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Embryogenesis: A New Start in Life

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INTRODUCTION

Embryogenesis effects the transition from the fertilized egg to the new multicellular generation, the seedling, which displays the basic body plan and organization of the plant. An apical–basal pattern along the main body axis of the embryo consists of a linear array of distinct elements, including the shoot meristem, cotyledons, hypocotyl, root, and root meristem. A radial pattern around the apical–basal axis is represented by the concentric arrangement of the primary tissues: epidermis at the periphery, ground tissue underneath, and conductive tissue in the center. During postembryonic development, the two primary meristems give rise to the elaborate structures of the adult plant (see Clark, 1997; Kerstetter and Hake, 1997; Schiefelbein et al., 1997, in this issue).

The basic body plan is established within the first one-third of embryogenesis and becomes fully apparent by the time dicot embryos reach the heart stage. Subsequent events include further growth of the embryo, morphogenesis, activity of the primary meristems, cell differentiation, and preparation of both embryo and seed for dormancy. Previous reviews have covered various aspects of embryogenesis, including the formation of embryo initials (Mordhorst et al., 1997), fertilization (Russell, 1993), endosperm development (Lopes and Larkins, 1993), somatic embryogenesis (Zimmerman, 1993; Emons, 1994), axis formation (Jürgens, 1995), gene expression (Thomas, 1993), and related topics (Goldberg et al., 1994; Laux and Jürgens, 1994; Yadegari and Goldberg, 1997).

In this review, we discuss how pattern formation generates different cell fates during embryogenesis. By drawing mainly on recent genetic and molecular studies in Arabidopsis, we first summarize what is known about the successive generation of cell fates and then discuss mechanisms that may underlie the establishment of diverse cell identities.

SUCCESSIVE GENERATION OF CELL FATES IN THE EMBRYO

The developing embryo consists of a growing population of cells, the fate of each of which must be determined in a position-dependent manner to form a functional organism. Here, we discuss the events that establish the basic body plan of the Arabidopsis embryo. Where informative, we include relevant data from other species. Arabidopsis embryogenesis has been described in detail previously (Mansfield and Briarty, 1991; Jürgens and Mayer, 1994).

Setting the Stage: Formation of the Apical–Basal Axis of the Embryo

A common feature of higher plant embryos is that their apical–basal axes are aligned according to the chalaza–micropyle axis of the ovule, suggesting an orienting influence of the surrounding maternal tissue. The embryo sac, egg cell, and zygote appear polarized in many higher plant species, including Arabidopsis (Esau, 1977; Willemse and Van Went, 1984; Mansfield and Briarty, 1991; Mansfield et al., 1991). In maize, for example, the cytoplasm and nucleus are shifted toward the apical end upon fertilization of the egg cell (Mòl et al., 1994). Although somatic embryos demonstrate that apical–basal polarity can be established without maternal information (Backs-Hüsemann and Reinert, 1970; Nomura and Komamine, 1985), the strict correlation between the orientation of the apical–basal axis of the embryo and the structure of the ovule suggests that such information could play an important role in zygotic embryogenesis.

The nature of such maternal information is unknown, although diffusable factors and/or physical constraints are valid possibilities. Moreover, maternal mutations affecting polarity and axis formation similar to those described in Drosophila (Johnston and Nüsslein-Volhard, 1992) have not been identified in plants. By contrast to higher plants, the apical–basal axis of the embryo is not oriented relative to maternal structures in the brown alga *Fucus*, in which the free-living zygote becomes polarized in response to external cues such as light (see Kropf, 1997, in this issue).

Another extra-embryonic tissue that may influence formation of the apical–basal axis of the higher plant embryo is the triploid endosperm, which is initiated after the fusion of the second sperm cell with the central cell of the female gametophyte (Mansfield and Briarty, 1991). The roles of the endosperm in embryogenesis appear diverse; they include nutrition of the embryo (Lopes and Larkins, 1993) and regulation of both embryo size (Hong et al., 1996) and fruit

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development (Ohad et al., 1996). However, there is no evidence that the endosperm plays an instructive role in embryo pattern formation.

The zygote generates the embryo and the extra-embryonic suspensor, which provides nutrients to the young embryo (Yeung and Sussex, 1979) and pushes it into the lumen of the embryo sac (Yeung and Meinke, 1993). Suspensor cells can initiate embryogenesis if the embryo is aborted or arrested (Gerlach-Cruse, 1969; Schwartz et al., 1994; Yadegari and Goldberg, 1997), suggesting that the embryo normally represses the developmental potential of the suspensor (Marsden and Meinke, 1985; Yeung and Meinke, 1993). In *twin* seeds, a suspensor cell can give rise to an additional embryo, although the primary embryo develops normally. The secondary embryo has normal or reversed apical–basal polarity (Vernon and Meinke, 1994), raising the possibility that the juxtaposition of embryonic and extra-embryonic cells may help to orient the apical–basal axis of the embryo. In this view, the juxtaposition of the wild-type embryo proper and the suspensor is instrumental in establishing the basal embryo pole. By contrast, *twin* embryos that initiate within the suspensor are flanked by suspensor cells on both sides, and thus, their basal pole may be established at random. Alternatively, the differences between the primary and secondary embryos may simply reflect that the former originates from a polarized zygote, whereas the latter arises from a suspensor cell that lacks information directing embryo polarity.

The Arabidopsis zygote divides asymmetrically, giving two daughter cells of different sizes and fates (Figure 1A). The smaller apical cell forms most of the embryo, whereas the larger basal cell contributes to the root of the embryo but principally gives rise to the suspensor. This difference in cell fate is highlighted by the accumulation of mRNA from the *ARABIDOPSIS THALIANA MERISTEM LAYER 1* (*ATML1*) gene in the apical but not the basal daughter cell of the zygote (Lu et al., 1996).

How the different fates of the apical and the basal cells are established is not known. Studies in other organisms suggest that cell fate determination may occur either before or after division of the zygote. For example, in *Fucus*, the zy-

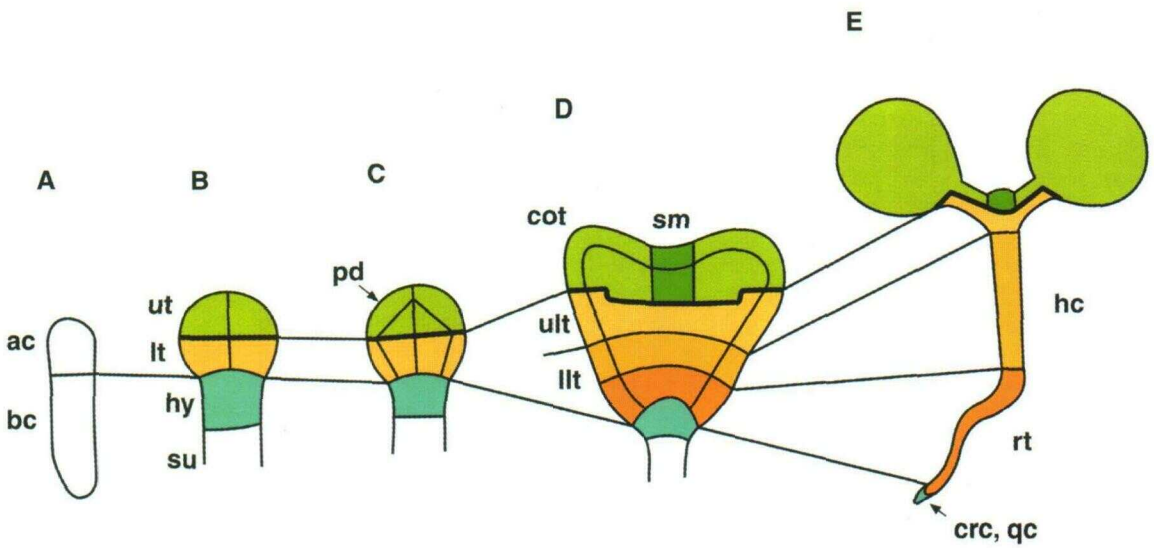

Figure 1. Establishment of the Arabidopsis Body Plan.

The Arabidopsis body plan is established in an ordered sequence of events. First, large regions are specified that will be subdivided later to give the structural elements of the seedling body (see text for details).

(A) Two-cell stage. A smaller apical cell (*ac*) and a larger basal cell (*bc*) have been formed by the asymmetric division of the zygote.

(B) Octant stage. The apical cell has given rise to four upper-tier (ut; light green) and four lower-tier (lt; gold) cells, and the basal cell has generated the hypophysis (hy; blue) and the suspensor (su; white).

(C) Dermatogen stage. Tangential cell divisions have separated the protoderm (pd) from inner cells.

(D) Heart-stage embryo. The apical domain, which is derived from the upper tier of cells, has been partitioned into cotyledon (cot; light green) and shoot meristem (sm; dark green) primordia. The central domain, which is derived from the lower tier of cells, has been subdivided into the upper-lower (ult; yellow) and the lower-lower (llt; orange) tiers.

(E) Seedling. The hypocotyl (hc), root (rt), and initials of the root meristem are derivatives of the lower-lower tier. The basal domain derived from the hypophysis has formed the quiescent center (qc; blue) of the root meristem and the initials of the central root cap (crc; blue).

Corresponding regions of the growing embryo and the seedling are colored. Individual cells are shown in **(A)** to **(C)** and groups of cells in **(D)** and **(E)**. The horizontal division plane of the octant-stage embryo and the boundary corresponding to this plane at later stages are shown as thick lines. Drawings are not to scale.

gote is polarized before cell division such that rhizoid cell fate determinants become associated with the cell wall at the basal pole and are inherited by the basal daughter cell (Berger et al., 1994; see also Kropf, 1997, in this issue). Asymmetric divisions of embryonic cells in the green alga *Volvox* give large generative and small vegetative daughter cells, and this cell fate segregation has been attributed to the postmitotic difference in cell size rather than to unequal distribution of molecules (Kirk et al., 1993). Finally, one reason the two daughter cells of the Arabidopsis zygote may acquire different fates in response to positional information could be because the basal but not the apical cell is attached to the surrounding maternal tissue. Whatever the underlying mechanism may be, the initial decision generates the distinction between embryonic and nonembryonic cell fates.

Of the Arabidopsis embryonic pattern mutants analyzed so far, only mutations in the *GNOM/EMB30* (*GN*) gene affect the apical–basal polarity of the embryo. The *gn* zygote does not elongate to the same extent as the wild-type zygote and tends to divide symmetrically (Mayer et al., 1993). Nevertheless, the reduced basal daughter cell does give rise to a shortened suspensor, and the apical cell forms an embryo proper, suggesting that an asymmetric division of the zygote is not required to establish the fate of the two daughter cells. Cell divisions are irregular in the developing *gn* embryo, and the expression of the *LIPID TRANSFER PROTEIN* (*LTP*) gene, which is normally restricted to the apical end of the later stage embryo, is variable along the apical–basal axis (Vroemen et al., 1996). This observation suggests that the polarity of the *gn* zygote, as expressed in the different fates of its daughter cells, may not be sufficient to establish the apical–basal axis of the embryo.

The *GN* gene appears to be expressed throughout development (Shevell et al., 1994), and the GN protein shows sequence similarity to two yeast proteins, Gea2p (Yec2p; Busch et al., 1996) and Gea1p, which are guanine–nucleotide exchange factors involved in vesicle transport between the endoplasmic reticulum and the Golgi complex (Peyroche et al., 1996). These findings raise the possibility that the GN protein participates in directional vesicle transport, which may function to stabilize the apical–basal axis of the embryo. Targeted vesicle fusion is also thought to play a role in axis stabilization in the *Fucus* embryo (see Kropf, 1997, in this issue).

Partitioning the Apical–Basal Axis of the Embryo

The apical–basal axis of the seedling is subdivided into five major components: shoot meristem, cotyledons, hypocotyl, root, and root meristem (Figure 1E). These components do not originate simultaneously by partitioning of the axis of the embryo but are established in steps. First, transverse cell divisions in the four-cell embryo result in upper and lower tiers, each with four cells (Figure 1B). The boundary between the two tiers can be followed throughout embryo development (Tykarska, 1976, 1979) and passes through the cotyledons (Figure 1; Scheres et al., 1994). Whereas the upper tier gives rise to the apical domain, which comprises the shoot meristem and most of the cotyledons, the lower tier generates the central domain, which contributes the "shoulder" to the cotyledons and also produces hypocotyl, root, and the proximal initials of the root meristem (Figure 1). The remaining parts of the root meristem, the quiescent center and the initials of the central root cap, are derived from the hypophysis, the uppermost derivative of the basal daughter cell of the zygote (Figure 1). Thus, the three domains established in the early embryo do not correspond to primordia of the seedling components. Nevertheless, analyses of mutant phenotypes argue that the early establishment of these domains plays a role in apical–basal patterning.

Proper Development of the Apical Domain Requires GURKE *Activity*

Mutations in the *GURKE* (*GK*) gene specifically affect the apical domain (Torres-Ruiz et al., 1996). Although cotyledon development appears to be more sensitive to the level of *GK* activity than does that of the shoot meristem, strong *gk* alleles abolish apical structures altogether and lead to the formation of a disorganized green mass of cells at the apical end of *gk* seedlings. Defects are first recognized in the apical domain of the heart-stage *gk* embryo, but defects in the central domain become obvious during later stages of embryogenesis. In the most extreme manifestation of the *gk* phenotype, the complete elimination of the cotyledons, the shoulders of which are derived from the central domain, and the reduction of the hypocotyl raise the possibility that *GK* is required not only in the apical but also in the central domain of the embryo. Alternatively, the reduction of the hypocotyl may be an indirect consequence of a primary defect in the apical domain, which would imply that cells from the central domain may be entrained to become incorporated into the incipient cotyledon primordia.

The MONOPTEROS *Gene Is Required in a Complementary Domain to* GURKE

monopteros (*mp*) seedlings lack roots and hypocotyls and also display defective vascularization of the cotyledons. The earliest deviation from wild-type development is observed at the eight-cell stage, when the *mp* embryo proper consists of four rather than two tiers of cells (Berleth and Jürgens, 1993). Subsequently, the cells of the central domain divide abnormally and fail to produce the elongated cell files that represent the hypocotyl and root primordia of wild-type globular embryos. In addition, the uppermost derivative of the basal cell, which normally would become the hypophysis and contribute to the root meristem, divides horizontally, similar to a suspensor cell, to generate a "central pile" of cells.

Thus, the *MP* gene could be required in both the lower-tier derivatives and the hypophysis or in only one of the two regions. Because the defect in the lower tier becomes apparent first, the embryo proper may normally signal to the uppermost derivative of the basal cell to become the hypophysis. If this is so, the root defect of *mp* embryos may be an indirect consequence of the aberrations in the lower-tier cells. This is consistent with the observation that wounded or bisected *mp* seedlings are able to form a root. Indeed, after root induction in tissue culture, *mp* seedlings can develop to the adult stage. However, the vascular cells of these plants are not arranged properly, which results in the formation of disrupted vascular strands (Przemeck et al., 1996). It has been suggested that the failure of vascular precursor cells to "axialize," that is, to establish a cellular axis, interferes with the hypothetical canalization process (Sachs, 1981; see also Nelson and Dengler, 1997, in this issue) by which cells along signal transport routes are induced to form files of conductive elements. Because the embryonic and the postembryonic defects are similar at the cellular level, the *MP* gene may play a primary role in cell axialization.

Partitioning the Central Domain of the Embryo

The lower tier of the embryo proper gives rise to the upper-lower and the lower-lower tiers of cells, from which different components of the seedling are derived (Figures 1D and 1E). Cells from the upper-lower tier contribute to the cotyledons, whereas those from the lower-lower tier give rise to the hypocotyl, root, and proximal initials of the root meristem (Scheres et al., 1994). Two single mutants, *möve* and *basal deletion*, lack roots and are also affected in hypocotyl development (Berleth et al., 1996). Although these mutants phenotypically resemble *mp* at the seedling stage, they may not be affected in the upper-lower tier of the globular embryo.

By contrast, mutations in the *FACKEL* (*FK*) gene specifically reduce the hypocotyl, resulting in seedlings in which the cotyledons appear to be directly attached to the root (Mayer et al., 1991). The defect becomes obvious at the midglobular stage, when *fk* mutants fail to undergo the asymmetric divisions that form the elongated vascular precursor cells of the hypocotyl. That no mutants specifically lacking the root have been identified may be related to the fact that the embryonic root is derived from two different groups of cells, only one of which originates from the root meristem (see below).

Primary Meristems of the Shoot and the Root

The primary meristems of the shoot and the root are established at opposite poles of the apical–basal axis of the embryo (Figures 1D and 1E). An important issue that has come into focus recently concerns the relationship between the meristems and differentiating tissues of the embryo: are the meristems autonomous pattern-generating machines, or do they need information from surrounding tissues to form new structures? A closer look at the embryonic origin of the primary meristems has given some clues.

Shoot Meristem

The primary shoot meristem of the seedling is organized in zones and layers (Steeves and Sussex, 1989; see also Clark, 1997; Kerstetter and Hake, 1997, in this issue). Although the shoot meristem is fairly inconspicuous in the Arabidopsis embryo, becoming histologically distinct from the differentiating tissues of the flanking cotyledon primordia only at the torpedo stage (Barton and Poethig, 1993), its organization has been inferred in a number of ways. For example, the analysis of mutants such as *wuschel* (*wus*) suggests that a central zone and a peripheral zone are established in the embryo (Laux et al., 1996). Furthermore, the shoot meristem L1 layer originates from the protoderm layer, which, as reflected by the expression pattern of the *ATML1* gene (Lu et al., 1996), is established in the octant-stage embryo (Figure 1B). Thus, the organizational features of the shoot meristem are essentially in place in the later-stage embryo.

Mutations such as *wus* (Laux et al., 1996), *zwille* (*zll*) (Jürgens et al., 1994; Endrizzi et al., 1996), and *pinhead* (*pnh*) (McConnell and Barton, 1995), which affect the development of the shoot meristem but not that of the cotyledons, suggest that these primordia are genetically distinct. The cotyledon primordia are first visualized in the late-globular-stage embryo by localized cell divisions at the flanks of the apical domain (Jürgens and Mayer, 1994). That the intervening cells are destined to form the shoot meristem was first suggested by clonal analyses (Christianson, 1986; Poethig et al., 1986).

This view is supported by the complementary expression patterns of two genes, *SHOOT MERISTEMLESS* (*STM*) and *AINTEGUMENTA* (*ANT*), within the apical domain of the globular embryo. The *STM* gene, which is involved in shoot meristem organization throughout plant development (Barton and Poethig, 1993; Clark et al., 1996; Endrizzi et al., 1996), is expressed between the cotyledon anlagen (Long et al., 1996). By contrast, the expression of the *ANT* gene at flanking sites presages cotyledon initiation (Elliott et al., 1996). The *ANT* gene, which is also required for the proper development of the flower, is expressed in the primordia of floral organs, cotyledons, and leaves (Elliott et al., 1996; Klucher et al., 1996). Thus, the apical domain of the globular embryo is partitioned into a central area that becomes the shoot meristem and a surrounding area from which the cotyledons develop.

The emergence of cotyledon primordia in the globular embryo is histologically similar to postembryonic leaf formation by the shoot meristem (Kaplan, 1969). Both processes are similarly affected in *stm* mutants (Endrizzi et al., 1996), suggesting that related mechanisms underlie the partitioning of the apical domain of the embryo and the initiation of leaves.

Moreover, organ primordia emerge during the initiation of adventitious shoot meristems before the cellular organization of the shoot meristem can be discerned, which is similar to the situation in the embryo during cotyledon initiation (Sussex and Rosenthal, 1973; Davis and Steeves, 1977; Tian and Marcotrigiano, 1993; see also Kerstetter and Hake, 1997, in this issue).

Before the onset of dormancy, the shoot meristem of the Arabidopsis embryo produces the first two leaf primordia perpendicular to the cotyledons, suggesting that the cotyledons serve as a reference point from which to establish the subsequent phyllotactic pattern. This view is supported by the correlation between defects in phyllotaxis and cotyledon number in *altered meristem program* (*amp1*; Chaudhury et al., 1993), *häuptling* (*hpt*; Jürgens et al., 1991), and *fass* (Torres-Ruiz and Jürgens, 1994) mutants. In these mutants, leaves are usually initiated between two adjacent cotyledons, irrespective of their number. The shoot meristem may also derive patterning information from the hypocotyl, because tissues added to the shoot during postembryonic development are contiguous with the tissue layers formed in the embryo. In conclusion, the shoot meristem may best be viewed as a population of dividing cells that are established in the early embryo and receive patterning information from differentiated tissues.

Root Meristem

The primary root meristem consists of two tiers of initials that surround a group of mostly mitotically inactive cells, the quiescent center (Figure 2; Dolan et al., 1993; see also Schiefelbein et al., 1997, in this issue). The proximal initials above the quiescent center add new cell tiers to the concentric layers of root tissues in a regular fashion: fixed numbers of initials each give rise to lateral root cap and epidermis, cortex and endodermis, and pericycle and vascular tissue. The distal initials below the quiescent center add cell tiers to the central root cap. The root meristem becomes active in the heart-shaped embryo, at which time the embryonic root begins to extend.

The embryonic origin of the root meristem has been analyzed in some detail, both histologically and by clonal analysis (Dolan et al., 1993; Scheres et al., 1994). The quiescent center and the initials of the central root cap derive from the basal domain of the embryo, which is established by the hypophysis. The hypophysis, in turn, originates from the basal daughter cell of the zygote (Figure 1). By contrast, the initials for the remaining root tissues derive from the lowest cell tier of the central domain and ultimately from the apical daughter cell of the zygote. Thus, a clonal boundary runs across the root meristem (Figure 2; Dolan et al., 1994; Scheres et al., 1994), suggesting that inductive events play a role in establishing the initials for the root tissues. Indeed, no root meristem is formed in the "hypophyseal group" of mutants (e.g., *hobbit*), in which the first recognizable defect is the aberrant development of the hypophysis (Scheres et al., 1996b). This suggests that a root meristem cannot be established unless the hypophyseal cell group is correctly specified (Scheres et al., 1996a). Hypophyseal group mutants display similar defects in adventitious root development, suggesting that root meristem specification involves the same regulatory mechanisms in different developmental contexts (Scheres et al., 1996b). Other mutations, such as *root meristemless* (Cheng et al., 1995) and *stump* (Berleth et al., 1996), do not affect the embryonic root meristem but specifically inhibit proliferation of both primary and lateral root meristems during postembryonic development. Thus, root meristem activity may be subject to different levels of control during embryonic and postembryonic growth.

The concentric arrangement of the root meristem initials precisely matches that of the mature tissues of the root. However, experimental evidence indicates that "the root appears to define the meristem, and not vice versa" (Scheres

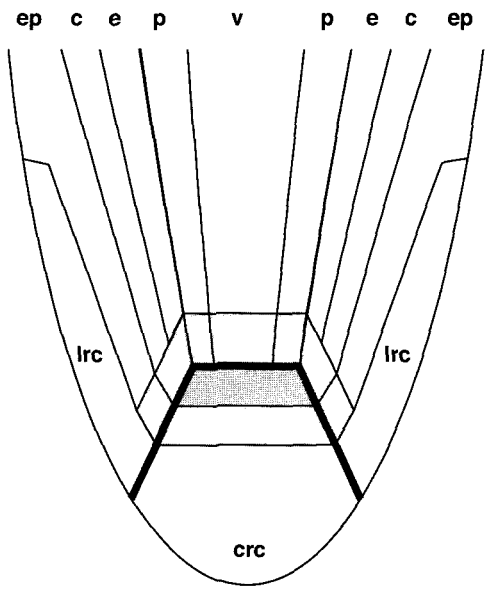

Figure 2. Radial Organization of the Root and Root Meristem.

A schematic representation of a median longitudinal section through the root tip is shown. The radial organization of the root consists of several concentric rings of tissue layers. From the center to the periphery, these are vascular elements (v), pericycle (p), endodermis (e), cortex (c), and epidermis (ep)/lateral root cap (lrc). The root meristem is composed of the quiescent center (darkly shaded), the proximal initials (above; lightly shaded), and the distal initials (below; lightly shaded), which give rise to the central root cap (crc). The endodermal and the cortical cell layers as well as the epidermal and the lateral root cap layers are each derived from common initials. A clonal border runs through the root meristem (thick line). The initials above this border are derived from the central domain of the embryo, whereas the quiescent center and the distal initials are derived from the hypophysis.

et al., 1996a). Indeed, after laser ablation of cortex/endodermis initials in the Arabidopsis seedling root, the underlying pericycle initials take over and function properly according to their new position. Furthermore, ablation of three adjacent daughter cells of cortex/endodermis initials resulted in abnormal cell files, suggesting that root tissue initials receive patterning information from mature root tissues (Van den Berg et al., 1995). In carrot, the upper part of transected somatic embryos regenerated root tissues before a new root meristem was formed (Schiavone and Racusen, 1991). This sequence of events is also seen during lateral root formation in Arabidopsis—that is, the lateral root primordium acquires a radial organization of tissue layers before the root meristem becomes active (Malamy and Benfey, 1997). Thus, the root meristem initials can be viewed as dividing cells that lack intrinsic patterning information.

Establishing the Radial Axis: Protoderm Formation

The radial axis of the embryo, which is defined as the concentric arrangement of tissue layers from the center to the periphery, becomes apparent after tangential cell divisions in the octant-stage embryo that partition the cell mass into an outer cell layer, the protoderm, and inner cells (Figures 1B and 1C). The protoderm gives rise to the epidermis by strictly anticlinal cell divisions, whereas the inner cells originate ground tissue and vascular elements. Subsequently, these primary tissues undergo further specialization.

Determination of Cell Fates along the Radial Axis

How are protoderm and inner cell fates segregated? By analogy with rhizoid cell fate segregation in the brown alga *Fucus* (Berger et al., 1994), cell fate information may be laid down in the cell wall of the zygote and passed on to all progeny with cell walls derived from the zygote wall. This idea was originally proposed for the determination of epidermal cell fate in *Citrus jambhiri* on the basis of the observation that the zygote is coated with a cuticle layer, which is a morphological marker for epidermal identity (Bruck and Walker, 1985). Nonepidermal cell fate would thus represent a developmental "ground state" corresponding to the absence of epidermal determinants derived from the zygote. In this model, cell fate segregation does not require strictly oriented cell divisions: any cell division that disconnects cells from the zygote-derived cell wall would suffice.

In support of this hypothesis, protoderm formation is not affected in embryos of the Arabidopsis *fass* mutant, which display an irregular cell division pattern (Torres-Ruiz and Jürgens, 1994). Moreover, the expression pattern of the *ATML1* gene is consistent with this model. *ATML1* is expressed in the apical daughter cell of the zygote and in all cells of the eight-cell embryo proper (Lu et al., 1996). However, after the tangential divisions, *ATML1* expression becomes restricted to the protoderm layer and is no longer detectable in the inner cells.

In an alternative model based on the action of morphogens in animals (Green and Smith, 1990), protoderm and inner cells could acquire different fates in response to positional information passing along the radial axis. Hypothetical signaling molecules may enter the inner cells from the suspensor and/or the protoderm cells from the endosperm. Although delivery of molecules to the embryo from both the suspensor and the endosperm has been discussed, there is no evidence that such substances affect cell fate determination in plant embryos. Whatever the mechanism of protoderm formation, the epidermal fate of the outer cell layer, once established, is stably maintained.

Stable Expression of Cell Fates Requires Physical Separation

Mutations in the *KNOLLE* (*KN*) gene perturb the segregation of protoderm and inner cell fate, a defect that has been correlated with incomplete cytokinesis (Lukowitz et al., 1996). Epidermal and subepidermal markers appear to be abnormally distributed in early *kn* embryos (Lukowitz et al., 1996; Vroemen et al., 1996), but at later stages, some inner cells stop expressing the epidermal marker and form vascular tissue. The attenuation of the mutant phenotype during later stages of embryogenesis may reflect either increasingly complete cytokineses, perhaps a result of the activation of redundant function(s), or the increasing distance of the inner cells from the embryo surface.

The *KN* gene encodes a syntaxin-like protein that is required for cell plate formation (Lukowitz et al., 1996). In *kn* embryos, with their incomplete cell walls, the hypothetical protoderm and inner cell fate determinants may not be fully segregated to adjacent cells but remain present within the adjoined cytoplasm. Injection experiments on root cells support the view that physical separation is necessary for proper cell differentiation (Duckett et al., 1994). For example, in the differentiated part of the root, fluorescent dye did not spread from the epidermis to the subepidermal layer but entered adjacent epidermal cells. In more general terms, before groups of cells can acquire different developmental fates, the uncoupling of their symplastic domains may be required to restrict the passage of cell type–specific molecules. Conversely, cells within any one tissue may use their symplastic continuity to disseminate cell-specific information for the specification of newly formed cells (see McLean et al., 1997, in this issue).

Elaboration of the Radial Pattern in the Central Domain of the Embryo

Although the protoderm forms around both the apical and central domains of the embryo, subsequent steps of radial

patterning are confined to the central domain (Figures 1 and 2). Periclinal divisions of the inner cells at the protoderm stage produce a layer of ground tissue that surrounds a central vascular primordium. After further periclinal cell divisions, this primordium gives rise to a layer of pericycle cells that encircle the conductive tissues (Scheres et al., 1995). It is only in the torpedo-stage embryo that the ground tissue splits into an outer cortex and an inner endodermis layer. This radial pattern is modified at two levels along the apical–basal axis: the hypocotyl primordium has two layers of cortical cells, and the lowest tier of the root primordium forms the outermost layer of lateral root cap cells after periclinal divisions in the epidermis layer.

Several mutations affect the radial pattern of the embryo. The asymmetric cell division of the ground tissue that generates the cortical and endodermal cell layers is absent in three mutants, resulting in a single cell layer instead of two (Scheres et al., 1995). The mutant cell layer appears to correspond to cortex in *short root* (*shr*) and to endodermis in *pinocchio* (*pic*) but displays both endodermal and cortical traits in *scarecrow* (*scr*). The *SCR* gene encodes a putative transcription factor and is expressed in both the cortex / endodermis initial of the root meristem and the endodermal cell layer (Di Laurenzio et al., 1996). Mutations in another gene, *WOODEN LEG* (*WOL*), result in a reduced number of vascular cells, all of which differentiate into xylem vessels (Scheres et al., 1995). All of these mutations affect the radial organization of the hypocotyl, root, and proximal initials of the root meristem, suggesting an intimate relation between root and hypocotyl development.

Is the absence of a specific cell layer the result of defective specification of cell fate or of a shortage of cells? This question has been addressed by double mutant analyses with *fass*, which causes an increased number of radial cell layers (Torres-Ruiz and Jürgens, 1994). The *fass* mutation was able to rescue the defects of *scr* and *wol*, suggesting that neither *SCR* nor *WOL* specifies cell fate (Scheres et al., 1995). Thus, the lack of phloem in *wol* mutants implies that the xylem must be formed before the phloem. A similar first-come-first-served mechanism has been suggested for the allocation of cells to floral organ primordia (Laux et al., 1996). By contrast, the *shr* defect was not suppressed by *fass*, suggesting that *SHR* specifies endodermal cell fate (Scheres et al., 1995). It should be noted that the mutations affecting the radial pattern of the embryo also display the same defects in lateral roots, implying that the same patterning mechanism operates during postembryonic development. This notion is also supported by corresponding patterns of marker gene expression (Malamy and Benfey, 1997).

In conclusion, the radial pattern of the embryo evolves sequentially. The initial separation of protoderm and nonprotoderm cell fates establishes polarity along the radial axis, which subsequently may be used for the induction of additional cell fates within the central domain of the embryo. That the radial pattern is elaborated differently along the axis suggests that apical–basal positional information modulates the response of cells to radial patterning signals.

MECHANISMS THAT ESTABLISH CELL FATE IN THE EMBRYO

Cell fate diversity can be generated by two different mechanisms: unequal division of a polarized cell to generate two daughter cells that assume different fates and intercellular communication to provide information for position-dependent cell fate determination. The former cell-intrinsic mechanism may apply in specific cases, such as the asymmetric divisions of the zygote and the hypophysis and possibly the tangential divisions of the octant-stage cells. But this cannot be a general mechanism (see below). Why then is the cell division pattern in Arabidopsis early embryogenesis so invariant?

Considering the pattern defects in *scr* and *wol* mutants, which result from a shortage of cells, it is tempting to speculate that pattern elements originate from a small number of founder cells so that the stereotyped cell division pattern of the Arabidopsis wild-type early embryo ensures that the complete body plan is established. Conversely, because all cell types are formed at the correct position in *fass* and *tonneau* (Traas et al., 1995), mutants that display highly irregular cell divisions, the orientation of cell division per se seems not to be instrumental in establishing the basic body plan. Thus, the stereotyped cell division pattern in the Arabidopsis wild-type embryo may reflect, rather than establish, cell fate specification.

Position-dependent cell fate specification was inferred from observations indicating that there are no cell lineages of fixed fate in plant development and that cells can adopt alternate fates if exposed to different developmental cues (Stewart and Dermen, 1975; Irish, 1991; Scheres et al., 1994). Although this flexibility may seem very different from the situation in animals, it should be noted that, for example, the cells in developing imaginal discs of Drosophila also continuously reassess their fate according to their position until they become irreversibly committed at the end of the proliferative period (Lawrence and Struhl, 1996). Thus, there may be some fundamental similarities in mechanisms that specify cell fate in animals and plants.

Positional Information: How Do Embryo Cells Sense What Makes Sense?

Plant development is integrated by long-range signals, such as growth factors, which are transported along the shoot–root axis (Lyndon, 1990; see also Creelman and Mullet, 1997; Kende and Zeevaart, 1997, in this issue). Auxin, for example, promotes the elongation of the embryo and cotyledon outgrowth (Schiavone and Cooke, 1987; Schiavone

and Racusen, 1990; Fischer and Neuhaus, 1996), and interference with auxin transport can result in the aberrant spacing and incomplete separation of cotyledon primordia (Okada et al., 1991; Liu et al., 1993). However, there is no direct evidence that growth factors play a role in the establishment of specific cell fates. The analyses of mutant phenotypes discussed above suggest instead that local interactions between adjacent cells or short-range signaling events are critical in this regard.

Several studies have suggested that the transport of molecules through plasmodesmata provides a route for cell–cell communication in plants. One example is the homeodomain protein KNOTTED1 (KN1), which is involved in regulating meristematic cell fate in the shoot meristem. The KN1 protein can be detected in all cell layers of the shoot meristem, whereas *KN1* mRNA is excluded from the epidermal layer (Smith et al., 1992; see also Kerstetter and Hake, 1997; McLean et al., 1997, in this issue). Moreover, ectopic *KN1* expression in vascular tissue alters cell differentiation in adjacent cells (Sinha et al., 1993). One explanation for these findings is that the KN1 protein is transported between cells. This hypothesis is supported by injection experiments in which KN1 protein appeared to move from cell to cell via plasmodesmata (Lucas et al., 1995). Injection of fluorescent dye into root cells supports the view that meristematic regions represent symplastic domains that exhibit facilitated plasmodesmal transport between cell layers. During differentiation, the tissue layers gradually become symplastically separated from each other (Duckett et al., 1994; McLean et al., 1997, in this issue).

In several cases, molecules have been identified as potential signals in plant development. For example, antigens recognized by the JIM8 antibody, which are localized in the cell wall of carrot culture cells, are necessary to sustain the development of JIM8-negative somatic embryo initial cells. This suggests that there may be an inductive interaction between JIM8-positive and embryogenic cells (Pennell et al., 1995). The active component can be washed off the cell wall by mild treatments and appears to contain carbohydrates and lipids. In the same experimental system, both Nod factors, which are *N*-acetylglucoseamine–containing lipopolysaccharides involved in bacteria–plant signaling during nodule formation, and the endochitinase EP3 can rescue arrested embryos of the *ts11* mutant (De Jong et al., 1992, 1993). Although the substrate for EP3 has not yet been identified, it is conceivable that chitinases, such as EP3, could mobilize diffusible signal molecules, such as Nod-like factors, which are required for embryo development. Similar factors may also function during animal embryo development. For example, the DG42 protein, which is required during polarity determination in Xenopus embryos and is localized at the cell periphery (Rosa et al., 1988), catalyzes the synthesis of Nod-like components (Semino and Robbins, 1985).

Whereas the functions of the JIM8 antigen and Nod-like factors during plant somatic embryogenesis may be related to growth control, a role in cell differentiation has been suggested for extracellular arabinogalactan proteins (AGPs). The presence of specific AGPs in the extracellular matrix has been found to correlate with cell type and developmental stage (Pennell et al., 1995; Kreuger and Van Holst, 1996). Therefore, it is conceivable that specific sets of cell surface molecules, such as AGPs, define a context in which neighboring cells respond and differentiate (Pennell et al., 1995).

How are signals transported through the cell wall during embryo development? Expression of the *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE* (*SERK*) gene marks carrot cells that are competent to form somatic embryos and also occurs specifically during the early stages of zygotic embryogenesis (Schmidt et al., 1997). Although its function and cellular localization are not known, the predicted SERK protein sequence resembles leucine-rich repeat receptor kinases, suggesting that it may function in a signal transduction pathway that acts very early in embryogenesis.

Do Cells Remember Their Origin?

How do cells maintain their identity once it has been specified? There is no information available concerning the length of time that positional information must be supplied for cell fates to become stabilized, although clonal analyses suggest that cell types can be altered during development as long as cells are still dividing. One model, developed after studies of *Fucus* rhizoid development, posits that rhizoid cell fate determinants laid down at the cell wall of the basal end of the zygote signal to the nucleus. This signaling leads to perpetuation of the rhizoid component in a positive feedback mechanism (Berger and Brownlee, 1995). A similar scenario can be hypothesized for perpetuation of epidermal cell fate in the outer cell layer, where propagation of extracellular substances, such as in the cuticle, could serve as a "memory" mechanism.

CONCLUDING REMARKS

Our understanding of the formal principles governing pattern formation in the embryo has been considerably enhanced over the past couple of years. These advances have been based in large part on the analysis of specific mutant phenotypes. These experiments have established that single cells or cell groups in the Arabidopsis early embryo make predictable contributions to the seedling body plan. In a number of cases, the development of a mutant embryo deviates from wild type at or before the globular stage, suggesting that cell fates are specified, in gross terms, early in embryogenesis.

Molecular studies are now needed to test the genetic models. Although, in a few cases, early cell fate specification has been visualized by specific gene expression pat-

terns, it is hoped that molecular analyses of early patterning genes will not only give clues to their functions but also facilitate the testing of genetic models of cell interactions. A different kind of molecular approach may also circumvent one of the problems inherent in genetic analyses of development: some developmentally important genes may not readily mutate to cause specific phenotypes, due, for example, to functional redundancy, and may thus have been missed in the extensive screens for pattern mutants. With this in mind, it may be rewarding to isolate genes with specific expression patterns by using the enhancer or gene trap approach, or to isolate genes known to play regulatory roles in other systems and subsequently to search for insertions in those genes to determine their biological functions. By combining various approaches, eventually we will learn how a plant embryo gets organized.

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NOTE ADDED IN PROOF

In this article we have discussed zygotic mutations that affect embryo development. Recently, Ray et al. described variable maternal effects of the *short integument* mutation on embryo development. This mutation can affect cotyledon number and/or the embryonic shoot meristem (**Ray, S., Golden, T., and Ray, A.** [1996]. Maternal effects of the *short integument* mutation on embryo development in Arabidopsis. Dev. Biol. **180,** 365–369).