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REVIEW

Establishing the body plan of the *Arabidopsis* embryo

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INTRODUCTION

During plant embryo development, a single cell, the zygote, gives rise to a multicellular organism that contains diverse cell types and tissues organized properly into a basic body plan. The body organization, which is most easily recognized at the seedling stage, consists of two superimposed patterns, one along the apical–basal axis and the other perpendicular to this axis. The basic apical–basal pattern is a linear array of a few elements only: the shoot meristem, the cotyledons, the hypocotyl and the root system, including the root meristem. The radial pattern is comprised of the primary tissues; that is, the epidermis, the parenchymal ground tissue and the vascular system. Once the seedling organization is established, the root and shoot meristems are responsible for further plant development by tip growth (Steeves & Sussex 1989). The simplicity of the *Arabidopsis* seedling facilitates the analysis of pattern formation during embryogenesis. Depending on the experimental approach used, different levels of pattern formation are recognized: morphological patterns are the consequence of patterns formed at the cellular level which, in turn, may reflect the distribution patterns of molecules.

The genetic control of basic body formation in the embryo has been extensively studied in *Drosophila* where relatively few genes are specifically involved in establishing the basic body organization (Ingham 1988; Nüsslein-Volhard 1991). Although no comparable analysis has been done in any plant species yet, embryo development has been genetically studied (Sheridan & Clark 1993; Nagato *et al.* 1989; Meinke 1985). Progress has recently been made in the genetic dissection of pattern formation in the *Arabidopsis* embryo, making the isolation of relevant genes feasible (Mayer *et al.* 1991).

WILD TYPE DEVELOPMENT

Embryo development in *Arabidopsis* is very similar to that of *Capsella bursa-pastoris*, a well-known textbook example for dicot embryogenesis. Two periods can be distinguished: an early period covering about the first one-third of embryogenesis in which the basic body organization of the embryo is established, and the maturation period during which tissue and cell-type development dominate while the embryo prepares for dormancy (Jürgens & Mayer 1994). The early period lasts from the zygote until the heart stage at which the seedling body plan is recognizable.

Invariance of the early cell-division pattern

In *Arabidopsis* and other *Brassicaceae*, specific elements of the seedling body pattern can easily be traced back by histological analysis to cell groups in the early embryo, since cell-division patterns are precise and invariant during the early phase of embryogenesis (Schulz & Jensen 1968; Tykarska 1976, 1979; Mansfield & Briarty 1991; Jürgens & Mayer 1994). This precision and invariance suggests some mechanistic relevance of the cell-division pattern. It is unclear, however, when cell identities are specified in the early embryo and to what extent their fates become fixed. Cell and tissue transplantations, which have been used to address these questions in animals such as *Xenopus* or sea urchin, are not feasible in plants due to both the inaccessibility of the embryo and the rigidity of plant tissue. That cell fate might not be fixed in plants to a similar extent as in animals is suggested by the potential of many differentiated plant cells to undergo dedifferentiation and even to generate a new plant (e.g. Williams & Mahjeswaran 1986; Konar & Nataraja 1965).

The invariant relationship between early embryo regions and seedling body parts should, for several reasons, not be confused with 'cell lineages' of clonal origin. First, mutations in the *FASS* gene of *Arabidopsis* change cell-division patterns from very early embryogenesis such that primordia of seedling structures cannot be recognized at the heart stage; and yet, all structures of the seedling body are well differentiated (Mayer *et al.* 1991; Torres Ruiz & Jürgens 1994). Second, embryo development in other dicot species proceeds through very similar stages as in *Arabidopsis*, but the early cell-division patterns are often very different and even highly variable in some cases (Pollock & Jensen 1964; Sivaramakrishna 1978; Natesh & Rau 1984; Johri *et al.* 1992). Third, somatic embryos can develop by different cell-division schemes as compared to their zygotic counterparts (McWilliam *et al.* 1974). Fourth, genetic-sector analysis in maize has demonstrated predictable, but variable, relationships between cells in the early embryo and regions of the postembryonic plant (Poethig 1986).

These findings strongly suggest that the regular cell divisions of the early embryo may reflect pattern formation, but are not part of the pattern-forming mechanism. Cells thus

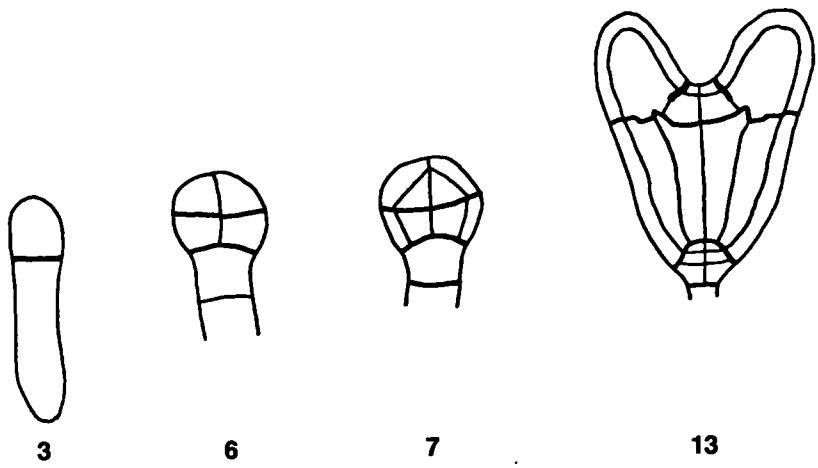

Fig. 1. Development of the seedling body plan in the early embryo. The zygote divides asymmetrically to give a small apical and a large basal cell (stage 3). At the octant stage (stage 6), the basic domains along the apical–basal axis are established: the upper tier giving rise to shoot meristem and cotyledons, the lower tier forming the hypocotyl and root, and the uppermost derivative of the basal cell (hypophysis) producing the basal end of the embryo. The primordium of the epidermis has been given off as an outer layer of cells at the dermatogen stage (stage 7). The body plan of the seedling is recognizable at the mid-heart stage (stage 13); stages after Jürgens & Mayer (1994).

seem to adopt specific fates according to their position in the plant embryo rather than to their clonal origin, suggesting that pattern formation involves some kind of cell–cell communication.

Establishing the body plan

The egg cell, which is located at the micropylar end of the mature embryo sac, is polarized such that its vacuole is towards the micropyle and its nucleus towards the chalaza (Mansfield *et al.* 1991). After fertilization, the zygote elongates before dividing transversely; this division is asymmetric, yielding a large basal and a small apical cell (Fig. 1, stage 3; Mansfield & Briarty 1991; Webb & Gunning 1991; Jürgens & Mayer 1994). The basal cell will contribute to root formation but mainly generates the extra-embryonic suspensor which attaches the basal end of the embryo to the wall of the embryo sac and might have a temporary role in conducting nutrients to the embryo (Yeung & Meinke 1993). The apical cell, which will give rise to most of the embryo body, undergoes three rounds of cleavage divisions, two longitudinal and one normal to the apical–basal axis. This last division partitions the now eight-celled embryo into two basic body domains, an upper tier and a lower tier (Fig. 1, stage 6). The upper tier will give rise to cotyledons and shoot meristem, whereas the lower tier will form the hypocotyl and most of the root system. The border separating the two domains is recognizable until late embryogenesis and provides a histological reference which has been called the 'O-line' in *Brassica napus* (Tykarska 1976, 1979). The apical–basal axis of the developing embryo is thus fixed by the asymmetric division of the zygote whose orientation correlates with the polarity of the embryo sac.

Tissue primordia are first separated when a round of tangential cell divisions in the octant embryo sets the protoderm apart from the ground tissue (Fig. 1, stage 7). Subsequent cell divisions in the outer cell layer are oriented anticlinally, thereby giving

rise to the epidermis in a polyclonal fashion. The innermost cells of the mid-globular embryo become recognizable by asymmetric divisions as the precursors of the vascular system while the remaining cells will form the parenchymal ground tissue. The transition from the globular to the heart-shaped embryo marks the completion of the basic body pattern. Two incipient lateral primordia reflect the subdivision of the apical domain (upper-tier derivatives) into cotyledons and epicotyl, which morphologically establishes the bilateral body symmetry. The lower-tier derivatives have formed the axis of the embryo by oriented cell divisions. The upper part of this domain corresponds to the hypocotyl while the lower end contributes to the root. The basal end of the root is provided by the descendants of the hypophysis which is derived from the basal daughter cell of the zygote (Fig. 1; Dolan *et al.* 1993; Benfey & Schiefelbein 1994).

In the heart-shaped embryo, the body plan of the seedling has been laid down (Fig. 1, stage 13). The radial pattern now consists of the precursors of epidermis, ground tissue and vascular system. The apical–basal axis has been partitioned into epicotyl, cotyledons, hypocotyl and root. Further development will include growth by cell division and cell enlargement, tissue differentiation by the formation of appropriate cell types, and preparation of the embryo for dormancy.

The shoot meristem remains inconspicuous until the bent-cotyledon stage, when it becomes recognizable as a bulge of small cells between the bases of the cotyledons (Barton & Poethig 1993). Genetic-sector analysis has demonstrated that in the mature *Arabidopsis* embryo, the initials of the first true leaves, although not visible, have been formed (Irish & Sussex 1992; Furner & Pumfrey 1992). After germination, the shoot and root meristems give rise to the adult plant body by tip growth. The body pattern laid down in embryogenesis; that is, apical–basal polarity and radial organization, is maintained and reiterated. In addition, the cotyledons appear to provide reference points for positioning of leaf primordia by the shoot meristem, as mutants with altered cotyledon number or spacing show analogous changes in leaf phyllotaxis (Chaudhury *et al.* 1993; our unpublished observations).

It is debatable whether patterning in early embryogenesis differs qualitatively from postembryonic development which involves meristematic growth. In one view, the apical domain of the globular embryo behaves like the shoot meristem after germination; the cotyledons are thus regarded as the first lateral organs of the shoot meristem, which is consistent with their possible evolutionary relationship to leaves (Haccius 1972; Meinke 1992; Rosenblum & Basile 1984). The alternative view maintains that shoot meristem and cotyledons originate by partitioning of the apical domain. This view is based on both histological and genetic criteria: the characteristic three-layered organization of the postembryonic shoot meristem is not evident in the embryo when the cotyledonary primordia are initiated (Barton & Poethig 1993) and mutants have been isolated that lack any signs of a shoot meristem in the embryo, but develop normal cotyledons (see below).

The body plan of a seedling is established during early embryo development, in a succession of events. Which steps belong to the initial formation of the basic body pattern and which steps might rather represent pattern elaboration? For the purpose of this review, we consider the following elements to be generated by the embryonic patterning process: shoot meristem, cotyledons, hypocotyl and root along the apical–basal axis, and epidermis, ground tissue and vascular system as radial elements perpendicular to the axis. In order to reveal mechanisms generating the body plan,

questions such as the following need to be addressed: How are axes defined, elements arranged and tissues initiated? How are positions distinguished and cell fates determined?

NON-GENETIC APPROACHES

Different approaches have been taken in a variety of plant species in order to reveal principles of embryonic pattern formation. Some of these studies are included in order to discuss general aspects of pattern analysis.

Histological studies

Careful histological studies of zygotic embryogenesis in many plant species form the basis for some conceptual insights into pattern formation (Natesh & Rau 1984; Johri *et al.* 1992). Being encased in rigid walls, plant cells cannot move about in the developing embryo. Thus, the growing embryo changes its shape only by organized cell activities, including differential rate of division, oriented division, asymmetry of division and shape changes. All these cell activities act in concert to bring about morphogenesis, generating the shape of the mature embryo. Although the underlying mechanisms of pattern formation have not yet been revealed, comparative studies suggest that none of these cell activities are instrumental in pattern formation (see above).

Molecular studies

Histological descriptions of embryogenesis have been extended to the molecular level by using specific spatial and temporal gene expression patterns as molecular markers for developmental processes. Molecular markers not only enable regions of the wild type embryo to be recognized independently of morphological criteria but also facilitate the analysis of aberrant development in mutant embryos. If the topography of the embryo is altered such that cells do not show distinguishing features, appropriate molecular markers could be used to ascertain their identity. For example, the expression of the soybean Kunitz trypsin inhibitor 3 (KTi3) gene is restricted to cells in lateral regions at the basal end of the globular embryo (Perez-Grau & Goldberg 1989) and can thus be used to identify cells which otherwise cannot be distinguished. A very useful marker for the radial pattern is the carrot EP2 gene which is exclusively expressed in the epidermis from the globular stage on (Sterk *et al.* 1991). Although few molecular markers are at present available in *Arabidopsis*, this situation might soon improve as more genes are being isolated by heterologous hybridization (e.g. Thoma *et al.* 1994), genetic approaches (see below) or enhancer trap experiments (Topping *et al.* 1991).

Molecular analysis can be used to study the developmental regulation of genes with interesting expression patterns. This approach has been used successfully in animals to identify *cis*-regulatory sequences of differentially expressed genes and to isolate cDNA-clones encoding DNA-binding proteins which regulate early embryonic gene expression (e.g. Calzone *et al.* 1991; Höög *et al.* 1991). It appears unlikely that very early events in *Arabidopsis* embryogenesis, such as axis definition and partitioning, can be addressed by this strategy since the factors involved in establishing the body plan might be very rare, the total embryo mass is small at this stage and isolating early embryos is not an easy task.

In-vitro *studies*

One way of analysing mechanisms of pattern formation is to experimentally perturb embryogenesis. Zygotic embryos have been explanted and grown in tissue culture where they become accessible to experimental manipulation. Depending on the developmental stage of the explanted embryo, different supplements of nutrients and hormones have been demonstrated to be required for completion of embryogenesis *in vitro* (e.g. Wu *et al.* 1992; Liu *et al.* 1993a; Steeves & Sussex 1989). The suspensor was found to be beneficial for embryo development only at early stages while embryos explanted later became increasingly independent (Yeung & Sussex 1979). Explanted embryos were also exposed to auxins and auxin-transport inhibitors, which had various effects on embryogenesis *in vitro* (Schiavone & Cooke 1987; Liu *et al.* 1993b).

Another experimental strategy takes advantage of the remarkable capacity of some single plant cells to generate somatic embryos (Backs-Hüsemann & Reinhart 1970; Nomura & Komamine 1985, 1986). Somatic embryos pass through similar stages as zygotic embryos although their cell-division patterns may differ (Halerin 1966; McWilliam *et al.* 1974); specific gene expression patterns may be identical (Sterk *et al.* 1991; Perez-Grau & Goldberg 1989). Using carrot somatic embryos as an assay system, extracellular glycoproteins have been found that are essential for embryo development (de Jong *et al.* 1993; Van Engelen & de Vries 1992).

Somatic embryos have also been used for microsurgery experiments to reveal rules for pattern regulation (Schiavone & Racusen 1991). By excising different parts of the carrot embryo, it has been shown that upon further cultivation, the shoot pole is capable of regenerating the root pole but not, to the same extent, *vice versa* (Schiavone & Racusen 1990). However, if only the very root pole of torpedo-stage embryos is cultured under appropriate conditions, cotyledons will be regenerated without formation of a typical shoot meristem.

Taken together, *in vitro* experiments have given valuable information on general requirements of the embryo throughout development; however, regulatory mechanisms of pattern formation have not been identified.

GENETIC DISSECTION OF PATTERN FORMATION

Genetic dissection appears to be the most promising approach to identify mechanisms that govern development and to elucidate the function of regulatory genes. Relevant genes can be identified by specific mutant phenotypes which reveal features of the developmental processes perturbed by mutation. In order to infer the possible role of the gene in wild type development, it is necessary to isolate a series of mutant alleles which enable the lack-of-function (null) phenotype to be ascertained. We will nonetheless include in our discussion some single mutants if they show interesting phenotypes, although the wild type functions of the genes cannot reliably be inferred. Once genes for specific developmental processes have been identified by their mutant alleles, their interactions can be studied and the genes can be isolated.

The favourable features of *Arabidopsis* for genetic analysis have already led to a detailed understanding of pattern formation in flower development, thus demonstrating the power of genetic dissection for studying developmental regulation in plants (Coen & Meyerowitz 1991). In *Arabidopsis*, T-DNA from *Agrobacterium* (Feldmann 1991) as

well as the Em/I and Ac/Ds transposon systems (Aarts *et al.* 1993; Bancroft *et al.* 1993; Long *et al.* 1993) from maize have been used to generate mutations by insertion. Once an interesting mutant has been identified, the inserted element provides a tag for isolating the gene. T-DNA insertion mutants, for example, have been used successfully to isolate developmental genes, such as the homeotic floral gene *AGAMOUS* (Yanofsky *et al.* 1990). In other cases, however, the inserted T-DNA underwent rearrangements, which makes isolation of the gene more difficult (Castle *et al.* 1993). The main disadvantage of T-DNA insertional mutagenesis in *Arabidopsis* has been a relatively low mutation rate which makes it difficult to tag a specific gene (Feldmann 1991). By contrast, chemical mutagens like EMS can give high mutation frequencies, rendering genomic saturation feasible, and they do not show preferences for specific genome regions. EMS also produces a wide range of mutant alleles, including weak (hypomorphic) alleles which, if ordered in an allelic series, help to infer how the gene normally exerts its effect. Genes identified by EMS-induced alleles have to be isolated by chromosome walking, which is feasible in *Arabidopsis* (e.g. Arondel *et al.* 1992; Giraudat *et al.* 1992).

What mutant phenotypes are indicative of embryonic patterning defects? Two alternatives are to be considered: embryonic-lethal mutants, which fail to complete embryogenesis, and seedling pattern mutants which are able to germinate. Embryonic-lethal mutants display a wide range of phenotypes, with development being arrested at different stages; the majority, however, fail to undergo the transition from globular to heart shape (Castle *et al.* 1993). In some mutants, the suspensor starts to proliferate when embryo development is arrested, and even to undergo some tissue differentiation, suggesting that the developmental potential of the suspensor is normally repressed by the embryo proper (Marsden & Meinke 1985; Yeung & Meinke 1993). This interpretation is consistent with similar observations made after damaging the embryo by irradiation (Gerlach-Cruse 1969; Yeung & Meinke 1993). Although genes for body patterning might also mutate to embryonic-lethal phenotypes, most embryonic-lethal mutations presumably affect genes required for general cell metabolism, so-called 'household' genes, especially when the arrest occurs in the early embryo. In fact, one of the few characterized embryonic-lethal mutants is defective in biotin biosynthesis (Schneider *et al.* 1989). Genes that may have specific effects on embryo development might be found among lethal mutants that proceed relatively far through embryogenesis, to the heart stage or later, before arrest occurs. One embryo mutant originally classified in this group, *emb30* (Meinke 1985), is an allele of the early acting patterning gene, *GNOM* (Mayer *et al.* 1993; see below). It appears reasonable to assume, however, that late-stage embryonic-lethal mutants might more often be defective in processes such as organogenesis, since the basic body pattern is already established at the heart stage.

Searching for pattern mutants at the seedling stage was based on the assumption that at least some defects in patterning do not interfere with the viability of the embryo (Jürgens *et al.* 1991) since this concept had been used very successfully for isolating pattern mutants in *Drosophila* (Ingham 1988; Nüsslein-Volhard 1991). Once identified by its seedling phenotype, embryogenesis of the putative pattern mutant is studied to determine whether the defect is visible in the heart-stage embryo when the pattern has normally formed. This strategy was employed to isolate, in *Arabidopsis*, a set of mutants that affect two different aspects of seedling body organization: apical–basal pattern and radial pattern (Mayer *et al.* 1991).

Mutations affecting the apical–basal pattern

Mutations in four genes alter pattern formation along the apical–basal axis of the early embryo; each gene is represented by several mutant alleles, which indicates that these genes are specifically involved in the process (Mayer *et al.* 1991). Mutations in the *GNOM* (*GN*) gene affect basal as well as apical structures of the seedling; while the root is always missing, the apical defects are variable (Mayer *et al.* 1993). The earliest stage at which *gn* embryos can be recognized is the zygote: the plane of cell division is shifted basally as compared to wild type. It has been postulated that the *GN* gene is required for the asymmetric division of the zygote and, possibly indirectly, for partitioning of the apical–basal axis. Mutations in the *GURKE* (*GK*) gene specifically affect the apical domain, including cotyledons and shoot meristem, and this defect is recognizable in the heart-stage embryo (Mayer *et al.* 1991; Torres Ruiz & Jürgens 1994). Mutant *fackel* (*fk*) seedlings seem to lack the hypocotyl, leaving the cotyledons directly attached to the root (Mayer *et al.* 1991). This phenotype has been traced back to the globular embryo (Mayer 1993; Jürgens *et al.* 1994). Mutant *monopteros* (*mp*) seedlings mainly show a deletion of basal structures, including root and hypocotyl, which has been correlated with abnormal cell divisions in the lower-tier derivatives of the globular embryo (Berleth & Jürgens 1993). Mutant *rootless* seedlings resemble *mp* seedlings but allelism has not been determined (Barton & Poethig 1993). Since these mutant phenotypes become recognizable earlier than the primordia of seedling structures, these genes might be required for establishing, or maintaining, regions along the apical–basal axis. As judged by the earliest deviation from wild-type development, the *GN* gene may act before *FK*, *GK* and *MP*. In fact, double-mutant analysis has demonstrated that *gn* is epistatic to *mp* (Mayer *et al.* 1993).

In tissue culture experiments, wounded *gn* seedlings did not respond to root-inducing conditions but formed callus instead (Mayer *et al.* 1993) whereas *mp* seedlings were capable of forming roots under comparable conditions (Berleth & Jürgens 1993). Thus, the *GN* function does not appear to be confined to the embryo. By contrast, the *MP* gene seems to be specifically required for basal development in the embryo.

While *gk* mutations affect the entire apical region, two other classes of mutation affect distinct elements of the apical domain, that is, cotyledons and shoot meristem. Mutants with an altered cotyledon number often show pleiotropic effects and/or reduced penetrance. The number of cotyledons is increased to three or four in *altered meristem program* (*amp1*) (Chaudhury *et al.* 1993) and *häuptling* (*hpt*) (Jürgens *et al.* 1991; S. Ploense & G. Jürgens, unpublished) mutants which later display other phenotypic aberrations, including altered phyllotaxis. Several mutants with only one cotyledon have been observed, however the penetrance of the phenotype usually was extremely low (our own unpublished observation). The *pin1-1* mutant shows defects in auxin transport (Okada *et al.* 1991), and a minority of mutant embryos have a single 'fused' cotyledonary primordium, which is consistent with the effects of auxin-transport inhibitors in cultured *Brassica juncea* embryos (Liu *et al.* 1993b).

Several mutants have been identified that lack a shoot meristem in the mature embryo. The *shoot meristemless-1* (*stm-1*) mutant is defective from the late-torpedo stage, lacking any sign of normal epicotyl development (Barton & Poethig 1993). Mutant *stm-1* seedlings also fail to initiate a proper shoot meristem after germination and the *stm*-1 gene might thus be required for shoot meristem formation *per se*. *wuschel* (*wus*) mutants also do not form a shoot meristem in the embryo; however, they produce

an abnormal shoot after germination (T. Laux, K. F. X. Mayer & G. Jürgens, unpublished). By contrast, *zwille* (*zll*) mutants fail to produce a shoot meristem during embryogenesis but initiate wild type like shoots postembryonically (Jürgens *et al.* 1994; T. Laux & G. Jürgens, unpublished). Thus, the *ZLL* gene appears to be specifically required for proper shoot meristem formation in the embryo. All these mutants which lack the shoot meristem have no other obvious phenotypic defects; in particular, the cotyledons are completely normal, suggesting that cotyledons are not formed by an 'embryonic shoot meristem'. This is consistent with the absence of a three-layered shoot meristem during cotyledon initiation both in the heart-stage embryo and in regeneration experiments (Barton & Poethig 1993; Schiavone & Racusen 1990). However, an alternative possibility is that shoot meristem development in these mutants is discontinued after cotyledon initiation.

The mutations described so far delete specific elements of the apical–basal body pattern. Although the selection of phenotypes may have been biased, it should be noted that the domains defined by mutant phenotypes coincide with the visible morphological units of the seedling. Two other mutants, which might not affect pattern formation but perturb embryo development in an interesting way, are *embryonic flower* (*emf*) and *leafy cotyledons* (*lec*). Mutations in the *EMF* gene cause the shoot meristem of the embryo to generate floral structures (Sung *et al.* 1992). The single *lec* mutant forms, in place of cotyledons, leaf-like lateral organs which feature trichomes and lack storage vesicles (Meinke 1992). Since these mutations are recessive their phenotypes indicate that pathways of shoot development are negatively regulated in the wild type embryo.

Mutations affecting the radial pattern

The radial pattern of the seedling, which consists of the primary tissues, such as epidermis, ground tissue and vascular strands, originates in the globular-stage embryo and is maintained during postembryonic development. While present in all plant organs, the primary tissues may undergo further specializations, including the formation, for example, of endodermis or pericycle, or of specialized cell types, such as trichomes or stomatal guard cells in the epidermal layer.

The radial pattern is distorted in *keule* (*keu*) and *knolle* (*kn*) seedlings; the deviations from wild type can be seen as early as the globular stage of embryogenesis (Mayer *et al.* 1991). In *keu* globular embryos, the outer cell layer consists of grossly enlarged cells while the internal cells appear to be fairly normal, suggesting that epidermal development is affected. By contrast, in *kn* embryos, cell enlargement is not restricted to the outer cell layer but also takes place in inner tissue. Mutant *kn* embryos can be recognized by this feature at the octant-stage which does not undergo the tangential divisions that would normally give rise to the epidermis primordium (Mayer 1993). Changes in cell size have also been observed in some embryonic-lethal mutants (Patton & Meinke 1990; our unpublished observations), and cell death in tissue culture often is accompanied by cell enlargement. Although *keu* and *kn* mutants share some cellular aspects with embryonic-lethal mutants, they are different in that the embryo remains viable, and the bloated-cell phenotype may thus not be correlated with cell death. It is not clear at present whether the two genes, *KEU* and *KN*, are directly involved in radial patterning or whether their mutant epidermal phenotype is an indirect consequence of a more general developmental defect. Molecular studies following gene isolation should help to clarify the role of *KEU* and *KN* genes in early embryo development. An internal cell layer is deleted in *short root* (*shr*) seedlings which lack the

root endodermis (Benfey *et al.* 1993). This defect has been traced back to the embryo, suggesting that tissue organization, rather than histogenesis, is affected by the *shr* mutation (Benfey & Schiefelbein 1994).

CONCLUSIONS

Few genes direct pattern formation in the Arabidopsis *embryo*

Mutagenesis experiments have identified a small number of genes that appear to be specifically involved in the embryonic pattern formation. Mutations in few genes mainly delete specific parts of the apical–basal pattern, and mutations affecting the radial tissue organization are even rarer. Although this situation is reminiscent of *Drosophila* where also a relatively small number of all essential genes direct the formation of the basic body pattern (Ingham 1988; Nüslein-Volhard 1991), it cannot be excluded that important genes have not been identified. For example, none of the mutations identified specifically affects the vascular system although, interestingly, mutations in several patterning genes, such as *GN, MP* and *GK*, disrupt vascular strands (Mayer *et al.* 1993; Berleth & Jürgens 1993; Torres Ruiz & Jürgens 1994). Mutations in as yet unidentified patterning genes might render the embryo lethal or cause inconspicuous seedling phenotypes. Some of these genes however may be identified in mutagenesis experiments using specific mutant backgrounds. Mutants alleles of the *CAULIFLOWER* gene, for example, have only been isolated in a mutant background of the flower homeotic gene, *APETALA1* (Bowman *et al.* 1993).

The mutant phenotypes of the patterning genes identified give some clues about pattern formation in the embryo. First, the pattern mutants analysed have specific phenotypes, which supports the notion that genes directing pattern formation in the *Arabidopsis* embryo play specific roles. Secondly, only deletions but no other pattern phenotype, such as homeotic transformation, have been observed. Isolation of such mutants in the future notwithstanding, this finding might indicate that the elements of the basic body plan lack homeotic alternatives, such as is found in *Drosophila* embryonic patterning (Ingham 1988). It should be noted that, in *Drosophila*, homeotic alternatives only exist for the segmentally repeated pattern along the main body axis but not for the pattern elements along the dorso-ventral axis. Finally, genetic control of pattern formation occurs in successive steps from the earliest stages of *Arabidopsis* embryogenesis. Most pattern mutant embryos deviate from wild type development at or before the globular stage; the *GN* gene apparently acts in the zygote, preceding the later-acting region-specific genes such as *MP*. The body plan might thus result from a hierarchical succession of events, leading from global to local regulatory levels.

Arabidopsis *as a model for plant pattern formation*

How representative is *Arabidopsis* embryo development? Can we reasonably expect the results of its genetic dissection to be useful for understanding basic body pattern formation in other plant species? The global events in embryogenesis are similar in all angiosperms (Steeves & Sussex 1989). In dicots, embryogenesis proceeds roughly through the same morphological stages as in *Arabidopsis*. Species-specific differences include the extent of endosperm development, cell-division patterns or even the lack of invariant division schemes and the extent of shoot meristem activity before seed dormancy (Natesh & Rau 1984; Johri *et al.* 1992). The mature monocot embryo is much

more elaborated than its dicot counterpart, displaying various embryo-specific tissues and organs and producing a number of true leaves (Johri *et al.* 1992). However, early embryo development appears to follow similar routes in monocots as in dicots (Natesh & Rau 1984; Johri *et al.* 1992). Thus, there is a fair chance that the way the basic body pattern is established in the *Arabidopsis* embryo may be representative of plants in general.

Therefore, once the genes involved in establishing the basic body plan have been isolated in suitable plants such as *Arabidopsis*, heterologous hybridization or PCR-based amplification may be used to isolate their counterparts in other plants species. This approach has already been applied successfully to isolate flower patterning genes in *Arabidopsis*, using their homologs from the distantly related snapdragon (*Anthirrhinum*) as probes (Wiegel *et al.* 1992), as well as maize homologs, using *Arabidopsis* genes as probes (Schmidt *et al.* 1993). Subsequent molecular analysis may indicate whether developmental mechanisms in embryonic pattern formation are shared between different plant species.

Developmental alternatives to zygotic embryogenesis

What is the significance of embryonic pattern formation for subsequent plant development? In normal development, the shoot and root meristems use the embryo body pattern as reference to form the postembryonic plant. However, there are alternative routes to the same end: somatic embryogenesis and regeneration from callus tissue.

As the development of somatic embryos in tissue culture shows, embryo pattern formation does not strictly require maternal factors although the apical–basal axis of the zygotic embryo is oriented with respect to the polarity of the embryo sac and surrounding maternal structures (Mansfield *et al.* 1991; Webb & Gunning 1991). Polarity is established in somatic embryos even when grown in liquid culture (Dudits *et al.* 1991; Nomura & Komamine 1986), which suggests that axis formation might result from random inhomogeneities either within cells or in their micro-environment. In the extreme case, even embryogenesis appears to be dispensable for establishing the adult body organization. Although the details of regeneration from callus tissue are not clear, this process leads, via formation of a shoot meristem, to a defined apical–basal as well as radial organization of the plant (Walker *et al.* 1989; Feldmann & Marks 1986).

To what extent these alternative pathways of development involve similar mechanisms for generating the body pattern can be assessed once the genes directing pattern formation in zygotic embryos have been isolated.

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