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Pattern formation in the *Arabidopsis* embryo: a genetic perspective

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SUMMARY

During embryogenesis, a single cell gives rise to different cell types, tissues and organs which are arranged in a biologically meaningful context, or pattern. The resulting basic body organization of higher plants, which is expressed in the seedling, provides a reference system for postembryonic development during which the meristems of the shoot and the root produce the adult body. The seedling may be viewed as the superimposition of two patterns: one along the apical–basal axis of polarity and the other perpendicular to the axis. To analyse mechanisms underlying pattern formation in the embryo, a genetic approach has been taken in *Arabidopsis*. Mutations in a small number of genes alter one or the other of the two patterns. The mutant phenotypes suggest that early partitioning of the axis is followed by region-specific development, including the formation of the primary shoot and root meristems. The cloning of two genes involved in pattern formation provides a basis for mechanistic studies of how cells adopt specific fates in the developing embryo.

1. INTRODUCTION

Sexually reproducing multicellular organisms pass through a single-cell stage in their life cycle. The selective advantage of being multicellular, which involves the division of labour among the different cell types, tissues and organs of the adult form, is reached by development. Starting from the fertilized egg cell, embryogenesis has to build up the body organization. Although in animals this process of pattern formation is confined to embryogenesis, higher plants follow a two-step strategy: embryogenesis generates an intermediate form, the seedling, which is transformed into the adult plant by the addition of new structures from localized growth centres, or meristems (see Steeves & Sussex 1989, for review). In contrast to the species-specific adult forms, seedlings from different species share essentially the same body plan, which may reflect developmental constraints on pattern formation in the embryo. The primary shoot and root meristems also originate at defined positions in the embryo and may acquire specific organizational features that guide their activities during post-embryonic development.

Here, we discuss pattern formation in the plant embryo from a genetic perspective, using *Arabidopsis* as a model. Studying pattern formation in the *Arabidopsis* embryo has two main advantages. First, cell divisions are very regular in the early embryo such that seedling structures can be traced back to their origins (Mansfield & Briarty 1991; Jürgens & Mayer 1994). Second, *Arabidopsis* is well-suited for a genetic dissection of pattern formation as a first step towards the analysis of underlying mechanisms.

2. THE EMBRYONIC ORIGIN OF THE SEEDLING BODY

The seedling body may be viewed as the superimposition of an apical–basal pattern along the main body axis and a radial pattern perpendicular to this axis. The apical–basal pattern is a top-to-bottom array of distinct structures: shoot meristem, cotyledons, hypocotyl, root and root meristem. The radial pattern is arranged in concentric layers of tissue types: epidermis, ground tissue (cortex, endodermis), vascular tissue (pericycle, xylem and phloem). These two simple patterns are laid down during embryogenesis, with the decisive events taking place early.

(*a*) *Development of the apical–basal pattern*

The zygote divides asymmetrically, giving two daughter cells of unequal sizes and different fates (see figure 1). The small apical cell will generate almost the entire embryo whereas the large basal cell will contribute to the root meristem but mainly produce a filamentous extra-embryonic suspensor. The apical cell undergoes three rounds of cleavage divisions, two vertical and one horizontal, resulting in a two-tiered proembryo. The basal cell has meanwhile produced, by repeated horizontal divisions, a file of about seven cells of which the uppermost one (hypophysis) participates in embryogenesis. Thus, three regions can be distinguished along the apical–basal axis of the early embryo: apical and central, which correspond to the two tiers of the proembryo, and basal, which is founded by the hypophysis (see figure 1*b*). As the globular embryo is growing, these three regions develop in

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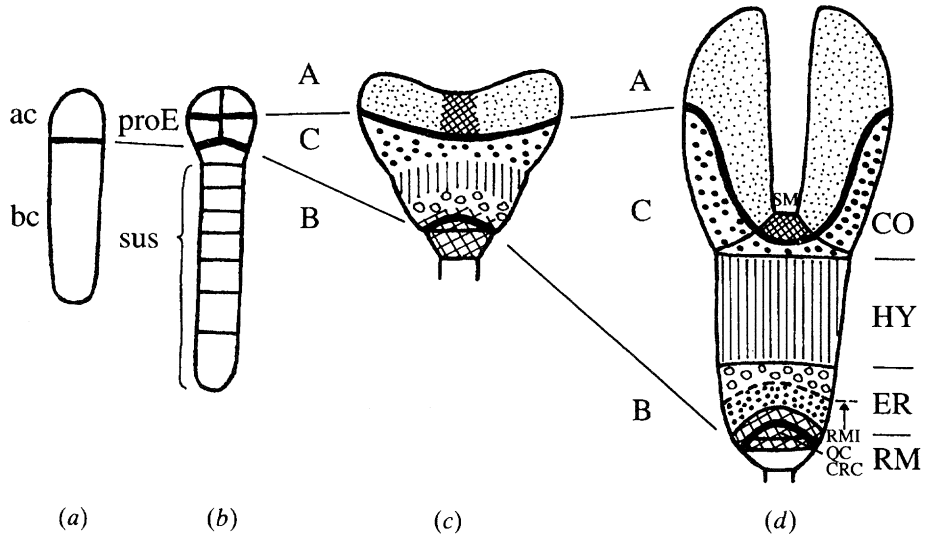

Figure 1. Development of the apical–basal pattern in the *Arabidopsis* embryo. (*a*) Asymmetric division of the zygote gives a small apical (ac) and a large basal (bc) cell. (*b*) Eight-cell stage. The proembryo (proE) consists of two tiers each of four cells (A, C) and is connected to the extra-embryonic suspensor (sus) via the founder cell of the basal region (B) of the embryo. (*c*) Heart-stage embryo. Approximate locations of primordia of seedling structures are indicated. (*d*) Torpedo-stage embryo. The dashed line indicates the upper end of the embryonic root derived from the root meristem initials (RMI). Below the quiescent centre (QC) of the root meristem are the initials of the central root cap (CRC). Early regions: A, apical; B, basal; C, central (separated by bold lines). Primordia of seedling structures: CO, cotyledons; HY, hypocotyl; ER, embryonic root; RM, root meristem; SM, shoot meristem.

distinct ways. The cells of the apical region divide without preferential orientation. By contrast, the cells of the central region produce files of cells which elongate the apical–basal axis. The founder of the basal region divides asymmetrically, giving a small lens-shaped cell, which is the precursor of the quiescent centre of the root meristem, and a larger trapezoidal cell, which generates the initials of the central root cap.

The end of the early phase of embryogenesis is marked by a change of symmetry from radial to bilateral, which is associated with the appearance of cotyledonary primordia (see figure 1*c*). This change is initiated by mitotic activity at two sites within the apical region. Continuing cell divisions cause the outgrowth of the cotyledonary primordia, rendering the embryo torpedo-shaped (see figure 1*d*). At this stage, the presumptive shoot meristem can be distinguished as a small group of cells between the bases of the cotyledonary primordia (Barton & Poethig 1993). The axis of the embryo elongates further by oriented cell divisions and, increasingly, by the addition of cell tiers from the root meristem initials above the quiescent centre (see figure 1*d*). The meristem-derived root is marked by a surface layer of lateral root cap cells. Below the quiescent centre, the initials of the central root cap produce cell layers which merge with the lateral root cap.

How the three early regions (apical, central and basal) relate to the elements of the apical–basal pattern in the seedling has been studied by clonal analysis (see figure 1*c*, *d*; Scheres *et al.* 1994*a*). The apical region gives rise to the shoot meristem and most, but not all, of the cotyledons. Specifically, the lower edge of the cotyledons ('shoulder' region) is derived from the central region. The hypocotyl and the upper part of the root ('collet') are produced by intercalary cell divisions in the central region. By contrast, the remainder of the root results from mitotic activity of the root meristem initials which themselves are the lower-most descendants of the central region. The basal region provides the quiescent centre of the root meristem and the initials that produce the layers of the central root cap (Dolan *et al.* 1994). Because the cotyledons and the root meristem each derive from two clonally distinct regions cell ancestry appears not to play a major role in forming the apical–basal pattern of the seedling. Moreover, *fass* (*fs*) embryos do not display the very regular cell divisions that characterize the wild-type embryo and yet give the normal seedling pattern (Torres Ruiz & Jürgens 1994). Thus *Arabidopsis* seems to be akin to other plant species in that the same overall seedling pattern is established regardless of how the cells divide in the embryo (see Johri *et al.* 1992, for overview).

(*b*) *Development of the radial pattern*

Radial patterning involves two choices of cell division: periclinal (radial), which gives a new tissue layer, and anticlinal (circumferential), which expands the tissue layers already established (see figure 2). Initially, periclinal divisions within the eight-celled proembryo give an outer layer of epidermal precursor cells and an inner cell mass (see figure 2*a*). Anticlinal divisions then maintain the integrity of the outer cell layer in the growing embryo. Further development of the radial pattern is confined to the central region of the apical–basal axis (see figure 2*b–d*). Shortly after the formation of the epidermis primordium, the inner cells divide periclinally to give vascular precursor cells in the centre and a surrounding layer of ground tissue (see figure 2*b*). Although the ground tissue undergoes

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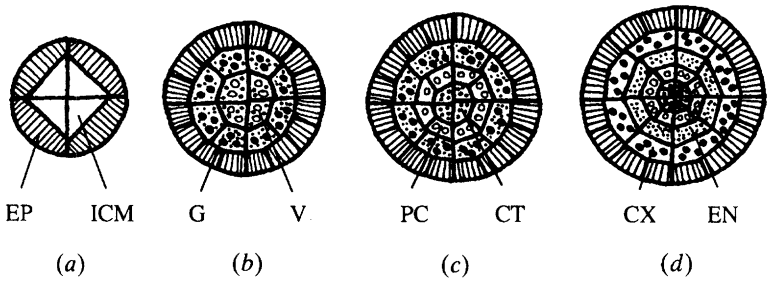

Figure 2. Development of the radial pattern in the *Arabidopsis* embryo. (*a*) Dermatogen stage. Periclinal (radial) divisions within the eight-celled proembryo (see figure 1*b*) give the outer epidermis layer (EP) and an inner cell mass (ICM). (*b*) Globular embryo. The inner cell mass has split into the ground tissue (G) and the centrally located vascular primordium (V). (*c*) Heart-stage embryo (see figure 1*c*). The pericycle layer (PC) surrounds the primordium of the conductive tissue (CT). The basic organization of the radial pattern is complete. (*d*) Torpedo-stage embryo (see figure 1*d*). Periclinal divisions in the ground tissue generate an outer cortex (CX) and an inner endodermis layer (EN). Schematic cross-sections through region C (see figure 1) of embryo (in *a*, *b*) and through the root primordium (in *c*, *d*).

anticlinal cell divisions, the vascular precursor cells produce a layer of pericycle cells surrounding the presumptive conductive tissue (phloem and xylem). This basic organization of the radial pattern is complete by the heart stage (see figure 2*c*; Scheres *et al.* 1995). Cell numbers may increase within the tissue layers, depending on the apical–basal level, and the presumptive hypocotyl forms an additional layer of ground tissue. Finally, the ground tissue gives rise to an inner layer of endodermis and an outer layer of cortex cells (see figure 2*d*).

(*c*) *Development of the primary meristems in the embryo*

The two patterns converge in the polar regions of the embryo where the primary meristems of the root and the shoot are formed. The root meristem initials display essentially the same basic organization of the radial pattern as the embryonic root: one layer each of epidermis, ground tissue and pericycle surrounding a core of conductive precursor cells (Dolan *et al.* 1993). Within the outer two layers, each initial generates, by periclinal division, two adjacent cells of different fates which then produce cell files: epidermis and lateral root cap or cortex and endodermis; each lateral root cap cell divides again to initiate two neighbouring cell files (Dolan *et al.* 1994). The postembryonic root meristem merely continues to produce the same set of cell files, thus elongating the primary root. Below the quiescent centre, the initials of the central root cap give successive layers of cells which merge with the layers of the clonally separate lateral root cap (Dolan *et al.* 1994).

The postembryonic shoot meristem is organized in layers and zones which perform specific functions (Steeves & Sussex 1989). The three layers, L1–L3, originate at different times in the embryo. The L1 layer derives from the outer layer of epidermal precursor cells within the upper tier of the eight-celled proembryo. The internal two layers derive from subepidermal tissue located in the centre of the apical region which underwent periclinal divisions at the heart stage of embryogenesis (Barton & Poethig 1993). By contrast, there is no morphological evidence for zonation within the small shoot meristem of the embryo.

3. INSIGHTS FROM MUTANT ANALYSES

Attempts to analyse pattern formation in the *Arabidopsis* embryo were initiated with the isolation and characterization of pattern mutants (Jürgens *et al.* 1991; Mayer *et al.* 1991). The primary interest was to learn about the logic of the pattern-forming process by determining how the embryo responds to the removal of single, genetically defined components. The long-term goal is to analyse, at the molecular level, the roles of the genes identified by mutation. Mutations in putative patterning genes affect specific aspects of the seedling body organization: the primary shoot meristem, the apical–basal pattern or the radial pattern (see table 1).

(*a*) *Origin and organization of the primary shoot meristem*

The shoot meristem of the mature *Arabidopsis* embryo is a small group of cells protruding between the bases of the cotyledons (Barton & Poethig 1993). Soon after germination, the shoot meristem acquires characteristic features of the active state, including histologically distinct zones and bulging leaf primordia on the flanks (Steeves & Sussex 1989). Mutations in three genes appear to eliminate the shoot meristem of the mature embryo but have different effects on the post-embryonic shoot meristem (see table 1). The *shoot meristem-less* (*stm*) mutant apparently fails to form a shoot meristem not only in the embryo but also during regeneration (Barton & Poethig 1993). Mutations in the *ZWILLE* (*ZLL*) gene interfere with the formation of the shoot meristem in the embryo but do not block the formation of adventitious shoot meristems (Jürgens *et al.* 1994; T. Laux & G. Jürgens, unpublished data). Thus the *ZLL* gene is specifically required for setting up the primary shoot meristem in embryogenesis. By contrast, the *STM* gene may confer shoot meristem identity, like the maize *Knotted-1* gene which is

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Table 1. *Genes involved in pattern formation of the* Arabidopsis *embryo*

process	*gene*	late phenotype	primary defect in embryo	reference
shoot meristem formation	*SHOOT MERISTEM-LESS* (*STM*)	no functional shoot meristem	shoot meristem not recognizable	Barton & Poethig (1993)
	ZWILLE (*ZLL*)	adventitious shoot meristem formation	shoot meristem not recognizable	Jürgens *et al.* (1994)
	WUSCHEL (*WUS*)	repetitive formation of defective shoot meristems	shoot meristem not recognizable	Laux *et al.* (1995)
apical–basal patterning	*GNOM* (*GN*)	no root and variable apical defects	zygote abnormal	Mayer *et al.* (1993)
	GURKE (*GK*)	no shoot meristem and cotyledons	apical region defective	Mayer *et al.* (1991)
	MONOPTEROS (*MP*)	no hypocotyl, root and root meristem	8-celled proembryo abnormal	Berleth & Jürgens (1993)
	FACKEL (*FK*)	no hypocotyl	central region abnormal	Jurgens *et al.* (1994)
	HOBBIT	no meristem-derived root(?)	hypophysis abnormal	Scheres *et al.* (1994*b*)
radial patterning	*KNOLLE* (*KN*)	no morphologically distinct epidermis layer	no periclinal cell divisions within 8-celled proembryo	Mayer *et al.* (1991)
	KEULE (*KEU*)	epidermis cells abnormally enlarged	epidermis precursors abnormal	Mayer *et al.* (1991)
	SHORT ROOT (*SHR*)	seedling root short	no endodermis layer in hypocotyl and root	Benfey *et al.* (1993); Scheres *et al.* (1995)

expressed in the shoot meristem and, if expressed ectopically, can cause shoot development on leaves (Sinha *et al.* 1993; Jackson *et al.* 1994). Mutations in a third *Arabidopsis* gene named *WUSCHEL* (*WUS*) affect the establishment of a central region within shoot and floral meristems (Laux *et al.* 1995). As the *wus* mutations also affect the embryonic shoot meristem it is very likely that pattern formation in the embryo establishes a functional organization of distinct zones, such as centre and periphery, within the primary shoot meristem. This idea is consistent with the formation of leaf primordia in maturation-defective embryos, such as *leafy cotyledon* (*lec*) or *fusca3* (*fus3*) (Keith *et al.* 1994; Meinke *et al.* 1994).

(*b*) *Patterning the apical–basal axis of the embryo*

The apical–basal pattern elements of the seedling originate from three regions of the early embryo. What is the significance of these regions for apical–basal pattern formation? Mutations in three genes, *MONOPTEROS* (*MP*), *GURKE* (*GK*) and *FACKEL* (*FK*), cause region-specific alterations in the embryo, resulting in the deletion of different seedling structures (see table 1, figure 3; Mayer *et al.* 1991). *mp* seedlings lack hypocotyl, root and root meristem whereas cotyledons and shoot meristem are present. This defect has been traced back to the octant stage: the *mp* proembryo has four rather than two tiers (figure 3; Berleth & Jürgens 1993). Subsequently the cells in the central and in the basal region divide abnormally, suggesting that the lower, but not the upper, tier of the octant-stage proembryo is affected. Thus the apical region appears to be sufficient for making shoot meristem and cotyledons.

The *gk* phenotype is essentially complementary to the *mp* phenotype: *gk* seedlings lack shoot meristem and cotyledons but not hypocotyl, root and root meristem (Mayer *et al.* 1991). This phenotype was traced back to the apical region of the heart-stage embryo (see figure 3; Mayer *et al.* 1991; R. A. Torres Ruiz & G. Jürgens, unpublished data). The *GK* gene may be required for proper organization of the apical region. As the apical region gives rise to only part of the cotyledons while the 'shoulder region' is clonally derived from the central region (Scheres *et al.* 1994*a*), the *gk* phenotype suggests that apical cells initiating cotyledonary primordia somehow 'entrain' adjacent central cells to participate in the formation of cotyledons.

The central region, which gives rise to hypocotyl, root and root meristem initials of the seedling, seems to develop as a single unit until the heart stage. However, mutations in the *FK* gene reveal two cell populations of different fates, hypocotyl versus root, within the central region before the heart stage (see figure 3). *fk* seedlings appear to lack the hypocotyl, with the root being directly attached to the cotyledons (Mayer *et al.* 1991), and this defect was traced back to the central region of the globular embryo (Jürgens *et al.* 1994). Because the basal region is not affected in *fk* embryos, in contrast to *mp* embryos, it seems likely that the basal region induces the adjacent cells of the central region to become root meristem initials which in turn make the root. This idea receives support from the seemingly complementary phenotype of the *hobbit* mutant which perturbs the cell division pattern of the hypophysis, resulting in a root defect (Scheres *et al.* 1994*b*). In summary, the early regions of the apical–basal axis may be viewed as developmental units that generate, by interaction, the pattern elements of the seedling.

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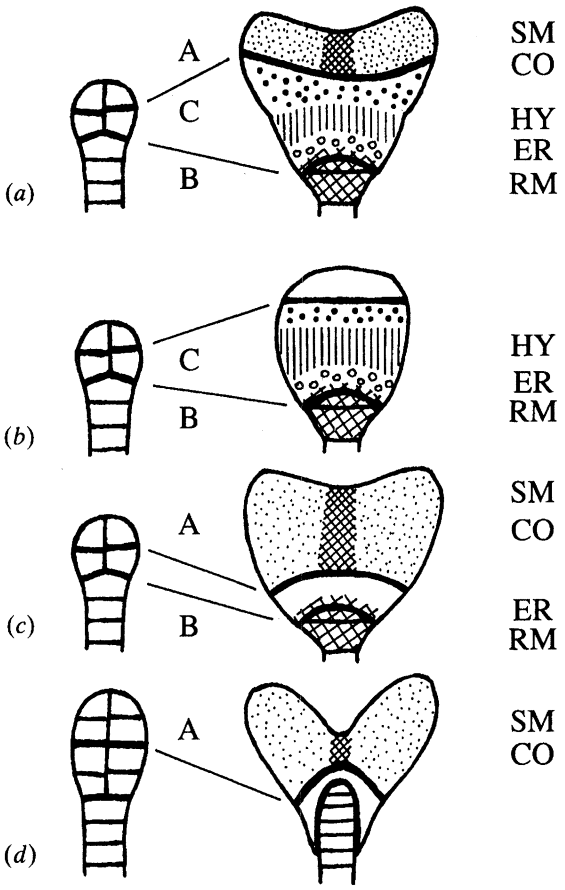

Figure 3. Region-specific alterations in the apical–basal axis of *Arabidopsis* mutant embryos. (*a*) Wild-type and (*b–d*) mutant embryos: (*b*) *gurke*, (*c*) *fackel*, (*d*) *monopteros*. Two stages each are shown, the octant stage (left) and the heart stage (centre). The structures present in seedlings are indicated on the right: SM, shoot meristem; CO, cotyledons; HY, hypocotyl; ER, embryonic root; RM, root meristem. The early regions (apical, A; central, C; basal, B) are separated by bold lines. Symbols of primordia as in figure 1. For details, see text.

How are the early regions established? The apical and the central region originate from transverse cell divisions that partition the four-celled proembryo derived from the apical daughter cell of the zygote. By contrast, the basal region is founded by the hypophysis which occupies the uppermost position in a cell file derived from the basal daughter cell of the zygote. In *mp* embryos, this cell file is initially indistinguishable from wild-type when the earliest defect is seen in the proembryo whereas subsequently, the uppermost derivative of the basal daughter cell behaves like a suspensor cell (see figure 3; Berleth & Jürgens 1993). Thus the presumptive hypophysis may lack some signal(s) that would normally emanate from the proembryo. The *MP* gene is not essential for root formation because bisected *mp* seedlings are capable of producing a root in culture (Berleth & Jürgens 1993). These observations suggest that the basal region is established by induction, in contrast to the apical and central regions which originate from partitioning of the apical daughter cell of the zygote.

Before dividing asymmetrically, the zygote elongates about threefold in the future apical–basal axis of the embryo (Mansfield & Briarty 1991). Concomitantly, the cortical microtubules which were previously oriented at random become aligned perpendicular to the axis (Webb & Gunning 1991). This change may reflect polarization of the zygote in response to some unknown signal(s). The formation of the apical–basal axis of the embryo may thus be initiated within the zygote which acquires an axis of polarity such that the opposite poles of the cell become different, and this difference is then fixed by the asymmetric division. This idea is supported by the analysis of mutations in the *GNOM* (*GN*) gene which affect the zygote and also alter the entire apical–basal axis of the seedling; in the extreme case, no apical–basal polarity is apparent (Mayer *et al.* 1991, 1993). The *gn* zygote expands but does not elongate before producing two daughter cells of nearly equal size (Mayer *et al.* 1993). The apical cell, which is enlarged at the expense of the basal cell, divides abnormally producing an embryo in which no regions can be discerned along the axis. Furthermore, the region-specific *MP* gene was shown to be ineffective in *gn* embryos, thus confirming the morphological interpretations (Mayer *et al.* 1993). How the *GN* gene acts at the molecular level has not been clarified although the *GN* gene (also called *EMB30*) was cloned by T-DNA tagging (Shevell *et al.* 1994) as well as by map-based cloning (Busch, U. Mayer, M. Kientz & G. Jürgens, unpublished data). The predicted 160 kDa GN protein contains a stretch of about 200 amino acids that has sequence similarity to other proteins in yeast (Sec7p; Achstetter *et al.* 1988) and human. However, the significance of this 'Sec7 domain' is not known nor does the remainder of the GN protein readily suggest a function within the cell. Whereas the yeast Sec7p protein is essential for cell viability, the GN protein is not: *gn* haploid gametophytes develop normally and *gn* diploid somatic cells grow well in culture. However, bisected *gn* seedlings produce callus from the cut edge, rather than making a root, under root-inducing culture conditions (Mayer *et al.* 1993). These observations as well as the phenotype of the *gn* zygote suggest that *gn* cells are defective in establishing a polarized organization. It will be interesting to determine whether or not the GN protein influences the cytoskeleton or some other component of the cell required for polarity and axis fixation.

(*c*) *Radial patterning and the origin of tissue types*

The radial pattern of the seedling consists of concentric layers of tissue types surrounding the conductive tissues (xylem and phloem). Mutant phenotypes suggest that the tissue layers are genetically distinct. For example, *keule* (*keu*) embryos have abnormally enlarged epidermis cells from the globular stage on (Mayer *et al.* 1991). Furthermore, an epidermis-specific marker gene, the LTP gene, is continuously expressed in the outer cell layer from the globular embryo on and throughout postembryonic development (Sterk *et al.* 1991; Thoma *et al.* 1994). Other mutants, such as *short root* (*shr*) or *wooden leg* (*wol*), have defects in internal tissues; the endodermis is

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absent in *shr* embryos while *wol* embryos have fewer vascular cells which all differentiate into xylem (Benfey *et al.* 1993; Scheres *et al.* 1995). However, the two mutations have different effects on the pattern, as revealed by double mutant studies with *fass* (*fs*): *fs* suppresses the *wol* phenotype but cannot restore the endodermis in *shr* embryos (Scheres *et al.* 1995). Because *fs* mutations do not interfere with pattern formation (Torres Ruiz & Jürgens 1994) but increase cell numbers and also cause supernumerary cortex layers due to irregular cell divisions, *shr* appears to have a tissue-specific effect whereas *wol* may only interfere with cell division (Scheres *et al.* 1995). Thus radial pattern formation may involve a position-dependent mechanism by which specific tissues and tissue-specific gene expression patterns are established sequentially from the periphery to the centre.

How is the radial pattern initiated in the early embryo? So far, only a single candidate gene, *KNOLLE* (*KN*), has been identified. In the eight-celled proembryo, no periclinal divisions separate the epidermis layer from the inner cell mass, and no morphologically distinct epidermis can be recognized in mutant seedlings (Mayer *et al.* 1991). Furthermore, the expression of the epidermis-specific LTP gene is uniform across the *kn* globular embryo, rather than being confined to the periphery (C. W. Vroeman, S. Langveld, G. Ripper, U. Mayer, A. Van Kammen, S. C. De Vries & G. Jürgens, unpublished data). Thus the periclinal divisions within the proembryo appear to be instrumental in separating the inner cell mass from the surrounding epidermis layer. How the *KN* gene influences these cell divisions is not known. The predicted KN protein shows sequence similarity to the syntaxin family of vesicle-docking proteins (W. Lukowitz, U. Mayer & G. Jürgens, unpublished data). However, this similarity does not immediately suggest a specific function of the KN protein within the cells of the proembryo.

4. CONCLUSIONS AND PERSPECTIVE

Pattern formation in the embryo establishes the basic body organization of higher plants. Two genetically independent processes can be distinguished, one along the apical–basal axis of polarity and the other perpendicular to the axis. The two patterns converge at the poles of the embryonic axis where the primary meristems are formed. The meristems thus acquire a functional organization in relation to embryonic pattern formation. This is obvious for the root meristem whose initials share the same radial pattern with the embryonic root (Dolan *et al.* 1993). It is less evident for the shoot meristem because the details of how it is established in the embryo are not known. Nonetheless, the necessary functional organization of the postembryonic shoot meristem for generating new structures, such as leaves, appears to be established in the embryo, as suggested by the *wus* phenotype. Furthermore, mutations in the *CLAVATA1* (*CLV1*) gene cause an opposite phenotype (Clark *et al.* 1993), suggesting that *WUS* and *CLV1* or related genes may interact in organizing the meristem. Continuous interaction between these specific genes could account for the apparent autonomy of the postembryonic shoot meristem observed in earlier studies (Steeves & Sussex 1989). Thus the body organization of the embryo appears to be a reference for making the adult plant during postembryonic development.

The two genetically distinct processes of pattern formation, apical–basal and radial, involve formally similar steps. Pattern formation, although initiated within cells, largely involves cellular interactions. In the apical–basal axis, for example, the proembryo appears to induce the basal region which, in turn, signals to adjacent cells of the central region. How the cells communicate remains to be determined. In radial patterning, tissue layers are formed successively from the periphery to the centre. Cell–cell communication as a means of establishing tissue layers would also account for the proper radial organization in 'secondary embryos' developing from the suspensor in some *Arabidopsis* mutants (Vernon & Meinke 1994; Schwartz *et al.* 1994; Yadegari *et al.* 1994). Cell–cell communication may later be inhibited between tissue layers, for example by restricting communication through plasmodesmata, as has been observed between the epidermis and the subepidermal layers of the seedling (Duckett *et al.* 1994).

The initial steps of pattern formation take place within cells. The intracellular events segregate different cell fates which may be prerequisites for subsequent cellular interactions. Recent cell-ablation studies on early *Fucus* embryos suggest that cell fates segregated by the asymmetric division of the zygote are imprinted into the cell wall (Berger *et al.* 1994). Whether higher-plant embryos use a similar mechanism is not known. The *Arabidopsis* zygote becomes polarized morphologically, like the *Fucus* zygote (Quatrano *et al.* 1991). Further analysis of the GN protein and its primary function may give clues to how cell fates are segregated within this cell. In contrast to the zygote, the cells of the proembryo within which radial patterning is initiated are not polarized morphologically. However, the outer walls of these cells are different from the inner walls in terms of origin and, possibly, composition: the outer, but not the inner, cell walls are derived from the cell wall of the zygote. If, for example, the zygote were an epidermis-like cell, as has been suggested for *Citrus* (Bruck & Walker 1985), the epidermis would be a developmental 'ground state' from which non-epidermal cell fates are to be segregated. Clearly, although the analysis of pattern formation in the *Arabidopsis* embryo has come a long way, further studies of pattern mutants as well as the molecular characterization of the genes affected are necessary to identify underlying mechanisms.

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