Programmed Changes in Form during Moss Development

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INTRODUCTION

Picture an organism that begins development as a germinated spore to give rise to a filament via tip growth. Within days of germination, and with the involvement of only a few cells and cell types, the pattern of growth changes dramatically from two dimensional to three dimensional as a leafy structure is produced on the filament. With this simple picture in mind, we can begin to think about vegetative development in moss. In this review, we introduce the reader to the processes involved in moss development, speculate on some of the underlying mechanisms, outline some of the advantages of using moss development to understand elements of general eukaryotic development, and identify areas that require additional research and clarification. We focus our discussion on the development of *Funaria hygrometrica* grown in culture, with additional information from studies using *Physcomitrella patens*.

The very early stages of moss development are characterized by cellular differentiation during filament growth. Spore germination leads to the formation of a filament that is made up of a tip cell (an apical cell) and a linear array of subapical cells that are produced by successive divisions of the tip cell. These subapical cells have walls that are perpendicular to the filament axis and filled with large round chloroplasts, hence their name, chloronema (Figure 1A). As is characteristic of tip-growing organisms, the subapical chloronema cells, which are ~100 μm in length, do not grow. The tip cell continues to elongate, reaches a maximum length of ~250 μm, and divides every 10 to 12 hr to extend the filament.

Chloronema filament growth continues until, in response to increases in light and auxin, the appearance of the chloronema tip cell begins to change, ultimately giving rise to the second filament cell type, the caulonema. Fully developed caulonema tip cells differ from chloronema tip cells in several important respects. They grow much longer (up to 400 μm), divide more often (every 5 to 6 hr), and possess fewer, smaller, and more elliptical chloroplasts. During the transition from chloronema to caulonema, the newly formed filament cells appear transitional, but after 5 to 6 days, the caulonema cells are long (~250 μm), nearly clear, and have cross walls that are oblique to the filament axis (Figure 1B).

Once caulonema cell formation has begun, a new axis of developmental polarity is set up as an initial cell is formed.

Soon after division of a caulonema tip cell, a small swelling is seen in the second subapical cell (the third cell of the filament). This swelling, which gives rise to an initial cell, appears at the apical end of the cell near the wide end of the oblique cross wall (Figure 1B, arrow). The apex of the swelling expands, and the division that produces the initial cell occurs 5 to 6 hr after visible evidence of initial cell formation is seen. Before this division, the nucleus from the filament cell migrates from the middle of the cell to the initial cell site, where it divides. One daughter nucleus moves into the nascent initial cell, and the second moves back to the middle of the filament cell. A cell wall, which is oriented in the direction of the longitudinal wall of the filament cell, then separates the fully formed initial cell from the filament (Figure 2A).

The caulonema initial cell has two developmentally distinct potential fates. In the absence of cytokinin, the initial cell continues to develop by tip growth to produce a new lateral filament, thus maintaining the two-dimensional growth habit. In the presence of cytokinin, however, the initial cell takes on a distinct morphology. This morphology is associated with the assembly of a bud and represents the transition to a three-dimensional growth habit. Within 2 to 3 hr of cytokinin perception, the initial cell swells dramatically and the chloroplasts within decrease in size (Figure 2B; compare initial cells in Figures 2A and 2B). An asymmetric division follows to produce a stalk cell and the bud proper (Figure 2C). Subsequent divisions of the bud give rise to a simple meristem. This meristem produces a shoot with simple leaves (Figures 3A and 3B) that eventually bears the sexual structures.

Given this description, we describe what is known about how a polar axis is established during caulonema cell differentiation and subsequent initial cell formation. Based on information from previous studies and comparable processes in other organisms, we also speculate on potential mechanisms underlying these processes and suggest directions for future study.

EXPRESSION OF POLARITY DURING MOSS DEVELOPMENT

In the differentiating cells of many organisms, cellular constituents, including plasma membrane domains, organelles,

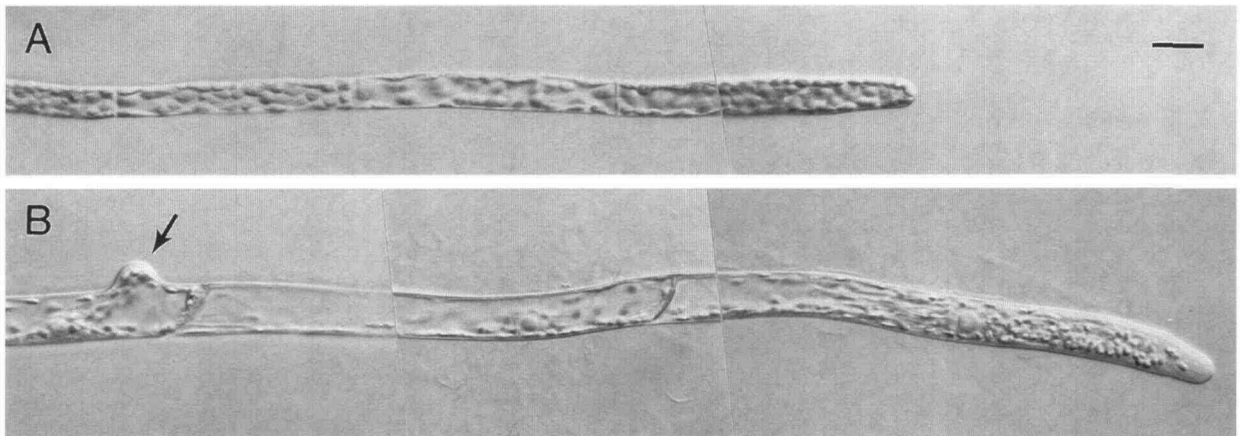[1] To whom correspondence should be addressed. E-mail schumake @ag.arizona.edu; fax 520-621-2012.

Figure 1. Light Micrographs Illustrating the Filamentous Stages of Moss Development.

(A) Chloronema filament.
(B) Caulonema filament. The arrow points to an outgrowth at the initial cell site.
Bar in **(A)** = 25 μm for **(A)** and **(B)**.

and cytoskeletal filaments, are organized asymmetrically. This polar distribution of cellular components ultimately leads to structural and functional diversity within the organism. Three processes during early moss development involve polar growth: growth of both chloronema filaments and caulonema filaments and establishment of initial cells. We focus here on the polar processes involved in caulonema cell differentiation and initial cell formation. We have chosen caulonema differentiation because this process is critical to initial cell formation and thus bud assembly and sexual reproduction. Equally important, caulonema differentiation produces cells that exhibit an extremely pronounced polar morphology with a distinct zonation of organelles, a *prominent tip zone, and a rapid growth rate* (Wacker at al., 1988; Walker and Sack, 1995). Initial cell formation is clearly a polar process because extension is restricted to a specific, predictable site on a cell that has otherwise ceased growing.

Caulonema Cell Differentiation

Auxin and cAMP

Research results produced over a span of >25 years have described the effects of auxin and cAMP on caulonema cell differentiation. We use the following criteria to provide a framework within which to evaluate the evidence implicating these compounds in moss development. Most importantly, auxin or cAMP (i.e., the effectors) should be present in the plant at the appropriate times during development and in the correct concentrations to elicit the response. This requires mechanisms that can alter the amounts of effector

(e.g., changes in rates of synthesis and/or catabolism, differential activation, and altered distribution) or the sensitivity of the plant to the effector. It should also be possible to show that altering the level of the effector in wild-type or mutant plants (e.g., addition of the effector or modulators of effector synthesis, catabolism, or action) changes the response.

In addition to a requirement for increased light, the conversion of a chloronema tip cell to a caulonema tip cell requires the presence of auxin (Johri and Desai, 1973; Atzorn et al., 1989a, 1989b). Auxin levels have been estimated in moss cells by using a number of techniques (Ashton et al., 1985; Jayaswal and Johri, 1985; Atzorn et al., 1989b). For example, by using HPLC enzyme immunoassays, Atzorn et al. (1989a) measured endogenous levels of auxin in tissues from the wild type and a caulonema cell–deficient mutant of *Funaria* (mutant 87.13). They showed that auxin levels increased concomitantly with the production of caulonema cells in the wild type and that the mutant had 50 to 70% less auxin at similar stages of development. Estimates of indole-3-acetic acid (IAA) levels in *pg-1* mutants of *Funaria* have provided additional evidence for auxin's involvement in regulating caulonema differentiation (Jayaswal and Johri, 1985; Atzorn et al., 1989b). The *pg-1* mutant produces predominantly caulonema filaments even in the absence of exogenous auxin (Handa and Johri, 1979). Based on fluorimetric assays, levels of IAA were 2.5-fold higher in *pg-1* than in wild-type tissue of the same age.

Although little is known about auxin biosynthesis in moss, analysis of additional *Funaria* mutants has provided insights into mechanisms that may be involved in the catabolism of endogenous auxin. The NAR-2 mutant produces caulonema cells only when treated with exogenous IAA or IAA precursors (Bhatla and Bopp, 1985). This mutant has lower endog-

enous IAA levels and shows a threefold increase in IAA oxidase activity relative to the wild type. This suggests that lower auxin levels in the mutant may be due to increased enzymatic degradation of the hormone and that the enzymes necessary for IAA catabolism operate in *Funaria*.

Additional evidence for auxin's role in caulonema differentiation comes from studies using exogenous auxin. Although caulonema differentiation in *Funaria* takes place independently of exogenous auxin under high light conditions, experiments performed under low light intensity (which slows normal conversion of chloronema to caulonema) have shown that the differentiation can be induced by external auxin. Furthermore, *Funaria* cells grown under the influence of exogenous auxin have been shown to produce initial cells, which are the target cells for cytokinin-induced bud assembly, suggesting that studies with exogenous auxin have relevance to normal moss development (Johri and Desai, 1973; Lehnert and Bopp, 1983). Conversely, removal of differentiating caulonema cells from auxin-containing media or treatment with IAA antagonists can prevent the differentiation of chloronema to caulonema and induce the dedifferentiation of caulonema to chloronema (Johri and Desai, 1973; Sood and Hackenberg, 1979; Bopp, 1980).

In 1976, it was shown that cAMP is present in moss tissue, and measurements in *Funaria* cells showed that cAMP levels are four- to sevenfold higher in chloronema cells than in caulonema cells (Handa and Johri, 1976, 1977). In addition, a molecule was identified in *Funaria* that stimulates the activity of protein kinase from rabbit skeletal muscle and cochromatographs with authentic cAMP (Handa and Johri, 1977). The ability of this molecule to stimulate kinase activity was eliminated in the presence of 3',5'-cyclic nucleotide phosphodiesterase in a manner similar to the ability of this phosphodiesterase to inactivate authentic cAMP. Subsequently, two cAMP phosphodiesterase activities were partially purified from chloronema cells of *Funaria* (Sharma and Johri, 1982a). One of these shows substrate specificity for 3',5'-cAMP, produces 5'-AMP from cAMP, is stimulated by imidazole, and is inhibited by methylxanthine-derived inhibitors of cyclic-nucleotide phosphodiesterases, suggesting that this moss cAMP phosphodiesterase is similar to animal cAMP phosphodiesterases (Sharma and Johri, 1982a).

Evidence that *Funaria* development is regulated by cAMP also exists. For example, caulonema differentiation was inhibited when cAMP was added to an auxin-containing medium, indicating that cAMP acts antagonistically to auxin and may participate in the regulation of caulonema differentiation (Handa and Johri, 1976, 1979). In addition, substituted xanthines mimicked the effect of cAMP in regulating chloronema differentiation (Handa and Johri, 1976). Further evidence indicating a role for cAMP in moss differentiation comes from studies in which the *pg-1* mutant was induced to produce chloronema filaments after the addition of cAMP to the medium (Handa and Johri, 1976, 1979). Taken together, the results suggest that cAMP enhances the differentiation of chloronema cells, whereas auxin inhibits chloronema differentiation and stimulates caulonema differentiation.

Caulonema Initial Cell Formation

Experimental evidence supports the presence of two distinct developmental stages in moss bud formation: (1) caulonema initial cell formation, and (2) assembly of the bud
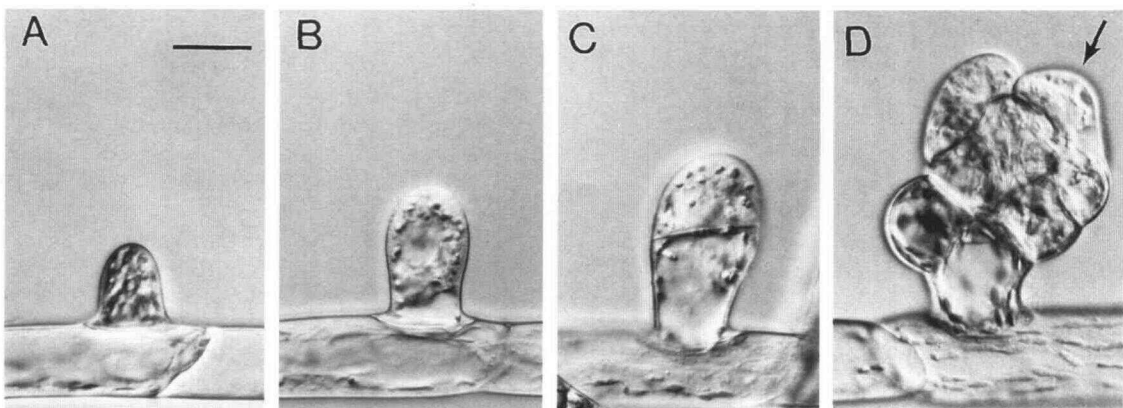

Figure 2. Light Micrographs Illustrating the Early Stages of Moss Bud Assembly.

(A) Fully formed caulonema initial cell.
(B) Caulonema initial cell ∼10 hr after the addition of cytokinin.
(C) Two-celled bud stage.
(D) Simple meristem. The arrow points to a leaf primordium.
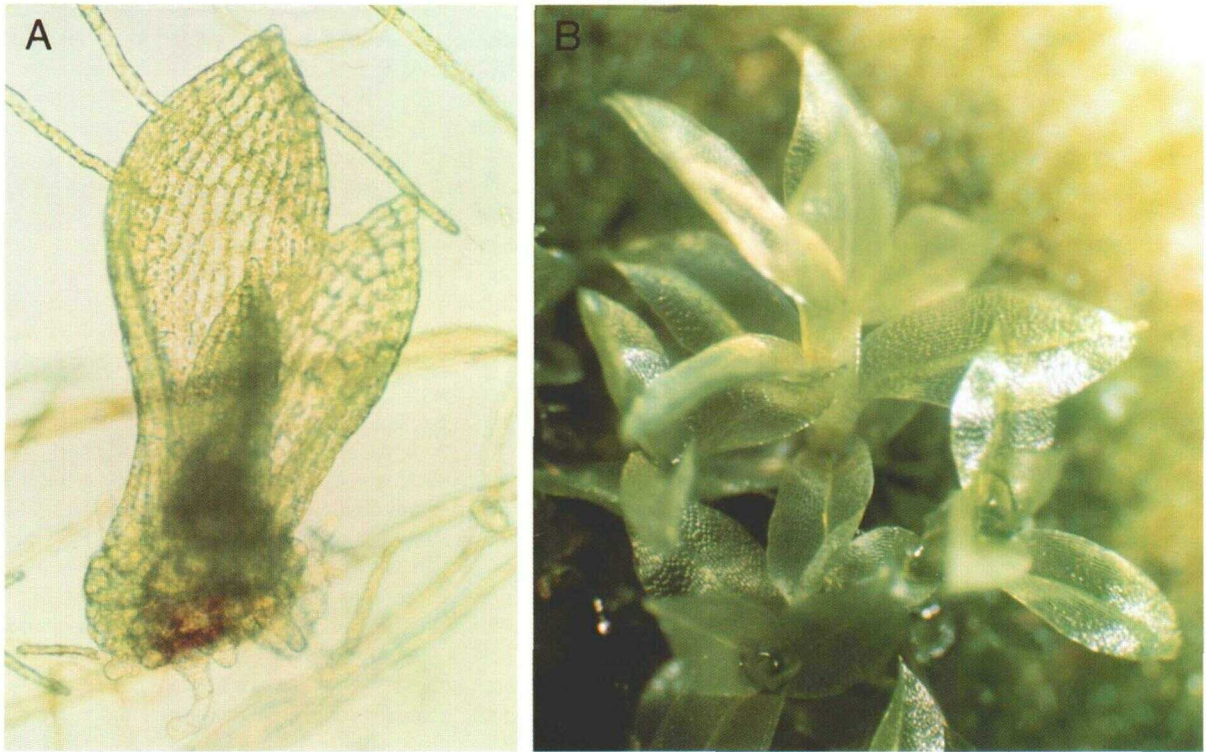Bar in **(A)** = 25 μm for **(A)** to **(D)**.

Figure 3. Light Micrographs Illustrating the Leafy Gametophyte.

(A) Young gametophyte. Magnification ×198.
(B) Mature gametophyte. Magnification ×40.

from the initial cell. Bopp and Jacob (1986) have shown that cytokinin is required for both processes. Picomolar levels of cytokinin induce a caulonema filament to produce initial cells, whereas micromolar concentrations are required for the assembly of a bud from an initial cell.

Calcium

Numerous studies have implicated calcium as an intracellular messenger in caulonema initial cell formation. In a series of reports, Saunders and Hepler (1981, 1982, 1983), Saunders (1986), and Conrad and Hepler (1988) performed experiments with *Funaria* that addressed the three criteria proposed by Jaffe (1980) to demonstrate that calcium is involved as an intracellular messenger in a physiological process. Specifically, the physiological response should be preceded or accompanied by an increase in intracellular calcium, blocking the natural calcium increase should inhibit the response, and the experimental generation of an increase in intracellular calcium should stimulate the response (Jaffe, 1980).

From the above-mentioned reports, the authors concluded that calcium was involved in bud formation; how-

ever, they did not distinguish between the processes of initial cell formation and the assembly of the bud from the initial cell. We believe that in most cases, the data point strongly toward a role for calcium in the establishment of the caulonema initial cell. Our conclusion is based on two observations. First, earlier studies used as their starting material tissue that had not produced the target cells for cytokinin-induced bud assembly; the addition of cytokinin to caulonema tissue that was not producing initial cells induced the formation of those targets. Second, the levels and distribution of calcium were measured immediately after the addition of cytokinin, which is during the time frame of initial cell formation and before targets of bud assembly would have been present.

Saunders and Hepler (1981) examined the temporal and spatial changes in membrane-associated calcium during initial cell formation by using the lipophilic fluorescent calcium chelating probe chlorotetracycline. They showed a localization of chlorotetracycline fluorescence in caulonema cells at the presumptive initial cell site within hours of cytokinin addition. By the time of the division separating the initial cell from the filament, the region was four times as fluorescent as the entire caulonema cell. Parallel measurements with the general membrane marker *N*-phenyl-1-naphthylamine

showed that the rise in membrane-associated calcium was not due to an increase in membrane density. The authors concluded that the increases in membrane-associated calcium reflected a localized increase in intracellular free calcium concentration.

Further evidence that calcium plays an important role in initial cell formation comes from studies measuring currents along a caulonema filament with a nonintrusive vibrating microelectrode (Saunders, 1986). In a caulonema cell, maximal inward current was measured near the nucleus (midway along the cell). After addition of cytokinin, the inward current increased twofold along the length of the cell, and within minutes, current decreased near the nucleus and at the basal end of the cell. At the same time, current increased at the apical end of the caulonema cell, predicting the location and preceding the formation of the initial cell. Inward current at the apical end fell to resting levels as the outgrowth of the initial cell began. The current was blocked by the lanthanide gadolinium, a competitive inhibitor of calcium transport. These results suggest that there is a calcium component to the current and that calcium entry at the apical end of the caulonema cell is critical to initial cell formation.

Saunders and Hepler (1983) prevented movement of calcium into cells treated with cytokinin to show that blocking the natural calcium increase inhibits bud formation. No buds formed when calcium was removed from the medium or when its movement into the cell was blocked by lanthanum. This inhibition could be reversed by adding calcium or by washing out the lanthanum and adding cytokinin. However, it is not clear whether initial cell formation and/or bud assembly was inhibited.

Clear support for a requirement for calcium in initial cell formation came from experiments in which the cell's ability to respond to cytokinin was prevented with the calcium channel blockers verapamil and D-600. In all cases, inhibitor activity was reversed and initial cells were formed when the external calcium concentration was increased or the calcium ionophore A23187 was added.

To address Jaffe's third criterion, that an increase in intracellular calcium should stimulate the response, Saunders and Hepler (1982) showed that initial cell formation can be induced in the absence of cytokinin by using A23187. For the most part, initial cells formed at the correct site; however, bud assembly did not take place. This suggests that other cytokinin-induced factors are required for complete formation of the bud. Removal of A23187 from the cells up to 5 hr after its application eliminated the response, suggesting that elevated intracellular calcium may need to be maintained over a period of time for normal development to proceed.

In 1988, Conrad and Hepler provided additional evidence that calcium is important in initial cell formation and that increases in intracellular calcium may take place through calcium channels on the plasma membrane in moss. By using 1,4-dihydropyridines (DHPs), which modulate calcium entry through voltage-dependent calcium channels in animal cells,

they showed that the DHP antagonists (−)202-791 and nifedipine inhibited bud formation by up to 94%. Reducing the concentration of the antagonist in the medium or photolysis of nifedipine to its inactive form reduced the ability of these compounds to block bud formation. Again, it is not clear whether initial cell formation or bud assembly (or both) was affected. Nevertheless, when cells were cultured in media containing the DHP agonists (+)202-791 or CGP 28392, initial cell formation was stimulated on virtually every cell. These experiments provided the first evidence that initial cell formation is due in part to increased calcium flux through voltage-operated channels.

Experiments from our laboratory have extended this work to show the presence of a DHP-sensitive calcium transport mechanism on the plasma membrane in moss (Schumaker and Gizinski, 1993, 1995, 1996). Studies with *Physcomitrella* have provided evidence that DHPs modulate calcium influx into protoplasts. For example, calcium influx was stimulated by DHP agonists and inhibited by DHP antagonists (Schumaker and Gizinski, 1993). As has been shown for DHP-sensitive calcium transport in animal cells, influx into moss cells was stimulated by a depolarization of the plasma membrane and was modulated by numerous classes of calcium channel blockers. We have also shown that there are abundant sites for DHP binding in the moss plasma membrane (Schumaker and Gizinski, 1995). In particular, DHPs bind with high affinity and specificity (Schumaker and Gizinski, 1995). This ligand–receptor interaction was stimulated by cytokinin at low concentrations and by heterotrimeric GTP binding proteins (Schumaker and Gizinski, 1995, 1996). Similarities in calcium influx and DHP binding (i.e., their inhibitor sensitivities and cation stimulation) between moss and animal cells suggest that calcium movement in these organisms shares a common molecular mechanism.

The Cell Wall, Plasma Membrane, and Cytoskeleton

The site of initial cell formation in caulonema cells is very predictable, but little is known about how the site is determined. Initial cell formation in caulonema filaments appears to be a consequence of divisions of the caulonema tip cell, and it has been proposed that the initial cell site is selected in a filament cell during the tip cell division that produces that filament cell (Schmiedel and Schnepf, 1979b; Doonan et al., 1986).

The cellular changes that take place during initial cell formation have been very well described using light and transmission electron microscopy (Schmiedel and Schnepf, 1979a; Conrad et al., 1986; McCauley and Hepler, 1990, 1992). During initial cell formation, there is a change in the morphological symmetry and polarity of the fine structure at the presumptive initial cell site, and selective growth at this site takes place via stratification of organelles. Schmiedel and Schnepf (1979a) showed that the apex of the outgrowth in *Funaria* contains Golgi bodies, Golgi vesicles, and subsurface endoplasmic

reticulum (ER) cisternae. They also showed that the outgrowth is filled with cytoplasm but contains only a few vacuoles and chloroplasts, which are positioned in the cell cortex (immediately adjacent to the plasma membrane). Conrad et al. (1986) verified the localization of Golgi bodies and associated vesicles in the initial cell outgrowth to directly beneath the plasma membrane and also showed that organelles were stratified in several subtending layers. Mitochondria were found to be directly adjacent to the vesicular layer, and the ER was organized beneath the mitochondrial zone and directly above the large central vacuole in the filament cell. From morphometric analyses of cells in various stages of initial cell formation, they showed that the relative volumes of Golgi bodies and vesicles adjacent to the initial cell apex increased during initial cell development coincident with a decrease in these organelles toward the base of the outgrowth. Mitochondria and chloroplasts followed the same pattern, although their highest relative volumes initially were farther from the initial cell apex and outside the initial site, respectively.

McCauley and Hepler (1990, 1992) monitored the pattern of ER distribution during initial cell formation in freeze-substituted cells or in cells treated with the lipophilic, fluorescent dye 3,3′-dihexyloxacarbocyanine iodide and visualized with confocal laser scanning microscopy. They showed that the cortical ER increases in quantity and reorganizes ("tightens") during initial cell formation and that these changes coincide with the apical swelling that constitutes the first visible sign of initial cell formation. Subsequently, the reorganized ER network extends into the outgrowth of the developing caulonema initial cell.

The organization of the initial cell during its formation is similar to the distinct zonation seen in the actively growing terminal regions of numerous tip-growing cells, including pollen tubes, root hairs, and fungal hyphae (Schnepf, 1986; Harold, 1990). However, unlike tip-growing cells in which there is perpetual stratification of organelles into distinct zones, the moss initial cell loses this organization after the division that separates it from the filament. In this case, mitochondria, chloroplasts, and small vacuoles become evenly distributed throughout the initial cell cytoplasm, whereas Golgi bodies and the ER occupy a cortical position (Conrad et al., 1986).

The role of the nucleus in establishing polarity during initial cell formation is unclear. The nucleus migrates to the outgrowth at the initial cell site concomitant with an increase in the number of microtubules between the nucleus and the base of the outgrowth. This increase in microtubules has been postulated to provide the physical basis for the directed movement of the nucleus to the initial cell site (Conrad et al., 1986; Doonan et al., 1986). Although the migration of the nucleus to a new division site is an important feature of cell patterning that influences the symmetry of the division products in a number of organisms, additional cellular components appear to be required during initial cell formation in moss. The initial cell site remains the same in cells that have been treated with the antimicrotubule drug cremart and then allowed to recover, suggesting either that an additional cellular component remains to connect the nucleus to the outgrowth or that microtubules are capable of repolymerizing in a biased direction from a particular site after removal of the drug.

Although it is possible that the peripheral cytoplasm and the nucleus determine the polar longitudinal axis, the question remains as to whether nuclear migration is a consequence of polarity establishment or if the nucleus determines the position of the new polar axis influenced by the longitudinal polarity already in place (Schmiedel and Schnepf, 1979b). The nuclear envelope may serve as a microtubule organizing center, and microtubules may connect the nucleus and plasma membrane to control polar distribution of functions in the plasma membrane. However, this may not be the major role of the nucleus during initial cell formation. If the initial cell site is determined at the time of caulonema tip cell mitosis, then the polar axis would have been established long before the nucleus migrates to the initial cell site (Doonan et al., 1986). In addition, the outgrowth can be seen before it is apparent that the nucleus has begun to migrate to the initial cell site.

It is evident that changes take place in cells of the caulonema filament before the first manifestations of initial cell outgrowth are visible. For example, Quader and Schnepf (1989) showed that a ringlike configuration of actin filaments localizes to the plasma membrane at the presumptive initial cell site in the first subapical cell. This polarized distribution of actin takes place long before initial cell development is visible. Organization of actin may be one of the early events of initial cell formation, and actin filaments may participate in the local differentiation of the plasma membrane at that site or in the vectorial transport of exocytotic vesicles to that site.

FUTURE PROSPECTS FOR THE ANALYSIS OF POLARITY IN MOSS

Caulonema Cell Differentiation

Although it is clear that auxin is essential for conversion of the filament tip from chloronema to caulonema, many questions remain about auxin's role in this process. For example, where is endogenous auxin made, and where does the perception that leads to caulonema cell differentiation take place? Is auxin made and perceived in the tip cell, or is it made in a subapical cell and perceived by the tip cell? The addition of auxin results in increased tip cell elongation, a more pronounced stratification of organelles, a change in the orientation of the filament cross walls, and a change in chloroplast size and shape. Why is continued exposure to auxin required to maintain this differentiated state? Is auxin causing the changes directly, or is it stimulating production of molecules that lead to the changes? Answers to these

questions are required before we can begin to develop a model for auxin action in caulonema differentiation.

Experiments monitoring auxin movement in moss should provide insight into the sites at which the endogenous auxin is produced and perceived. Auxin has been shown to move in a polar manner, from the tip of a caulonema filament to its base (Rose et al., 1983). Auxin accumulation is pH dependent and saturable, and auxin efflux is inhibited by a polar auxin transport inhibitor. Rose and Bopp (1983) have suggested that auxin transport in moss does not follow a symplastic route because polar transport of auxin persists in rhizoids when the cytoplasm of a cell in a filament between an auxin donor and receiver block is destroyed. It should be possible to determine whether exogenous auxin is perceived intracellularly or at the plasma membrane. This could be done, for example, by comparing early events in tip cell conversion in cells that have been treated with impermeant auxin with events in cells microinjected with auxin. Ultimately, identification of the sites of endogenous auxin synthesis and perception during normal differentiation will require the use of methods that allow quantitative measurement of auxin in situ.

Similar questions remain for the role of cAMP in caulonema cell differentiation. Sharma and Johri (1982b) provided evidence that ^{3}H-labeled cAMP is taken up and metabolized in chloronema cells of *Funaria*. However, it is unclear where along a chloronema filament cAMP is made and perceived during normal differentiation or how it stimulates chloronema and inhibits caulonema formation.

Caulonema Initial Cell Formation

From the cellular and biochemical studies outlined above and from parallel studies of polarity establishment in other organisms, we can begin to develop a model for the establishment of polarity during initial cell formation. During normal development, a change in the auxin and cAMP levels leads to sequential changes in the chloronema tip cell and ultimately results in the formation of a caulonema tip cell.

An initial cell site on a subapical cell is chosen possibly as early as during the formation of that subapical cell. In this caulonema cell, the expression of polarity may begin at the cell cortex via the assembly of axis markers at the selected initial cell site (Sanders and Field, 1995). The cell wall, plasma membrane, and cytoskeleton may organize the cortex and allow localization of specific proteins to the initial cell site. These proteins could direct the polarized reorganization of the cytoskeleton toward this site, change ionic gradients, alter the structure of the cell wall, increase turgor, and direct the transport of secretory vesicles containing new cell wall components and plasma membrane to the presumptive initial cell site. Microtubules at the leading edge of the nucleus, influenced by changes in cellular ion concentrations, may establish the proper nuclear orientation for the migration and cell division that give rise to the fully formed

initial cell. At each stage in this model, many important questions remain to be addressed. We identify some of these questions below and suggest approaches that may be used to answer them.

Are interactions between the cell wall, plasma membrane, and cytoskeleton important in organizing the cortex during initial cell formation? The cell wall is important in growth and differentiation and in the establishment of polarity in many organisms (Lord and Sanders, 1992; Henry et al., 1996). Recent evidence suggests that the cell wall influences cellular function in plants through interacting proteins that physically link the cell wall to the plasma membrane and cellular cortex (Kropf et al., 1988; Pont-Lezica et al., 1993; Henry et al., 1996; see Kropf, 1997, in this issue). It should be possible to determine if these interactions, termed adhesions, play a role as anchoring sites that provide a framework onto which other parts of the growth machinery may be assembled during initial cell formation. A combined biochemical and cell biological approach will allow visualization of adhesions in moss cells, determination of their role as part of the polarizing mechanism, and identification of molecules that form these adhesions. By taking advantage of the developmental progression that exists along a caulonema filament, these studies should also provide information about the spatial and temporal organization of adhesions during initial cell formation.

Are proteins involved in selecting and organizing the initial cell site? The processes taking place during initial cell formation in moss appear to be similar to those involved in bud site selection, polarity establishment, and bud growth in the budding yeast *Saccharomyces cerevisiae*. In both organisms, an asymmetric division follows localized directed growth, a polar axis is established well before the bud or initial cell outgrowth is apparent, and actin microfilaments are oriented toward the site of cell surface growth. Many genes and proteins involved in bud site selection and bud formation have been identified in *S. cerevisiae* (Adams and Pringle, 1984; Chant and Herskowitz, 1991). It should be possible to determine if homologs of these proteins exist in moss by using methods that rely on conserved sequences determined from analyses of genes in yeast or that enable the isolation of moss transcripts that functionally complement yeast polarity defects. Subsequent in vivo assays that alter the levels and distribution of any such molecules at the initial cell site should provide important insights into their role and regulation in the establishment of polarity during initial cell formation.

What is the nature of the information that is conveyed by changes in intracellular calcium concentration, and how are calcium levels regulated during initial cell formation? Numerous studies have shown that altered calcium levels affect the formation of the initial cell in moss (Saunders and Hepler, 1982, 1983; Conrad and Hepler, 1988). By using fluorescent calcium indicators, measurements of intracellular calcium in moss cells at specific times during development will provide insights into potential calcium-regulated processes. Cellular

calcium measurements used in conjunction with cell and molecular biological approaches will make it possible to determine the following: (1) if calcium is important in the regulation of proteins required for initial cell site assembly (e.g., by targeting proteins to or retaining proteins at the cell surface; Drubin, 1991; Nelson, 1992; Ohya and Botstein, 1994); and (2) if calcium is involved in structural changes at the initial cell site (perhaps by regulating the rate of wall synthesis and degree of wall extensibility at the growing initial cell site, serving as part of the polarizing mechanism required for selective targeting of vesicles to the apex, or by regulating localized exocytosis; Harold and Harold, 1986; Levina et al., 1995; see Cosgrove, 1997, in this issue). By monitoring intracellular calcium changes under conditions in which calcium channel activity can be regulated, it should be possible to determine if and when calcium channels are involved in moss development.

It is clear that development in moss provides the opportunity to study morphogenesis and differentiation in vivo in an intact, relatively simple organism. The large cell size and simple morphology allow specific developmental processes to be examined using approaches such as microinjection of single cells and whole-mount transcript and protein localization. By using liquid cultures of *Funaria* that are enriched for specific stages of development (Johri, 1974), it is possible to manipulate in vivo the developmental events of interest. Because cells of a single filament represent a developmental progression and because one can predict when and where polarity will be established, it will be possible to investigate the spatial and temporal distribution and regulation of molecules involved in this developmental process. Several laboratories, including ours, have successfully transformed moss cells (Schaefer et al., 1990; Kammerer and Cove, 1996; Zeidler et al., 1996); this technique should provide an additional approach with which to study gene function during moss development.

By using the approaches briefly outlined here and taking advantage of the cellular and biochemical information describing events taking place during initial cell formation, it should be possible to understand the molecular components that assemble a specific site into a polar structure. Because the basic mechanisms of polarity establishment appear to be common to a number of organisms, results from studies of moss development should provide important general information about mechanisms involved in establishing developmental polarity in eukaryotes.

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