

# ASYMMETRIC CELL DIVISION IN PLANTS

*Ben Scheres<sup>1</sup> and Philip N. Benfey<sup>2</sup>*

<sup>1</sup>Department of Molecular Cell Biology, Utrecht University, 3584 CH Utrecht, The Netherlands; and <sup>2</sup>Department of Biology, New York University, New York, NY 10003; e-mail: b.scheres@bio.uu.nl

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## ABSTRACT

Asymmetric cell divisions generate cells with different fates. In plants, where cells do not move relative to another cell, the specification and orientation of these divisions is an important mechanism to generate the overall cellular pattern during development. This review summarizes our knowledge of selected cases of asymmetric cell division in plants, in the context of recent insights into mechanisms underlying this process in bacteria, algae, yeast, and animals.

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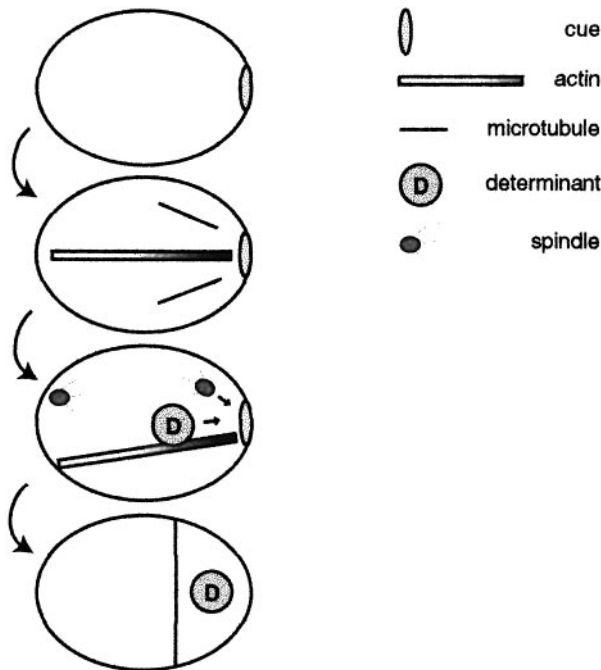
## INTRODUCTION

During the life cycle of plants and animals, a single cell produces a multicellular organism with many different specialized cell types. To generate the multitude of different cell types requires cell divisions in which daughter cells have different fates. These are called asymmetric cell divisions, whether or not asymmetry is morphologically evident at the time of division (48). Asymmetric cell divisions are traditionally divided into two flavors. The difference in daughter cells may be due to unequal partitioning of factors in the mother cell such that all or most are inherited by only one daughter. Alternatively, the division may result in daughter cells that have equal developmental potential at first but become different subsequently through interactions with each other or with different neighboring cells. In this case, extrinsic cues determine cell fate. The first can be considered a monarchy in which fate is passed down based on lineage, the second a modern democracy in which fate is determined by interest groups. Note that both mechanisms can involve external spatial information. In the intrinsic case, spatial information may direct the orientation of an asymmetric cell division prior to its occurrence, whereas in the extrinsic case this information acts to determine fate after the cell division. As highlighted in this review, actual development often involves a combination of these two strategies.

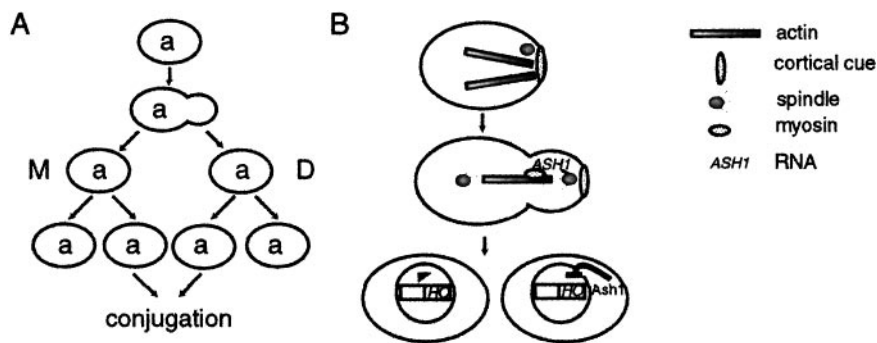
In plants, the cell wall prevents extensive rearrangement of cells, and common sense predicts that asymmetric division must be important to generate the abundance of diversified cells in the plant body. However, approximately correct tissue patterns can emerge from plants mutated in their ability to divide in the appropriate plane (102, 103). Moreover, the flexible fates of many plant cells (77a, 107) indicate that the determination of cell fates is not restricted to narrow time windows, as is often the case in animal development. Are these differences reflecting new mechanisms, or do they arise from subtle changes in the same underlying basic mechanisms? This review probes this question, making use of the wealth of information that has emerged recently on asymmetric cell division in organisms as diverse as bacteria, yeast, flies, and nematodes. We briefly highlight these findings and subsequently turn to selected cases in plants (for other recent reviews see 36, 51b, 84).

## YEAST AND DROSOPHILA: INTRINSIC DETERMINANTS

Two criteria should be met by an intrinsic factor that mediates asymmetric cell division: (a) The factor is preferentially distributed into one daughter cell during the cell division; (b) its presence or absence determines cell fate. The second criterion predicts that loss-of-function mutants in the factor-encoding genes lead to two cells of one type, and ectopic expression of the factor in both cells leads to two cells of the other type. In both yeast and *Drosophila*, gene products that meet these criteria have been recently identified. The common elements found in all cases are: (a) localized cues initiate polarization of the cytoskeleton; (b) factors are partitioned to one end of the cell; and (c) the spindle is oriented so that when the cell divides the factors end up predominantly in only one of the daughters (Figure 1). In each case we first discuss the intrinsic



*Figure 1* A schematic representation of an asymmetric cell division that involves partitioning of intrinsic determinants. An asymmetrically localized cue provides initial polarity; the cytoskeleton is oriented with respect to this polarity, leading to the preferential partitioning of cell fate determinants to one daughter cell, and concomitant orientation of the mitotic spindle.



**Figure 2** Asymmetric division in budding yeast results in cells with different mating-type switching abilities. **A.** Division pattern displaying the switches in mating types  $\alpha$  and  $\alpha$ . **B.** Concomitant distribution of *ASH1* RNA and spindle orientation.

factors or determinants, then associated proteins involved in their asymmetric distribution, and finally initial cues and their relation to spindle orientation.

### *Yeast: Ash1p as an Intrinsic Determinant*

In the budding yeast *Saccharomyces cerevisiae*, cells of two mating types exist. Upon spore germination, cell division is asymmetric: It gives rise to a “mother” cell capable of mating-type switching during the next cell cycle, and a “daughter” cell—which originates as a growing bud on the mother cell—that is not able to switch (Figure 2A). In this way, yeast spores generate cells of two mating types that can subsequently conjugate to produce a diploid cell.

This asymmetric cell division appears to be intrinsically determined (97). Mating-type switching is initiated by a site-specific endonuclease encoded by the *HO* gene. This gene is transcribed in mother cells only in a narrow time window at the G1 to S phase transition (72\*, 98). The difference in cell fates, with one daughter able to switch and the other not, is therefore determined by differential regulation of *HO* gene transcription. Recently, the *ASH1* gene was identified in two different genetic screens as a negative regulator of *HO* transcription (8, 93). The Ash1 protein, which is related to GATA transcriptional regulators, is partitioned into daughter cells. Its presence down-regulates *HO* expression and thereby determines fate, and loss-of-function and gain-of-function experiments demonstrate that this factor meets the criteria for being a cell-intrinsic determinant of cell fate.

The partitioning of Ash1 requires localization of *ASH1* mRNA, which is performed by an actin-based mechanism involving a myosin motor (62, 51). Although the precise cue responsible for Ash1 localization is unknown, it has been shown that *ASH1* RNA becomes anchored to a region of the cell cortex

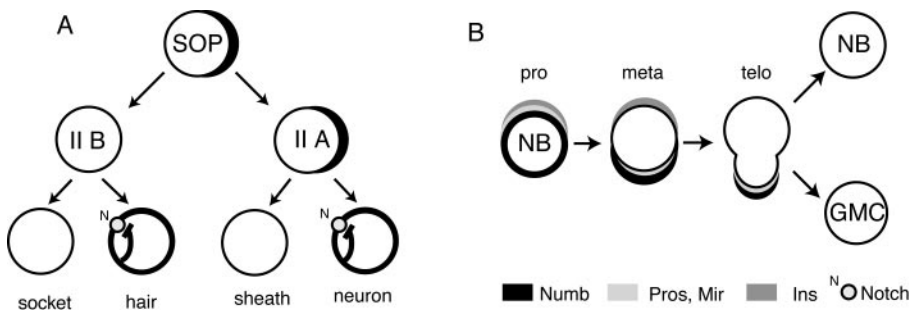
that will end up in the daughter cell (62). This may be a direct consequence of the polarization of the actin cytoskeleton in response to small GTPases that are asymmetrically activated in the cell cortex. The Bud3 protein, which marks a ring around the site of the previous cell division, initiates the activation of these GTPases (15). Bud3-dependent processes also orient the mitotic spindle by positioning one spindle pole body. Use of the same Bud3-dependent cue for localization of Ash1 and orientation of the spindle would ensure that Ash1 becomes partitioned into only the daughter cell (reviewed in 14, 86a).

In summary, in yeast a single (nonswitching) cell gives rise to two different cell types (switching and nonswitching) by actin-dependent preferential distribution of the Ash1 mRNA/protein, which acts as a cell fate determinant by repressing transcription of the *HO* gene. Initial polarity cues ultimately mediate partitioning of Ash1p and coordinate the orientation of the mitotic spindle to ensure proper segregation of fates (Figure 2B).

### *Drosophila: Numb and Prospero as Intrinsic Determinants*

In the fruit fly, precursor cells for both the central (CNS) and the peripheral nervous system (PNS) undergo asymmetric cell divisions. Two genes, *numb* and *prospero*, encode intrinsic determinants essential for the acquisition of cell fate.

During the development of the peripheral nervous system, sensory organ precursor (SOP) cells divide asymmetrically to produce a neuron/sheath precursor and an outer support cell precursor (Figure 3A). The membrane-associated Numb protein was shown to be both partitioned into one daughter cell and responsible for the eventual differences in cell fate (82, 105).



**Figure 3** Asymmetric cell divisions during *Drosophila* neurogenesis. A. External sensory organ formation. Distribution of Numb is shown (thick outline). SOP: sensory organ precursor cell. B. Neuroblast (NB) division to generate ganglion mother cells (GMC). Distribution of Notch, Prospero, Miranda, and Inscuteable are shown at three stages of mitosis.

While Numb itself is an intrinsic determinant, the mechanism by which it determines cell fate is through an interaction with neighboring cells. The fate of the SOP daughters requires signaling mediated by the transmembrane receptor Notch: Without Notch activity, only neurons arise (44a). Notch activity is inhibited in the daughter cell that inherits the Numb protein, so Numb biases cell fate decisions by interfering with Notch signaling (33, 42). This, then, is an example of an intrinsic determinant that acts by antagonizing a signal received from neighboring cells.

Recently, it has been shown that the orientation of Numb partitioning as well as the orientation of the SOP cell divisions along the fly's antero-posterior axis depends on signaling from the Frizzled transmembrane receptor, which binds ligands of the Wnt family. This indicates that extracellular signals provide the polarizing cue for both Numb localization and antero-posterior spindle orientation (37).

The second intrinsic determinant in neural precursor cells, the homeodomain protein Prospero, is expressed in the precursors of the *Drosophila* central nervous system. Neuroblasts divide asymmetrically to produce a ganglion mother cell and another neuroblast (Figure 3B). During neuroblast division, Prospero protein is retained in the cytoplasm and partitioned to the ganglion mother cell only, whereupon it is translocated to the nucleus (46). Prospero most likely controls ganglion mother cell fate by transcriptional regulation (25, 106).

What is the mechanism for partitioning Prospero and Numb to the appropriate daughter cell? During asymmetric divisions in CNS and PNS, which occur in apical-basal orientation, Numb and Prospero colocalize and form a crescent at the basal cell cortex, suggesting a common mechanism for their partitioning (53a) (Figure 2B). The actin cytoskeleton is essential for localization of Numb and Prospero (12, 54) whereas disruption of microtubules affects the placement of the crescent with respect to the division plane (53a, 55). Therefore, actin is involved in determining partitioning itself, whereas the microtubular network serves an as yet undefined role in aligning this segregation mechanism with the orientation of the spindle. Tethering of the nuclear protein Prospero to the membrane is mediated by its association with an asymmetrically distributed adapter protein, Miranda, which also interacts with Numb (90, 50, 89). Localization of Numb, Prospero, and Miranda in the basal crescent requires the *inscuteable* gene, encoding a protein with a putative SH3 binding domain, frequently found in proteins that interact with the cytoskeleton (55). Surprisingly, Inscuteable protein is localized apically, at the opposite side of the future Numb/Prospero/Miranda crescent (Figure 3B), and its localization is actin dependent. Furthermore, Miranda can also bind Inscuteable through a domain that is required for its asymmetric localization. Inscuteable is also required

to orient the mitotic spindle, suggesting that it coordinates asymmetric protein localization with spindle orientation (Figure 3B). Thus, Inscuteable localizes cell fate determinants in the apical-basal plane through its capacity to bind Miranda, and it orients the plane of cell division by anchoring centrosomes via an unknown mechanism.

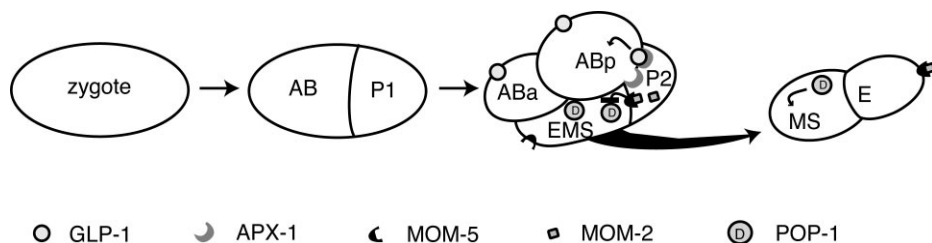
In summary, in fly neuroblasts two intrinsic determinants, Numb and Prospero, are partitioned basally through an actin-dependent mechanism that involves several other proteins that are themselves partitioned. The cue responsible for the initial polarization of this machinery is still unknown.

## *CAENORHABDITIS ELEGANS*: EXTERNAL SIGNALS REGULATE ASYMMETRIC CELL DIVISION

The key criteria for the involvement of external cell fate determination in an asymmetric division process are (a) inductive signals from neighboring cells determine cell fate and (b) both cells resulting from the division are equally responsive to these signals. An important prediction from the first criterion is that alterations in the cellular environment after the cell under investigation has divided can change cell fate. Manipulation of the cellular environment is feasible in the soil nematode *C. elegans*, and it has been demonstrated that inductive interactions can determine the fate of particular cells in the context of a nearly invariant cell division pattern. It will become evident that inductive processes can regulate asymmetric divisions in different ways. In the first case that we discuss, the division is initially symmetric and cellular interactions then bias one of the daughters to take a fate different from its sibling. The second case is highly analogous to the *Drosophila* neuroblast divisions, in that an external signal regulates the partitioning of intrinsic factors and spindle orientation.

### *GLP-1 Signaling Determines Daughter Cell Fate After Division*

The first division of the zygotic embryo produces two cells AB and P<sub>1</sub> (Figure 4). This asymmetric division partitions cell-intrinsic factors that are not discussed here, but it is interesting to note that the gene products required for the asymmetry again are localized in the cell cortex (reviewed in 10, 43, 86). The AB cell then divides to give two cells ABa and ABp with different fates. Only the ABp cell directly contacts P<sub>2</sub>, one of the daughters of P<sub>1</sub>. If ABa and ABp are switched such that ABa now contacts P<sub>2</sub>, a normal worm will result (79). Laser ablation of P<sub>2</sub> results in both AB daughter cells having the ABa fate (11). Furthermore, if ABp is not allowed to contact P<sub>2</sub> it will produce tissues normally produced by its sister, and if ABa is placed in contact with P<sub>2</sub> it will produce tissues normally made by ABp (68, 64). The conclusion from these



**Figure 4** Asymmetric cell divisions of blastomeres in early *C. elegans* embryos. Receptors and ligands of two extrinsic signaling pathways are shown, which are involved in mediating cell fate decisions. From left to right: zygote, two-cell embryo, four-cell embryo, and the two EMS daughters at the eight-cell stage.

experiments is that ABa and ABp initially have equivalent potential to adopt either fate and that interaction with P<sub>2</sub> is the determining factor in the fate adopted.

Nuclear factors that determine the difference between the ABa and ABp fates are not known, but some of the molecules involved in the interaction that establishes the difference between these cells have been identified. Mutations in the *glp-1* gene result in ABp following an ABa fate (4, 78). *glp-1* is a homolog of the *Drosophila Notch* gene and encodes a receptor that accumulates on the membrane of AB descendants but not P<sub>1</sub> descendants. The ligand for GLP-1 appears to be APX-1, which is a homolog of the Notch ligand, Delta (68). APX-1 is membrane-tethered to the P<sub>2</sub> cell and is localized to the junction between ABp and P<sub>2</sub> (69). The localization of these two interacting proteins, which is in turn determined by previous asymmetric divisions as well as membrane polarization, therefore determines which cell will take on the ABp fate.

### *MOM-2 Signaling Directs Asymmetric Divisions*

Inductive interactions can also occur prior to cell division. EMS, the sister cell to P<sub>2</sub>, normally divides to give an MS cell whose descendants will form muscle and pharyngeal tissue, and an E cell whose descendants generate intestine (Figure 4). If EMS is removed from the embryo and allowed to divide in culture, both daughters have the MS fate (38). It has been demonstrated that P<sub>2</sub> is the source of the information necessary for the E fate, and that its position relative to EMS determines which of the daughters will follow that fate (reviewed in 44). In this case, P<sub>2</sub> is responsible for setting up an asymmetry within the EMS cell.

One intrinsic factor that is required to establish the E fate, in conjunction with others, is the product of the *POP-1* gene, a nuclear protein with an HMG domain (59). Interestingly, POP-1 is required for the fate of the anterior cell in many cell divisions in the antero-posterior orientation, and it presumably interacts with different partners in each of these cases.



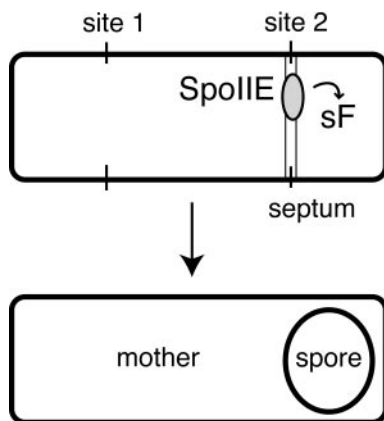
Analogous to their involvement in asymmetric cell division of the SOP precursor cell in the fly, components of the Wnt signal transduction pathway have been shown to be involved in the determination of E fate. *mom-2* encodes a protein with homology to Wnt, while *mom-5* encodes its probable receptor, a member of the Frizzled gene family (83, 100). *mom-1* has homology to a *Drosophila* protein involved in the processing and secretion of Wnt (83). It has been shown that the putative ligand *mom-2* and the processing factor *mom-1* act in P<sub>2</sub>, and it is assumed that the putative receptor *mom-5* acts in EMS (100). Downstream of the receptor/ligand interaction are the  $\beta$ -catenin homologue, *wrm-1*, and an APC (human adenomatous polyposis) related gene, *apr-1*, both implicated in Wnt signal transduction. It is hypothesized that the *wrm-1* and *apr-1* products form a complex with POP-1 that results in the latter's inactivation (44). One model is that the Wnt signal acts directly on the cytoskeleton to polarize the localization of POP-1 in the EMS mother cell, which results in the MS daughter having a higher concentration of POP-1 in its nucleus (44, 99). Interestingly, *mom-1,2,3,5* all affect both POP-1 localization and spindle orientation of the EMS cell division, whereas *wrm-1* only affects POP-1 localization (83, 100). Thus, the Wnt signal transduction pathway branches to coordinate spindle orientation and segregation of cell fate determinants.

In summary, the Notch signaling pathway can determine cell fate after cell division in *C. elegans*, and the Wnt signaling pathway can orient asymmetric cell divisions. Both pathways are also associated with asymmetric cell divisions in flies. Note the different utilization of the Notch pathway: In worms the position of a cell in relation to neighboring cells determines whether it receives a signal, whereas in flies an intrinsic determinant (Numb) is partitioned that inhibits Notch signaling.

## **BACILLUS SUBTILIS: A BACTERIAL SOLUTION TO ASYMMETRIC CELL DIVISION**

During vegetative growth, the bacterium *B. subtilis* divides symmetrically to form two equal-sized daughters with identical fates. Under conditions of nutritional stress, division in *B. subtilis* becomes asymmetric. Instead of a septum forming in the middle of the cell, a choice is made between one of two sites at either end of the cell (Figure 5). Division results in a larger mother cell and a smaller forespore that is eventually engulfed by the mother cell.

The specialized sigma factors act as determinants for mother cell and forespore fate. The association of a sigma factor with RNA polymerase enables the regulation of transcription of cell-specific genes, among which are those that lead to new sigma factors (87, 110).



*Figure 5* Asymmetric cell division in *B. subtilis*. Membrane-bound SpoIIE differentially activates the release of a sigma factor that mediates forespore-specific gene expression. Site 1, 2, the locations of two potential division sites, of which one is chosen.

One of the first steps in asymmetric cell division is the localization of a membrane-bound phosphatase, SpoIIE, to one of two potential division sites (Figure 5) (110). The choice of division site may be influenced by the previous site of vegetative division, or a case of “spontaneous symmetry breaking” (28, 47; reviewed in 110). In addition to marking the site of septum formation, SpoIIE initiates cell-specific gene expression after cell division. Its phosphatase activity dephosphorylates SpoIIAA, which then binds a kinase, SpoIIAB. This prevents the binding of SpoIIAB to sigma-F, releasing the specialized sigma factor subunit of RNA polymerase to activate gene expression in the forespore (1, 27, 31).

The pivotal question to understand asymmetry of the *B. subtilis* division is by what cue SpoIIE is differentially activated in the pre-spore cell. Preferential gene activation in one compartment is dependent upon accurate targeting of the SpoIIE protein to the septum membrane, which suggests that there are recognizable membrane domains in the bacterium (87). It is unknown whether SpoIIE is asymmetrically distributed in the septum membrane with its C-terminal phosphatase moiety facing the forespore compartment, or whether it is perhaps symmetrically distributed and its concentration (per cytoplasmic volume) is higher in the forespore due its smaller size (110). The latter possibility bears some relevance to asymmetric cell division in a distant plant relative, the multicellular alga *Volvox carteri*. Here, cell size appears to be the critical factor to distinguish between reproductive and somatic cell fate (53).

## LESSONS: A COMMON THEME AND VARIATION

What are we able to discern from this overview of asymmetric divisions? One obvious common theme can be extracted from the eukaryotic examples of intrinsic fate determination: Localized cues initiate the partitioning of intrinsic cell fate-determining factors and coordinate these with spindle orientation. It seems relevant that these cues depend on or organize the actin cytoskeleton in all cases discussed. Given the involvement of actin and its associated motor proteins in active directional transport, their role in asymmetric cell division can be easily pictured. However, the role of actin in the various examples of asymmetric cell division appears to vary, as microfilaments are sometimes polarized (as in yeast) and sometimes not. This may reflect an underlying diversity of mechanisms.

Important variations on the simple subdivision of intrinsic versus extrinsic asymmetric divisions have emerged. Intrinsic mechanisms for asymmetric cell division sometimes adhere closely to the simple definition, but in other instances represent a mixed case. Yeast budding and the role of Prospero in CNS division represent the simple case: A transcription factor is localized to one part of the cell prior to division; the factor then activates genes in the daughter cell to which it is partitioned, determining the fate of that cell. However, the activity of the Numb protein, itself partitioned by an intrinsic mechanism, depends on cell-cell signaling. Furthermore, before intrinsic factors establish differences prior to cell division, surrounding cells often play a role in directing the orientation of the asymmetry, and we have seen examples in *Drosophila* as well as in *C. elegans*, where cell-cell communication organizes partitioning of an intrinsic factor in the mother cell. It is noteworthy that these cell signaling events are mediated by receptors like Frizzled and Notch, whose use is not restricted to orientation of asymmetric cell division because they are involved in a large variety of signaling events.

The molecular identity of localized cues involved in intrinsic asymmetric cell divisions appears to be diverse. It remains to be seen whether components of the yeast machinery for budding site selection, the nematode PAR proteins, or *Drosophila* cues have plant counterparts. It is even conceivable that the asymmetric activation mechanism of fate-determining factors in *Bacillus* turns out to be more relevant to plants, given the resemblance of their division process.

## ASYMMETRIC CELL DIVISIONS IN PLANTS

Despite variations in cell division sequences, all plants generate a spectrum of different, regularly spaced cell types. Any cell division that generates daughters

with different fates is asymmetric by definition. Cell movement is limited in plants, and thus control of the cell division plane has traditionally been considered important for the formation of regular patterns. Oriented cell divisions certainly have a role in generating ordered files of cells. Is control of the orientation of cell division also related to mechanisms of cell fate determination? Below, we first compare and contrast mechanisms for orienting cell divisions in plants and animals. We then survey examples of asymmetric cell divisions in plants to probe whether they are regulated by intrinsic or extrinsic fate determining processes.

### *Orientation of Cell Division in Plants:*

#### *Role of the Cytoskeleton*

In animals, the location of the centrosomes—microtubule organizing centers (MTOCs) for the nucleation of the spindle microtubules (80a)—determine the direction of chromosome segregation and the orientation of cell division. Spindle alignment is an important aspect of asymmetric cell division in yeast and animals. It is thought to be regulated by capture of the microtubules emanating from one centrosome, or its yeast analog, by regions of the cell cortex (14, 112). In nematodes, stable actin patches, possibly remnants of the previous cell division site, are present in specific regions of the cortex, and the Bud3p protein in yeast similarly marks the previous division site.

Plants contain no centrosomes. Instead, antibody staining suggests that the plant nuclear surface has MTOC properties (17, 30, 108). Thus, a centrosome-based mechanism to orient cell division cannot operate in plants. Nevertheless, several lines of evidence point to a role of the microtubular network in plant cell division. Microtubules of nuclear origin can connect to, and may even contribute to, the formation of the preprophase band (PPB), a dense cortical array of microtubules that transiently marks the site of division in plant cells (41, 61a, 77).

The finding that the PPB predicts the orientation of cell division has been followed up by several studies on its formation, in an attempt to uncover mechanisms that operate in cell division site selection. A less condensed cortical microtubule network is present in the interphase cell well before PPB formation (23). These cortical microtubules are already oriented in the same plane as the future PPB, indicating that cytoskeletal polarity coinciding with the cell division plane is present before the division site is selected. Apart from the possible involvement of the nucleus in the deposition of the cortical microtubular network and the PPB, the principal spatial cues for division site selection remain unknown. Centrifugation experiments have shown that altering the location of the nucleus can direct the formation of a second PPB (69a, 71), which suggests that the mother cell nucleus may direct the position of PPB formation.

It is unclear whether this involves the nuclear MTOC activity or other polarized domains on the nuclear surface.

There is evidence that the PPB, in turn, may be important in regulating spindle formation (69a, 71). However, *Arabidopsis* mutants in preprophase band formation demonstrate that the PPB is not required for spindle formation per se. In *tonneau/fass* mutants there is a total lack of the preprophase band (103), but spindles are formed nevertheless. Division planes in the mutants appear random from the earliest stages of embryogenesis onward, and hence the orientation of cell division is severely affected (102, 103). One might expect that cell fate would be severely affected in these mutants. However, all tissues are present in the right places and cellular differentiation appears normal, although organ morphology is affected. The finding that random cell divisions do not fundamentally alter embryonic patterning can be explained in at least two different ways. First, asymmetric divisions in plants may be guided by extrinsic rather than intrinsic cues. Second, asymmetric divisions may not be dependent on PPB formation.

The PPB is not the single decisive cue for orientation of the spindle. In particular cells, the spindle axis may rotate during division due to space constraints. However, in general, the cell plate corrects for this so that a curved cell wall is again attached to the PPB-marked site (21, 75). In extreme cases, a new division site may be chosen, resulting in a change of the orientation of cell division (74a). The emerging, sketchy, picture of orientation of cell division in plants is that the nucleus may regulate cortical array and PPB formation. The PPB provides the cue for the direction of division, and geometric constraints, which may be dictated by the cell's autonomous elongation program but also by its neighbors, may in some cases influence the division plane at a later stage. The identification of the principal cues that guide the cortical microtubule array in plant cells is a major challenge for the future.

The PPB disappears prior to the onset of mitosis. Yet there is evidence for a "memory" function that may involve the phosphorylation of cortex proteins by a P34<sup>cdc2</sup> kinase that localizes to the PPB (21a, 69a), an "actin depleted zone" (5, 20, 60), and the formation of an actin network between nucleus and division site.

While most animal cells form a contractile ring that, as its name suggests, contracts to pinch off the two daughter cells, most higher plant cells form a cell plate in between daughter nuclei. The cell plate serves as the assembly site of a new cell wall (reviewed in 2, 32, 96). The first step in cell plate formation is the assembly of a phragmoplast, another plant-specific structure, which consists of endoplasmic reticulum, Golgi-derived vesicles, microtubules, and microfilaments. Maturation of the cell plate requires continuous delivery of material via vesicle transport. The plate grows from the center outward until

it reaches the sites previously marked by the PPB. The maturation process involves several stages: First there is a tubular network, then a fenestrated sheet, and eventually new cell walls are formed with plasma membrane on either side. A notable exception to this process is found in microspore mother cells that use a contractile ring for division (74b, 75a).

In the *Arabidopsis* mutants *knolle* (63) and *keule* (3) as well as in the pea mutant *cyd* (61), there appears to be a defect in completion of the cell plate resulting in cells with multiple nuclei. The *KNOLLE* gene encodes a member of the syntaxin family of integral membrane proteins involved in vesicle docking and fusion (63). Immunolocalization of *KNOLLE* protein and ultrastructural analysis indicates that it serves a cell plate-localized role in vesicle fusion (58). The availability of single components of the cell division machinery such as the *KNOLLE* protein will enhance our abilities to investigate molecular aspects of cytokinesis in plants.

### *A Survey of Asymmetric Cell Divisions During the Plant Life Cycle*

Starting from the plant zygote, a large number of asymmetric divisions occurs to generate the mature plant with female and male gametes. Some of these not only give rise to different progeny but they also generate cells of different sizes. Examples of such asymmetric cell divisions in *Arabidopsis* are: (a) the first division of the zygote (65); (b) the embryonic division that gives rise to the lens-shaped progenitor cell of the quiescent centre (25a); (c) the male microspore division; and (d) divisions during stomatal complex formation (57). The physical asymmetry of these cell divisions indicates that the differences in fate of the daughters are defined during the process of cell division, which makes these divisions candidates for being determined by intrinsic factors. In later paragraphs, we discuss the evidence for intrinsic mechanisms.

Other asymmetric cell divisions lead to different cell types, but the differences are not yet evident during cell division. Important divisions of this class are the oriented periclinal divisions in the early embryo that separate the progenitor cells for the three main tissues, epidermis, ground tissue, and vascular tissue (51c). The stem cell divisions that separate differentiation-competent daughter cells and new stem cells in the root meristem are another example in this category (25a, 107). For this class of asymmetric cell divisions, the importance of intrinsic versus extrinsic mechanisms cannot be assessed without further analysis, and we discuss relevant experiments later.

The separation of the main organs (cotyledons, shoot apical meristem, hypocotyl, root, and root apical meristem) during embryogenesis is not correlated with early cell divisions, even in species like *Arabidopsis* with regular cell lineages (85). Thus, organ boundaries are not caused by asymmetric cell

divisions but rather arise from the patterning of groups of cells, as in the well-investigated case of floral organ patterning. This does not preclude the involvement of asymmetric cell divisions in the generation of a coarse pre-pattern for embryonic organ specification. For example, the separation of the *Arabidopsis* embryo proper into apical, central, and basal regions may turn out to yield examples of asymmetric cell division as well (51a).

In summary, one class of asymmetric cell divisions in plants may be regulated by intrinsic mechanisms, and in that case orientation of cell division and partitioning of cell fate determinants should be coupled. Asymmetric divisions of a second class may be regulated entirely by external cues. Nevertheless, oriented cell division can still be an important parallel process, required for the establishment of the regular cell arrangements that accompany the asymmetric cell divisions.

## THE FIRST DIVISION OF THE PLANT EMBRYO

The first division of the plant zygote produces two daughter cells with different fates. This asymmetric division has been investigated in lower and in higher plants, which show similarities in their early embryo development despite the large evolutionary distances. Lower plants such as the brown alga *Fucus* have free-living zygotes, and the early steps of embryogenesis can be investigated by direct experimental manipulation. In higher plants, where the developing zygote is buried in maternal tissues, genetic manipulation is an important tool and we focus on genetic analyses in *Arabidopsis*.

### *Fucus: Vesicle Transport and the Cell Wall*

The *Fucus* zygote divides to give rise to two cells with different fates (Figure 6). The apical cell will form the stipe and fronds of the mature plant, whereas the basal cell will give rise to the holdfast, a support structure. Of major significance is the finding that apical and basal cell fates after the two-cell stage are determined by the cell wall, as shown by laser manipulations whereby the progeny of the apical and the basal cell after the first zygotic division were allowed to contact different cell walls (7). While this opens up the possibility that cell fate determinants can be deposited into the cell wall, nuclear factors that execute the differences in cell fate have to be identified. Studies on cell fate at later stages of *Fucus* development indicate that a role of the cell wall in cell fate may be restricted to the zygotic division (9). Hence wall components may only play a role in a small number of asymmetric cell divisions.

How is the orientation of this asymmetric cell division controlled? Prior to the first cell division, the orientation of the apical-basal axis is determined by extrinsic cues such as sperm entry, gravity, and light. This labile polarization is

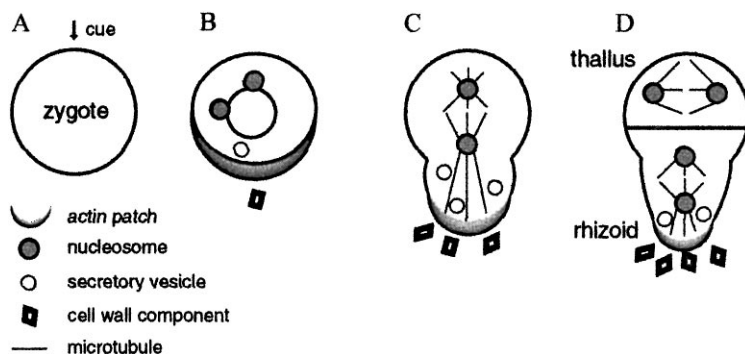


Figure 6 The asymmetric division of the *Fucus* zygote, and the associated acquisition of cellular polarity. The initial cue here is drawn as a light vector, but other cues are possible.

associated with changes in ion currents and deposition of various compounds, among which is filamentous actin, at the presumptive basal ("rhizoid") pole (56). It is not known which of these changes causally relate to the orientation of the apical-basal axis. The axis becomes fixed only just before germination, and this stage is marked by F-granules, Golgi-derived vesicles that accumulate at the basal cortex and are thought to deposit cell wall material (80). Inhibition of actin polymerization and Golgi transport block axis fixation, and also the deposition of basal-specific cell wall material. Thus, vesicle transport, most likely mediated by the actin cytoskeleton, serves to fix the main axis of polarity, possibly through one or several secreted cell wall components.

Two processes occur after axis fixation: Polar growth initiates from the marked pole, and the spindle is aligned with the apical-basal axis by the preferential stabilization of centrosome-derived microtubules at the rhizoid pole, such that the first division occurs perpendicular to the main axis. It is possible that the basally localized actin patch could play a role in this stabilization, but more research is needed to clarify this issue. If actin patches play a role, an interesting analogy emerges with the involvement of actin in spindle rotation in *C. elegans* blastomeres (112). Interestingly, in *Fucus*, randomly oriented cell divisions could occur when a Golgi transport blocker was transiently added, whereas the polarity cue remained capable of instructing polar growth (88). Thus, spindle orientation and cell division can be uncoupled from the initial cue that polarizes the cell. Hence, the Golgi transport process appears to serve two purposes: fixation of initial polar information, and alignment of the spindle. Important questions are which, if any, of the identified factors is required for the asymmetric cell division to occur, which cell wall component(s) specify the apical and basal cell types, and whether these are already laid down in the zygotic cell wall.



Overall, the first asymmetric cell division in *Fucus* appears to adhere to the general principle of intrinsically determined asymmetric division, in that a cortical target site initiates the segregation (or, in this case, deposition) of intrinsic factors as well as spindle rotation. The intriguing possibility that the intrinsic factors are not transcription factors, like yeast Ash1, nor membrane proteins like *Drosophila* Numb, but secreted cell wall molecules, may add another item to the list of cell fate segregating mechanisms.

### *Arabidopsis: Vesicle Transport Again?*

The division of the higher plant zygote produces the apical daughter cell, giving rise to the majority of the later embryo, and the basal daughter that forms the extraembryonic suspensor. Which factors determine cell fate of the two zygotic daughter cells in angiosperms? Genes whose mutant phenotype adheres to the criteria for intrinsic factors involved in cell division (i.e. two daughters of one type upon loss-of-function, and two daughters of opposite type upon gain-of-function) have not been identified so far. Moreover, the analysis of *twin* (*tnw*) mutants in *Arabidopsis* has demonstrated that possible fate differences established after the first zygotic division are reversible. In *tnw* mutants, a second embryo develops from the basal cell-derived suspensor region (109, 115). However, the *TWN* genes do not encode cell fate-determining factors. For example, *tnw2* mutants display altered expression of a valyl-tRNA-synthase, which arrests development of the apical cell progeny. One model states that the apical cell (progeny) normally suppresses the capacity of the basal cell (progeny) to form an embryo, and that the basal cell will only express this potential when this control is absent (Figure 7) (109). An important corollary of this model is that some *tnw*-like mutants could be in genes directly involved in this control, but these have not yet been reported.

Even though the determinants that differentiate apical and basal cell fates are still unknown, the histological asymmetry of the zygotic division allows one to ask which factors orient it. The apical-basal axis is aligned with the micropylar-chalazal axis of the female gametophyte (26), suggesting that positional cues from the haploid phase of the life cycle may guide zygotic asymmetry. Furthermore, the oocyte is frequently polarized prior to fertilization (74). Nevertheless, only sparse genetic evidence has surfaced in favor of maternal control of zygotic polarity (e.g. 81), but it should be noted that no systematic screen for maternal-effect mutations has been reported in plants to date. Tissue- and cell-culture studies reveal that plant embryos can develop in the absence of maternal tissue. These studies can be taken to suggest that maternal cues are also not required for the asymmetric division of the zygote, but it is difficult to assess their implications until more is known about the mechanism of in vitro embryogenesis and its relation to zygotic embryogenesis.

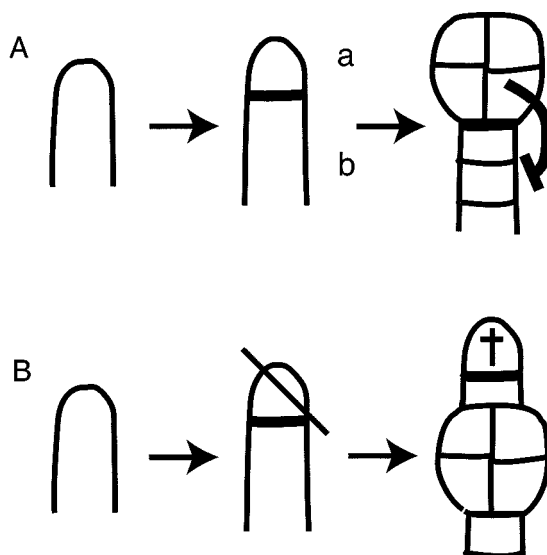


Figure 7 *Arabidopsis* embryogenesis. Zygote, one-cell, and octant stage are shown. A. Model for the maintenance/determination of suspensor identity by a negative signal from the embryo proper. B. In *twm* mutants, the signal is lacking, allowing the formation of secondary embryos.

Although the cues that orient the asymmetric cell division of the zygote are so far unknown, the *GNOM* gene (also known as *EMB30*) has emerged as a candidate for a role in axis fixation. In *gnom* mutants, the first division of the zygote is frequently skewed or symmetric, and early apical marker genes such as *LTP1* may be expressed with reversed polarity (i.e. in the basal region) later on (66, 109a). Later divisions in *gnom* mutants are also abnormal, indicating that the GNOM protein is a part of a general machinery for correct orientation of cell division. The *GNOM* gene encodes a protein homologous to yeast guanine nucleotide exchange factors involved in vesicle transport to the Golgi network (13, 70, 76, 91). It is tempting to contemplate similarities between a requirement for vesicle transport in axis fixation of higher plants, and the involvement of Golgi-derived vesicles in the same process in algae. More work is needed to assess whether both higher and lower plants do indeed utilize a similar mechanism to stabilize the axis for the asymmetric division of the zygote.

In *Arabidopsis*, the zygotic nucleus is positioned in the subapical region and the subsequent division produces a smaller apical and a larger basal cell. Is this difference in size instrumental in determining cell fate, as it appears to be in the green alga *Volvox* and perhaps also in *Bacillus* (see above)? A survey of the first zygotic cell division in higher plants provides circumstantial evidence

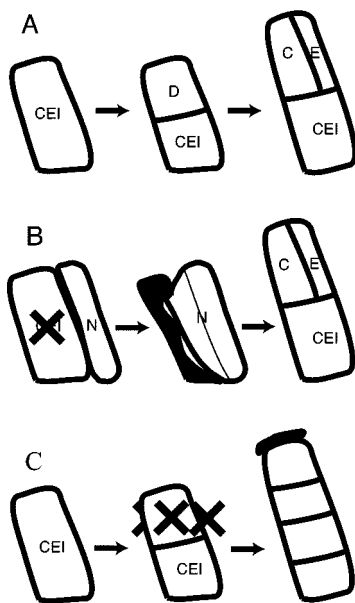
against this view. The size difference of the apical and basal cells is absent or opposite in a significant number of angiosperms, indicating that it is not of general importance for the segregation of cell fates (94).

Taken together, much of the control of the first zygotic cell division in higher plants remains unknown. It is not evident whether intrinsic or extrinsic cues are utilized, although pre-existing polarities suggest the presence of intrinsic mechanisms. However, the flexibility in fate of the zygotic daughters in *twn* mutants strongly suggests that a simple scenario with a differentially segregated intrinsic fate determinant is not sufficient to explain the different fate of the two daughters: Subsequent signaling events of unknown nature can modify cell fates later on. Identification of components involved in the zygotic division of lower plants, and extension of these findings to higher plants can be one way to address the many open questions. Another approach may be a rigorous genetic screen to define early steps in zygote development, perhaps aided by novel tools like early marker genes.

## RADIAL PATTERNING OF THE ROOT

In the root meristem, all initial cells undergo stem cell-like asymmetric divisions as they regenerate themselves and produce a daughter that differentiates. Some daughter cells go through additional asymmetric divisions when they divide to form the precursors of different cell lineages. In *Arabidopsis*, with its regular cell lineages, these divisions can be followed with ease (25a). An example is the cortex/endodermal initial cell (CEI) that gives rise to the cortex and endodermal lineages. In the *Arabidopsis* root meristem, this cell divides first anticlinally to regenerate an initial with the same stem cell properties and a daughter cell that divides asymmetrically in a periclinal orientation to form cells of two types, cortex and endodermis (Figure 8A).

Candidate genes encoding determinants for the cortical/endodermal fate should, in the ideal case, lead to symmetric cell divisions in the ground tissue. However, mutations in the *SHORT-ROOT* and *SCARECROW* genes, which affect cell fate in the ground tissue, concomitantly result in the loss of the periclinal asymmetric division of the CEI. The fate of the daughter cell differs for the two mutants. In *short-root* (*shr*) the descendants of the resulting cell lack endodermal features, suggesting that SHR is responsible for initiating or maintaining the pathway leading to the endodermal cell fate (6). In *scarecrow* (*scr*), the remaining cell layer has differentiated features of both cortex and endodermis, indicating that SCR is not required for cell fate, per se, but rather plays a role in effecting the asymmetric division (24). Consistent with these interpretations is the result of combining *shr* with *fass* in which there are supernumerary ground tissue layers. The *shr,fass* double mutant contained no



**Figure 8** Asymmetric cell division to generate cortex and endodermis in *Arabidopsis*. CEI, cortical-endodermal initial; D, daughter of the initial; E, endodermis; N, neighboring cell. Crosses represent ablations, and black regions are cell corpses. **A.** Formation of the daughter cell from the initial, and asymmetric division to generate cortical and endodermal progenitor cells. **B.** Ablation of the CEI cell leads to replacement from a neighboring cell layer, demonstrating that CEI fate is position-dependent. **C.** Asymmetric division is controlled by more mature D cells.

identifiable endodermal tissue, confirming that *SHR* is indeed required for the formation of endodermis (85).

Isolation and characterization of the *SCARECROW* gene indicates that it may act as a transcription factor (24). Its expression pattern was particularly revealing. It is expressed in the CEI prior to the first division, then in only one of the two daughter cells resulting from the second division. This would be consistent with its being required for the asymmetric division. Although there is no direct evidence to date as to whether the expression of *SCR* in only the endodermal daughter cell is due to partitioning, indirect evidence argues against it. The *SCR* promoter directs marker gene expression in a pattern identical to that of the endogenous RNA, indicating that transcriptional regulation is sufficient to account for the endodermal daughter-specific expression pattern (24).

How might *SHR* and *SCR* function to regulate the asymmetric division? A simple model is that *SHR* is or produces an endodermal determinant, which, in turn, induces *SCR* to effect the asymmetric cell division. This model predicts

that: (a) *shr* should be epistatic to *scr*; if SHR is required to induce SCR activity, then in the absence of SHR it should not matter whether SCR is present; (b) SCR activity may be induced by SHR at either a transcriptional or posttranscriptional level. Molecular analysis should enable one to untangle the involvement of SCR and SHR in determination of cell fate and in cell division.

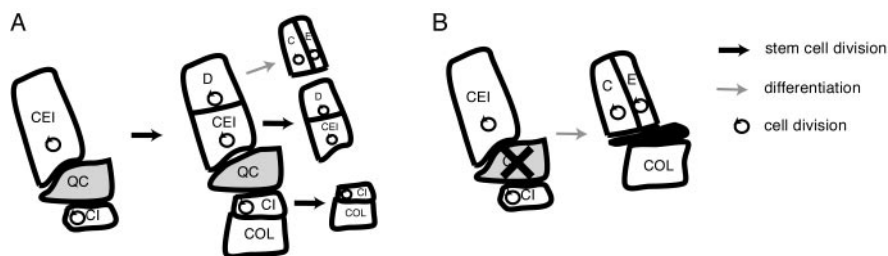
There is evidence that at least parts of the process that regulates a specific asymmetric division in roots are conserved in shoots. Mutations in SCR and SHR result in the loss of a normal "starch sheath," a layer occupying an analogous position to the root endodermis, surrounding the vascular tissue in the shoot (34). Thus, these genes appear to be generally required to define ground tissue cell layer(s) around the vascular bundle. Analysis of the ontogeny of shoot-derived ground tissue layers and concomitant expression analysis of SCR and of SHR should help to determine if similar asymmetric divisions occur in shoot and root.

What orients asymmetric cell divisions in the root meristem? The regularity of cell lineages like those giving rise to cortex and endodermis in the *Arabidopsis* root could be taken as an indication that cell fate is determined by intrinsic factors. Laser ablation of cells within the root was used to test this hypothesis. Contrary to expectations, there was no evidence for cell lineage irreversibly committing a cell to a particular fate. Rather, when any one of the initial cells or their immediate descendants was ablated, a neighboring cell of a different lineage would expand into the empty space, then divide, and one of the daughters would take on the fate of the ablated cell (Figure 8B) (107). Ruling out lineage leaves only position as a source of cell fate information. Further ablations revealed a possible source of the information required for at least one type of asymmetric division. When three adjacent cells that were the upper daughters of the first division of the CEI were ablated, the middle initial was unable to make the asymmetric periclinal division but was able to continue the anticlinal divisions (Figure 8C). This indicated that information from more mature cells above the initial was required for the cell to perform the asymmetric division to separate the cortical and endodermal fates. Analysis of SHR and SCR mutants had shown that when these asymmetric divisions were defective in the root tip, corresponding divisions were defective in the complete embryonic axis. Combining the ablation and genetic results led to the hypothesis that an embryonic pre-pattern instructs asymmetric divisions within the root meristem in a "top down" direction (85, 107). As in the EMS/P2 interaction in *C. elegans*, an extrinsic signal may result in the polarization of an internal determinant. SCR expression remains on in the entire endodermal lineage (24), which suggests the possibility that SCR could be involved in "top-down" transfer of information. This is not necessarily inconsistent with its role as a transcription factor given the clues for non-cell autonomous behavior of the

KNOTTED homeobox protein (62a) and of the transcription factors GLOBOSA and DEFICIENS (75b). Outstanding questions now are how signaling events of whatever nature can direct the separation of the two cell fates and the orientation of cell division.

## STEM CELL DIVISIONS IN THE ROOT MERISTEM

Meristems maintain themselves throughout plant development by retaining a stem cell reservoir, a pool of relatively undifferentiated cells. Cells in the *Ara-bidopsis* root meristem divide with sufficient regularity to localize the stem cells ("initials") for the different cell lineages, which surround the mitotically almost inactive quiescent center (QC) (25a). Stem cells perform asymmetric cell divisions, as they produce one daughter that will proceed to differentiate, while the other daughter remains a stem cell. The daughters that retain stem cell characteristics are those that are in closest proximity to the QC (Figure 9A). This spatial relationship suggests a function for the QC in the maintenance of stem cells, and thus the influence of QC cells on the surrounding initials was investigated by laser ablation studies (107). When single QC cells were ablated, contacting initial cells of the columella root cap and the cortex/endodermis lost the stem cell status and proceeded with the activity of the daughter cell (Figure 9B). This defect in initial cells contacting eliminated QC cells could not be rescued by intact QC cells, which were at a distance of a few microns. It was concluded that a short-range signal of the QC maintained the stem cell status of cells in its immediate proximity. Phrased in more general terms, an extrinsic cue from the QC biases the fate of the daughters of the asymmetric stem cell divisions. Identical ablation experiments in mutants that lack postembryonic cell division in the root showed that the QC is required in these mutants as well to maintain the initial status. This indicated that cell



**Figure 9** The quiescent center (QC) controls stem cell identity of the cortical-endodermal initial (CEI) and of the columella initial (CI). **A.** Activities of the stem cells contacting the QC. **B.** Result of QC ablation.

differentiation status is controlled directly without being a result of control of cell division.

No determinants are yet identified that specify the stem cell status, and promoter trap or enhancer trap screens for genes expressed specifically in stem cells may be one way to proceed. What signaling pathways can be expected for the maintenance of stem cells? The apparent contact-dependency of the signaling between the QC and the surrounding stem cells reminds one of cell-contact-dependent signaling involving tethered ligands and their transmembrane receptors, such as *Drosophila* Notch and its nematode counterpart GLP1. An important further parallel exists in the shoot apical meristem, where a distinct stem cell population cannot be recognized by anatomical criteria, but where the *CLV* genes are required to regulate the balance between differentiated and undifferentiated cells. *CLV1* encodes a putative transmembrane receptor with an extracellular leucine-rich-repeat domain and an intracellular kinase (19). The *CLV3* gene shows close genetic interactions with *CLV1* and its product is a good candidate to act as a *CLV1* ligand (18). Interestingly, a distinct class of *CLV1* homologues is expressed in the root meristem (R Heidstra & B Scheres, unpublished data), and it will be interesting to investigate whether members of this small gene family are candidates to be a part of the QC signaling pathway.

## ASYMMETRIC DIVISION DURING POLLEN DEVELOPMENT

During pollen development, each of the products of meiosis undergoes an asymmetric division. The division of the microspore, known as pollen mitosis I (PMI), produces a larger vegetative cell (VC) and a smaller generative cell (GC) that becomes completely engulfed in the VC cell cytoplasm (Figure 10) (reviewed in 104). This process is reminiscent of engulfment of the smaller forespore by the larger mother cell in *B. subtilis*. The GC will later divide to form the two sperm cells, while the VC produces the pollen tube (67).

The two different cell types resulting from PMI of isolated, in vitro cultured microspores are fully functional (29). This provides evidence that the asymmetry of the division is the consequence of the partitioning of intrinsic factors as

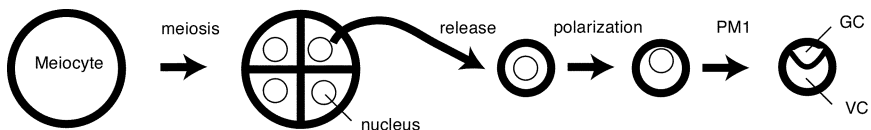


Figure 10 Pollen mitosis is an asymmetric cell division. GC, generative cell; VC, vegetative cell. Where is this called out in the text?

opposed to post-divisional cell-cell contact. The *gemini* (*gem*) mutation results in various division defects, including two-cell pollen in which both cells express VC markers (104). The symmetric division in *gem* does not occur precociously. This may suggest that in *gem* the mutation is in a cell fate determinant. However, a similar phenotype is observed when microspores are cultured in low levels of colchicine, which is expected to perturb the machinery for asymmetric cell division itself. More detailed analysis of the division process in the *gem* mutant will be required to clarify its role in the asymmetric cell division.

What orients the asymmetric cell division in microspores? Evidence for internal polarization of the microspore prior to the asymmetric division comes from histological observations, which reveal an unequal distribution of cytoplasm and organelles, with the lion's share going to the vegetative cell after division. The spindle is also polarized, forming a blunt end at the GC pole and a sharp end at the VC pole (45a). The polarized aspect of the spindle may be a direct result of the localization of the microspore nucleus very close to the wall closest to the future GC. Changing nuclear localization through cold, caffeine treatment, or centrifugation can result in symmetric divisions (99a). Treatment of isolated microspores with the microtubule inhibitor colchicine blocks cell division. The resulting cell has the phenotypic properties of a vegetative cell, indicating that VC differentiation does not require the presence of a generative cell (29). Treatment with lower doses of colchicine results in the occasional occurrence of symmetric divisions in which both daughters express a VC-specific marker. This provides evidence that intact microtubules are required for the asymmetric division. Remarkably, the PMI asymmetric division can still occur in *tetraspore* and in the allelic *stud* mutants that fail to separate the four products of meiosis (95, 49). This suggests that early cues have demarcated multiple domains at the tetrad stage (possibly dependent upon the cytoskeleton), and that these domains are still present in the *tetraspore* mutant (95). The nature of these cues remains to be elucidated.

In addition to *gemini*, there are other mutants in which the asymmetric division is defective. In *sidecar pollen* (*scp*) some pollen grains are formed that contain two VC and one GC (16). Analysis of the division process indicates that the microspore first divides symmetrically to produce two microspores, of which one can follow the normal asymmetric division process while the other differentiates to become a VC. This suggests that the *SCP* gene product may be involved in partitioning of a cytoplasmic determinant such that in the mutant, random distribution occurs in the first division and the daughter cell that happens to get more then goes through the normal asymmetric division process. Another model would have *SCP* involved in preventing precocious cell division. In the mutant, premature division would result in symmetric fates, but the normal processes that ready the cell for asymmetric division would still take place, allowing for a later asymmetric division in one of the two cells GC (16).



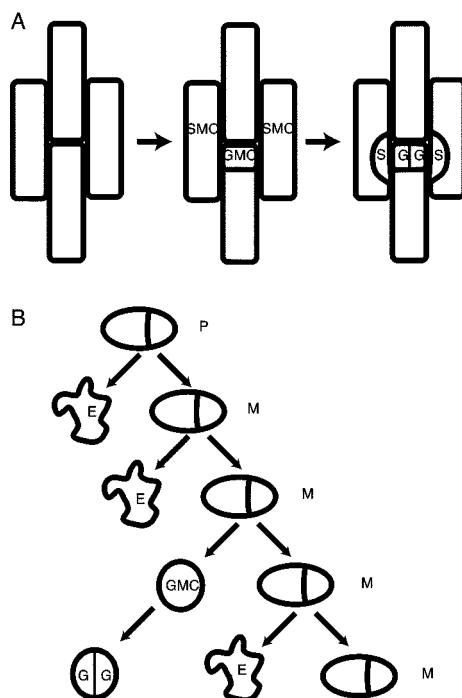
Pollen grains resulting from the *solo* mutation have only a single cell that expresses VC markers (29). The role of the corresponding gene in asymmetric cell division is not yet clear, but it is interesting to note the similarity between *solo* and *shr* mutants: Both block an asymmetric cell division and result in one of the two possible cell types.

Collectively, the genetic and experimental data indicate that VC is the default state (104). There is circumstantial evidence that the difference in cell fate between the vegetative and generative cells is dependent upon an intrinsic factor whose partitioning requires intact microtubules. Whether this unidentified factor is an activator or a repressor of cell fate is unknown, as is the machinery that partitions it.

## THE FORMATION OF STOMATA

The formation of stomata, pairs of guard cells that regulate gas exchange, is accompanied by several asymmetric cell divisions that yield cells of unequal size with different fates, as recently reviewed by Larkin et al (57). In monocotyledonous plants, one asymmetric division yields guard mother cells (GMCs) that divide symmetrically to produce guard cell pairs; another asymmetric cell division is subsequently induced in epidermal cells of the adjacent file to give subsidiary cells (Figure 11A). In dicots, a stem cell–like sequence of asymmetric divisions gives rise to stomata. A protodermal cell divides asymmetrically to produce a “meristemoid,” a stem cell–like stomatal initial cell capable of generating either epidermal pavement cells or GMCs. The GMCs produce guard cell pairs by symmetric divisions (Figure 11B). The orientation of several of these asymmetric divisions is guided by external cues. First, in monocots, the asymmetric divisions are perpendicular to the proximo-distal axis of leaf polarity, with the GMC as the distal daughter (101). Second, in dicots, the first asymmetric division that produces a meristemoid appears to be randomly oriented, but further asymmetric divisions are oriented with respect to developing GMCs (35). Furthermore, both daughters of a scheduled asymmetric division can become epidermal pavement cells to prevent direct contact of GMCs (52). The nature of the proximo-distal cue in monocots and the GMC-related cues in dicots is unknown. The direct accessibility of the epidermis for experimental interference (see 22), and the possibility of monitoring cell division patterns with epidermal peels (52, 113) may provide tools to investigate the nature of the cues involved.

Genetic analysis in *Arabidopsis* may enable one to identify genes involved in the asymmetric divisions that accompany stomatal development. Although candidate genes encoding determinants have not yet been identified, some mutants that affect stomatal patterning have been described. *four lips* mutants, for example, form guard cell pairs by two instead of one symmetric division of



**Figure 11** Stomatal complex formation. *A.* In monocots, guard mother cells (GMCs) arise from asymmetric cell divisions oriented along the apical-basal axis, and subsidiary mother cells arise from asymmetric cell divisions that are likely to be induced by the neighboring GMC or its daughters, the guard cells (G). *B.* Repetitive asymmetric divisions retain cells capable of further division, the meristemoids (M). These develop into GMCs that give rise to guard cell pairs or to interstitial epidermal pavement cells (E).

the GMC, and one interpretation is that the corresponding gene is involved in establishment or maintenance of GMC identity (57, 114). While it is uncertain whether this gene will provide an entrance to the mechanisms of asymmetric cell division, promoter/enhancer traps that specifically mark stomatal development should facilitate direct screening for genes involved in asymmetric cell divisions of the epidermis. Analysis of the contribution of intrinsic and extrinsic factors has to await the identification of such genes.

## SUMMARY

Although no single asymmetric division has been analyzed with the rigor that has recently been applied to yeast and animal systems, there is mounting

evidence that cell fate determination after asymmetric divisions in plants occurs both through the partitioning of cellular determinants and by cellular interactions. The asymmetric division of pollen grains into vegetative and generative cells has all the hallmarks of a strictly intrinsic process. While there is strong evidence that positional information plays an important role in other asymmetric divisions, too little is known to decide to what extent it combines with intrinsic mechanisms. Candidates for intrinsic factors that can be modulated by signaling are the *SCR* and *SHR* gene products, and detailed analysis of their function should provide entrances into at least one asymmetric cell division soon. Overall, it is quite possible that "mix and match" combinations of signaling and intrinsic determinants described in other organisms will also be found in plants.

It is not yet possible to know if most of the signaling will be short distance, cell-to-cell, or longer-distance signaling. To date there is little evidence for a role of the classical plant hormones in regulating specific asymmetric divisions, although this does not rule out that hormonal influences will be found when the different examples of asymmetric cell division are carefully examined. The recent ablation results in *Fucus* indicate a role for factors that travel apoplastically. Alternative signaling pathways might use plasma membrane receptors with tethered diffusible ligands or factors transported through plasmodesmata. The evidence from laser ablation of root meristem cells indicates a role for continuous signaling to maintain the separation of cell fate that is first initiated in the embryo. More generally, the remarkable plasticity of plant cells suggests that once an asymmetric division has occurred the resulting cell fates probably also require active maintenance. In floral development, the gene *CURLY LEAF*, with homology to the polycomb group of cell fate maintenance factors in *Drosophila*, has been shown to provide such a function (39).

To further our understanding of the mechanisms that regulate plant asymmetric divisions, a first step will be to analyze in depth a few of those currently under investigation. We feel that two requirements should be met to face this task.

First, the paradigms that have emerged from yeast and animal systems need to be appreciated by our community of plant scientists. As we have aimed to demonstrate here, these paradigms provide an overall conceptual framework for classification and the interpretation of experimental results, while leaving sufficient space for an open view towards the peculiarities of plant development that will undoubtedly surface.

Second, improvement in techniques for basic cell biological analyses is called for. We need better ways of visualizing plant cell divisions in planta, for example, by using markers that allow the visualization of structures such as the PPB, spindle, and phragmoplast. A step in this direction is the fusion of Green Fluorescence Protein (GFP) to a dynamin-related protein that localizes to the

phragmoplast (40). To ascertain the extent to which signaling plays a role in asymmetric divisions, it would be extremely useful to be able to change the location of a plant cell. This may not be as difficult as once imagined, if in vivo cell-specific marking can be successfully combined with micromanipulation and laser microsurgery.

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