coordination with other Ca^{2+} activated proteins, leads to localized activation of cellular responses, many of which depend on protein phosphorylation cascades. Reproduced from reference 22 with permission.

Axis Formation

Regardless of the mechanisms of signal perception and transduction, the polar zygote results from localization of macromolecular determinants to the different ends of the developing embryonic axis, causing regional cytoplasmic differentiation. Identification of localizations within the young zygote has received much more attention than signal perception and transduction, and so it constitutes the bulk of this review. Localizations of polysaccharides in the cell wall, organelles in the cytoplasm, the secretory apparatus, ionic fluxes, and cytoskeleton have all been investigated. Some of the localizations are temporally associated with formation of a labile axis, whereas others occur as the axis is irreversibly fixed in space.

Axis formation can be separated temporally from axis fixation using two sequential pulses of unidirectional light (4). An axis formed in accordance with the first light pulse can be realigned by a second light vector from another direction, provided that the second treatment is given sufficiently early. If, however, the second light is delayed, the developmental axis becomes irreversibly set in place in accordance with the first light pulse. Analyzing the position of rhizoid outgrowth provides an easy assay for determining whether the axis was fixed at the time of light reversal. Such experiments yield the time courses of axis formation and fixation (Fig. 2) and can also be useful in determining whether localization of a particular cytoplasmic constituent correlates with axis formation or fixation. If the localization can be repositioned by application of a second light, it must be associated with axis formation. If, however, localization does not occur until axis fixation, it will be insensitive to repositioning.

There are surprisingly few detectable changes in the cytoarchitecture of a young zygote as it establishes a labile embryonic axis. Cytoskeleton, cell wall, and general cytoplasmic organization has been investigated in relation to axis formation. There is no detectable asymmetry in the cell wall; cellulose, fucan, and alginic acid, the most prevalent wall polysaccharides of polarizing zygotes, are spread evenly over the cell surface (112). Microtubules detected by indirect immunofluorescence remain uniformly distributed around the nuclear membrane (81), and actin filaments, visualized by fluorescent phalloidins, are distributed uniformly in the cell cortex (17, 78). Thus, establishment of polarity does not involve gross rearrangements of cytoskeletal filaments, but subtle changes might not be detected by using these techniques. Preservation of microtubules and microfilaments for electron microscopy (EM) has proved difficult, so high-resolution images of the cytoskeleton are not available. EM studies of general cytoplasmic organization also failed to uncover localization of any structural component during axis formation (109), except for the observation that the sperm eyespot and mitochondria persist in the perinuclear region and could mark the sperm entry site (18). Despite this lack of structural asymmetry, zygotes clearly acquire functional polarity and they establish an axis. Only two processes, jelly secretion and ionic transport, are known to become localized.

Polarized secretion. Zygotes secrete an amorphous polysaccharide jelly outside the cell wall, and after germination this thick layer of jelly surrounds the growing tip. Most importantly, the polarized secretion of jelly markedly precedes growth. Fertilized eggs incubated continuously in unilateral light begin to secrete jelly at the presumptive rhizoid by 4.5 h, yet they do not germinate until approximately 9 h (133). If the direction of unilateral light is changed by 90° at 4.8 h, jelly secretion becomes localized on the new shaded hemisphere and the rhizoid grows from that point. Thus, localized jelly secretion is associated with a labile axis well before it becomes spatially fixed. To the best of my knowledge, this is the earliest asymmetry thus far detected in polarizing zygotes.

Soon after jelly secretion, at approximately 5 to 6 h postfertilization, a zone of cortical clearing can be observed at the future growth site. This zone can be observed by light microscopy as a translucent arc between the plasma membrane and cell wall (101) and by freeze fracture techniques as an area where the plasma membrane is not tightly appressed to the wall (106). It is present when the axis is still labile, as judged by experiments in which it is repositioned by a second unilateral light treatment (101). The final position of the clear zone accurately predicts the site of rhizoid emergence. Cortical clearing apparently results from the local fusion of vesicles at the presumptive rhizoid, pushing the plasma membrane away from the wall. The plasma membrane at this site contains many patches lacking imprints of cell wall fibrils, and each patch is interpreted to be the signature of a recent vesicle fusion event (106). The presumptive thallus region contains many fewer smooth patches, such that by "about 5 h after fertilization, fusion sites are present exclusively at the presumptive rhizoid end" (147).

Localized cortical clearing may be related to the observation that zygotes plasmolyze preferentially from the rhizoid hemisphere. In zygotes treated for 1 h with unilateral light of 280 nm and then plasmolyzed by incubation in seawater containing sucrose, the cytoplasm pulls away from the cell wall at the presumptive rhizoid while staying closely appressed to the wall at the thallus end (120). Polarized plasmolysis is evident 15 min after the 1-h light treatment and so is probably correlated with a nascent, labile axis. Vesicle fusion, which pushes the membrane from the wall and leads to cortical clearing, might also be expected to

make this region more sensitive to plasmolysis owing to loss of wall-membrane contacts. Thus it seems that asymmetric jelly accumulation, cortical clearing, and polarized plasmolysis may all be consequences of localized secretion at the presumptive rhizoid site as an axis is established.

The vesicles responsible for polarized secretion have not been identified. Eggs and young zygotes are filled with vesicles of various shapes and sizes, so much so that it has been proposed that a reticular vesicular network might extend from the Golgi in the perinuclear region out to the cell surface (143). Vesicle sizes range from 40 nm to 1 to 2 μm, but the functions of the various classes have not been sorted out (18). Some of these vesicles are clearly secretory and contain cell wall precursors, including alginate and fucans, for assembly into the wall (141). Others bear cellulose synthase complexes embedded in the membranes; once inserted into the plasma membrane, the complexes synthesize microfibrils on the outer membrane surface (106). Yet cell wall polymers are symmetrically distributed around polarizing zygotes, so it is unlikely that these classes of vesicles are involved in early polarized secretion. The source of the polarly secreted vesicles is also unknown. Surprisingly, the secretory apparatus (endomembranes and F-actin) shows no marked polarity at the time of axis formation; not until the axis is fixed in space is there a clear accumulation of vesicles and hypertrophied Golgi on the rhizoid side of the nucleus (109).

Transcellular current. The other phenomenon that can be correlated with axis formation is the initiation of a transcellular electrical current. For clarity, it is worthwhile defining what is meant by the term "transcellular current." It simply means that net positive charge flows into one region of a cell and out of a different region. In fucoid zygotes, positive charge flows in at the rhizoid pole and out at the thallus pole. Because current loops must be complete, positive charge flows through the cytoplasm from rhizoid to thallus and back through the extracellular medium (Fig. 5). In solution, charge is carried by ions, so in its simplest form a transcellular current can be thought of as localized cation influx at one end (rhizoid) and localized efflux from the other (thallus). Because cation efflux is generally an active process (requiring energy) and influx is passive, it is often stated that the current results from the spatial segregation of ion pumps from ion leaks in the plasma membrane. However, several factors complicate this simple model. First, pumps and leaks need only be functionally segregated, and not necessarily asymmetrically distributed. That is, a transcellular current may result from local regulation of transporters such that they are active at only one end of the zygote. Furthermore, it is often not possible to determine whether anions or cations are flowing across the plasma membrane. An anion circulation in one direction is electrically equivalent to a cation circulation in the other orientation; thus, the current in fucoid algae could be generated by anions preferentially flowing into the thallus and out of the rhizoid. Finally, the inward and outward charge fluxes need not be carried by the same ion. For example, in growing pollen tubes the inward current is mainly K^+ influx and the outward current is due to proton pumping (149).

The first indication that a transcellular current might be involved in axis establishment was reported in 1923. Lund showed that zygotes grown in an electric field germinate and grow rhizoids toward the anode and suggested that "an electrochemical polarity of some sort may possibly be a fundamentally associated condition for the development of morphological polarity" (89). However, these putative endogenous currents remained speculative for the next four decades because the electric field generated by single cells proved too small to measure. In 1966, L. F. Jaffe conducted an ingenious experiment to measure the small voltages. He placed fertilized eggs in a capillary and oriented them by shining light from one end of the tube. This effectively connected the cells in series (rhizoid to thallus), and the voltage signal across the entire capillary represented the sum of the voltage drops across all the individual cells (62). By amplifying the voltage signal in this way, he was able to show that zygotes generate transcellular currents at the time of germination. Even so, this approach was too crude to detect the small currents generated by polarizing zygotes.

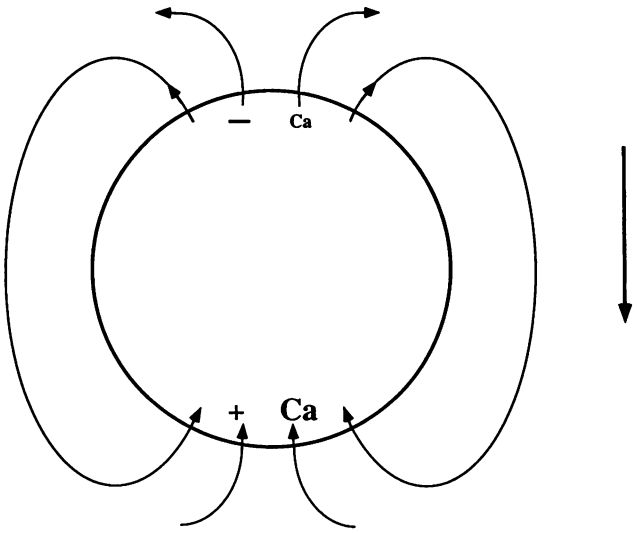

FIG. 5. The transcellular ionic current may establish ionic and voltage gradients in the cytoplasm. The direction of positive current flow is indicated by arrows. Leakage of Ca^{2+} preferentially into the presumptive rhizoid site is postulated to make the local cytoplasm relatively high in calcium (indicated by the size of the lettering) and electropositive. This local calcium may be free in the cytosol or stored in internal membrane systems. Calcium efflux from the presumptive thallus may leave that cytoplasm relatively low in calcium and electronegative. Rather than a smooth morphogenetic gradient, elevated Ca^{2+} levels may be restricted to a narrow zone just beneath the membrane (66).

The development of a vibrating probe sensitive enough to detect currents surrounding single cells revolutionized the study of transcellular ionic currents (103). The electrode vibrates at a frequency of a few hundred hertz over a distance of approximately 30 μm and measures the voltage difference between the ends of its sweep. The noise of the instrument is much reduced by monitoring the signal only at the frequency of electrode vibration. Sensitivity is in the nanovolt range, orders of magnitude better than for stationary electrodes. The measured voltages are converted to current by use of Ohm's law. By using this instrument, patches of inward and outward current can be detected as early as 30 min after fertilization of *Pelvetia* eggs, but these patches are unstable and shift position (101). Even so, inward current settles down at the presumptive rhizoid site more than 1 h before germination and often as early as 5 or 6 h postfertilization. That is, inward current precedes growth and accurately predicts the site of rhizoid outgrowth. Localization of the inward polarization current occurs during axis formation, before axis fixation; when the developmental axis

is rotated by changing the direction of unilateral light, the inward current shifts to the new rhizoid position (101). At this time, the measured steady current is small (0.06 $\mu A/cm^2$) compared with the steady growth-associated currents (0.13 $\mu A/cm^2$) detected a few hours later (101) and is minuscule when compared with the current pulses (>1 $\mu A/cm^2$) generated by growing embryos (103).

Localization of inward current and initiation of polarized secretion occur simultaneously, and it is difficult to assess cause and effect. Inward current was detected at the future rhizoid site before cortical clearing was apparent at that position (101), but this may be due to the disparity in sensitivity of the assays. Cortical clearing is a rather crude measure of localized secretion, whereas the vibrating probe is a very sensitive means of detecting current. As a case in point, by using the capillary method, current was not detected until germination, well after polarized secretion. As Nuccitelli points out, the results "cannot conclusively answer the question of cause or effect because current measurements and electron microscopy have not been done on the same egg for optimal temporal resolution" (101).

The manner in which localized secretion might give rise to the transcellular current is relatively straightforward. If ion transporters (channels?) carrying the inward current are present on Golgi vesicles, they would be incorporated into the plasma membrane at the future rhizoid site by localized vesicle secretion. Current would preferentially enter this site, regardless of the distribution of transporters carrying current outward. Such a mechanism has been proposed to account for the large current pulses generated by growing rhizoids (42), but has yet to be investigated in polarizing zygotes.

The mechanism by which the current might be causal to axis formation has been carefully considered in numerous research and review articles (63, 69, 102). The current, like all imposed vectors, is thought to generate polarity by establishing cytoplasmic localizations in the cell. Current flowing into one end and out of the other end of the cell could establish both voltage and ionic gradients. Jaffe et al. have argued that the developmental significance of these putative gradients depends on which ion carries the current (69). Unfortunately, the ion(s) carrying the bulk of the polarization current is not known, although radioactive tracer data indicate that some of the inward current is carried by Ca^{2+}, Na^+, and K^+ influx (69). Much of the attention has focused on the Ca^{2+} component of the current, which is estimated to make up 2% of the total electrical current flowing through polarizing zygotes (101). By growing zygotes on a nickel screen in unilateral light, Robinson and Jaffe were able to measure $^{45}Ca^{2+}$ fluxes into and out of the presumptive rhizoid and thallus hemispheres (126). Ca^{2+} preferentially enters the presumptive rhizoid and exits the thallus. At 6 h postfertilization, the time of axis formation and stabilization of the steady ionic current, nearly six times as much Ca^{2+} enters the presumptive rhizoid hemisphere as enters the presumptive thallus, whereas efflux is three times greater from the thallus (126). The tracer flux data are supported by experiments with the vibrating probe. Increasing external Ca^{2+} or decreasing external Na^+ levels increases the magnitude of the electrical current (101). Both treatments are known to stimulate Ca^{2+} influx (123), consistent with the interpretation that Ca^{2+} carries part of the inward electrical current at the presumptive rhizoid.

Calcium circulation through the cell would be expected to establish a cytoplasmic Ca^{2+} gradient, high at the site of influx (presumptive rhizoid) (Fig. 5). Because Ca^{2+} is a second messenger regulating numerous processes, such a gradient could induce unique local differentiation at the two ends of the cell. An array of Ca^{2+}-dependent enzymes (kinases, proteases, etc.) may be activated only at the presumptive rhizoid. Dynamic assembly and disassembly of microfilaments and microtubules are regulated by Ca^{2+} and Ca^{2+}-dependent binding proteins (135), so cytoskeletal assemblies at the two developmental poles may be quite different. Vesicle fusion with the plasmalemma requires Ca^{2+}, and the putative gradient may thereby restrict secretion to the presumptive rhizoid. These are but a few of the possible ways in which a cytoplasmic Ca^{2+} gradient may bring about structural, biochemical, and physiological polarity within the zygote.

Localized Ca^{2+} fluxes may also generate an intracellular electric field (Fig. 5). It has been calculated that, owing to the tight binding of Ca^{2+} to fixed negative charges on macromolecules, the Ca^{2+} circulation may generate a cytoplasmic Donnan potential on the order of 1 mV, rhizoid side positive (69). Such a field could, in principle, provide the driving force for localization of negatively charged constituents to the rhizoid cytoplasm by a process termed self-electrophoresis (69). The possibility that secretory vesicles are localized by self-electrophoresis is supported by studies of vesicle surface charge in *Fucus* zygotes. Griffing and Quatrano (44) showed that polarly transported vesicles possess a higher negative surface charge than the randomly secreted vesicles. Membrane proteins and glycoproteins could also be redistributed in the plane of the membrane (lateral electrophoresis) by the internal or external endogenous voltage gradients (65). Imposed fields do indeed cause the redistribution of concanavalin A receptors on embryonic muscle cells (107). Thus, it is possible that both cytoplasm and plasmalemma are rearranged under the influence of endogenous fields.

What evidence supports this hypothesis that a Ca^{2+} circulation is fundamental to axis formation? First, as stated above, a Ca^{2+} circulation is present at the appropriate time. In addition, imposed gradients of voltage (89), Ca^{2+} (127), and Ca^{2+} ionophore (124) polarize zygotes. The imposed voltage gradients need be only a few millivolts per cell (147), not much greater in magnitude than the putative endogenous cytoplasmic electric field calculated from endogenous current flow (69). Zygotes germinate toward the high end of a Ca^{2+} ionophore gradient but toward the low end of a Ca^{2+} gradient. These seemingly contradictory findings may be explained by the fact that Ca^{2+} influx is sevenfold greater at 1 mM than at 10 mM Ca^{2+} (123). Hence, Ca^{2+} entry into the zygote may be greater on the hemisphere facing the lower end of the imposed Ca^{2+} gradient (147). These findings are therefore consistent with the hypothesis that the site of maximal Ca^{2+} influx determines rhizoid position.

Of course, one would like to measure directly the putative Ca^{2+} and voltage gradients in the cytoplasm. Brownlee et al. have used Ca^{2+}-selective electrodes and a fluorescent Ca^{2+} indicator (fura 2) to address the question of Ca^{2+} gradients. After germination there is clearly a gradient, with the highest Ca^{2+} concentration at the tip, detected by both techniques (24, 25). Similar results were obtained with chlorotetracycline, a probe for membrane-associated Ca^{2+} (82). However, more pertinent to this discussion, are there gradients at the time of axis formation? Using fura 2, Brownlee detected what might be a slight transcytoplasmic gradient in 5- and 7-h-old *Fucus* zygotes but concluded that the data were equivocal (20). If present, the difference in Ca^{2+} concentration is less than 100 nM between rhizoid and thallus regions,

and the physiological significance of such a small difference is dubious. Gradients of membrane-associated Ca^{2+} were also not detectable in polarizing zygotes (82). The putative voltage gradient of 1 mV or less across a cell is too small to measure reliably with microelectrodes. Clamping the cytoplasm to constant voltage is another possible approach to investigating the importance of an endogenous electric field. One would like to know whether a zygote can photopolarize without an internal electric field.

A second approach to investigating Ca^{2+} currents, gradients, and voltages is to ask whether they are dispensable. This approach has the advantage of investigating function, rather than correlating spatial and temporal events with development. It should be noted that this approach can determine whether a phenomenon is absolutely essential but cannot assess the relative importance of nonessential functions. This point is significant because it is becoming increasingly clear that development and differentiation often make use of redundant molecules and pathways, none of which is essential. This is perhaps best exemplified by gene knockout experiments in yeasts, cellular slime molds, and other organisms, for which it has been shown that deleting presumed essential genes, such as those coding for myosin, has very little physiological effect (39, 75, 88). Redundancy may in fact provide an evolutionary advantage.

Even so, much has been learned concerning fucoid embryogenesis from experiments aimed at identifying processes that are physiologically indispensable. Recently Speksnijder et al. investigated the requirement for cytoplasmic calcium gradients by injecting Ca^{2+} buffers (BAPTA buffers) with dissociation constants near that of cytoplasmic Ca^{2+}. They argue persuasively that this treatment collapses the cytoplasmic Ca^{2+} gradient rather than clamping the cytosolic Ca^{2+} to different values. Buffer injection prevented rhizoid outgrowth without cytotoxic side effects (134). The results indicate a role for Ca^{2+} in growth, but, unfortunately, do not address the issue of whether a Ca^{2+} gradient is needed for axis formation. That is, the injected zygotes may have possessed an axis oriented by the unilateral light, yet were unable to extend a rhizoid. To test this possibility, zygotes must be released from the BAPTA block so that axis formation and fixation can be assessed (21).

The requirement for Ca^{2+} in the medium during the various stages of early development, including axis formation, has recently been investigated. Surprisingly, lowering the free external Ca^{2+} levels by withholding Ca^{2+} from artificial seawater (ASW) and adding ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) has very little effect on axis formation. *Fucus* (82) and *Pelvetia* (54) zygotes photopolarize quite well even at external free Ca^{2+} levels of 10^{-10} M and less. *Fucus* zygotes also photopolarize in the presence of Ca^{2+} channel blockers (verapamil, D600, La^{3+}). These results indicate that Ca^{2+} influx, and therefore a transcellular circulation of Ca^{2+}, is not essential for axis formation. They do not, however, rule out the need for a cytoplasmic Ca^{2+} gradient; the gradient could be maintained by internal stores (endoplasmic reticulum, mitochondria, or vacuole) in the absence of influx. When Ca^{2+} is removed from ASW and EGTA is added, the internal stores detected by chlorotetracycline (82) and the cytoplasmic levels of free Ca^{2+} (25) fall quite rapidly. Recent measurements by Brownlee with dextran-linked fura 2 show that cytosolic levels recover relatively rapidly, presumably at the expense of internal stores (23). It thus appears that zygotes can form an axis with little or no Ca^{2+} influx, but the need for a cytosolic gradient remains controversial.

In light of this controversy, a critical reevaluation of the evidence presented above implicating Ca^{2+} in axis formation is warranted. The calcium hypothesis rests on two major lines of evidence: (i) a Ca^{2+} circulation is present at the appropriate time and correlates with the flow of electric current, and (ii) imposed Ca^{2+} and Ca^{2+} ionophore gradients induce polarity in young zygotes. The first point is made less convincing by the Ca^{2+} chelation studies which indirectly show that Ca^{2+} circulation is not essential for axis formation. The second line of evidence (polarization induced by Ca^{2+} and Ca^{2+} ionophore gradients) is difficult to interpret because many other gradients, seemingly unrelated to Ca^{2+}, also induce polarity. These include ionic gradients such as K^+ and pH (8), as well as pH ionophore gradients (150). In sum, the role of calcium in axis formation is unresolved; a Ca^{2+} circulation is present quite early in development, but whether it is the cause or consequence of polarity has not been settled. In this light, it should be kept in mind that Ca^{2+} currents and gradients may be causal to polarity only under specific conditions. Zygotes may possess other pathways by which polarity can be established in the absence of Ca^{2+}. That is, Ca^{2+} may be optional, not mandatory, for axis formation (45). If, under certain conditions, Ca^{2+} does play a central role in polarization, it now seems likely that it will not involve a transcytoplasmic Ca^{2+} gradient that constitutes a morphogenetic field. Instead, there may be a narrow "zone of high calcium under the future growth pole" (66) that determines this position as rhizoid. In fact, growing *Pelvetia* rhizoids show just this distribution of membrane-associated Ca^{2+} (82).

What is the role of other ions in seawater? Axis formation is not much affected by elimination or replacement of ions in ASW, except for Na^+ (54). Na^+ replacement by *N*-methylglucamine causes a slight concentration-dependent reduction in polarization. One interpretation is that Na^+ drives cotransport systems in zygotes (see below), and perhaps in other marine plants, but this is speculation at this point. On the whole, it seems that axis formation is not contingent on the circulation of any one particular ion through the cell. When more extensive salt removal experiments were conducted, it was found that potassium salts on their own are most effective in supporting polarization (the particular anion is unimportant). KCl (10 mM) containing sucrose as the osmoticum is sufficient for polarity establishment and constitutes a minimal medium. The ability of other monovalent cations to support polarity reflects their ability to substitute for K^+ ($K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$). (The ability of Na^+ to substitute for K^+ explains why K^+ removal from ASW does not prevent polarization.) It is now critical to determine whether zygotes generate a transcellular current in this minimal KCl medium; if so, it must be carried by K^+, Cl^-, or H^+ (or some combination thereof).

During early development, net KCl uptake serves to generate turgor pressure. The egg lacks a cell wall and therefore has no turgor pressure. Within minutes of fertilization it synthesizes a nascent wall, and by 3 or 4 h turgor pressure begins to build (1). Prior to growth the zygote generates approximately 7 to 8 atm (0.7 to 0.8 MPa) of pressure (93a), which is needed for rhizoid elongation. This increase in turgor pressure is driven mainly by uptake of large amounts of KCl (1). The membrane potential (V_m) of unfertilized eggs is roughly −60 mV (12), and the egg membrane is somewhat nonselectively permeable to K^+, Na^+, and Cl^- (125). At fertilization V_m falls rapidly to about −25 mV and constitutes a fast block to polyspermy (12); it then slowly rises over a period of hours to −70 mV and

stabilizes (5, 148). Concomitant with the slow hyperpolarization, the K^+ permeability rises sixfold and the Na^+ and Cl^- permeabilities fall dramatically. The fall in chloride permeability makes Cl^- efflux undetectable (125), so that Cl^- accumulates in the young zygote. The increase in potassium conductance allows potassium to accumulate passively (driven by V_m) at a rate of 15 to 20 mM/h (1, 125). The net result is an increase in the internal KCl concentration of approximately 200 mM between fertilization and germination, causing a substantial rise in internal osmotic potential (1). The influx of water accompanying this increase in osmotic potential generates turgor pressure.

Perhaps the ability of K^+ salts to support axis formation is related to the massive KCl uptake at this stage of development. That is, turgor pressure may be needed not only for rhizoid growth, but also for axis formation. Just how a scalar entity such as turgor pressure might function in establishing polarity is unclear, but it may regulate ion channels (stretch activated channels) or maintain cortical integrity by keeping the plasma membrane closely appressed to the cell wall. It should be noted that this putative role for turgor pressure in axis formation is not supported by earlier research showing that zygotes form and stably fix an axis in hyperosmotic seawater (110, 140). Resolution of this matter will require direct measurement of turgor pressure in zygotes polarizing in normal and hyperosmotic media.

Despite electrophysiological studies of ion fluxes and electrical potentials, it is not at all clear how fucoid zygotes generate a membrane potential. In higher plants, V_m is typically more negative than -100 mV, and this large potential is generated directly by an electrogenic proton ATPase (138). The animal motif is characterized by a less negative potential governed by an electrogenic Na^+-K^+-ATPase and a high K^+ conductance. The resting V_m is set mainly by K^+ diffusion out of the cell. V_m in fucoid algae more closely resembles the animal motif in that it is a potassium diffusion potential (5, 148) but the primary pumps that set up the ionic gradients are unknown. There is no evidence for a Na^+-K^+-ATPase or an electrogenic proton pump. An alternative not yet explored is the possibility that an inwardly directed Cl^--ATPase contributes to V_m. There is precedent for such a Cl^- pump in marine algae, most notably *Acetabularia* species (38, 57, 129), in which V_m is set by a combination of Cl^- pumping and passive K^+ diffusion. In fucoid zygotes Cl^- is accumulated well beyond equilibrium with V_m, whereas K^+ remains very near equilibrium (148). These findings are consistent with a hypothesis in which Cl^- is electrogenically pumped into the polarizing zygote at the expense of ATP and the absence of substantial Cl^- efflux causes Cl^- accumulation (125, 147). The increasing permeability to K^+ allows K^+ to be accumulated passively for charge compensation. This model is purely speculative at present, but merits serious research.

It is equally unclear which ion energizes secondary active transport (symport, antiport). Animals typically use Na^+, whereas plants, bacteria, and fungi use H^+. In *Pelvetia fastigiala* the proton motive force across the plasma membrane is quite small (<-50 mV [personal observation]) and would be unable to energize substantial nutrient accumulation. A similar argument can be made for Cl^-. Instead, it seems most likely that Na^+ drives cotransport. Seawater contains roughly 0.5 M Na^+, whereas cytoplasmic Na^+ (ignoring accumulation in vacuoles and vesicles) is approximately 25 mM (1). Combined with a membrane potential of -60 mV, the electrochemical driving force on Na^+ is well in excess of -120 mV, directed inward. Thus, of the major monovalent ions, Na^+ is furthest from equilibrium. It may be that one way in which fucoid algae and other marine organisms have adapted to excessive Na^+ in their external environment is to harness the Na^+ electrochemical gradient to energize cotransport.

Cytoskeleton. Only two conditions that must be met during axis formation have been identified: (i) as discussed above, K^+ or a related ion must be present in the medium, and (ii) the F-actin network must be functional. Recall that zygotic RNA and protein synthesis are required, but they can be uncoupled from axis formation. If zygotes are grown for 8 h before being exposed to unilateral light, they photopolarize in the presence of transcriptional and translational inhibitors (79). This is because all of the protein and RNA synthesis needed to finish the first cell cycle is completed in the first 8 h of development.

By contrast, cytochalasins prevent photopolarization. In both *Fucus* (110) and *Pelvetia* (97) zygotes, 50 to 100 μg of cytochalasin B per ml added during unilateral light treatment completely inhibits photopolarization, regardless of the treatment period. That is, when the inhibitor is removed and zygotes are allowed to germinate, the rhizoids grow out in random directions. These results suggest that actin is involved in generating cellular asymmetry, probably by localizing cytoplasmic or membrane determinants or both. One intriguing possibility is that F-actin is involved in setting up the transcellular ionic current. A preliminary report indicates that cytochalasins prevent the establishment of current flow in accordance with an imposed light vector (17).

Microtubules, on the other hand, are dispensable. Zygotes incubated continuously in microtubule-depolymerizing drugs photopolarize and germinate on their shaded hemispheres under conditions where no microtubules can be detected by indirect immunofluorescence (13, 81). The nucleus is displaced from its central location by these treatments, implying that nuclear positioning is a microtubule-dependent process. This hypothesis is supported by the location of microtubules in normal, polarizing zygotes. Microtubules initiate uniformly over the surface of the nuclear envelope and radiate into the cortex as if anchoring the nucleus (81).

Attempts to visualize actin have been less conclusive. EM studies of the cytoarchitecture of young zygotes have failed to detect microfilaments (13, 18, 19, 109), except for one report of putative microfilaments associated with the cleavage furrow (15). Attempts to label F-actin with heavy meromyosin also proved unsuccessful (11). Instead, fluorescent phalloidins have been used to study F-actin distributions in formaldehyde-fixed zygotes. Phalloidins are a class of fungal toxins that bind specifically to polymerized actin. Using nitrobenzoxadiazole (NBD)-phallacidin, Brawley and Robinson detected F-actin in the cortex of polarizing zygotes (17). Unfortunately, the background autofluorescence prevented visualization of endoplasmic filaments. Even so, they were unable to detect any asymmetry in cortical F-actin immediately after zygotes were photopolarized for 1.5 h (sufficient to establish an axis). This finding was confirmed in *Fucus* zygotes labeled with rhodamine phalloidin; cortical F-actin remained uniformly distributed throughout axis formation (Fig. 6) (78). In both studies, F-actin eventually localized to the presumptive rhizoid, but the timing of localization correlated with the period of axis fixation. However, it must be shown that localized F-actin is not susceptible to repositioning by a second light pulse before one can be certain that localization correlates specifically with axis fixation. These experiments must be conducted on

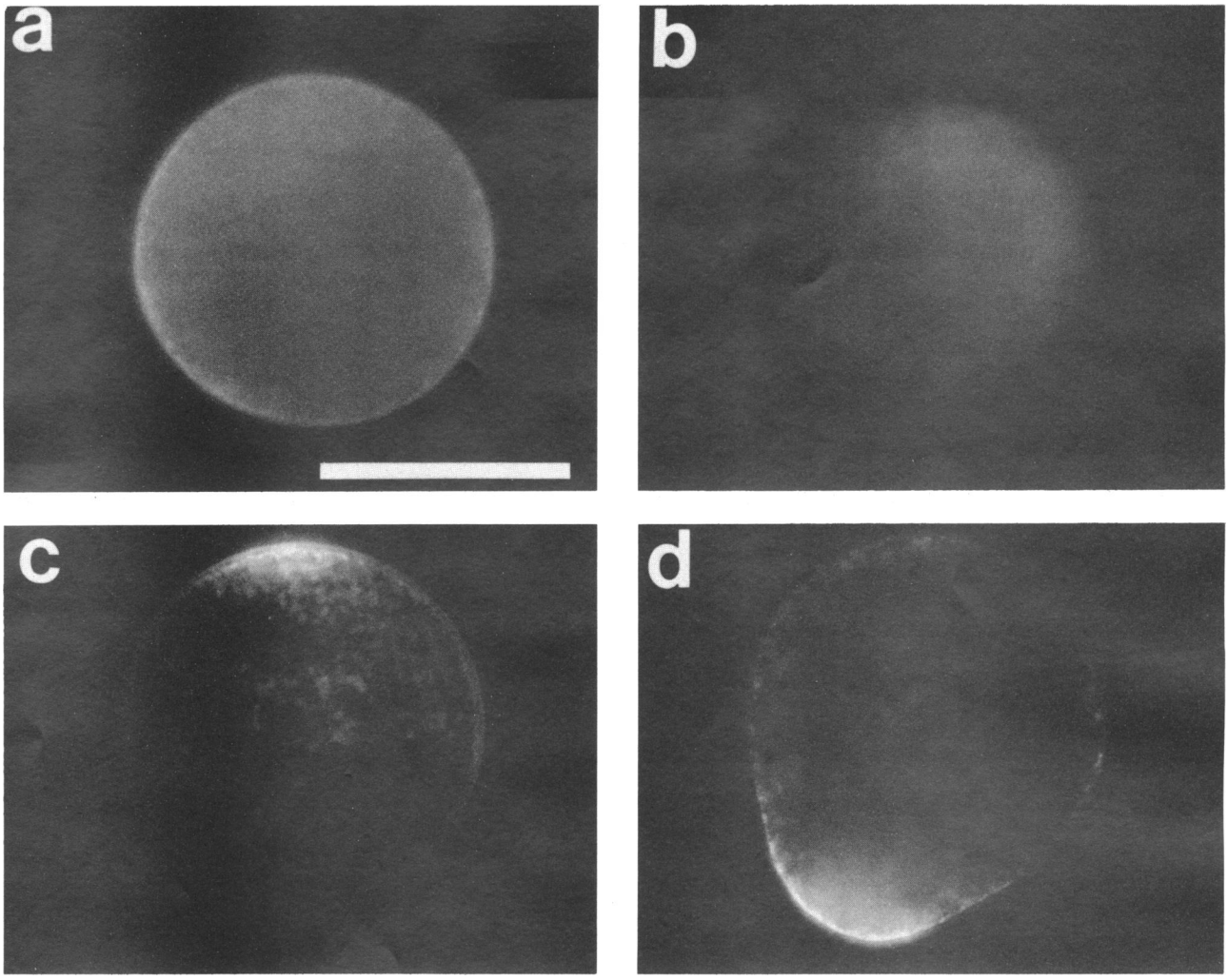

FIG. 6. Cortical F-actin distribution during first cell cycle in *Fucus* species. (a) Before and during axis formation, cortical F-actin is uniformly distributed (7-h-old zygote). (b) KI (0.6 M) disrupts this network and eliminates cortical staining. (c) At axis fixation, cortical F-actin becomes localized to one region of the cortex and marks the position of subsequent rhizoid growth (11-h-old zygote). (d) As the rhizoid elongates, F-actin remains in the apical cortex (16-h-old zygote). Bar, 50 μm. Reproduced from reference 78 with permission.

living cells, and as yet in vivo labeling of F-actin has not been attempted with fucoid zygotes.

The pattern of F-actin staining in chemically fixed zygotes is difficult to interpret. With both rhodamine phalloidin and NBD-phallacidin, staining is very diffuse throughout the cortex and endoplasmic filaments have not been visualized (Fig. 6). Confocal microscopy shows that rhodamine phalloidin appears to label smoothly the surface of large vesicles and other organelles. No filamentous structures or cables can be detected. Similar patterns have been observed in other lower eukaryotes, especially in tip-growing cells. Diffuse apical caps and subapical spots and plaques have been reported in fungi (for a review, see reference 46), but the physiological significance of these patterns remains unclear (58). One attractive but unsubstantiated possibility is that cortical microfilaments are arranged in a fine meshwork which cannot be resolved and so appears diffuse. We are left with the observation that some process dependent on F-actin is essential to axis formation, yet staining of cortical actin does not provide any clues to what that process might be.

Axis formation model. Coherent models of axis formation should take into account the major findings discussed above; namely, (i) polarized secretion begins within 4 hours of fertilization, (ii) an ionic current (partially carried by Ca^{2+}) accompanies axis establishment, (iii) disruption of F-actin prevents photopolarization, and (iv) the medium need contain only K^+ salts (or salts of monovalent cations that substitute for K^+). In the past two decades, a number of hypotheses have been proposed to account for axis formation (17, 111, 114, 117). In large part they have been quite similar. The basic assumptions are that the initial asymmetries are set up in the cortex or plasma membrane and that these weak asymmetries are amplified and eventually give rise to endoplasmic polarity. A summary version of the basic model is presented in Fig. 7A. On the basis of the impressive studies of ionic currents, it is assumed that unilateral light leads to segregation of ion pumps and leaks in the plasma membrane. The most straightforward way for this to occur would be by actively moving transporters in the plane of the membrane such that channels end up at one pole. Cortical F-actin is a prime candidate for effecting this redistribution (45). Linking of the membrane transporters to cortical F-ac-

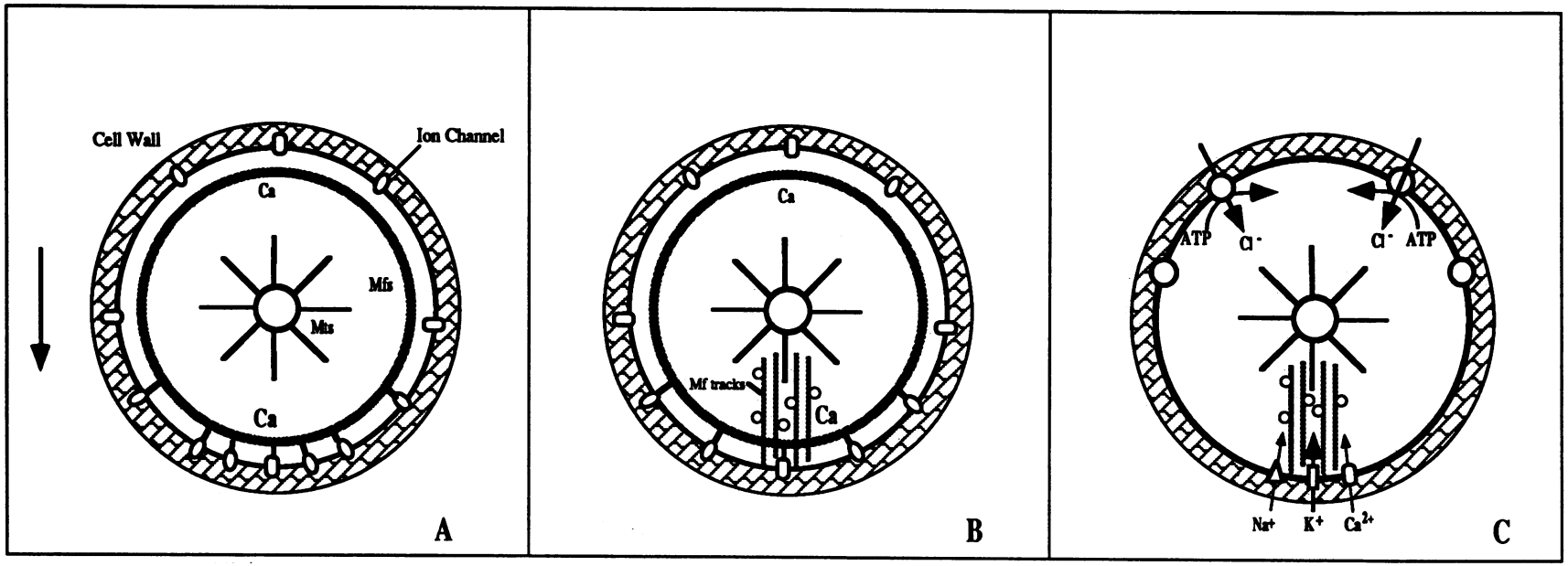

FIG. 7. Working model for axis formation. (A) Unilateral light induces channel migration and accumulation at the presumptive rhizoid end of the cell. Receptors are anchored to the microfilament cytoskeleton at the presumptive rhizoid but are free to diffuse on the lighted side. Channel asymmetry gives rise to an intracellular Ca^{2+} gradient. The arrow indicates the direction of unilateral illumination. (B) Elevated Ca^{2+} level at the rhizoid pole stabilizes endoplasmic microfilaments, which deliver vesicles containing more Ca^{2+} channels. This amplifies the initial weak asymmetry in ion fluxes. (C) The bulk of the transcellular electric current may be carried by active Cl^- influx at the presumptive thallus (outward current) and passive K^+, Ca^{2+}, and Na^+ at the presumptive rhizoid (inward current). Coordinated K^+ and Cl^- fluxes set the membrane potential, provide the driving force (osmotic potential) for turgor pressure, and may also make up most of the transcellular current. The bold arrow indicates that the K^+ channel is more conductive than the Ca^{2+} or Na^+ channels. Drawing is not to scale. Panel A reproduced from reference 17 with permission.

tin (perhaps via actin-binding proteins) would allow movement generated by contraction of the cortical F-actin network (or some other F-actin mediated movement) to generate asymmetries in pumps and leaks, much like patching and capping of membrane receptors on lymphocytes. However, there is no evidence for accumulation of cortical F-actin at the presumptive rhizoid during axis formation. Brawley and Robinson therefore modified the model such that membrane transporters are tethered to cortical actin in unpolarized zygotes and unilateral light severs the link on the lighted side, allowing ion channel diffusion (17). Channel diffusion leads to a slight net accumulation at the presumptive rhizoid, where channels remain tethered (Fig. 7A). If a PI cycle is involved in transduction of the light signal, its effectors (kinases, etc.) may be involved in breaking the tethers.

Some of the localized leaks are channels carrying Ca^{2+} into the presumptive rhizoid, and the resulting transcellular Ca^{2+} current would give the zygote a weak polarity which would be rapidly amplified as follows. A local increase in the Ca^{2+} concentration in the cytoplasm at the presumptive rhizoid pole causes F-actin stabilization at that site (Fig. 7B). Golgi vesicles containing more Ca^{2+} channels are transported from the perinuclear region to the presumptive rhizoid along the nascent actin filaments. Local secretion inserts Ca^{2+} channels, which allows for more local Ca^{2+} entry and stimulates further F-actin assembly and vesicle secretion. This amplification loop progressively strengthens the cellular asymmetry. Opening of voltage-gated channels may also be part of amplification. Voltage-gated Ca^{2+} channels at the rhizoid may be activated by membrane depolarization (caused by the inward limb of the transcellular current), which would stimulate further Ca^{2+} entry (21). Voltage-gated Ca^{2+} channels have recently been identified in unfertilized *Fucus* eggs (23). Once cytoskeletal stabilization, vesicle secretion, and perhaps channel activation have amplified the signal, polarity is firmly established but is still labile in its orientation.

As with all models, this formulation has strengths and weaknesses. It accounts for polar secretion and transcellular current as early phenomena, and local secretion of vesicles containing ion channels explains why the intensity of the steady current increases gradually prior to germination. F-actin is justifiably assigned a central role in generating both membrane and cytoplasmic asymmetries. The model correctly predicts that with cytochalasins the ion current will not be established owing to free diffusion of unanchored channels in the plane of the membrane. Cytochalasins would also be expected to break the amplification loop by inhibiting vesicle transport. However, several important aspects of the model lack experimental support. There is no direct evidence that membrane transporters redistribute during development, and it is possible that the ionic current is the result of localized channel activation rather than channel redistribution (21, 22). Also, endoplasmic F-actin at the presumptive rhizoid has not been detected, but improved techniques for preserving and visualizing actin must be developed before this issue can be resolved. Several features of the model dealing with the Ca^{2+} circulation do not fit well with the available evidence. (i) One might expect a relatively extensive zone of elevated Ca^{2+} concentrations in the cytoplasm at the presumptive rhizoid if it is to stabilize endoplasmic F-actin, but measurements of cytosolic Ca^{2+} concentrations do not support this view. (ii) A Ca^{2+} amplification loop predicts steadily increasing Ca^{2+} fluxes, but the measured Ca^{2+} fluxes remain constant during photopolarization, and their asymmetry actually decreases (126). (iii) An even more important point is that zygotes establish an axis without Ca^{2+} in the medium. It may well be that internal stores (mitochondria, endoplasmic reticulum) of Ca^{2+} can support the elevated levels at the shaded pole, but there is no evidence to support this proposal.

Identification of the ion(s) carrying the bulk of the current and the need for K^+ (or other alkali metal) in the medium are not addressed in the model, the latter because the data are relatively new. The model can accommodate these features by postulating that the polarly transported vesicles contain not only Ca^{2+} channels but also K^+ and perhaps Na^+ channels (Fig. 7C). The highly conductive K^+ channels carry most of the inward current into the presumptive rhizoid. The outward current may be driven by active Cl^- uptake, probably by an ATPase which is not present in the Golgi vesicles and so is excluded from the putative rhizoid. The Cl^- pump and K^+ leak are the same transporters postulated to set V_m and generate turgor (see above). If they are asymmetrically distributed (or differentially regulated), the substantial KCl uptake that generates turgor during polarization may also be the basis of the transcellular current. As discussed above, turgor (and, by inference, K^+) may be needed for regulation of channel activity, for communication between plasma membrane and cell wall, or for maintenance of polarized secretion. This modification of the model is supported by tracer flux data. K^+ and Ca^{2+} preferentially enter the future rhizoid end during axis formation, and, after germination, Na^+ is taken up preferentially into the growing tip. By contrast, Cl^- is preferentially pumped into the thallus of germinated zygotes (69). Unfortunately, localized fluxes of Na^+ and Cl^- have not been reported for polarizing zygotes. Even so, calculations show that the total fluxes of K^+ and Cl^- into ungerminated zygotes are sufficient to account for all of the polarization current (125). In other words, segregation of K^+ channels to the future rhizoid and exclusion of Cl^- pumps from this region could account for the transcellular current. Insertion of K^+ channels via vesicle secretion also provides a mechanism to explain the large increase in K^+ conductance after fertilization (148).

A major assumption of the model is that an ionic current precedes and causes localized secretion. However, this need not be the case. Cause and effect have not been untangled for secretion and ionic current, and it may be that localized secretion causes the current flow. Localized secretion is observed at least as early as an ionic current, and perhaps localized secretion of vesicles containing ion channels, in particular conductive K^+ channels, sets up the current flow (Fig. 7C). Perception of environmental gradients may be transduced into endoplasmic polarity, perhaps via kinases, etc., activated by the PI cycle. Local stabilization of endoplasmic actin filaments and vesicle secretion at the presumptive rhizoid would then generate the transcellular current. This hypothesis is consistent with the finding that the transcellular circulation of specific ions is not essential to axis formation; in fact, these fluxes may be epiphenomena resulting from polarized secretion. It would also account for the symmetry in cortical actin throughout axis formation, a feature not easily accommodated in traditional models. According to this hypothesis, the essence of axis formation is polarized secretion, not rearrangements of transport proteins in the plasmalemma.

In sum, we do not yet understand enough about the relationships and interactions between the participants in axis formation to be able to formulate the rules of the game.

Ca^{2+} gradients, electric fields, cortical actin rearrangements, assembly of localized endoplasmic actin, and polarized vesicle secretion all participate, but the chain of causality in this web is obscure. And, to make matters more complex, the precise pathway by which polarity is established may not be invariant but may depend upon the environmental conditions. In the words of F. M. Harold, "I would not be surprised to learn that the *Fucus* embryo is more nimble than we, and can use more than one kind of field to mark its polar axis" (45).

Axis Fixation

The initial axis remains labile for a time (hours in *Pelvetia* zygotes, much less in *Fucus* zygotes) and then becomes irreversibly set in place just prior to rhizoid outgrowth. A population of *Fucus* zygotes fix their axes between 9 and 12 h postfertilization, whereas *Pelvetia* zygotes do so between 8 and 10 h (82) (Fig. 2). The period between axis fixation and rhizoid outgrowth is a time of latent but stable polarity, which lasts only 1 h or less (82, 95). The time course of axis fixation has been measured in two ways, one by using a single unilateral light pulse and another by using two antagonistic pulses (95). In the first approach, a single pulse is given at different times during the period of interest. Pulses applied in the photosensitive period polarize nearly all zygotes, but pulses at slightly later times (prior to germination) have progressively less effect. This lack of response is taken to mean that the axis was fixed prior to light treatment. The percentage of cells not responding to the light is then a measure of axis fixation. In the other method, a short unilateral light pulse is followed by a second pulse oriented 90° or 180° to the first. Cells that germinate in response to the second light pulse had labile axes at the beginning of the second light treatment, whereas those that germinate in accordance with the first light pulse must have fixed their axes in place prior to the start of the second treatment.

Approaches to investigating axis fixation have been very similar to those described above for axis formation. Localizations which correlate temporally with stabilization of the axis have been sought, and inhibitors have been used to probe the coupling between specific processes and axis fixation. The latter approach has identified a number of factors that are not fundamental to axis fixation. Quatrano found that fixation was unaffected by cycloheximide or colchicine and so does not depend on protein synthesis or an intact microtubule array (110). Microtubule staining is consistent with the inhibitor data; microtubules remain uniformly distributed around the circumference of the nucleus and apparently play no role in axis fixation (81). As discussed above, protein synthesis is needed only during the first hours of development (79, 108). Zygotes also stably fixed an axis in ASW made hypertonic with sucrose (110), but direct measurements of turgor pressure under these conditions are needed. Finally, removal of Ca^{2+} or addition of Ca^{2+} transport inhibitors does not prevent fixation (82) and internal gradients of Ca^{2+} cannot be detected during fixation (20, 82).

Axis fixation is dependent on two organellar systems, the microfilament network and the cell wall. Cytochalasins block axis fixation (110), as does removal of the cell wall. Enzymatic treatments have been developed to remove completely the cell wall from 6-h-old *Fucus* zygotes (72), and the resulting protoplast does not divide as long as the cell wall is absent. These protoplasts can form an axis in response to unilateral light; when allowed to regenerate a wall and grow, the rhizoid emerges from the hemisphere that was shaded when the protoplast was illuminated (80). However, the protoplast lacking a wall cannot irreversibly stabilize the axis. If, after 2 days of constantly illuminating protoplasts with unilateral light, the light treatment is reversed (rotated 180°) during regeneration, the rhizoid emerges in accordance with the second light pulse (80). Thus, axis fixation is prevented by wall removal. These findings raise the possibility that cortical actin and cell wall interact to stabilize the nascent axis.

Cortical actin undergoes a rather remarkable rearrangement during axis fixation (Fig. 6). As mentioned above, cortical actin accumulates at the presumptive rhizoid pole during axis fixation in *Fucus* (78) and *Pelvetia* (17) zygotes. In *Fucus* zygotes the time course of fixation and actin localization coincide remarkably well, beginning around 8 h and finishing by 12 h (78). Cytochalasins block the actin localization, but little is known about the mechanism of localization. Actin depolymerization at the presumptive thallus (30), contraction of a stress-bearing cortical F-actin network (45), and actin/myosin-based movement are all possibilities. The redistribution of actin occurs without any change in total actin content or synthesis. Three actin isoforms can be detected on two-dimensional blots, and their amounts and rates of synthesis stay constant from fertilization through the first days of embryogenesis (78).

Cell wall. Considerable effort has been devoted to investigating localizations in the cell wall during early development, and the importance of the cell wall in axis fixation warrants a short digression on its biochemistry (for more detailed discussions, see references 36, 73, 111, 112, 114, 117, and 141). The mature fucoid cell wall is composed mainly of three polysaccharides, alginic acid (alginate), cellulose, and fucans (fucoidins), which make up more than 95% of the dry weight (118). Their proportions are roughly 60% alginate, 20% cellulose, and 20% fucans. Cellulose, a (β1-4)-linked glucose polymer, needs no introduction. Alginic acid is a heteropolymer of (β1-4)-D-mannuronic acid (M) and α-L-guluronic acid (G), which differ only by the position of the carboxy at C-6. Alginate polymers contain regions rich in M (M blocks) or G (G blocks), as well as regions containing both (MG blocks) (112, 142). The proportions in embryo walls are 69% G blocks, 27% M blocks, and 4% MG blocks (84). Alginate polymers are synthesized as pure M residues, some of which are later converted to G by an epimerase. The gelling properties of alginate are conferred by G blocks which are cross-linked in the presence of Ca^{2+} (141).

Fucans are a broad group of fucose-containing polymers that are often sulfated in the brown algae. Three fucan classes are present in embryonic walls; F1 is composed mainly of uronic acid and xylose with little fucose or sulfate, F3 is an (α1-2)-linked fucose polymer containing high levels of sulfate, and F2 appears to have an F3 backbone with F1-like side groups (117). In studying the wall of mature plants, Medcalf and Larsen (92) termed the F1 component ALC (ascophyllanlike component) and the F2 component FC (fucan complex). Proteins are linked to fucans but make up little of the total cell wall by weight.

Fucoid zygotes have served as models for cell wall biosynthesis because the egg is a natural protoplast and fertilization induces rapid and synchronous wall synthesis (29). Although unfertilized eggs are surrounded by an amorphous jelly layer, they contain no structural cell wall and no detectable alginate, cellulose, or fucan (136, 141). Alginate can be detected cytochemically at 15 min postfertilization,

and cellulose is detectable by 30 min (118, 136). The nascent wall is composed of approximately equal parts cellulose and alginate. F1 is first incorporated into the wall at 1 h, and at this time the wall becomes birefringent (indicating ordered cellulose microfibrils) and can be isolated as an intact organelle (112). Wall integrity is probably acquired initially by Ca^{2+} cross-linking of G blocks in alginate and later by cellulose deposition (141). Nearly all of the cellulose present in 24-h-old embryos is synthesized and deposited during the first 4 to 5 h postfertilization (136) and is responsible for maintaining the spherical cell shape of the young zygote (112). Even so, the percentage of cellulose in the wall falls during the first few hours owing to fucan deposition (112). F1 is first deposited at 1 h postfertilization, and F3 becomes stably incorporated into the wall by 4 h, but there is no detectable F2 until just prior to germination (112). After germination, fucans finally make up approximately 20% of the embryonic wall, roughly equal by weight to cellulose.

Critical analysis of wall assembly requires specific probes for each wall polymer. To circumvent the lack of specificity of cytological stains, Vreeland et al. have developed antibodies against alginate (144) and constructed fluorescent oligosaccharide hybridization probes for G blocks (142). These probes were used to show that alginates are synthesized intracellularly, probably in precursor form, and delivered to the cell surface. Intracellular transport is mediated by vesicles derived from perinuclear Golgi (141). Vesicle secretion delivers alginate precursors to the nascent wall, where they presumably gel rapidly on exposure to the high calcium levels (10 mM) in seawater. Sulfated fucans (F2 and F3) follow a similar pathway to the surface. Pulse-labeling with $^{35}SO_4^{2-}$ shows that fucan sulfation occurs in perinuclear Golgi vesicles, which move to the cell surface during a short chase period (14).

Unlike alginate and sulfated fucans, cellulose microfibrils are synthesized at the cell surface by protein complexes in the plasma membrane. When cells are fractionated into wall and cytoplasm, all of the cellulose is found in the wall while the cytoplasm has barely detectable levels (136). Freeze fracture analysis has revealed putative cellulose synthase complexes embedded in the plasma membrane and the membranes of Golgi vesicles (106). These complexes are arranged in strings and are attached to the wall microfibrils. In unfertilized eggs, synthase complexes are found in Golgi vesicles but not in the plasma membrane. Fertilization induces these vesicles to fuse with the plasma membrane, where the synthases begin to deposit cellulose microfibrils within minutes (106). This initial microfibril deposition is independent of zygotic transcription and translation, indicating posttranslational control of cellulose synthesis (136). (Prolonged treatment with cycloheximide does, however, eventually block wall biosynthesis [100].)

Most important for this discussion, the nascent wall is apolar. Secretion of alginate (144) and fucans (F1 and F3) (117) and deposition of cellulose microfibrils (106) are roughly uniform over the cell surface. As best as can be determined, the cell wall remains symmetrical throughout axis formation and for most of axis fixation. However, just before germination, a novel sulfated fucan (F2) is secreted locally at the site of incipient growth. The localization of F2 and its role in polarization have been studied intensively by Quatrano and coworkers.

F2 is a class of compound formed by addition of ALC side chains (rich in uronic acid, low in sulfate) to an F3 backbone (fucan sulfate) via guluronic acid links (117). The timing of synthesis and the distribution of F2 are unique among the major wall polymers. Although F1 and F3 are sulfated early and deposited within hours of fertilization, F2 is not detectable until 10 h postfertilization, when it first becomes sulfated (50, 116). Sulfation may precede or follow the coupling of ALCs to F3 (117). Whatever the substrate, sulfation occurs on a preexisting backbone (116, 117) and takes place in the Golgi (28, 31, 91). In contrast to the uniform distribution of F1 and F3, F2 is deposited preferentially at the rhizoid tip of germinated zygotes (14, 100, 116). This localization is first demonstrable at axis fixation, before growth begins. Zygotes grown in hypertonic ASW fix an axis but do not initiate rhizoid growth and so remain spherical, yet F2 localizes to the determined rhizoid site (99, 145). Localized deposition is accomplished by the unidirectional transport of F2-containing vesicles (F granules) to the rhizoid site (14).

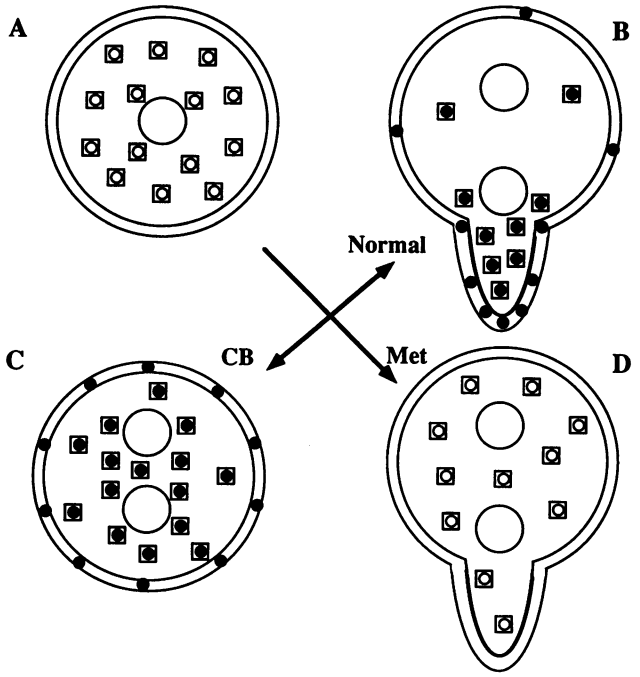

FIG. 8. Distribution and secretion of F2 in normal and treated embryos. Squares represent the distribution of F granules, and the circles within denote the state of F2 sulfation; solid circles indicate sulfated F2, and open circles represent unsulfated F2. (A) In young zygotes (4 h postfertilization), F granules are uniformly distributed and contain unsulfated F2, which is not secreted into the wall. (B) Untreated zygotes in ASW have germinated and completed mitosis by 20 h. F2 is sulfated and secreted preferentially into the rhizoid wall, and F granules are concentrated in the rhizoidal cytoplasm. (C) Treatment with cytochalasin B prevents germination but not mitosis, so by 20 h the zygote has two nuclei but remains spherical. F2 is sulfated and secreted randomly into the wall, and F granules accumulate around nuclei. (D) Met embryos germinate and undergo mitosis but do not adhere. F2 is neither sulfated nor secreted, and F granules are not localized. Reproduced from reference 113 with permission.

Localization to the rhizoid depends on two factors: sulfation of F2 and an intact microfilament network (Fig. 8). F2 sulfation is inhibited when zygotes are grown in a medium in which sulfate is replaced by methionine (Met embryos) (32). In Met embryos vesicles containing unsulfated F2 are not localized to the rhizoid and F2 is not secreted into the wall (14, 51, 113, 114). F granules from normal embryos have a higher electrophoretic mobility than those from Met em-

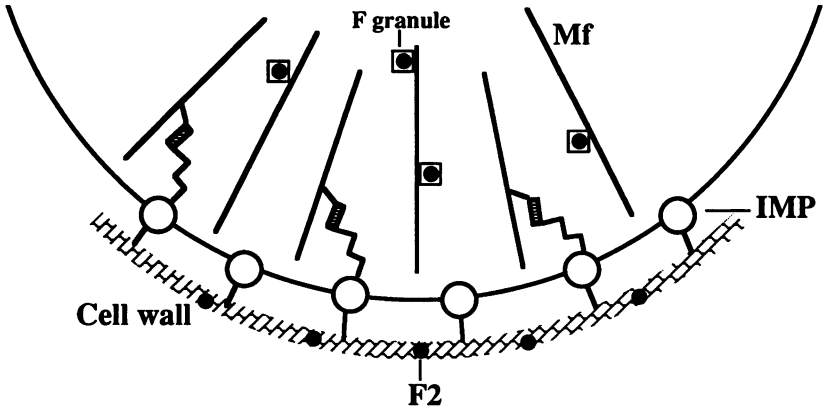

FIG. 9. Working model for axis fixation. An axis-stabilizing complex is formed by a transmembrane bridge indirectly linking the cell wall to the microfilament (Mf) cytoskeleton. Cell wall polymers are connected to integral membrane proteins (IMPs), perhaps via a vitronectinlike molecule. The integral membrane protein may be a member of the integrin family. On the cytoplasmic face, a series of interacting proteins (possibly actin-binding proteins) anchor the integral membrane protein to cortical microfilaments. Microfilaments are also postulated to transport F granules to the presumptive rhizoid, where F2 is incorporated into the wall. Reproduced from reference 113 with permission.

bryos (117), consistent with the hypothesis that F granules are moved to the tip by electrophoresis through an endogenous electric field (Fig. 5). Sulfation of F2 may add sufficient negative charge to the surface of F granules so that they are driven to, and accumulate at, the anodal pole in the rhizoidal cytoplasm. Sulfation of F2 may also facilitate vesicle secretion and embryo attachment because Met embryos do not secrete F2 or adhere to the substratum (113).

Cytochalasin B also prevents directed F-granule transport and F2 localization but does not affect sulfation, secretion, or incorporation of F2 into the wall (114). F granules of cytochalasin-treated zygotes contain sulfated F2 and yet are secreted uniformly over the surface of the cell (Fig. 8) (14). This indicates that, although necessary, sulfation of F2 is not sufficient for localization. F granules accumulate in the perinuclear region after cytochalasin treatment, which can be interpreted in two ways (114). First, cytochalasins are known to disrupt the endogenous current (17) and may thereby prevent localization by self electrophoresis. Alternatively, microfilaments may be directly involved in vesicle transport. Vesicles containing F2 may be transported along microfilament tracks from the perinuclear Golgi to the presumptive rhizoid (117). Although the data available do not discriminate between these two mechanisms of transport, the weight of evidence from other organisms favors transport along actin cables. Organellar transport along actin cables is the basis of cytoplasmic streaming in plant cells (70), with myosin on the surface of the organelles serving as an ATP-dependent motor (48, 139). Isolated pollen tube vesicles are capable of motility along actin cables of the giant internodal cells of Charaeceaen algae (77), and it would be informative to test the ability of F granules to move on these cytoskeletal tracks. Self-electrophoresis might be testable under voltage clamp conditions; however, these experiments may prove difficult owing to the rather small magnitude (1 mV) of the putative voltage gradient across a fertilized egg (69).

Regardless of the transport mechanism, localization of F2 during axis fixation is not essential for differentiation of two-celled embryos. Met embryos, in which F2 is neither sulfated nor secreted into the wall, germinate and divide normally, albeit slowly (Fig. 8) (32). Thus, F2 localization does not cause establishment of polarity but instead is an early expression of polarity. In sum, the cell wall must be present to irreversibly stabilize the nascent axis but the relevant cell wall components involved in axis stabilization have not been identified. It may be that uniformly distributed wall polysaccharides (cellulose, alginate, F1, and/or F3) or cell wall proteins stabilize the axis.

Axis fixation model. Two basic requirements for axis fixation have been identified: (i) the microfilament network must be intact, and (ii) the zygote must possess a cell wall. Furthermore, cortical F-actin localizes to the presumptive rhizoid site during fixation. On the basis of these findings, it has been proposed that "the formation of transmembrane bridges between cytoskeletal filaments and wall fibrils at the future rhizoid site" (80) is at the heart of axis fixation. The concept of a transmembrane bridge has evolved in stages. The idea that the cytoplasmic part of this bridge might be involved in axis fixation was formulated quite clearly over a decade ago by Quatrano et al. (114), who suggested that "membrane patches at the presumptive rhizoid site are stabilized in the membrane or to the underlying cytoplasm by a cytoskeletal component, possibly microfilaments. This corresponds to polar axis fixation." The more recent finding that the cell wall is also needed for fixation (80) has led to the extension of this model to include links to wall polymers, thus forming a transmembrane bridge or axis-stabilizing complex (Fig. 9). This indirect linkage may be restricted to the rhizoid site owing to localization of one or more of its components (excluding wall polysaccharides). Functionally, the axis-stabilizing complex may anchor relevant proteins (e.g., cortical actin or plasma membrane molecules) at the presumptive rhizoid and prevent their redistribution in response to subsequent environmental gradients.

The concept of a transmembrane bridge has ample precedent in animal cells, and the animal motif has served as a framework for formulating models of the axis-stabilizing complex in fucoid zygotes (113, 115). Animal cells in culture adhere to the substratum at specialized sites termed focal contacts or adhesion plaques, much as the rhizoid attaches a fucoid embryo to a rocky substratum in the intertidal zone. Focal contacts consist of a transmembrane bridge from cortical actin to the extracellular matrix. Many components of focal contacts have been identified (no doubt many more are yet to be discovered), and the in vitro binding properties

of each are being investigated (for a review, see reference 27). Briefly, vitronectin and fibronectin in the extracellular matrix bind to receptors in the plasma membrane. These receptors, or integrins, are heterodimers of α and β subunits and make up a family of closely related proteins. On the cytoplasmic face, focal adhesions are composed of a series of proteins in a chain, each interacting specifically with its neighbors. Integrin binds talin, which binds vinculin, which in turn binds α-actinin. The α-actinin cross-links cortical actin filaments. This bridge anchors the cytoskeleton to the substratum (extracellular matrix) and gives the focal contact its adhesive properties.

Quatrano and coworkers are now investigating the possibility that these components are part of the axis-stabilizing complex in *Fucus* species. Using protein immunoblotting, they have shown that *Fucus* zygotes contain proteins which cross-react with antibodies to vitronectin, vinculin, and the β-1 subunit of integrin (115). Recent evidence indicates that the material cross-reacting with the vitronectin antibody is localized to the rhizoid tip (146). The vitronectin homolog may bind to F2 at the rhizoid tip, much as human vitronectin binds the F2-like molecule, heparin sulfate (137). Vitronectinlike proteins and mRNAs have recently been discovered in higher plants (130, 132). These exciting findings suggest that the axis-stabilizing complex in fucoid zygotes may be a plant homolog of focal adhesions.

Somewhat surprisingly, zygotes bearing a fixed axis require neither actin nor cell wall to maintain their developmental polarity. Cytochalasin B addition (110) or cell wall removal (80) after fixation does not affect the preformed axis. Thus, the putative axis-stabilizing complex is needed only transiently, and soon after fixation other localized factors permanently imprint polarity on the zygote. What might these factors be? It has been suggested that they may include localized mRNA or localized metabolic processes (17). Although no evidence for localized mRNA has been obtained, there are indications of localized processes which accompany axis fixation. In particular, the endomembrane system in the perinuclear area becomes highly polarized. Fingerlike projections radiate from the nuclear envelope toward the presumptive rhizoid, and "mitochondria, ribosomes, osmiophilic bodies, densely fibrillar vesicles and what appears to be Golgi derived vesicles filled with finely fibrillar material" are localized in the perinuclear region on the rhizoid side of the nucleus (109). This indicates that the entire secretory apparatus is polarized before growth begins and probably accounts for local delivery and insertion of F2 at the presumptive rhizoid. Once secretion is committed to the presumptive rhizoid, it may be that the axis-stabilizing complex need not be maintained.

Metabolic processes also become asymmetrically distributed at the time of axis fixation. An extracellular pH gradient is detectable hours before growth, and 1 to 2 h before germination (i.e., during axis fixation) it stabilizes such that most of the surface of the zygote is acidic, except for the presumptive growth site, which is slightly alkaline with respect to the medium (40). Inhibitor data indicate that the extracellular acidity is a result of secretion of metabolic acids and that the pH gradient is a reflection of localized metabolism. Like localization of F2, establishment of the extracellular pH gradient is not essential for embryonic polarization; rather, it is one of the earliest expressions of stable polarity within the zygote.

EXPRESSION OF EMBRYONIC POLARITY

The remainder of this review will consider expression of stable polarity. To reiterate the timing of events, establishment and stabilization of polarity occur during the first half of the cell cycle, roughly 10 of 20 h in *Pelvetia* zygotes and 12 of 24 h in *Fucus* zygotes. This polarity then serves to orient developmental events occurring in the second half of the cell cycle. Expression of polarity has received considerably less attention than its establishment, as will be evident in the following discussion.

Germination and Tip Growth

Soon after the axis is stably fixed in place, morphological polarity is first expressed in the form of localized growth from the presumptive rhizoid pole. This denotes germination. The previously spherical zygote becomes pear shaped (Fig. 1), and the emerging rhizoid attaches the zygote more firmly to the rock substratum. Germination is nothing more than the initiation of tip growth; therefore, mechanistically they are identical processes and will be considered together. The rhizoid extension rate varies from 1 to 5 μm/h, which is quite slow in comparison with other tip-growing cells and organisms. Pollen tubes and fungal hyphae can elongate at tens of micrometers per minute. Even so, the mechanism of tip growth in fucoid algae does not appear to be fundamentally different from that in the faster-growing cells; its essence is unidirectional vesicle transport to, and local insertion at, the growing tip. (For a comprehensive consideration of tip growth in fungi and plants, see references 45, 47, and 135.) As with most tip-growing cells, rhizoid elongation is extremely sensitive to manipulation and is inhibited by many treatments (99). We often observe embryos that have divided many times without ever synthesizing a rhizoid. This is probably because tip growth is a very delicate process involving the endoplasm, cortex, membrane, and cell wall and depends on localized ionic fluxes, polarized F-actin, and carefully regulated turgor pressure. It should be pointed out that in older, multicellular embryos the common growth habit is intercalary growth and only the apical cell of the rhizoid elongates by tip growth.

Cytoarchitecture. The delicate nature of tip growth is manifest in wall structure. As in all tip growers, the apical wall of the elongating rhizoid is thinner than in subapical regions, soft enough to allow for turgor-driven expansion yet strong enough to resist lysis. This may be accomplished in part by controlling the gelling state of alginate (141), but measurements of specific wall parameters have not been made. There is some evidence that alginate at the apex is different from that of the rest of the zygote. G blocks at the tip are not available for specific antibody binding (10), implying a unique gelation state within the apical wall. Despite the unique nature of the apical wall, the distribution of polymers in the rhizoid wall reflects the distributions set up before growth. Alginate and cellulose remain uniformly distributed in growing rhizoids (100), whereas F2 is localized to the growing tip, where it probably functions in attachment (51, 96, 100, 114).

In fact, much of what goes on during tip growth can be viewed as a direct extension of the previously established polarity. That is, the polarity of cytoplasm, membrane, and cell wall set in place during the first half of the cell cycle becomes manifest as morphological polarity in the form of rhizoid growth (Fig. 10). For example, localized growth is the result of vesicle fusion at the rhizoid pole and is a

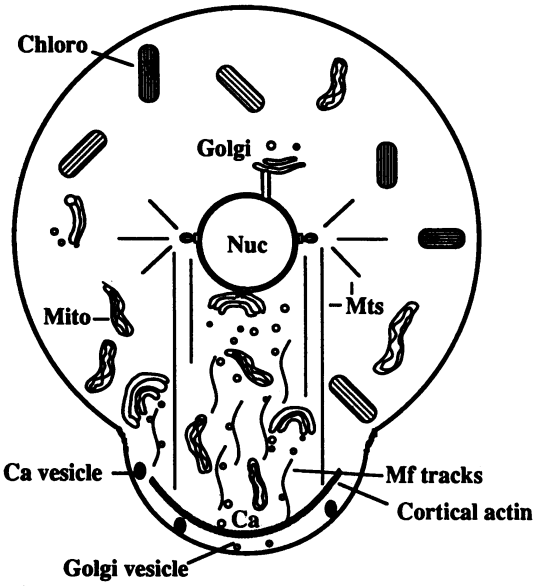

FIG. 10. Rhizoid elongation. The cytoplasm is highly asymmetric with mitochondria, cortical actin, Golgi, Golgi vesicles, and Ca^{2+} vesicles preferentially in the rhizoid region. Calcium vesicles are just beneath the plasma membrane, and the free cytosolic Ca^{2+} concentration is high in this zone. Golgi vesicles are transported along endoplasmic actin to the rhizoid cortex, and exocytosis is Ca^{2+} mediated. Mitochondria are highly convoluted. Microtubules extend between the nucleus and rhizoid cortex; their minus ends reside in MtOCs containing centrioles buried in pockets in the nuclear envelope. The axis defined by the MtOCs is initially perpendicular to the growth axis. Chloroplasts with axial grana are preferentially in the thallus hemisphere. Based on a drawing kindly provided by S. Brawley.

consequence of the extreme polarity in the endomembrane system, which first becomes apparent late in axis fixation (109). Cortical actin, which also localizes to the presumptive rhizoid during axis fixation, remains in the apex of the growing rhizoid (17, 78), where it may stabilize the fragile tip against the forces of turgor pressure during vesicle fusion and wall assembly (135). Delivery of vesicles to the tip depends on polarized endoplasmic F-actin connecting the Golgi to the apex (14), and presumably this F-actin was set in place during axis formation (Fig. 7B) and was involved in F-granule transport during axis fixation (Fig. 9). Together, endomembranes and actin arrays can be considered a highly polarized secretory complex that was assembled in the stages prior to growth. In this context, early development can be viewed as a continuum of increasingly polarized secretion. Secretion is at first uniformly distributed in the fertilized egg but becomes weakly polar at axis formation and then strongly asymmetric and irreversibly oriented at axis fixation. Finally, the rate of localized vesicle fusion intensifies dramatically at germination and is maintained at high levels during apical growth. In light of this need for constant vesicle synthesis and delivery, it is not surprising that rhizoid elongation is the stage of early development which is most dependent on protein synthesis. Inhibition of protein synthesis by cycloheximide treatment stops elongation of a preexisting rhizoid and prevents germination if applied a few hours in advance (108, 110).

In fact, the only cellular structure which actually takes on a new distribution as growth begins is the microtubule cytoskeleton. The previously uniform distribution of microtubules radiating from the perinuclear region into the cortex is supplanted by microtubules which preferentially extend from the nucleus to the elongating tip, along the polar axis of the cell (Fig. 10 and 11A) (81). These microtubules would be excellent candidates as tracks for secretory vesicle transport were it not for the fact that tip growth continues in the absence of all detectable microtubules (13, 81). That is, zygotes polarize, germinate, and grow in the presence of microtubule-depolymerizing drugs. In many of these treated zygotes the rhizoid is much broader than normal, indicating some role for axial microtubules in determining rhizoid

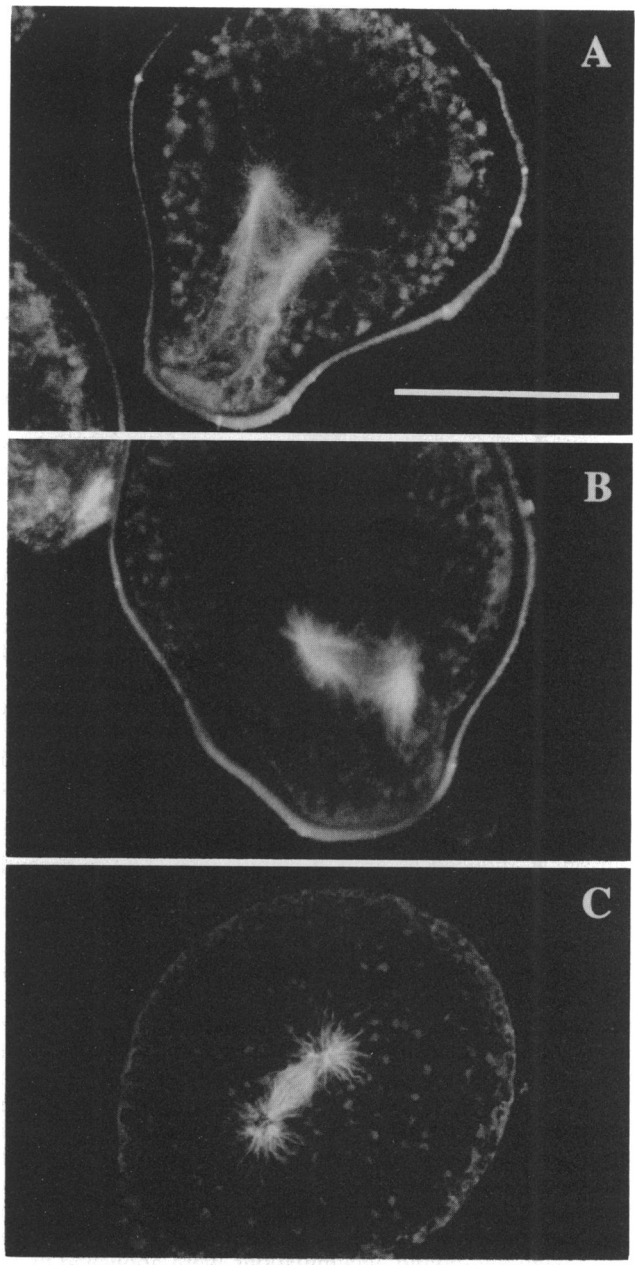

FIG. 11. Microtubule distributions late in the first cell cycle. (A) Microtubules extend into the tip as soon as growth begins. (B) MtOC axis is rotating in the germinated zygote. (C) After rotation is completed, the mitotic spindle forms in alignment with the growth axis. Bar, 50 μm.

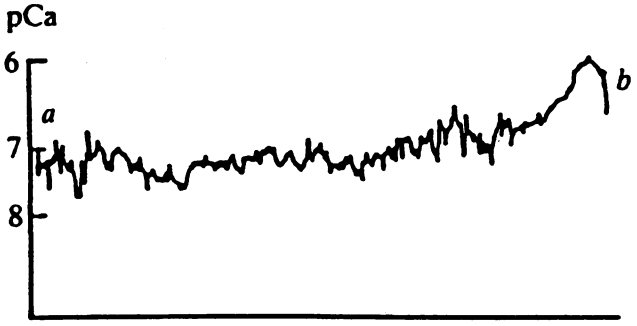

FIG. 12. Distribution of cytosolic Ca^{2+} in a growing rhizoid. A rhizoid was microinjected with the fluorescent Ca^{2+} indicator fura 2, and the free Ca^{2+} levels were measured along the length of a rhizoid by ratio imaging. Position *a* is the most basal region, and position *b* is at the tip. The subapical Ca^{2+} concentration is less than 0.1 μM, whereas the concentration at the tip approaches 1 μM. Reproduced from reference 24 with permission.

morphology. It is interesting that in the absence of microtubules the rhizoid elongates to approximately the length it would normally obtain by the onset of cytokinesis and stops there. Without division elongation ceases, indicating coordinate control of these processes.

Ca^{2+} gradients. Although the role of the transcellular current in polarity establishment is controversial, there is ample evidence that it has physiological function during tip growth. The magnitude of the steady current intensifies at germination (62), and part of this current is carried by Ca^{2+}. Although flux data indicate that the magnitude of the Ca^{2+} current is reduced as growth commences (126), a recently developed Ca^{2+}-specific vibrating electrode easily detects Ca^{2+} current flowing into the growing tip (83). (A Ca^{2+} current in younger, ungerminated zygotes could not be measured.) As growth begins, cytoplasmic Ca^{2+} accumulates at the elongating tip (Fig. 12), and this is the earliest stage at which an asymmetry in Ca^{2+} distribution has been unequivocally detected. Low-temperature autoradiography was initially used to show an accumulation of $^{45}Ca^{2+}$ in the rhizoid (41, 59). More recently, free cytoplasmic (24, 25) and membrane-bound (82) Ca^{2+} have been measured, and in both cases Ca^{2+} levels are highest at the apex, just beneath the plasma membrane (67). By using ion-selective microelectrodes, free cytosolic Ca^{2+} concentrations of 2.6 μM at the tip and 0.4 μM in the subtip were measured (25). Both free and membrane-bound accumulations were abolished by removal of Ca^{2+} from ASW or addition of Ca^{2+} channel blockers, and, concomitantly, rhizoid elongation ceased (82). This suggests that a localized Ca^{2+} flux establishes the Ca^{2+} gradient and that apical Ca^{2+} has a physiological role in rhizoid growth. To test further the need for apical Ca^{2+}, Speksnijder et al. used injection of Ca^{2+} buffers (BAPTA derivatives) to dissipate gradients and make the cytoplasm uniform in Ca^{2+} concentration (134). Injections of buffers with a dissociation constant of about 5 μM prevented rhizoid outgrowth without causing cell death, presumably by shuttling Ca^{2+} from regions of high concentration to regions of low concentration. These data provide the best evidence for a physiological role for the transcellular current, in particular Ca^{2+} circulation, in embryonic development.

In other tip-growing organisms, Ca^{2+} currents and elevated apical Ca^{2+} levels are commonly associated with growth (60, 121, 122), but exceptions have been noted (49). It is postulated that the elevated apical Ca^{2+} controls differentiation of the cortical cytoplasm by locally activating Ca^{2+}-dependent processes (67). A high apical Ca^{2+} concentration, perhaps as high as 10 μM in fucoid zygotes (134), is thought to activate protein kinases via calmodulin-dependent and -independent pathways. (Calmodulin has been identified in fucoid zygotes [16].) The resultant phosphorylation of cortical proteins at the apex may regulate assembly and disassembly of cytoskeletal components, mediate fusion of secretory vesicles with apical plasma membrane, or control any number of biochemical pathways (135). In addition to these putative functions, the Ca^{2+} current in germinated fucoid embryos is involved in turgor regulation. As in all cells enclosed in a wall, turgor pressure provides the driving force for expansion and must be finely regulated. Too little, and growth ceases; too much, and the cell lyses. In growing *Pelvetia* rhizoids, turgor pressure is maintained around 0.8 MPa (8 bars) (93a).

Turgor pressure in growing rhizoids is regulated in part by ion transport, which can be detected as current pulses. Prior to rhizoid outgrowth the transcellular electric current is a steady current of low intensity, but later a large pulsatile current is superimposed on the steady current. Current pulses last from seconds to a few minutes, with an average frequency of 1 to 5/h, and carry up to 30 $\mu A/cm^2$ into the tip (103). Because of its large size and long duration, the pulse current has been investigated in detail. The inward current is carried mainly by Cl^- efflux from the rhizoid (anion efflux corresponds to inward current), and the outward current is K^+ efflux from the rest of the cell (104). The net result is KCl efflux, which lowers the osmotic potential of the cytoplasm and, by osmosis, lowers turgor pressure. As expected, zygotes increase pulsing rates in hypoosmotic treatments and reduce them in hyperosmotic media. Pulsing can be stimulated by decreases in external osmotic pressure as small as 3%.

Ion deletion experiments indicate that pulsing is regulated by Ca^{2+}. Treatments which are known to stimulate Ca^{2+} entry, such as reductions in Na^+, Mg^{2+}, or K^+ concentrations, trigger pulsing, and Ca^{2+} removal suppresses pulsing (105). Nuccitelli and Jaffe (105) proposed that osmotic stress allows a small amount of Ca^{2+} to enter the tip and that this opens Cl^- channels. Cl^- exits through the channels, causing depolarization of the membrane potential, which drives K^+ out of the cell. Recently, freeze fracture analysis of ultrarapidly frozen embryos has uncovered numerous 0.5-μm disk-shaped vesicles lying in the cortical cytoplasm (42). Hypoosmotic treatment causes these vesicles to fuse with the plasma membrane. Gilkey and Staehelin (42) propose that these vesicles contain Cl^- channels and that fusion inserts the Cl^- channels into the apical plasma membrane, allowing Cl^- efflux, which carries inward current. The small Ca^{2+} entry which triggers pulsing may cause fusion of these vesicles with the plasma membrane. A combination of Cl^- channel regulation and addition of new Cl^- channels by vesicle fusion would allow the rapid and reversible turgor regulation needed to control tip growth. In the final assessment, it seems that in fucoid zygotes, as in other walled tip-growing cells, growth is driven by a scalar quantity, turgor pressure, and growth position is determined by vectorial secretion (45).

Rotation of the MtOC Axis

At germination, the microtubular network is reorganized into a polar array in which microtubules emanate from the perinuclear region and focus on the rhizoid tip (81). Initially

polar microtubules are nucleated from the entire rhizoid-facing hemisphere of the nuclear envelope, but within 2 h of germination (12 to 14 h postfertilization of *Pelvetia* species) they originate from two well-defined foci. The foci serve as MtOCs (2) and presumably form by coalescence of the perinuclear material. A network of parallel microtubules runs from each MtOC toward the rhizoid cortex. Two centriole pairs associated with the nuclear envelope have been observed in electron micrographs (15), and each MtOC probably contains one pair. (Nuclei of brown algae typically contain centrioles buried in a pocket in the nuclear envelope [98].) The axis defined by the two MtOCs is initially perpendicular to the growth axis but rotates 90° prior to mitosis and comes to align with the growth axis (Fig. 11B). Rotation occurs in either direction and probably involves rotation of the entire nucleus. In midrotation, the majority of the microtubules radiating toward the tip are associated with the MtOC moving apically. Inhibitor studies suggest that rotation is dependent on microtubules and microfilaments, and it is proposed that the force driving rotation is provided by microtubules connecting the MtOCs to the apical cortex and perhaps by endoplasmic F-actin (2). Although the precise mechanism of rotation is unknown, it may be similar to centrosome rotation in *Caenorhabditis elegans* embryos (55, 56), in which the microtubules emanating from the two centrosomes appear to pull against one another and the network which pulls the hardest wins out and its centrosome moves apically.

Cortical microfilaments may also be involved in MtOC rotation but in a more indirect way. Cytochalasins prevent microfilaments from localizing in the rhizoid cortex during axis fixation (17, 78) and disrupt proper rotation of the MtOC axis (2). Allen and Kropf (2) propose that localized cortical microfilaments provide positional information to the endoplasm regarding the location of the tip. Endoplasmic microtubules emanating from MtOCs make use of this spatial information and anchor in the rhizoid cortex. Once anchored, the endoplasmic microtubules (and perhaps F-actin) provide force to rotate the nucleus. After rotation has been completed, the MtOCs are aligned along the rhizoid/thallus axis and the zygote enters mitosis.

Mitosis and Cytokinesis

Mitosis begins approximately 16 h after fertilization in *Pelvetia* species and a few hours later in *Fucus* species. The rotated MtOCs serve as spindle poles, ensuring that the spindle is properly aligned along the growth axis (Fig. 11C). Mitosis of the first cell cycle in *Fucus* zygotes was initially described in 1896 by Farmer and Williams (37) and was described in more depth in 1909 by Yamanouchi (152). These early workers accurately described many events associated with mitosis, including centrosomes (MtOCs) in contact with the nuclear envelope just prior to mitosis. Early in mitosis, microtubules originating from these centrosomes were seen penetrating the nuclear envelope and coursing into the nucleoplasm. Nuclear envelope breakdown was observed not to be completed until metaphase or later. Analysis of metaphase structures revealed a haploid chromosome number of 32. Much of this early work has been confirmed and extended by using transmission electron microscopy (15). At all stages of mitosis the spindle is surrounded by membranous material, including rough endoplasmic reticulum, annulate lamellae, and vesicles. By early anaphase the nuclear membrane has broken down, the nucleolus has disappeared, and microtubules radiate from the spindle poles into the nucleoplasm. Paired centrioles reside at each spindle pole, as is typical in the order Fucales (85). At telophase, the newly re-formed nuclei are relatively far apart (30 to 50 μm) and are surrounded by Golgi and mitochondria (15). A perinuclear cap of microtubules is present on the distal end of each daughter nucleus, presumably a remnant of the spindle pole (81). Initially there is no clear ultrastructural distinction between the daughter nuclei, but axial microtubules soon connect the rhizoid nucleus to the elongating tip (81). Golgi and mitochondria increasingly associate with the rhizoid nucleus, while the thallus nucleus is surrounded by rough endoplasmic reticulum and a radially symmetrical array of microtubules (15).

The first division of *Fucus* zygotes is an unequal, deterministic cleavage. As zygotes enter cytokinesis, an aggregation of chloroplasts marks the plane of cell division (15); this plane bisects the spindle and is perpendicular to the growth axis. Partitioning occurs predominantly by furrowing, but not strictly so, since vesicles do coalesce with the growing partition membrane (15). In general, cytokinesis by furrowing involves an F-actin, and filamentous structures possibly representing F-actin were reported in the chloroplast-rich region prior to partitioning (15) and in the cleavage furrow (17) of *Fucus* zygotes. However, cytochalasin D prevents cytokinesis only if added 4 h or more prior to division (2). When added just before cytokinesis, cytochalasin D has little effect, indicating that furrowing may not be F-actin dependent. It may be that microfilaments bring chloroplasts to the furrow site in preparation for cytokinesis but do not actively participate in furrowing. Instead, inhibitor studies indicate that cytokinesis is immediately dependent on microtubules (2), as in higher plant cells. Although microtubules were not observed near the furrow by EM (15), recent confocal images do show microtubules extending from the daughter nuclei toward the division site where the furrow meets the parental wall (2). However, these microtubules are not organized into a phragmoplast. Interestingly, 2-h treatments with either cytochalasin or nocodozole applied any time prior to cytokinesis disrupt the orientation of the division plane, indicating that positioning in some way involves both F-actin and microtubules (2).

In the final stage of cytokinesis, cell wall is formed between the partition membranes. The composition of the partition wall has not been investigated, but most probably it contains alginate, cellulose, and perhaps fucans. Although formation of the wall has been reported to obliterate plasmodesmata (15), rhizoid and thallus cells remain coupled electrically (148) and small molecules move freely between cells (24). Even so, the rhizoid and thallus cells differ markedly from one another in morphology and content. Golgi bodies, mitochondria, and rough endoplasmic reticulum are more abundant in the rhizoid cell (15). The numerous hypertrophied Golgi bodies in the perinuclear region presumably supply vesicles that fuel tip growth. Chloroplasts, on the other hand, are more numerous and divide more actively in the thallus cell and its derivatives. Chloroplasts begin to degenerate in the rhizoid cell, and the continue this process as development proceeds and a holdfast is formed. The uniqueness of the rhizoid and thallus cells implies that, despite their electrical and dye coupling, they are differentiated cells with predetermined fates. That is, the first unequal division appears to be a deterministic cleavage. However, the developmental potential of the two cells has not been investigated directly, and so the degree to which they are committed is unclear.

DIRECTIONS FOR FUTURE RESEARCH

Fucus and *Pelvetia* zygotes have long been investigated as models for the establishment and expression of cellular polarity. From these studies, we have gained a rather detailed description of the cellular and physiological phenomena which accompany these developmental processes. Because of this progress, fucoid zygotes now serve as a paradigm for cellular polarization during development. However, the mechanisms which cause polarity have proved elusive, and clearly "it is time to disentangle the web of causality by simple manipulative experiments" (45). The following is an attempt to pose important questions and, where possible, formulate experiments which may help disentangle the web.

(i) Perception of environmental gradients and transduction of the stimuli into developmental polarity have received relatively little attention. Does a PI cycle involving Ca^{2+} transients and protein phosphorylation couple environmental signals to establishment of cellular polarity?

(ii) Nowhere is the need to establish function more acute than in the area of ionic currents. The transcellular current is clearly present early in development, but its role is as unclear today as it was nearly 20 years ago when it was first detected. Perhaps the most pressing issue is whether current flow is fundamental to polarity and growth or whether it is an epiphenomenon. Are the putative voltage and ionic gradients set up by transcellular current flow causal to developmental polarity? In this endeavor, careful experiments must be conducted to assess the role of Ca^{2+} in axis formation and fixation, distinct from its function in tip growth. One approach would be to define conditions in which polarity and ionic currents are uncoupled; for example, does an ionic current flow in the minimal KCl medium? The question whether the ionic current is the result of asymmetric channel distribution or local channel regulation can be addressed by using patch clamp studies to compare the membrane at the putative rhizoid with that at the putative thallus. At a more general level, we need to know just how the plasma membrane is energized. What are the primary pumps (H^+-ATPase, Cl^--ATPase, and/or Na^+-K^+-ATPase), and what is their role in generating V_m? Finally, which energized ion drives cotransport? If it is Na^+, how is it pumped out of the zygote? Answers to these more general questions will provide a broader electrophysiological perspective in which to interpret the ionic current.

(iii) Although progress has been made concerning cytoskeletal structure and function, much remains to be done. Accessory proteins putatively involved in rearranging membrane transporters during axis formation, building an axis-stabilizing complex during axis fixation, and rotating MtOCs in preparation for mitosis must be identified. Animal cells possess a staggering array of actin-binding accessory proteins and microtubule-associated proteins, and many of these proteins (or their functional equivalents) probably play important roles in cellular polarization in fucoid zygotes. Also, the organization and localization of F-actin must be clarified. Is there a fine meshwork of cortical actin? Do endoplasmic F-actin filaments course from the perinuclear Golgi to the putative rhizoid zite, and do they transport secretory vesicles? If so, are the endoplasmic microfilaments localized by 5 h, when polarized secretion can be detected? High-resolution techniques such as immunogold labeling of filaments for visualization by EM may be necessary in this effort. Finally, visualization of dynamic rearrangements of the cytoskeleton in living cells would be very illuminating and should be feasible by using microinjection of fluorescent probes.

(iv) The possibility that turgor pressure is fundamental to polarization must be clarified. If it is true, it would identify a novel role for turgor pressure in plant cells. Use of a pressure probe to monitor single cells under conditions which are permissive or inhibitory to axis formation and fixation should shed light on this matter.

(v) Features of cellular ultrastructure, not revealed by earlier work on glutaraldehyde- and formaldehyde-fixed zygotes, may be visualized by modern techniques of rapid freezing and freeze substitution. In particular, a reinvestigation of ultrastructure may provide additional information on localization of organelles (including cytoskeleton), vesicles, and other subcellular components and may provide some insight into the origin, content, and function of the multitude of seemingly diverse vesicles packed into the egg and zygote cytoplasm.

(vi) The application of molecular techniques should open avenues of research not tractable by cellular and physiological approaches. It should be possible to identify proteins and mRNAs that are localized to either the rhizoid or thallus end of the cell prior to the first division and then to study the timing of redistribution and the mechanism by which the localized molecules are transported intracellularly. After division, rhizoid- and thallus-specific genes, including putative sequences that specify tissue-specific expression, can be investigated.

ACKNOWLEDGMENTS

I thank C. Brownlee, F. M. Harold, M. Slocum, and R. S. Quatrano for critically evaluating an early draft of the manuscript. J. Jordan, B. Gibbon, and V. Allen helped prepare figures and were very patient during my absence from the laboratory.

Preparation of the manuscript and some of the research presented were supported by NSF grant DCB-8904770.

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