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Genetic Regulation of Embryonic Pattern Formation

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INTRODUCTION

During plant embryogenesis, a simple body plan is established that consists of shoot meristem, cotyledons, hypocotyl, root, and root meristem along the apical–basal axis and a concentric arrangement of epidermis, subepidermal ground tissue, and central vascular cylinder along the radial axis. To establish this organization, the cells of the embryo need to become specified and must differentiate into cell types in an integrated manner. The genetic regulation of this process is addressed here. We focus on data from Arabidopsis but also refer to other species where helpful. For information on other aspects of embryo development, readers are referred to excellent reviews (Natesh and Rau, 1984; Goldberg et al., 1994; Mordhorst et al., 1997; Yadegari and Goldberg, 1997; Chaudhury et al., 2001).

REGIONALIZATION OF THE EARLY ARABIDOPSIS EMBRYO INTO TRANSCRIPTIONAL DOMAINS

In addition to being widely used as a genetic model organism, Arabidopsis lends itself to studies of embryonic development because of a fixed pattern of cell divisions in early stages, which makes it possible to trace the origin of seedling structures back to regions of the early embryo (Mansfield and Briarty, 1991; Jürgens and Mayer, 1994).

First, the egg cell and zygote display a polar organization, with a large vacuole at the basal end and most of the cytoplasm and the nucleus at the apical end (Mansfield et al., 1991). Notably, in maize, a fraction of egg cells appear nonpolarized and the nucleus and cytoplasm shift toward the apical end only after fertilization (Mol et al., 1994). In Arabidopsis, the zygote elongates and then divides asymmetrically to form daughter cells of different sizes and cytoplasmic densities (Figure 1). The apical daughter cell after two rounds of longitudinal and one round of transverse divisions gives rise to an eight-cell embryo proper (Figure 1). At the same time, the descendants of the basal daughter of the zygote divide transversely to form the suspensor and the uppermost cell, the hypophysis. At the eight-cell stage, four regions with different developmental fates can be recognized: (1) the apical embryo domain, composed of the four most apical cells of the embryo proper, will generate the shoot meristem and most of the cotyledons; (2) the central embryo domain, consisting of the four lower cells of the embryo proper, will form the hypocotyl and root and contribute to cotyledons and the root meristem; (3) the basal embryo domain (hypophysis) will give rise to the distal parts of the root meristem, the quiescent center, and the stem cells of the central root cap; and (4) the extra embryonic suspensor pushes the embryo into the lumen of the ovule and provides a connection to the mother tissue. The boundary between the apical and the central embryo domains can readily be recognized and serves as a histological reference point throughout embryo development (Tykarska, 1976, 1979).

In agreement with the regular cell division pattern, clonal analyses confirm that the contribution of each cell to the seedling body plan is highly predictable. However, rare variations in the cell division pattern do occur. In such cases, each cell differentiates according to its final position, in agreement with the well-established observation that developing plant cells are flexible and assume their fate corresponding to positional information (Poethig et al., 1986; Saulsberry et al., 2002).

Gene expression studies indicate that already at the earliest stages of embryogenesis, specific transcription programs are initiated in single precursor cells of embryo pattern elements (Lu et al., 1996; Weterings et al., 2001; Friml et al., 2003; Haecker et al., 2004). For example, both the egg cell and the zygote express a mixture of mRNAs encoding *WUSCHEL HOMEOBOX2* (*WOX2*) and *WOX8* transcription factors specific for early apical and basal embryo development, respectively (Haecker et al., 2004). The asymmetric division of the zygote separates these mRNAs, thereby establishing two cells of different identities and setting up the apical–basal axis of the embryo. At this stage, additional genes are asymmetrically expressed, indicating that both daughter cells of the zygote rapidly assume different transcriptional profiles. Subsequently, the boundaries of transcriptional domains are refined by interregional communication, resulting in the progressive elaboration of region-specific expression programs. Thus, embryonic patterning is marked by the early establishment and subsequent refinement of transcriptional domains.

MATERNAL INFLUENCES IN DEVELOPMENT

In animal embryogenesis, information from the mother is crucial for embryonic patterning. Is this also the case in plants? The observation that plants can form complete organisms from cultured cells in a process that resembles zygotic embryogenesis argues against a strict requirement for maternal information (Backs-Hüsemann and Reinert, 1970; Nomura and Komamine, 1985; Mordhorst et al., 2002). Nevertheless, several findings imply that in normal development maternal tissues do affect embryo patterning. For example, the apical–basal axis of the

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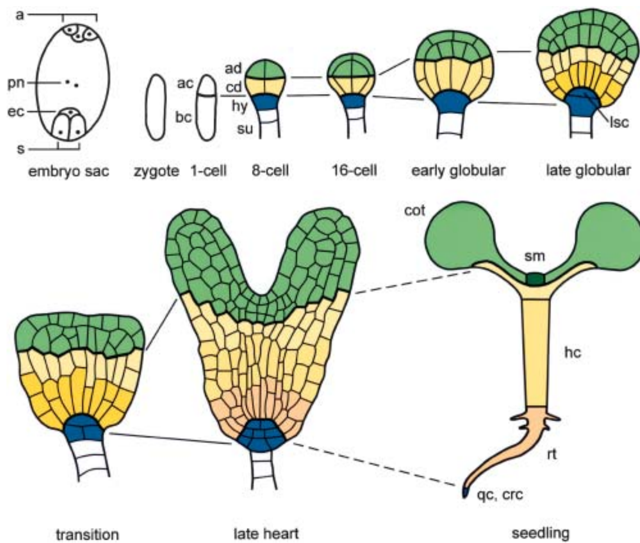

Figure 1. Apical–Basal Arabidopsis Embryo Development.

Schemes of longitudinal median sections. The upper and lower thick lines represent clonal boundaries between the descendants of the apical and basal daughter cells of the zygote and between the apical and central embryo domains, respectively. See text for details. a, antipodes; ac, apical daughter cell; ad, apical embryo domain; bc, basal daughter cell; cd, central embryo domain; cot, cotyledons; crc, central root cap; ec, egg cell; hc, hypocotyl; hy, hypophysis; lsc, lens-shaped cell; pn, polar nuclei; qc, quiescent center; rt, root; s, synergids; sm, shoot meristem; su, suspensor.

embryo is invariably aligned parallel to the chalazal–micropylar axis of the ovule, suggesting that the polarity of the embryo is guided by the surrounding maternal tissue (Esau, 1977; Mansfield and Briarty, 1991; Mansfield et al., 1991). Below, we discuss a number of genetic studies indicating that information from the female sporophyte and the female gametophyte contributes to zygotic embryogenesis.

Maternal Effects from the Female Sporophyte

Evidence for effects of the diploid mother sporophyte on the embryo were found by analyzing hypomorphic mutant alleles of the *DICER-LIKE1* (*DCL1*) gene (Ray et al., 1996; Golden et al., 2002). Although putative null alleles of *DCL1* (named *sus1* in previous studies) show a zygotic embryo-lethal phenotype (Schwartz et al., 1994), ~10% of embryos homozygous or heterozygous for a weaker *DCL1* allele (named *sin1*) display various defects in apical development (Ray et al., 1996). The latter can be rescued if the mother plant is heterozygous, but not homozygous, for *sin*, indicating a maternal component of *DCL1* function in embryo development. DCL1 localizes in the nucleus, where it is required for the production of short micro-RNA molecules that presumably are involved in gene-silencing mechanisms (Papp et al., 2003), similar to the function of DICER in animals (Bernstein et al., 2001; Ketting et al., 2001). Such micro-RNAs produced in the mother plant then could affect embryo development as direct signals or via a more indirect mechanism.

Maternal Effects from the Female Gametophyte

Several observations suggest that the two parental gene copies contribute differentially to early plant embryogenesis as a result of parent-specific imprinting. For example, heterozygosity for mutations in the *MEDEA* (*MEA*) gene results in 50% aborted embryos that cannot be rescued by one or even two copies of the paternal wild-type allele, indicating that *MEA* supports embryo development only when supplied from the female gametophyte (Chaudhury et al., 1997; Grossniklaus et al., 1998). *MEA* encodes a member of a Polycomb group protein complex that acts largely through transcriptional repression of the MADS box gene *PHERES1* (Vielle-Calzada et al., 1999; Kohler et al., 2003a, 2003b). *MEA* is expressed specifically in the central cell, the egg cell, and the synergids of the female gametophyte, but not in the male gametophyte (Vielle-Calzada et al., 1999). After fertilization, the maternal copy of MEA continues to be expressed in the developing endosperm, the early embryo, and the suspensor but is repressed thereafter. Repression of *MEA* might be mediated by an inhibitory chromatin structure, because mutations in the chromatin remodeling factor gene *DECREASE IN DNA METHYLATION1*, which results in genome-wide DNA hypomethylation, lead to activation of the paternal *MEA* gene copy in the early embryo. *MEA* expression appears to be induced again in the female gametophyte through the introduction of nicks in its promoter by the DNA glycosylase DEMETER (DEM), suggesting that activation could occur through nucleosome sliding and changes in chromatin structure (Choi et al., 2002). *DEM* expression is restricted to the central cell and the synergids, suggesting that *DEM* activates *MEA* expression in these cells directly, whereas expression of *MEA* in the egg cell may be attributable to a non-cell-autonomous function of *DEM*. Once activated, *MEA* expression may be propagated after fertilization in the embryo and the endosperm until renewed establishment of a repressive imprint.

How common is imprinting in plants? Analysis of a set of maternally expressed genes revealed the majority to have the paternal copy silenced (Vielle-Calzada et al., 2000). However, there also are examples in which both parental copies clearly were not silenced and each contributed to zygotic expression (Springer et al., 1995, 2000; Baroux et al., 2001; Weijers et al., 2001). Therefore, imprinting is unlikely to be a general mechanism; more likely, it is a specific mechanism that affects the expression of some but not all genes during plant embryo development.

What is the significance of imprinting? Analysis of imprinted loci as well as reciprocal crosses between individuals of different ploidies suggest that increased gene activity from the maternally derived genome results in the inhibition of mitosis and low seed weight, whereas extra paternally derived activity causes the opposite. However, when both copies are hypomethylated and hence active, no pronounced effect on plant development is observed (Adams et al., 2000). This is reminiscent of the ''parental conflict'' hypothesis proposed for mammals, which holds that imprinting serves to balance the allocation of

resources from the mother to the offspring (Moore and Haig, 1991) and supports the notion that imprinting is not strictly required for embryo development per se (Jaenisch, 1997).

APICAL–BASAL AXIS FORMATION

One of the earliest patterning events in plant embryogenesis is the establishment of the apical–basal axis, which can be traced back to the egg cell and the zygote. Below, we discuss our current knowledge concerning the mechanisms that regulate this process.

The First Division of the Zygote

What is the significance of the asymmetric division of the zygote? Are the morphological and/or transcriptional differences of the daughter cells linked to their different developmental fates?

One of the genes that is expressed asymmetrically in the daughter cells of the zygote, *PINFORMED7* (*PIN7*), encodes a member of the PIN family, which presumably are part of the auxin efflux transport machinery (Gälweiler et al., 1998; Friml, 2003). PIN7 is restricted to the basal daughter cell of the zygote, where it is localized at the apical cell wall and mediates the efflux of auxin into the apical cell (Friml et al., 2003). In *pin7* mutants, the failure to do so appears to cause aberrant apical cell division patterns. Mutations in *WOX2*, which is expressed specifically in the apical daughter cell, results in similar phenotypes (Haecker et al., 2004). Considering the invariant cell division pattern during early Arabidopsis embryogenesis, the timing and orientation of cell divisions is an important aspect of cell identity. Thus, asymmetric *PIN7* and *WOX2* expression is important to establish apical cell identity at this stage. In both cases, however, no marked defects can be detected after the globular stage, and mutant seedlings appear normal. The reason for this ''rescue'' of embryo development is elusive. However, because *pin7* defects are enhanced and the apical–basal organization of seedlings is severely disturbed if additional *PIN* genes are mutant, genetic redundancy might be an explanation.

The phenotype of the *gnom* (*gn*) mutant argues against the possibility that the different sizes of the daughter cells per se are important for apical–basal development: here, the zygote is less elongated and divides more randomly to give daughter cells of variable sizes (Mayer et al., 1993), but the basal cell nevertheless generates a shortened suspensor and the apical cell forms an embryo proper. Later in development, *gn* embryos can give rise to ball-shaped seedlings without any signs of apical–basal organization in the most extreme cases (Vroemen et al., 1996). *GN* (also called *EMB30*; Shevell et al., 1994) encodes a guanine nucleotide exchange factor that is required for the transport of PIN1 from an endosomal compartment to the membrane at one side of a cell (Steinmann et al., 1999; Geldner et al., 2001, 2003). One interpretation of this finding is that the subcellular localization of PIN1 determines the site of auxin efflux and thus the direction of auxin flow in the embryo. In this view, GN affects apical–basal embryo axis formation as an essential component of the vesicle transport machinery. However, because the loss of PIN1 results in defects much less severe than those in *gn*, GN function also might be important for the intracellular transport of additional yet unknown factors involved in apical–basal patterning, such as other PIN proteins.

Why do most descendants from the basal daughter cell of the zygote form a suspensor rather than an embryo, as the descendants of the apical daughter do? Several observations indicate that it is the embryo itself that represses embryonic development in the suspensor. Experimental abortion of the embryo can induce the formation of a secondary embryo from the suspensor cells (Gerlach-Cruse, 1969). Moreover, mutations in several genes result in the same effect either after the primary embryo arrests development or even if it continues to develop, resulting in polyembryony in the latter case (Schwartz et al., 1994; Vernon and Meinke, 1994; Zhang and Somerville, 1997). Interestingly, the apical–basal polarity of suspensor embryos in *twin1* mutants can be reversed to that of the primary embryo (Vernon and Meinke, 1994), suggesting that polarity information available to the primary embryo is not functional in suspensor cells.

Organization of the Shoot Apex

The shoot apical meristem is the center of postembryonic organ formation in the shoot. It can be subdivided into regions with different properties and functions (Figure 2). The central zone (CZ) contains relatively slowly dividing cells that are slightly larger and more vacuolated than the surrounding cells. It harbors the stem cell niche, consisting of the stem cells in the outermost three cell layers and the signaling niche cells, termed the organizing center (OC) (Mayer et al., 1998). *WUSCHEL* (*WUS*) activity in the OC maintains the stem cells in an undifferentiated state. The stem cells in turn express CLAVATA3 (CLV3), the putative ligand for the CLV1 receptor kinase signaling pathway (Fletcher et al., 1999), which limits the size of the OC by restricting *WUS* expression (Brand et al., 2000; Schoof et al., 2000). This regulatory feedback loop between stem cells and the OC provides a mechanistic framework to explain how the plant could dynamically assess and adjust the size of the stem cell pool (Schoof et al., 2000).

The CZ is surrounded by stem cell daughters that are unvacuolated and divide more rapidly and, based on gene expression, initiate differentiation. However, the outgrowth of lateral organs is still suppressed by *SHOOTMERISTEMLESS* (*STM*) (Long and Barton, 1998), possibly allowing the stem cell daughters to amplify to sufficient numbers before becoming allocated to organ primordia (Lenhard et al., 2002). Eventually, *STM* becomes downregulated in the organ precursor cells and the expression of organ-specific genes is initiated, resulting in the outgrowth of organ primordia from the flanks of the meristem. By contrast, outgrowth remains repressed in the cells between the lateral organs.

How is the shoot apex established during embryogenesis? Histologically, the first sign is the outgrowth of the cotyledonary primordia from the flanks of the late globular embryo. Somewhat later, the shoot meristem becomes apparent between the cotyledons by its typical three-layered structure (Barton and Poethig, 1993). Genetic and gene expression studies revealed, however, that shoot apex development is initiated much earlier and can be formally divided into three steps: (1) specification of

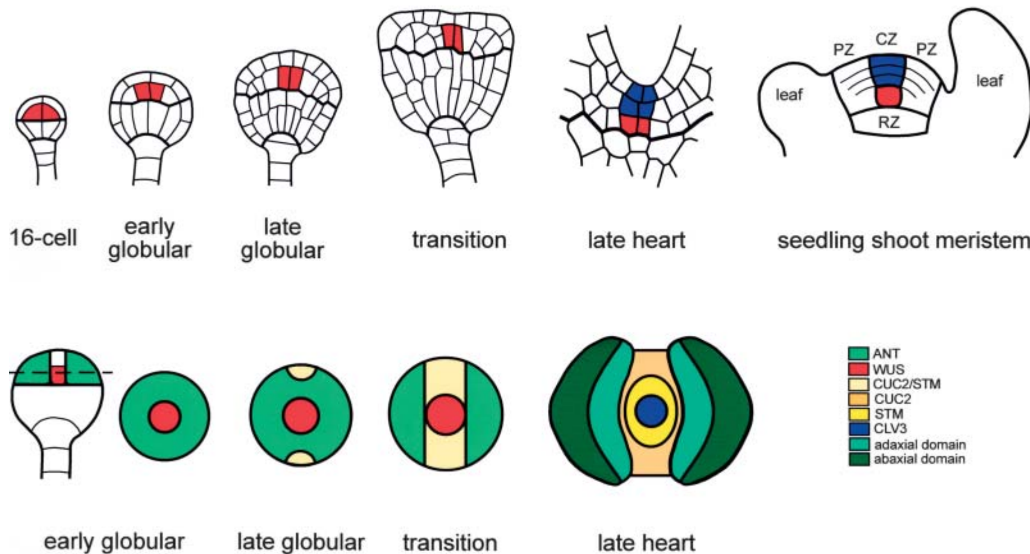

Figure 2. Development of the Apical Embryo Domain.

The top row shows schemes of longitudinal median sections. The upper and lower thick lines represent clonal boundaries between the descendants of the apical and basal daughter cells of the zygote and between the apical and central embryo domains, respectively. The bottom row shows cross-sections of the same stages as indicated by the dashed line at left. CZ, central zone; PZ, peripheral zone; RZ, rib zone. The expression domains of early genes in the apical region are shown in color as indicated. See text for details.

the apical domain, (2) initiation of the stem cell niche, and (3) central–peripheral patterning into shoot meristem and cotyledonary primordia.

Specification of the Apical Domain

Mutations in genes that are involved in the specification of the apical embryo domain are predicted to disrupt both cotyledon and shoot meristem development. This is the case in the *gurke* (*gk*) mutant, in which neither the shoot meristem nor cotyledons form properly (Torres-Ruiz et al., 1996). However, the precise role of *GK* is unknown.

Elegant temperature-shift experiments using the temperature-sensitive Arabidopsis mutant *topless* (*tpl*) demonstrated a remarkable flexibility of embryonic region identity: the apical embryo domain can be respecified until approximately the transition stage to make a root even after it had already initiated shoot development (Long et al., 2002). These apical roots morphologically resemble normal roots; however, their formation differs from that of normal embryonic roots in that they are not affected by the *monopteros* (*mp*) mutation (see below) and do not express an auxin-responsive reporter gene. Thus, it is possible that apical root formation in *tpl* embryos might use mechanisms similar to the de novo induction of postembryonic roots, for which *MP* also is dispensable (Berleth and Jürgens, 1993).

Initiation of the Stem Cell Niche

The first indication of embryonic shoot meristem initiation is the onset of *WUS* expression in the four subepidermal apical cells of the 16-cell embryo (Figure 2) (Mayer et al., 1998). Subsequently, these cells divide asymmetrically several times, establishing the *WUS* expression domain at its correct position within the developing shoot meristem primordium. *WUS* function is required for embryonic shoot meristem formation and for the expression of *CLV3* or *STM* in mature embryos (Laux et al., 1996; Mayer et al., 1998; Brand et al., 2002). Correct spatial *WUS* expression in turn requires CLV3 activity from the heart stage on, suggesting that the feedback loop that regulates meristem homeostasis already is functional at this stage (Schoof et al., 2000). The reason why *WUS* is expressed earlier is unknown, yet a plausible model is that WUS function prevents the precursor cells of the stem cell niche from entering other embryonic developmental pathways (Mayer et al., 1998; Groβ-Hardt and Laux, 2003).

Central–Peripheral Patterning of the Apical Domain

The central–peripheral patterning of the apical embryo domain delineates a central region that includes the incipient meristem primordium from peripheral regions, from which the cotyledonary primordia grow out (Figure 2).

In the center of the apical domain, outgrowth is repressed by *CUP-SHAPED COTYLEDON1* (*CUC1*), *CUC2*, and *CUC3*, allowing for the separation of the two cotyledonary primordia. Single mutants of either gene display only weak and infrequently occurring aberrant phenotypes. In double mutant combinations, however, the cells that normally separate the margins of opposite cotyledons exhibit ectopic outgrowth, which results in the fusion of the cotyledons and the loss of the shoot meristem (Aida et al.,

1997, 1999; Vroemen et al., 2003). The dynamics of the *CUC* expression pattern are intriguing. *CUC1* and *CUC2* are first expressed in isolated patches of apical cells before expression spreads into a stripe across the embryo apex that divides it into a central and two peripheral zones (Aida et al., 1999; Takada et al., 2001). This suggests that *CUC* expression does not simply reflect a preexisting pattern but may be involved in generating bilateral symmetry. The *CUC* genes encode putative transcription factors homologous with the petunia NO APICAL MERISTEM (NAM) protein, which also affects cotyledon separation and shoot meristem formation (Souer et al., 1996). Both *cuc* and *nam* mutants show similar defects during flower development, indicating related mechanisms for organ separation in embryos and flowers.

The spatial expression patterns and the functions of *CUC1* and *CUC2* require *MP* and *PIN1* activities, which are both implicated in auxin signaling (Aida et al., 2002). This indicates a role for auxin signaling in the patterning of the apical embryo domain, consistent with previous findings from physiological experiments (Liu et al., 1993; Hadfi et al., 1998). CUC functions, on the other hand, activate *STM* expression in the central stripe across the embryo (Aida et al., 1999). *STM* in turn promotes *CUC1* activity together with *PIN1* and is necessary for the correct spatial expression of *CUC2* (Aida et al., 1999, 2002). In heart-stage embryos, *STM* and *CUC* genes eventually assume complementary expression patterns that reflect the delineation of the shoot meristem primordium within the central stripe, with *STM* being restricted to the incipient shoot meristem and *CUC* to the boundaries between the shoot meristem and the cotyledons. Concurrently, the expression of *AINTEGUMENTA*, which is found in a ring around the circumference of the apical domain in globular embryos, becomes restricted to the incipient cotyledonary primordia, and organ-specific gene expression is initiated (Long and Barton, 1998).

Together, these activities establish a regulatory network of transcription factors within the apical embryo domain separating a central stripe in which organ-promoting genes are repressed and a peripheral zone in which outgrowth of the cotyledonary primordia occurs. Even though many similarities exist, this process differs from that of postembryonic leaf formation in that the cells of the cotyledonary primordia are not derived from the stem cell niche but are initiated simultaneously.

Competence to Form a Shoot Meristem

How is the position of the shoot meristem determined? Several lines of evidence indicate that signals from surrounding cells, the cotyledonary primordia and the underlying vascular primordium, are crucial for this process.

Soon after cotyledonary primordia bulge out, their adaxial–abaxial polarity becomes evident by specific gene expression patterns (Siegfried et al., 1999; Kerstetter et al., 2001; McConnell et al., 2001; Otsuga et al., 2001). Mutations that transform adaxial fates into fates normally restricted to the abaxial side interfere with shoot meristem formation. By contrast, a gain-of-function mutation in *PHABULOSA* (*PHB*) that promotes adaxial cell fates increases the size of the embryonic shoot meristem and can partially rescue organ formation in the *stm* mutant (McConnell and Barton, 1998). Together, these data suggest that adaxial cells of the adjacent cotyledonary primordia provide meristem-promoting signals or, alternatively, block meristem-inhibiting signals emanating from cells at the abaxial side. The nature of such signals is unknown. However, the presence of putative lipid/sterol binding domains in REVOLUTA and PHB suggests that small-molecule signals might be involved (McConnell et al., 2001; Otsuga et al., 2001).

The shoot meristem is localized on top of the developing vasculature, which plausibly links the future transport of nutrients to the apical growth point (Figure 3). Interestingly, during the divisions of OC precursor cells, *WUS* expression always is restricted to those daughter cells next to the vascular primordium. This raises the question of whether communication between the forming vasculature and the shoot meristem coordinates their development.

Analysis of *ZWILLE* (*ZLL*; *PINHEAD*) function might allow this question to be addressed. *zll* embryos display a range of phenotypes in which *STM* is expressed aberrantly and differentiated structures are formed in place of the shoot meristem (Jürgens et al., 1994; McConnell and Barton, 1995; Moussian

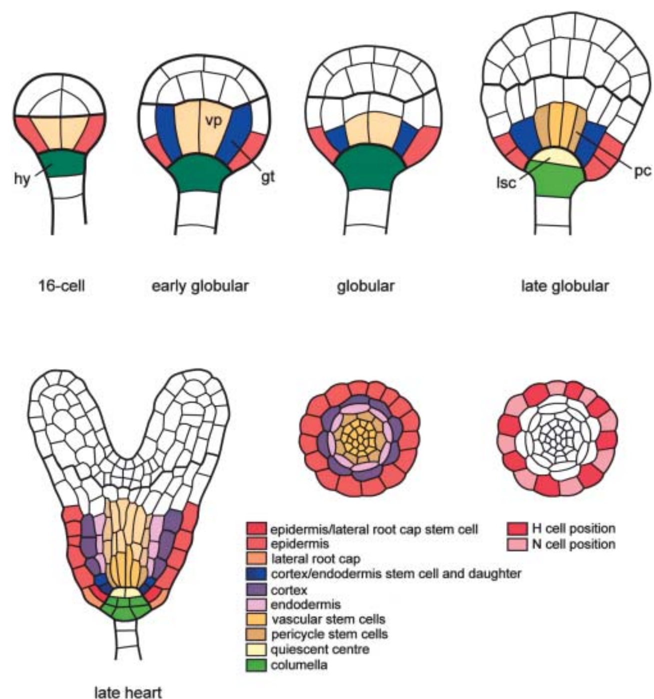

Figure 3. Development of the Radial Pattern.

The top row and the illustration at bottom left show schemes of longitudinal sections; the other illustrations in the bottom row show schemes of cross-sections through a root. The upper and lower thick lines represent clonal boundaries between the descendants of the apical and basal daughter cells of the zygote and between the apical and central embryo domains, respectively. Cell types are shown in color as indicated. Vascular and pericycle cells are shown in lighter colors than stem cells. See text for details. gt, ground tissue; hy, hypophysis; lsc, lens-shaped cell; pc, pericycle; vp, vascular primordium.

et al., 1998; Lynn et al., 1999). Ectopic *ZLL* expression on the abaxial side of cotyledon primordia in a *zll* background results in the transformation of cotyledons into shoot axes with a meristem at its tip (Newman et al., 2002). In the wild type, *ZLL* is expressed initially in all cells of the early embryo and later becomes restricted to the vascular primordium (strong expression) and to the shoot apex and the adaxial sides of the cotyledons (weak expression). *ZLL* encodes a member of the PIWI ARGONAUTE ZWILLE (PAZ) family, which is conserved in animals and plants and several members of which have been implicated in RNA interference (Cerutti et al., 2000; Carmell et al., 2002). Embryos that are doubly mutant for *ZLL* and the ubiquitously expressed related *ARGONAUTE1* (*AGO1*), which is involved in post-transcriptional gene silencing (Fagard et al., 2000), display a synergistic phenotype, suggesting that both proteins have partially overlapping functions (Lynn et al., 1999). Together, these data indicate that ZLL activity in surrounding cells promotes a favorable environment for meristem cell fates in the embryo apex.

In summary, patterning the embryo apex involves the successive establishment of the stem cell niche and of the network that regulates organ formation. Both processes appear to be initiated independently, based on expression studies of *WUS* and *STM*, but each requires the other for ongoing shoot meristem activity (Mayer et al., 1998). About halfway through embryo development, the components that govern postembryonic meristem activity are in place and, as judged by the appearance of the first leaf primordia, the shoot meristem commences its activity.

The Central Embryo Domain

The cells of the central embryo domain first divide horizontally to give apical descendants that will contribute to the base of the cotyledons and basal descendants that will form hypocotyl, embryonic root, and proximal stem cells of the root meristem (Figure 1) (Scheres et al., 1994).

Analysis of *FACKEL* (*FK*), *HYDRA1*, and *STEROL METHYLTRANSFERASE1/CEPHALOPOD* activities indicate important roles of sterols for proper embryo development (Topping et al., 1997; Diener et al., 2000; Jang et al., 2000; Schrick et al., 2000, 2002). The earliest defects in the respective mutants are the failure of cells in the central embryo domain to elongate and to divide asymmetrically to form the vascular primordium. Later in development, mutant embryos also show abnormalities in the apical and basal domains and often form multiple shoot meristems and cotyledons.

In which way could sterols be involved in embryo development? One possibility is that they act as structural membrane components, exemplified by disturbed cell polarity and auxin transport in mutants defective for the biosynthesis of major membrane sterols (Willemsen et al., 2003). Alternatively, steroid molecules may function as signaling molecules. The finding that the brassinosteroid receptor BRASSINOSTEROID-INSENSITIVE1 (Sakurai and Fujioka, 1997) acts downstream of FK to promote postembryonic growth is in agreement with this possibility (Schrick et al., 2002). However, because *FK* acts independently of other genes involved in brassinosteroid synthesis, sterol signals other than brassinosteroids could be involved in cell expansion in the vascular primordium.

The Basal Embryo Domain (Hypophysis)

In the root meristem, the quiescent center (QC), a group of nondividing cells, maintains the undifferentiated state of the surrounding stem cells by local signaling (Figure 3) (van den Berg et al., 1997; Sabatini et al., 2003). Therefore, the QC appears to fulfill an analogous function to that performed by the OC in the shoot meristem. The position of the QC, on the other hand, depends on the presence of an auxin maximum in the columella, termed "distal organizer," which is maintained by basipetal auxin transport (Sabatini et al., 1999; Friml et al., 2002, 2003).

Each stem cell gives rise to a file of differentiating cells. Accumulating data support the notion that it is not the stem cells, but signals from adjacent more mature cells in each file, that determine the fate of the differentiating daughters (van den Berg et al., 1995; Malamy and Benfey, 1997). Thus, root meristem activity is maintained by a balance between signals from the QC inhibiting stem cell differentiation and opposing signals from the more mature tissues instructing differentiating descendants.

During embryonic root meristem formation, the proximal stem cells are derived from the most basal cells of the central embryo domain, whereas the QC and the stem cells of the central root cap are derived from the basal embryo domain, the hypophysis (Figure 3) (Mansfield and Briarty, 1991; Jürgens and Mayer, 1994; Scheres et al., 1994). The establishment of the root meristem coincides with an accumulating auxin maximum in the hypophyseal region. Failure to properly establish this auxin maximum results in aberrant specification of root meristem cell fates (Sabatini et al., 1999; Friml et al., 2002, 2003). At the globular embryo stage, the hypophysis undergoes an asymmetric cell division, producing an upper lens-shaped cell that will form the QC and a lower daughter cell that will give rise to the columella stem cells. This asymmetric division is marked by the expression of *SCARECROW* (*SCR*) and *WOX5*, first in the hypophyseal cell and subsequently only in the lens-shaped cell and the QC, suggesting that QC identity is established at approximately this time (Wysocka-Diller et al., 2000; Haecker et al., 2004). Early defects in QC development often are followed by abnormal differentiation of the neighboring stem cell precursors (Willemsen et al., 1998), suggesting that the QC signaling not only maintains stem cell identity in the active root meristem but also is crucial for the initiation of stem cells in the embryo. These observations suggest that the stem cell niches of the root and the shoot pole not only are functionally equivalent but also share molecular and developmental similarities.

Genetic studies of two genes involved in auxin response, *BODENLOS* (*BDL*) and *MP*, suggest that early root development depends on gene activities in the embryo proper. *MP* encodes a putative transcription factor related to auxin response factors (Hardtke and Berleth, 1998). MP is thought to be kept inactive by binding to the indoleacetic acid (IAA) protein BDL, which is degraded upon intracellular auxin signaling, allowing MP to activate its target genes (Hamann et al., 2002). The earliest defects in *mp* and *bdl* mutants appear in the apical daughter cell of the zygote, which divides horizontally instead of vertically,

leading to octant-stage mutant embryos consisting of four instead of two tiers of cells (Berleth and Jürgens, 1993; Hamann et al., 1999). Additionally, in the eight-cell embryo, *WOX9* expression fails to be shifted from the hypophysis to the central embryo domain, as it is in the wild type, suggesting *WOX9* as a target of BDL/MP-dependent signaling (Haecker et al., 2004). Later, the cells of the central domain divide abnormally and the hypophysis fails to produce the lens-shaped cell, resulting in seedlings lacking the root, the root meristem, and the hypocotyl in the strongest phenotypes. Because *MP* and *BDL* are expressed only in the embryo proper at the time when the hypophysis develops aberrantly in the mutants, the MP- and BDL-mediated auxin response in embryo proper cells appears to be required for signaling from the embryo proper to the subjacent hypophysis to allow for normal root development.

Not surprisingly, auxin response appears to be important also in the basal cell lineage for root development. In contrast to *mp* and *bdl*, the first defects in *hobbit* (*hbt*) and *auxin-resistant6* (*axr6*) embryos are aberrantly orientated cell divisions in the derivatives of the basal daughter cell of the zygote; subsequently, no root meristem is formed (Willemsen et al., 1998; Hobbie et al., 2000). *HBT* encodes a CDC27 homolog that in yeast and animals is part of an anaphase-promoting complex that controls M-phase progression through targeted proteolysis. *hbt* mutants accumulate the IAA17 repressor of auxin response and show reduced expression levels of an auxin-inducible reporter gene, suggesting a functional link between proper cell cycle progression and differentiation (Blilou et al., 2002). *AXR6* encodes a subunit of the ubiquitin protein ligase SKP1/CULLIN/F-BOX PROTEIN and could be involved in the degradation of IAA proteins in response to auxin signaling (Hellmann et al., 2003).

FORMATION OF THE RADIAL PATTERN

The first manifestation of a radial (inner–outer) pattern consisting of vasculature, ground tissue, and epidermis is apparent in octant-stage embryos when tangential cell divisions delineate the protoderm from the inner cells (Figure 3). Subsequently, periclinal divisions in the basal tier of cells in the central embryo domain successively establish concentric layers of tissue primordia in the developing hypocotyl and root. The first divisions produce the central vascular primordium and a layer of ground tissue that surrounds it. Later, the vascular primordium splits into the central vasculature and a surrounding layer of pericycle cells, and the ground tissue splits into an inner endodermis layer and an outer cortex layer (Scheres et al., 1995). This radial pattern is modified at two positions along the apical–basal axis: in the hypocotyl region, additional periclinal divisions result in two layers of cortex, and in the root meristem, the epidermal stem cell divides periclinally to give the epidermis and the lateral root cap.

The specification of root cell types is independent of the appearance of the root meristem, consistent with the view that patterning information is imposed on the root meristem stem cells by more differentiated cells (Scheres et al., 1995; Malamy and Benfey, 1997). Once established, the radial pattern is precisely maintained during postembryonic growth by orientated cell divisions.

Establishment of the Radial Axis

The first critical event in radial patterning that can be observed are the cell divisions that separate protoderm from inner cells. Why do the cells derived from these divisions enter different pathways? One interesting observation made in *Citrus jambhiri* is that the zygote and subsequently all cell walls at the surface of the embryo are coated with a cuticle layer that serves as a morphological marker for epidermal identity (Bruck and Walker, 1985). An attractive hypothesis derived from this observation holds that an as yet unidentified component conferring epidermal fate to the attached protoplasts is contained within the cell wall of the zygote and propagated to every progeny cell having a cell wall contiguous to that of the zygote, whereas all inner cells assume subepidermal fates. A role of cell wall–located components in cell fate regulation also has been proposed based on ablation experiments in *Fucus* embryos (Berger et al., 1994).

The early expression patterns of two Arabidopsis homeobox genes, *ARABIDOPSIS THALIANA MERISTEM LAYER1* (*ATML1*) and *PROTODERMAL FACTOR2* (*PDF2*), are consistent with this hypothetical mechanism. Both genes are expressed at early stages in all embryo proper cells that possess an outer cell wall contiguous with the cell wall of the zygote (Lu et al., 1996; Abe et al., 2003). Upon the tangential cell divisions at the eight-cell stage, the protoderm continues its expression, whereas the inner cells gradually cease expression. Later expression is confined to the epidermis of the shoot apex and young primordia. Although the single mutants are aphenotypic, double-mutant embryos have an irregular surface in the apical domain and lack the layered organization of the shoot meristem. Upon germination, the double mutants appear to lack an epidermis, resulting in leaves with mesophyll cells at the surface (Abe et al., 2003). *ATML1* and *PDF2* encode homeodomain transcription factors containing a START domain, which is implicated in lipid/sterol binding. Both factors bind to an 8-bp *cis*-regulatory element that confers epidermis-specific gene expression and that is present also in the promoters of *ATML1* and *PDF2*, suggesting that both genes regulate their own expression in addition to that of other target genes. Therefore, it is plausible that epidermal cell identity is stably propagated by a positive feedback loop, which would necessitate, however, the active repression of both genes in the subepidermal cells after protoderm formation.

Radial Organization of the Root and Hypocotyl

Embryos mutant for *WOODEN LEG* (*WOL*) omit one round of tangential cell divisions in the vascular primordium, resulting in a reduced number of cells in the vascular cylinder of the root and hypocotyl. All cell files differentiate into protoxylem, whereas phloem is missing (Scheres et al., 1995; Mahonen et al., 2000). *WOL* encodes a two-component hybrid signal transducer that is expressed in the vascular primordium of the embryo and later in the vascular cylinder and pericycle (Mahonen et al., 2000). It is allelic to *CYTOKININ RECEPTOR1* (*CRE1*) (Inoue et al., 2001) and is activated by extracellular cytokinin (Hwang and Sheen, 2001). In analogy to bacterial two-component signaling pathways, *WOL/CRE1* after perceiving cytokinin signals on the cell surface could transmit the signal to the nucleus to regulate the

expression of genes that control cell divisions in the vascular primordium.

The vascular cylinder and the pericycle in turn regulate the development of the surrounding ground tissue by producing SHORT ROOT (SHR), a putative transcription factor (Helariutta et al., 2000). Elegant experiments suggest that it is SHR itself that moves into the ground tissue, where it activates the expression of the related transcription factor *SCR* (Di Laurenzio et al., 1996; Nakajima et al., 2001). The movement appears to be restricted to cells directly adjacent to those that transcribe *SHR*, explaining why only the immediate neighbors of the stele, but not the next cell layer, differentiate into endodermis. *scr* and *shr* mutants lack asymmetric cell divisions of the ground tissue, resulting in a single cell layer where normally endodermis and cortex are formed.

Generally, the failure of a mutant to produce a given pattern element may be attributable to either the inability to provide the correct cell fate information or the failure to generate the cells necessary for that element. Genetic studies between radial patterning mutants and the *fass* (*fs*) mutant, which causes an increased number of radial cell layers (Torres-Ruiz and Jürgens, 1994), address this issue. The *fs* mutation is able to rescue the defects of *scr* and *wol*, suggesting that neither *SCR* nor *WOL* is required for cell fate specification (Scheres et al., 1995; Mahonen et al., 2000); rather, they are needed to regulate the number of cell layers. In agreement with this argument, the single cell layer in *scr* expresses characteristics of both cortex and endodermis (Di Laurenzio et al., 1996). The lack of phloem in *wol* mutants could be explained by a hierarchical first-come–first-served mechanism in which the specification of one cell type (xylem) precedes that of another (phloem) and in which the latter cannot occur if all cells are consumed by the former. By contrast, *fs* rescued only the reduced number of cell layers in *shr* mutants but not endodermal cell fate (Scheres et al., 1995). Along with the observation that the single layer in *shr* mutants has only cortex identity, this finding indicates that SHR acts both to promote the asymmetric cell divisions via SCR and to specify endodermal cell fate via a yet unknown mechanism (Helariutta et al., 2000).

Patterning the Circumference

By the heart stage, both the apical–basal and the radial organization of the embryo have been defined. Yet another patterning event distinguishes between epidermal cell fates in root and hypocotyl with respect to their circumferential position: cells that are in contact with the intercellular space of two underlying cortex cells (the H position) will differentiate into hair cells in the root and stomata in the hypocotyl, whereas cells that are in contact with only one cortical cell (the N position) differentiate into nonhair cells and nonstomatal cells (Figure 3). The origin of this patterning information is unknown, but one possibility is that signals emanating from the intercellular space between cortex cells set up the initial difference. Subsequently, a network of genes is activated that specifies non-root-hair fate while at the same time laterally suppressing it in its neighbors (Schiefelbein, 2003). One of the components of this network, the homeodomain transcription factor *GLABRA2* (*GL2*), has been used to monitor epidermal patterning during embryogenesis (Lin and Schiefelbein, 2001; Costa and Dolan, 2003). In seedlings, *GL2* is expressed specifically at the N position and confers nonhair fate to root cells and nonstomata fate to the hypocotyl. During embryogenesis, *GL2* is expressed first in single protodermal cells at the root pole in heart-stage embryos. At the torpedo stage, expression in the hypocotyl region already is confined to the N position. By contrast, expression in the root region expands through the entire epidermis and only later becomes restricted to the N position, indicating that initially equivalent cells become diverse in response to positional cues.

MECHANISMS THAT ESTABLISH CELL FATE IN THE EMBRYO

Generally, two mutually nonexclusive mechanisms can be envisioned for how different cell fates segregate during embryonic cell divisions: (1) asymmetric divisions generate daughter cells that inherit different developmental determinants, and (2) initiation of different developmental pathways in initially equal daughter cells by differential positional cues. Which of these mechanisms functions in plant embryo patterning?

Differential Inheritance of Determinants

The polar localization of molecules before cell division and their asymmetric segregation into daughter cells have been demonstrated to initiate different developmental fates in bacteria, fungi, and animals (Jacobs and Shapiro, 1998). In plants, the divisions of the zygote, the hypophysis, and the octant-stage cells are examples of asymmetric divisions in the embryo that generate daughter cells with morphological differences that develop into different cell types. In several cases, the division of cells expressing mRNAs of developmental regulators, such as *WUS* and *SCR*, segregates one daughter cell that continues to express the mRNA and one that lacks its expression (Mayer et al., 1998; Wysocka-Diller et al., 2000). If initial symmetric expression in both daughter cells is never observed, it is plausible that mRNAs or regulators of their expression have been localized in a polar manner in the dividing cell and subsequently inherited asymmetrically by its daughters. Thus, only indirect evidence suggests differential inheritance of molecules during cell division in plants to date, and polar localization of such components before cell separation has yet to be demonstrated. It is noteworthy that such a mechanism would not imply a cell-autonomous mode of cell fate specification, because polarization of the dividing cell conceivably can depend on positional cues.

Pathways of Cell–Cell Communication

In many cases, daughter cells of embryonic cell divisions initially appear equal and only subsequently seem to establish different gene expression patterns. How do early embryo cells perceive information that enables them to enter different developmental pathways?

In animals, cell–cell communication involving extracellular signals and cell surface–bound receptors plays an important role in cell fate decisions during embryogenesis (Johnston and

Nüsslein-Volhard, 1992). Probably the best characterized signaling events in embryo patterning are those that involve auxin as a signaling molecule. Many examples demonstrate an important role for cellular auxin response in different parts of the embryo and at different stages, raising the question of how auxin is distributed in space and time during embryogenesis. Recent analysis of the PIN protein family and monitoring of auxin and the auxin response provided significant novel insights into this issue. A first auxin maximum is established in the apical daughter cell of the zygote by PIN7-mediated transport of auxin from the basal cell (Friml et al., 2003). At the globular embryo stage, the direction of net auxin flow appears to become reversed from apical to basipetal, establishing a new auxin maximum in the hypophysis. This step is accomplished by the dynamic localization of PIN1, PIN4, and PIN7 (Steinmann et al., 1999; Friml et al., 2002, 2003). Later in embryo development, auxin flow appears to be directed toward the tips of the cotyledons and other organs, which supports a role for auxin as a general distal organizer, and this accumulation is reflected by apically orientated PIN1 localization (Sabatini et al., 1999; Benkova et al., 2003; Reinhardt et al., 2003). Although the source of auxin often remains elusive, these results indicate that a dynamic distribution of auxin during embryogenesis is accomplished by the changing localization of the cellular auxin efflux machinery.

How can diverse effects be explained by the action of a simple molecule such as auxin? One possibility is that cells are already predetermined to respond to auxin in a cell-specific manner and that the presence of auxin triggers this preset pathway. The large variety and the specific expression patterns of proteins involved in the auxin response are consistent with this hypothesis (Kepinski and Leyser, 2002). Alternatively, auxin may induce only a few generic primary cellular responses, such as cell elongation, which in turn could direct the timing and orientation of cell divisions and thus contribute to patterning.

Stable Separation of Cell Fates

Plant cells are effectively coupled via plasmodesmata that permit the facilitated exchange of molecules, including even transcription factors (Lucas et al., 1995; Wu et al., 2003). This poses the question of how important is the separation of cells for cell fate segregation and to what extent does a single cell or nucleus represent a functional unit in plant development.

Cellular separation is disturbed in *knolle* (*kn*) and *keule* mutants that fail to properly form a cell plate during cytokinesis, resulting in embryos with multinucleate cells (Lukowitz et al., 1996; Assaad et al., 2001). Both genes encode components of the vesicle fusion apparatus, suggesting a role in the transport of material to the site of cell wall formation during cytokinesis. Based on marker gene expression, the segregation of protoderm and inner cell fate are affected in early *kn* embryos (Lukowitz et al., 1996; Vroemen et al., 1996), suggesting that cell separation is a prerequisite for the stable segregation of cell fate determinants. Studies of cell–cell transport demonstrated that plasmodesmal coupling of cells is not always present but is region specific and dynamic (van der Schoot and Rinne, 1999) and that movement through plasmodesmata is possible for some molecules but excluded for others (Lucas et al., 1995). Therefore, it is conceivable that preferential and selective coupling of embryo cells restricts the movement of cell fate determinants to cells within a given group, allowing the acquisition of a developmental fate different from that of an adjacent group of cells.

PERSPECTIVES

Although analyzing embryo development in plants is not easy because of the inaccessibility of the embryo, our understanding of the mechanisms underlying plant embryogenesis has been enhanced considerably during the last decades by using Arabidopsis as a model.

A curious observation is that many mutants disturbed in embryonic patterning display abnormal cell divisions as their earliest defect. In many cases, however, these can be compensated for, at least in part, at later stages when the embryo has reached a higher cell number by the activation of alternative pathways that are known to function in postembryonic tissue regeneration. This is consistent with long-standing observations derived from plant regeneration studies that the ability to organize into a body plan is a more general property of plant cells that is executed during Arabidopsis embryogenesis in a stereotypic manner but that can be reached in many alternative ways. In this view, the stereotypic embryonic cell division pattern might simply ensure that sufficient numbers of cells are present to allow for the most rapid and early establishment of the body plan that could provide an advantage in natural habitats.

Many important functions likely remain to be detected as a result of functional redundancy or embryo lethality. Molecular and reverse genetics approaches have begun to add new players to an increasingly comprehensive regulatory network. In the future, global RNA and protein profiling will enable us to assess the molecular basis of cellular states (i.e., in embryonic versus differentiated cells), and improvement of the tools used to visualize intracellular processes in embryos will allow the assessment of the dynamics of regulatory processes. Future challenges will be to identify the origin of patterning information, such as the mechanisms that establish specific transcriptional zones and regulate cell fate segregation, and to identify effector processes that translate pattern information into cell types.

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