

Embryogenesis: Pattern Formation from a Single Cell

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Summary

During embryogenesis a single cell gives rise to a functional multicellular organism. In higher plants, as in many other multicellular systems, essential architectural features, such as body axes and major tissue layers are established early in embryogenesis and serve as a positional framework for subsequent pattern elaboration. In Arabidopsis, the apical-basal axis and the radial pattern of tissues wrapped around it is already anatomically recognizable in very young embryos of approximately a hundred cells. This early axial pattern seems to provide a coordinate system for the embryonic initiation of shoot and root. Findings from genetic studies in Arabidopsis are revealing molecular mechanisms underlying the initial establishment of the axial core pattern and its subsequent elaboration into functional shoots and roots. The genetic programs operating in the early embryo organize functional cell patterns rapidly and reproducibly from minimal cell numbers. Understanding their molecular details could therefore greatly expand our ability to generate plant body patterns de novo, with important implications for plant breeding and biotechnology.

1. Introduction

The generation of a functional organism from a single cell requires the spatially coordinated acquisition of numerous cell identities. Molecular genetic studies of embryo development in animal species have not only elucidated many of the underlying processes, but have also greatly advanced the general understanding of eukaryotic cell signaling and gene regulation. Because of the fundamental differences between animal and plant cells, the study of plant embryo pattern formation may have a similar dual impact. In addition to improving our understanding of the organized growth of plant cells and consequently our ability to manipulate these patterns, research in this field may also reveal entirely novel modes of eukaryotic cell communication.

In animal species, the importance of understanding pattern formation in the embryo is self–evident, as the mature embryo usually constitutes a miniature version of the adult organism. In plants, by contrast, embryogenesis generates only a less complex core structure, the seedling (Figure 1G), while virtually the entire adult plant morphology is generated by the activities of the apical meristems. This raises the question of why, in plants, should emphasis be placed on pattern formation in the embryo. As we will discuss in this review, the seedling is not merely an unstruc-

tured support device for autonomously patterning apical meristems. Rather, it can be regarded as the initial unit of a reiterative patterning process, in which existing structures serve as positional references for those that follow (Figure. 2). Moreover, the seedling pattern is itself generated with reference to an early axial embryonic pattern, which is already visible in young embryos comprising approximately a hundred cells. Uniquely therefore, the study of early embryo pattern formation may elucidate mechanisms through which plant cells are able to generate functional body patterns in the absence of pre-structuring multicellular templates.

De-novo generation of body patterns from individual cells other than the zygote is a well-known and widely exploited property of plant cells. Gardeners as well as entire industries exploit the amazing regenerative capacities of plant cells, but the process remains stochastic and its molecular basis elusive. Only a few cells in an aggregate participate in the initiation of a multicellular pattern and species specific differences in regeneration properties are still poorly understood. By contrast, the genetic program initiated in zygotic embryos creates a highly reproducible axial pattern from a single cell often within ten cell divisions. In Arabidopsis, embryos of the late globular stage (Figure 4D) are overtly structured in the apical-basal and radial dimensions and are in the process of developing two properly spaced cotyledons. In this review, we will focus on the establishment of this axial pattern in the early embryo and on mechanisms, through which it acts as a foundation for shoot and root development. Other aspects of embryogenesis and overlapping activities of the apical meristems will be addressed in chapters on ovule, seed and apical meristem development, respectively. Further aspects of angiosperm embryo development have also been discussed in a number of thoughtful reviews (Mansfield and Briarty, 1991; Bowman, 1994; Meinke, 1995; Kaplan and Cooke, 1997; Mayer and Jürgens, 1998; Raghavan, 2000; Jürgens, 2001), as has somatic embryogenesis (Dejong et al., 1993; Schmidt et al., 1994).

2. Stages of Arabidopsis embryo development

The embryo develops from a fertilized egg cell, positioned within the embryo sac, which is itself embedded in the protective maternal tissue of the ovule (Figure 3A, reviewed in Gasser et al., 1998; Schneitz, et al., 1998). All three structures are elongated and polarized along the long axis. The

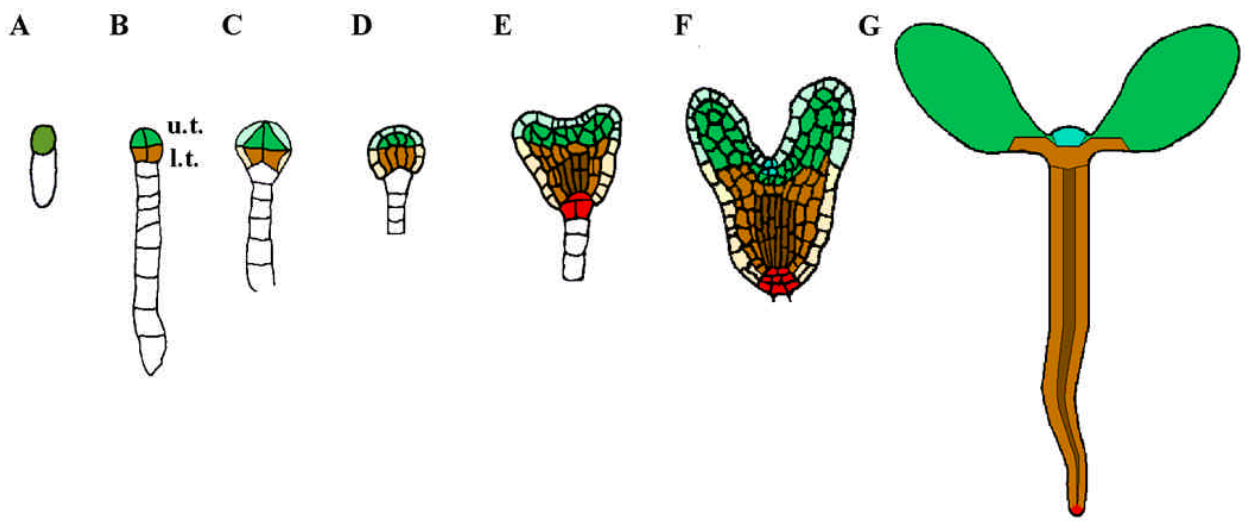

Figure 1: Embryonic origin of seedling structures.
The reproducibility of Arabidopsis embryo development enables tracing the origin of seedling organs and tissues to progenitor cells in the early embryo. Colors identify corresponding regions in embryo and seedling. A detailed description of embryonic stages is given in Figure 4. Radial subdivision; grayscale: vascular tissues, dark; ground tissue, plain color; epidermis, lightly shaded (missing in G). Apical-basal subdivision; upper and lower tier descendants in green and brown, respectively. The most distal part of the root meristem (red) originates from the uppermost suspensor cell (hypophyseal cell. Section 4.1.2).

polar organization of the Arabidopsis ovule comprises a small nucellus, harboring the embryo sac, and the chalaza, from which two integuments of unequal size grow out to enclose most of the embryo sac, leaving only a small opening at the opposite end, the micropyle. Within the embryo sac the egg cell and the synergids are located at the micropylar end, while the antipodal cells occupy the opposite position (chalazal end). The egg cell displays its own intracellular polarity with a major vacuole located near its micropylar pole and dense cytoplasm towards the cha-

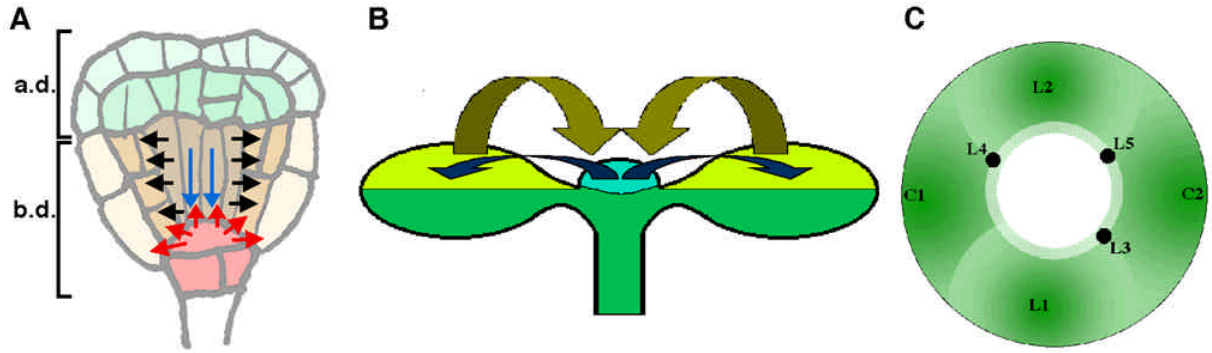

Figure 2: Positional references provided by the early embryo pattern
Arrows indicate presumed pattern transmission mechanisms, which are discussed in sections 4 and 5. **A**: Triangular-stage embryo with central vascular cylinder (narrow cells in the center). Black arrows indicate signaling from the vascular cylinder to induce radial patterning in the overlying ground tissue (Section 5.3.); blue arrows indicate the likely dependence of hypophyseal cell fate acquisition on apical signals (Section 4.1.2.). Signals from hypophyseal derivatives (red arrows) confer stem cell identity in the root meristem (a.d. and b.d. = the apical and basal domains respectively. Section 4). **B**: Signals from the shoot meristem promote adaxial-abaxial polarity in leaves, while conversely, adaxial cell fate in leaf primordia promotes shoot meristem development, yellow arrows (Section 4.2.3). **C:** Positioning of lateral shoot organs. Primordia are restricted to the peripheral zone of the meristem. Initiated with an arbitrarily positioned cotyledon primordium (C1), graded lateral inhibition (indicated by green gradients) could restrict C2 to a position opposite C1. As subsequent leaf primordia (L1-L5) are produced, less synchronous differential inhibition would lead to a gradual transition of phyllotactic angles from 180˚ to 137˚ (Section 4.2.2.).

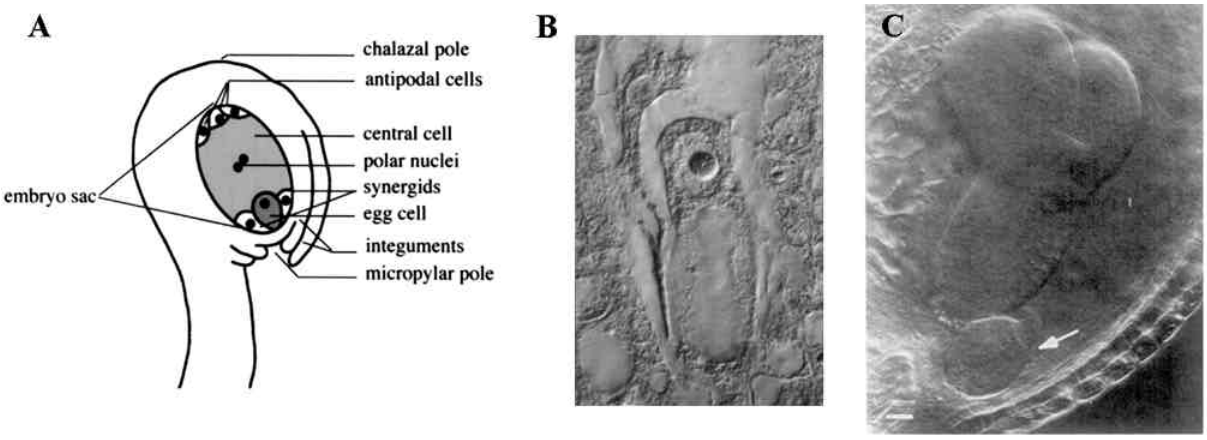

Figure 3: Maternal polarity and embryo development
A: The egg cell develops at the micropylar end of the embryo sac and its basal pole points towards the outside. B: Ultrastructure of a polarized egg cell from the related crucifer *Capsella bursa pastoris*. Apical-basal embryo patterning can be uncoupled from egg cell polarity. C: For example, *twin* mutants develop two embryos of opposite polarity. Arrow points at the basal end of a second embryo developing from a suspensor cell. A: from Mordhorst et al., 1997. B: courtesy of R. Raghavan. C: from Vernon and Meinke, 1994.

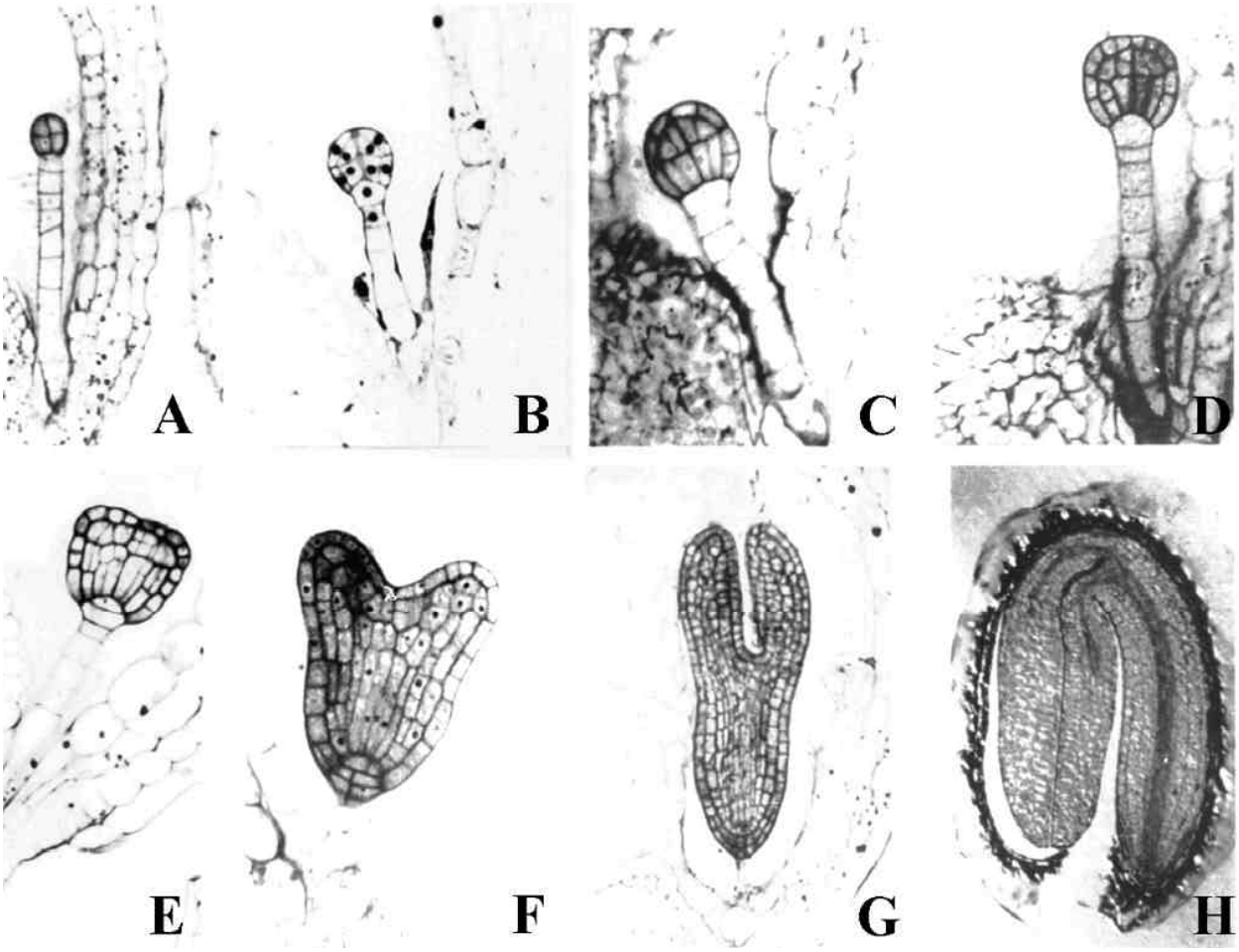

Figure 4: Stages of Arabidopsis embryogenesis.
A: Octant stage; four of eight cells (darkly stained) in two tiers are visible. Cells of the upper and lower tier (*u.t.* and *l.t.*) of the octant will give rise to specific parts of the seedling (see Figure 1). Together with descendants of the uppermost suspensor cell (hypophyseal cell) the eight 'octant' cells will form all the structures of the seedling. **B**: Dermatogen stage. A tangential division of each of the eight 'octant' cells produces inner cells and epidermis (protoderm) cells. **C**: Early globular stage; the divisions of the inner cells immediately after the dermatogen stage are oriented in the apical-basal dimension, endowing the embryo with a morphologically recognizable axis. **D**: Procambial stage: Differences in the orientation of cell divisions in the center and periphery of the *l.t.* have generated narrow procambial cells. At this stage, *u.t.* cells remain isodiametric. **E**: Triangular stage; now a polarized pattern of major elements is recognizable (see text): *u.t.* cells have generated two symmetrically positioned cotyledon primordia and *l.t.* cells a radially patterned cylinder (comprising epidermis, ground tissue and vascular tissue). Additional divisions distinguish the 'hypophyseal cell' from other suspensor cells. Its descendants will ultimately form the quiescent center of the primary root meristem and the columella initials. **F**: Heart stage; cotyledon outgrowth. Subsequently, cells not contributing to cotyledon formation initiate the primary shoot meristem (not visible in **F**). Divisions of the hypophyseal cell have generated three tiers. The quiescent center of the primary root meristem being derived from the uppermost tier. **G**: Mid-torpedo stage; enlargement of cotyledons and hypocotyl and further elaboration of the radial pattern. Vascular differentiation in the cotyledons is visible. **H**: Bent cotyledon stage embryo with elaborated radial pattern in different organs. In the cotyledons a single adaxial sub-epidermal layer of elongated cells (palisade mesophyll) can be distinguished from underlying mesophyll cells. The radial pattern of the hypocotyl is comprised of; a single epidermal, layer, two cortical layers, one of endodermis and one pericycle layer enclosing the vascular cylinder. (From Przemeck et al., 1996).

lazal pole (Figure 3B). To complete fertilization, the pollen tube enters the ovule through the micropyle and delivers two haploid nuclei, one of which fuses with the nucleus of the egg cell, while the other combines with the central cell. This double fertilization event initiates the development of two intimately interconnected multicellular structures, the embryo and the endosperm, which are derived from the zygote and the fertilized central cell, respectively. The development of the endosperm is amazingly complex (Berger, 1999; Brown et al., 1999), but because there is no evidence to support its direct involvement in specifying cell fates in the Arabidopsis embryo it will not be discussed in this review.

In Arabidopsis embryogenesis the pattern of cell division follows the *Capsella* variation of the Onagrad type, commencing with an approximately 3-fold elongation of the zygote and followed by an asymmetric cell division, yielding a smaller apical and a larger basal cell (Mansfield and Briaty, 1991. See Figure1A). These two cells differ profoundly in their internal composition and in their subsequent division patterns. The apical cell contains dense cytoplasm and is the site of very active protein synthesis, whereas the basal cell and its descendants are highly vacuolated. Through perpendicular shifts during three successive rounds of cell division, the apical cell gives rise to a sphere composed of eight cells, the octant (Figure 1B and 4A). The sequence of these early divisions of the apical cell descendants is highly reproducible. Two vertical and one horizontal division produce the octant, comprising an upper and a lower tier (*u.t.* and *l.t.* in the following), which will later give rise to the apical and much of the basal portion of the seedling respectively. By contrast, the basal cell divides horizontally and thereby produces a filamentous structure, the suspensor. Most of the mature embryo is thus derived from the apical cell. However, parts of the root apex originate from the basal cell, as the uppermost suspensor cell (the hypophysis) becomes incorporated in the formation of the embryonic root meristem (Figure 1A-F and Section 4.1.2.).

In the octant stage embryo, a single round of tangential divisions separates an outer layer of eight epidermal precursor (or protoderm) cells from eight inner cells (Figure 1C and 4B). Protoderm and inner cells soon become histologically distinguishable and due to predominantly anticlinal divisions in the outer layer, the protoderm remains essentially separated from inner cells throughout development. While the external shape of the embryo remains globular for a while, cell divisions of inner cells already reveal axis formation and regional differentiation. Initially, all inner cells adopt a common orientation of cell division, in which newly formed cell walls are aligned along the apical basal axis (Figure 1D and 4C). Therefore, the inner cells remain organized in two tiers, but amplify the number of cells in each tier. Further, the common orientation of division endows the still globular embryo with an anatomically recognizable apical-basal axis. At this stage, there is little indication of differential cell behavior along this axis (*u.t.* versus *l.t.*). Thus, the embryo proper at this stage can be considered as 'axialized' but not yet asymmetrically 'polarized' in the apical-basal dimension. However, immediately succeeding rounds of oriented cell division generate narrow cells specifically in the center of the *l.t.* (Figure 4D). Thereby, in addition to radially subdividing the emerging cylindrical cell pattern into central vascular and surrounding ground tissue, these divisions also reflect apical-basal polarity through differential cell behavior of *u.t.* and *l.t.* cells. Further divisions of *l.t.* descendants are strictly oriented either parallel or perpendicular to the apical-basal axis, generating continuous cell files in increasing numbers of concentric cell layers (Figure 4E to 4H). Cell divisions of *u.t.* descendants are less strictly oriented and these cells also remain largely isodiametric prior to the initiation of the cotyledons (Figure 4E). At late globular stage, when the number of cells has increased to more than a hundred, the embryo gradually assumes a triangular shape due to localized growth at two opposite positions in the apical region (Figure 4E). The early heart stage embryo (also referred to as 'triangular' stage) comprises approximately 200 cells and the primordia of most major seedling organs, cotyledons, hypocotyl and primary root, as well as the basic tissue types, provascular, protoderm and cortex are anatomically discernible.

The development of the suspensor is somewhat variable**,** but the two terminal cells adopt invariant fates. The uppermost cell, the hypophysis, undergoes a sequence of reproducible divisions giving rise to part of the primary root meristem, comprising the quiescent center and the central (columella) root cap initials (Scheres et al., 1994). The most basal suspensor cell enlarges dramatically and has abundant contact with surrounding maternal tissues, likely facilitating the supply of nutrients to the embryo. It is interesting to note that in a number of species no plasmodesmata connecting the embryo to maternal tissue have been observed, in sharp contrast to the extensive symplastic connections within the embryo (Lyndon, 1990). Therefore, it is unlikely that high molecular weight molecules of maternal origin can be transferred to the embryo.

Further refinement of the embryonic pattern occurs during succeeding developmental stages, in which the embryo adopts sequentially "heart", "torpedo" then "bent cotyledon" shapes (Figure 4F, G and H). In post heart-stage embryos the shoot meristem becomes discernible as three distinct cell layers that will subsequently attain a tunica-corpus organization (Barton and Poethig, 1993). In torpedo and bent-cotyledon stage embryos, provascular tissues also become recognizable within cotyledon primordia and the cellular organization of hypocotyl and root is completed (Figure 4H). Although cells in most tissues

will complete differentiation after germination, the complexity of the tissue pattern in the bent-cotyledon stage embryo basically equals that of the seedling.

3. Conclusions from variable development

3.1. Is there maternal control?

Maternal influences on embryonic development in Arabidopsis have been reported (Ray, 1998), but apparently these are not involved in specifying basic architectural features of the embryo, such as the establishment of apical-basal polarity. There is obvious polarity alignment within the reproductive apparatus (Figure 3A), and it therefore seems reasonable to suggest that the polarity of the ovule impinges on the polar orientation of the embryo sac, the egg cell (Figure 3B) and thereby the zygotic embryo. However, apical-basal pattern formation in the embryo is not dependent upon this external influence and can be uncoupled from it. This can be demonstrated not only in somatic embryos, which display properly ordered apical-basal patterns in the absence of any fixed spatial relationship to maternal structures, but also in zygotic embryos, which can develop normally in abnormal orientation relative to maternal structures. In 'twin' mutants for example (Schwartz et al., 1994; Zhang and Somerville, 1997), multiple embryos from the same zygote can develop inverted apical-basal patterns (Vernon and Meinke, 1994. Figure 3C), excluding the possibility that the polarity of the zygote stringently predisposes the polarity of the embryo.

3.2 Lineage or position?

The reproducible sequence of cell divisions in the Arabidopsis embryo effectively results in the specification of cell fates along predictable cell lineages. For example, characteristic early divisions suggest a hierarchy of partitioning events, in which early established lineages contribute only to particular structures in the mature embryo (Section 2; Figure 1). Does this predictability reflect lineage-imposed cell fate restriction? Obviously, lineage-imposed fate restriction cannot be recognized in an invariant system, since here its consequences cannot be distinguished from those of reproducible positional specification. In a number of Arabidopsis mutants however, the regular sequence of cell divisions is dramatically disturbed and yet major organs and tissues remain properly positioned (Torres Ruiz and Jürgens, 1994; Figure 5). These observations suggest that the specification of the basic embryonic pattern is largely, if not entirely, based on positional cues, which are surprisingly indifferent to cell boundaries, cell numbers and overall dimensions. The interpretation is consistent with the highly variable development observed in embryos of certain angiosperm species (Johri, 1984) and of Arabidopsis embryos developing in culture (Luo and Koop, 1997). However, it does not exclude a role for lineage-dependent cell fate specification in local patterning processes at later stages.

3.3. A second level of pattern control?

If the sequence of cell divisions has no bearing on the resulting pattern, why then is embryonic cell division so invariant? What evolutionary pressure could have generated a second, tighter level of control specifying each individual division so precisely? Perhaps the most attractive explanation is that the stereotyped pattern of divisions in unperturbed Arabidopsis development constitutes the product of an optimization process to produce functional cell patterns from a minimum number of cells. While it seems possible to generate a seedling pattern in various ways, it is hard to imagine how this can be accomplished with fewer cells. In the Arabidopsis seedling, individual tissue layers are often only one cell wide, which obviously constrains the variability of a functional patterning system. Consistent with this interpretation, the generation of the seedling pattern through variable cell divisions is typically associated with larger cell numbers. Therefore, in small embryos there could be a second level of control, probably involving numerous short-range interactions, which together specify the course of embryo development in great detail.

4. Generating the apical-basal pattern

The seedling pattern is often viewed as the superimposition of an apical-basal and a radial pattern. In this review, we will follow this scheme as a suitable formal categorization, but it should be emphasized that the available evi-

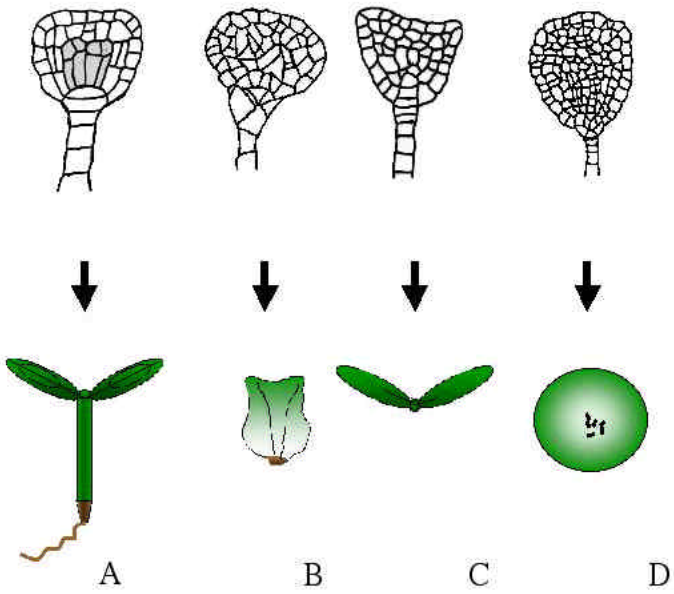

Figure 5: Cell pattern independent signaling in embryo pattern formation
A: The wild type Arabidopsis embryo pattern is generated in a highly invariant sequence of cell divisions (triangular stage, top; seedling pattern, bottom). **B**: Although mutations in the *FASS/TON2* gene distort cell dimensions and result in irregular cell patterns in early embryos, functional seedlings still develop. These are short and stout, but all major organs and tissues aFre properly positioned. By contrast, patterns remain abnormal in two other mutants (*monopteros*, **C**) and *(embryo defective 30/gnom*, **D**) presumed to be defective in auxin transport and auxin signal transduction, respectively.
Gray shading in **A**, early procambial tissue. Lines in seedling schemes **A, B** and **C** symbolize continuous vascular strands, dark dots in **D** isolated, randomly oriented vascular cells. **A-D** adapted from (Berleth, 2001), original data from Torres Ruiz and Jürgens 1994, **B**; Berleth and Jürgens 1993, **C**; Mayer et al., 1993, **D**)

dence does not support a strict separation of patterning cues along these axes. Rather, it appears that individual genes can be involved in patterning events along both axes (Sections 4.1.1. and 5.2.) and that patterning in a single dimension may involve the piecemeal action of independent signaling processes (Section 5.1. and 5.2.).

The main elements to be positioned along the apical-basal axis are the shoot apical meristem (SAM), cotyledons, hypocotyl, radicle and the root apical meristem (RAM). For the most part, SAM and parts of the cotyledons originate from the *u.t.*, hypcotyl and radicle (including most RAM initials) from the *l.t.*, and the quiescent center and columella from the hypophyseal cell (Scheres et al., 1994; Figure 1). As will be discussed below, the primary shoot and root meristems are generated as integral parts of signaling processes defining an apical and a basal embryo domain, respectively. In the following sections, we will discuss signaling processes within the basal and the apical domain and then apical-basal polarity itself.

4.1. Patterning the basal domain

The basal domain comprises the hypocotyl, the radicle and the primary root meristem (Figure1 and 2A). Essential steps in basal patterning are the formation of a radially structured cylinder in the late globular-stage embryo and the subsequent establishment of a complex stem cell system at the basal end of this cylinder (Figure 6A). We will refer to these two processes as the formation of the embryo axis and of the primary root meristem, respectively. Despite signaling overlap, they will be discussed in separate sections.

4.1.1. Formation of the embryo axis

Most of the body of the seedling takes the form of a cylinder comprised of concentrically arrayed tissue layers. The hypocotyl and radicle are sections along this cylinder, with distinguishable anatomical and physiological properties. The initiation of the embryo axis occurs through strictly oriented divisions in the early globular embryo, probably reflecting some sort of vectorial signaling in the apical-basal direction (compare Section 2. Figure 1D and 4C). Divisions oriented along the apical-basal axis predominate among *l.t.* cells and generate a cylinder of parallel cell files. This cylinder becomes radially structured by further divisions in the center, which generate narrow procambial cells of the embryonic stele (Figure 4D). Mutations in three genes, *MONOPTEROS* (*MP*), *BODENLOS* (*BDL*) and *AUXIN RESISTANT 6* (*AXR6*) interfere to varying degrees with the formation of the embryo axis, recognizable by less oriented divisions in the basal domain (Figure 6B) (Berleth and Jurgens, 1993; Hamann et al., 1999; Hobbie et al., 2000). In addition to an apparent deficiency in responding to axial cues, all three mutants fail to initiate a primary root meristem, have severely reduced vascular systems and variably fused cotyledons (Figure 6D-F). Axial cell alignment, vascular differentiation and root induction could have a common derivation in defective responses to polarly transported auxin (Sachs, 1991; Przemeck et al., 1996). This interpretation is supported by independent evidence, which implicates all three genes in auxin signal transduction. Mutations in *BDL* and *AXR6* are associated with defective response to exogenous auxin (Hamann et al., 1999; Hobbie et al., 2000) and *MP* encodes a transcriptional regulator of the 'Auxin Response Factor' (ARF) family (Hardtke and Berleth, 1998). This family of transcription factors can confer auxin responsiveness to downstream genes by binding to conserved 'auxin response' promoter elements (Ulmasov et al., 1997; Guilfoyle et al., 1998), apparently modulated in activity by interaction with nuclear proteins of the AUX/IAA family (recently reviewed in; Hagen and Guilfoyle, 2001; Liscum and Reed, 2001; Leyser, 2001). As a polarly transported, non-cellautonomous signal, auxin could mediate coordinated axis formation and polarization of cell groups (Figure 7). Therefore, failure to differentiate along the apical-basal axis could be an embryonic feature indicative of defects in auxin signal transduction or auxin transport. Interestingly, a mutation in the Arabidopsis gene encoding *AUXIN BINDING PROTEIN 1* (*ABP1*) results in embryos comprised of misoriented and poorly elongated cells (Chen et al., 2001). The auxin binding properties of ABP1 have been charac-

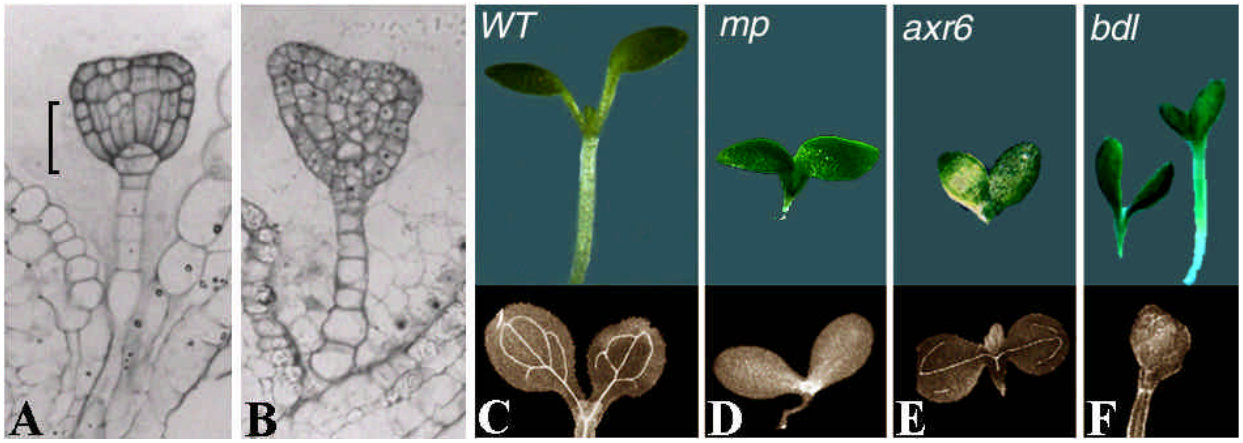

Figure 6: Embryo axis formation and auxin signal transduction
A: Basal domain of the wild type triangular-stage embryo (marked by bracket) comprises files of narrow cells in the center (embryonic stele) and a characteristically dividing uppermost suspensor cell ('hypophyseal' cell), whose derivatives form the QC and columella initials of the primary RAM. **B**: Triangular-stage *mp* mutant, cell divisions are not oriented along the apical basal axis, narrow cells of the embryonic stele are missing and the uppermost suspensor cell divides abnormally. **D-F**: Seedlings mutant for the presumed auxin signal transduction genes *MONOPTEROS (MP), AUXIN RESISTANT 6 (AXR6)* and *BODENLOS (BDL)* are defective in embryo axis formation and vascular differentiation. Hypocotyl and root are missing in *mp* and *axr6* mutants (**D** and **E**) and are variably reduced in *bdl* mutants (**F**).
A and **B**: from Berleth and Jürgens, 93. **C-E**: Seedling phenotypes and dark field view of xylem strands of indicated genotypes reproduced from Berleth et al., 2000; Hobbie et al., 2000 and Hamann et al., 1999, respectively.

terized extensively (summarized in Venis and Napier, 1995) raising the possibility that ABP1 could act as an auxin receptor. Future studies will directly assess whether mutant cells are defective in auxin perception. Overall therefore, mutant phenotypes suggest that auxin signal transduction is required for oriented cell division and vascular differentiation in the basal domain, and these differentiation events may constitute prerequisites for subsequent radial differentiation (Section 5.2.) and root meristem formation (Section 4.1.2.).

Defects in sterol synthesis also impinge on the formation of the embryo axis. Mutations in the *FACKEL* (*FK*) gene, which encodes a sterol C-14 reductase, result in seedlings with extremely reduced hypocotyls, which nevertheless produce cotyledons as well as shoot and root meristems (Jang et al., 2000; Schrick et al., 2000). *FK* encodes a sterol C-14 reductase and mutations are associated with abnormal sterol composition, various defects in cell elongation and division, shoot meristem programming and adult organ shape. Mutants cannot be rescued by application of brassinosteroids and it remains to be determined whether *fk* mutant defects reflect new types of sterol signals, or whether they are due to changes in general cell properties, such as abnormal sterol compositions in cell membranes.

4.1.2. Formation of the primary root meristem

The RAM cell pattern consists of concentrically arrayed stem cells (initials in the following) that extend the radial pattern in the growing root, the four cells of the quiescent center (QC), which divide only infrequently, and most distally, the initials of the central root cap (columella) (Figure 8A). This highly organized cell pattern is not only generated in the embryo, but also post-embryonically in the formation of lateral roots. As these emerge from the pericycle at considerable distance from the meristem of the higher order root, the cell pattern seems to be generated de novo in each lateral root primordium, but nevertheless in response to shoot derived signals.

Patterning the RAM involves the establishment and maintenance of a stable stem cell pool at the root tip, specification of proper tissue identity of initials in their respective positions and, in order to get the system started, some mechanistic link to a localized signal from the shoot (Figure 8A). It has long been suspected that cell fate in the RAM is largely controlled by positional cues and more recently single-cell ablation has been employed to trace the origin of some of these cues (summarized in Scheres and Heidstra, 1999). One, originating from the QC, seems to confer stem cell identity to cells surrounding

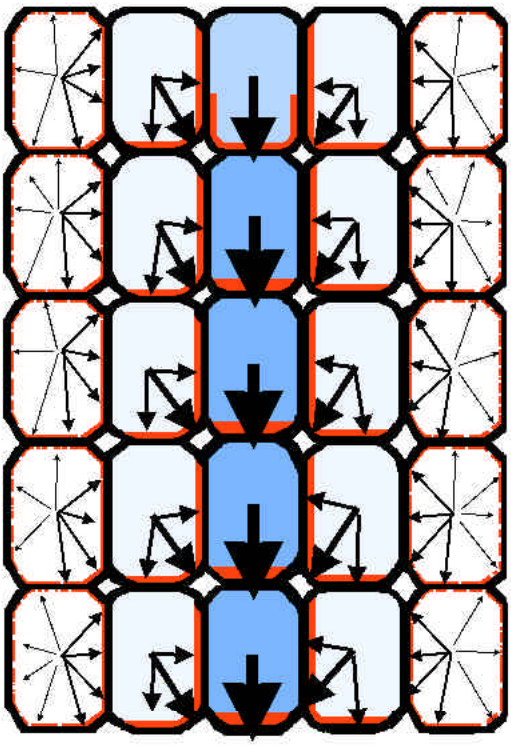

Figure 7: Integration of cell polarity through auxin transport
A highly schematic view. Rectangles represent cells and arrows of different strength represent the intensity of auxin flow. For simplicity it is assumed that intensity and direction of auxin flow is solely controlled through the quantity and distribution of auxin efflux carriers (orange) in the plasma membrane. Routes of preferred auxin transport have been associated with sites of vascular differentiation (blue).
The central proposition is that auxin flow and cell polarization are connected in a positive feed-back loop, illustrated here by restricting auxin efflux to the basal side of each cell as an expression of cell polarization. Thereby, cells in a given region, including cells newly formed by division, would integrate polarity. The feed-back system could further include the stabilization of auxin sources or sinks. Note that the same the cellular feed-back mechanism would progressively enhance initial differences in auxin conductivity leading to the specification of different cell types in the radial dimension (drawn after Sachs, 1991).

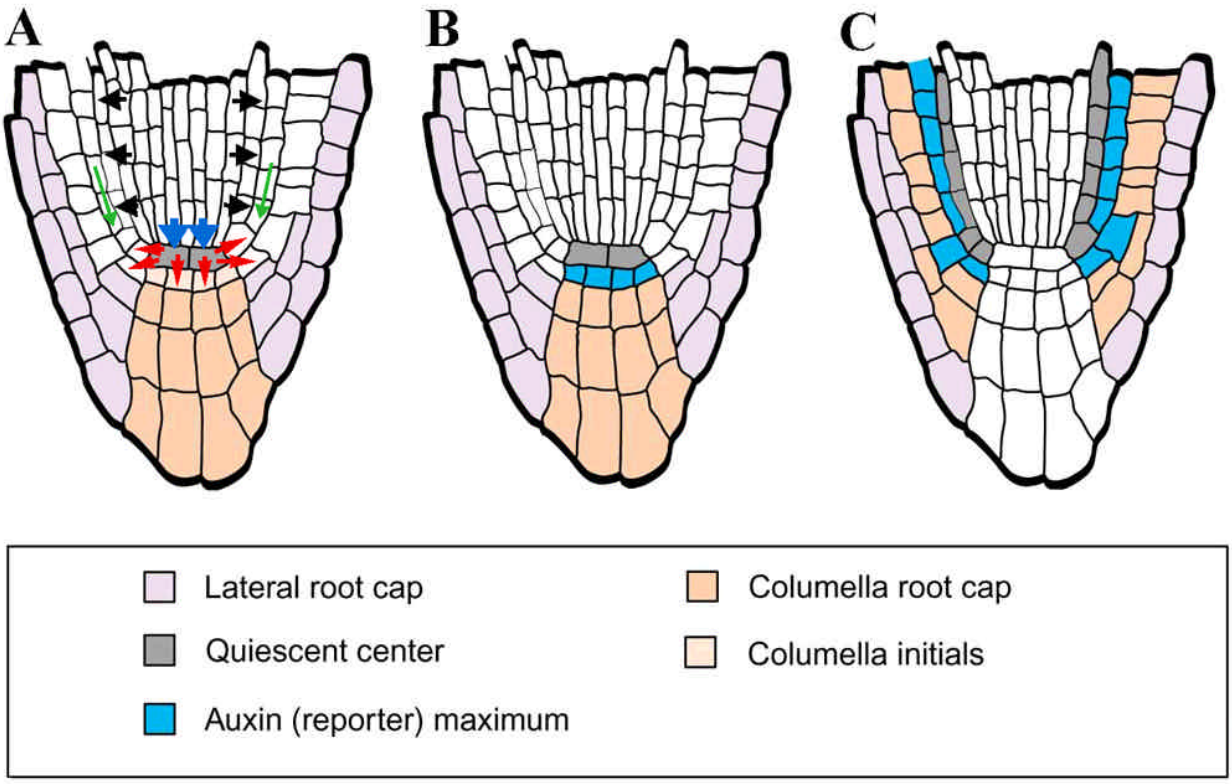

Figure 8: Cell fate specification in the root meristem
A: Organization of cell types in the root meristem. Centrally located QC cells (grey) are flanked by initials of various tissues: initials extending tissue layers in the growing root and, laterally and basally, initials replenishing cells in the lateral (violet) and central root cap (orange). Blue arrows indicate that the acquisition of QC cell fate seems to be dependent on signals from the shoot (compare Figure 2A); red arrows the dependence of stem cell fate on signals from the QC. Black arrows represent endodermis inducing signals from the stele (Figure 18) and green arrows the stabilization of tissue identity within each layer. B: An auxin-response reporter gene detects a maximum (blue) at the position of the columella initial cells. C: When the auxin response maximum is displaced (e.g. because of auxin transport inhibition), the positions of all three cell types in relation to the stele and auxin response maximum are maintained, suggesting an important role for auxin distribution in root meristem patterning. From Scheres, 2000.

the QC (Figure 8A). Ablation of individual QC cells is associated with the loss of stem cell identity in neighboring cells, which instead acquire characteristics of their differentiated daughter cells (van den Berg et al., 1997). Short-range signaling from the QC would obviously be a very suitable mechanism to ensure that a stem cell pool of constant size is maintained at the root tip.

Another type of positional signal seems to help integrate the tissue identity of all cells in a given layer (Figure 8A). Cell ablation experiments revealed that cortex tissue continuity is required to maintain the identity of the cortex-endodermal initial, suggesting that signals from more mature cells are transmitted within a tissue layer to reinforce tissue identity of less mature cells (van den Berg et al., 1995). Many Arabidopsis tissues comprise only single cell layers, but their functions often critically depend on tissue continuity. Therefore, integrating signals passed along individual tissue layers (in combination with highly flexible radial patterning mechanisms, Section 5.3.) could help stabilize an otherwise fragile radial pattern.

If the QC has a central role in conferring stem cell identity on neighboring cells how is the QC itself positioned? A recent study suggests that auxin may act as a positional signal in proximal-distal patterning in the RAM, specifying the positions of the QC and columella initials relative to the vascular cylinder (Sabatini et al., 1999). When the intensity

of an auxin response was visualized through reporter gene expression under control of an auxin-response element, an auxin-response maximum was found associated with the position of the columella initials, adjacent to the QC (Figure 8B). Interestingly, any shift in the localization of this maximum relative to the vascular cylinder, through genetic or experimental interference with auxin transport, was associated with a correlated shift in the pattern of distal cell fates, suggesting an instrumental role of auxin signals in root meristem patterning (Figure 8C). Auxins have long been known to stimulate root formation and could therefore serve as signals to coordinate root initiation with shoot requirements. Although the experiments do not directly address the origin of the auxin signal, it seems possible that the situation in the embryo parallels signaling to induce lateral or adventitious root formation in an adult plant. Auxin supplied from apical sources could specify QC fate, and thereby indirectly a zone of stem cell activity close to the distal end of the stele (Figures 6A and 8A).

Consistent with this interpretation, the uppermost suspensor cell fails to adopt 'hypophyseal' identity in embryos mutant for any one of the auxin signal transduction genes of the *MP*; *BDL*; *AXR6* class (Figure 6B). The function of these genes could be required for either cell differentiation crucial for delivering the auxin signal (e.g. via generation of the stele), for auxin signal transduction within the uppermost suspensor cell itself, or for both processes. While this question is presently unanswered, there is another class of mutants, termed 'hypophyseal group' mutants, whose embryonic defects resemble those in *mp*; *bdl*; *axr6* mutants specifically in the hypophyseal region (Scheres et al., 1996; Willemsen et al., 1998). The 'hypophyseal group' genes could therefore be involved in the local perception and or interpretation of an auxin signal.

In summary, the largely cylindrical architecture of the basal domain seems to be initiated in response to directional signals from the apical domain, and apical signals also seem responsible for inducing QC identity. A properly localized QC may in turn anchor the position of initials elongating the cell files of the growing root.

4.2. Patterning the apical domain

The formation of the apical pattern comprises; (I) generation of cotyledons and SAM (4.2.1.), (ii) proper spacing of lateral shoot organs 4.2.2.) and (iii) adaxial-abaxial patterning within each lateral organ primordium (4.2.3.). Although the two latter processes do not fall under apical-basal patterning in a strict sense, the gene activities organizing all three aspects of the apical pattern are integrated to such a degree as to preclude independent discussion.

4.2.1. Generating the cotyledons and the shoot apical meristem

Cotyledons can be considered as embryonic lateral shoot organs, although they differ from post-embryonic leaves in many ways. Lateral shoot organs are typically highly adapted to their stage specific function, but their common origin in a vegetative ground state can be demonstrated genetically. This is also true for cotyledons, which acquire many properties of rosette leaves, if genes relaying late-embryonic signals are defective (Meinke 1992; Keith et al., 1994; Conway and Poethig, 1997). Loss-of-function mutants impaired in the formation of cotyledons have been identified and define the *GURKE* (*GRK*) (Torres Ruiz et al., 1996) and *PASTICCINO* (*PAS*) (Faure et al., 1998; Vittorioso et al., 1998) genes. However, the phenotypes of these mutants are relatively pleiotropic and their gene products are either unknown or cannot easily be placed in a signaling context. Therefore, there is presently no molecular model for the very early organization of the apical domain.

Formation of a permanent shoot apical meristem within the apical domain requires localized signals to confer stem cell identity to a small population of cells between the cotyledon primordia (Figure 1F, G; Barton and Poethig, 1993). Continuous meristematic activity further requires stable zonation within the SAM, with stem cell identity restricted to cells within a small central zone, while cells displaced to the periphery undergo differentiation (Figure 9). The delicate balance between the pools of stem cells and differentiating cells in the permanent SAM is maintained through negative feed-back interactions between antagonistic activities (reviewed in Clark, 2001; Lenhard and Laux, 1999; Waites and Simon, 2000 and discussed in detail in the chapter on shoot meristem development). Since the shoot is embryonically initiated with the formation of lateral organs, it may not be surprising that the two genes identified as required for SAM initiation, *SHOOT MERISTEMLESS* (*STM*) and *WUSCHEL* (*WUS*), also function as permanent positive regulators of stem cell identity in the SAM.

STM encodes a maize KNOTTED-type homeo-domain transcription factor and is expressed in the position of the incipient SAM from late globular stage onward (Figure 10) (Long et al., 1996). Loss-of-function mutations in *STM* result in embryos, which display slightly fused cotyledons (4.2.2.) and fail to produce a SAM (Barton and Poethig, 1993). Post-embryonically, *STM* is expressed in the SAM

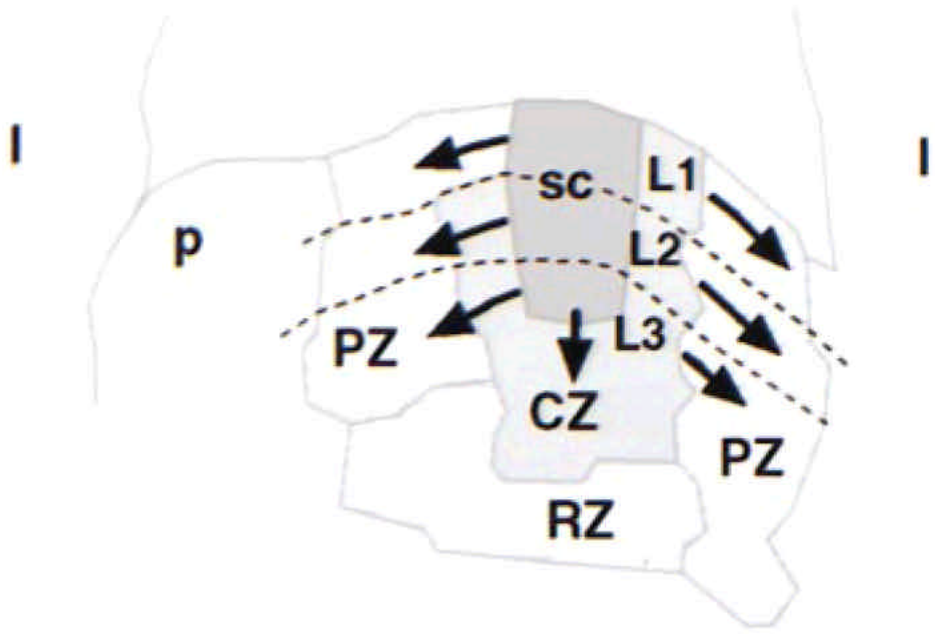

Figure 9: Zonation of the shoot apical meristem
Stem cells (sc) in the SAM are restricted to the central zone (CZ), while cells displaced to the peripheral zone (PZ) differentiate and are incorporated into lateral organ primordia (P). CZ central zone, domain of *WUS* expression; RZ, rib zone, origin of the pith; l, leaf; L1, L2, L3 cell layers. From Mayer et al., 1998.

center and turned off as cells are recruited towards the formation of lateral organ primordia (Figures 10 and 11). The *MYB* domain transcription factor *ASYMMETRIC LEAVES 1* (*AS1*), which has been implicated in specifying organ founder cell identity in lateral organ primordia, is expressed in a reciprocal pattern and in situ hybridization analysis suggests that *AS1* expression in the center is suppressed by *STM* (Byrne et al., 2000). Interestingly, properly positioned SAMs are formed in *stm;as1* double mutants, indicating that *STM* is not strictly required for SAM formation in the apical domain. Instead, *STM* could primarily be involved in locally counteracting *AS1* activity to maintain undifferentiated cell fates in the meristem center. Since *AS1* is already expressed in the cotyledons, this counteracting function could explain why *STM* is required for the embryonic initiation of the SAM (Figure 10).

Another positive regulator of stem cell identity, *WUSCHEL* (*WUS*), encodes a novel homeo-domain transcription factor, which is continuously expressed in the established SAM beneath the zone of proliferating stem cells (Figure 9; Mayer et al., 1998). In this case, the signal antagonizing *WUS* activity originates from the stem cells themselves and is passed through the *CLAVATA* pathway negatively regulating *WUS* expression (details reviewed in Lenhard and Laux, 1999; Waites and Simon, 2000; Clark, 2001). In *wus* mutants, the stem cell pool is reduced, but not absent and the formation of the SAM is delayed, although SAMs will eventually arise in abnormal positions after germination (Laux et al., 1996). Therefore, *WUS* activity is obviously crucial for the positioned initiation of the SAM in the embryo, but there seem to be other positive regulators of stem cell identity that can partially substitute for *WUS* activity. Consistent with a function in initiating the SAM within the apical domain, *WUS* is expressed in a conspicuous pattern in the very early embryo (Figure 12) and *WUS* expression seems to be sufficient to confer stem cell identity at least in a variety of positions (Schoof et al., 2000). Therefore, *WUS,* or regulators of apical W*US* expression, seem to be crucial in anchoring shoot development in the apical domain.

4.2.2. Positioning of lateral organs

Lateral shoot organs are placed around the circumference of the SAM in amazingly regular patterns termed phyl-

lotaxis. The mechanisms underlying this regularity have been subject to intensive research, which is summarized in a number of insightful reviews (Richards, 1951; Steeves and Sussex, 1989; Callos and Medford, 1994; Jean, 1994; Reinhardt and Kuhlemeier, 2001). Available evidence indicates the existence of an inhibiting influence originating from existing primordia on the formation of new ones (lateral inhibition), directing their formation to positions of lowest inhibition (Figure 2C). The embryo seems to provide the initial positional references in the form of two cotyledons at opposed positions on the apex, evidenced by the fact that abnormal cotyledon positions have predictable effects on vegetative phyllotaxis. If two cotyledons are properly spaced, the observed positions of the first two rosette leaves at maximum distance from the cotyledons and the gradual transition to spiral phyllotaxis are consistent with a lateral inhibition model (Figure 2C).

How is this phyllotactic system set-up initially? How are the cotyledon primordia positioned precisely relative to one another, if there is no prior reference point in a radially symmetric embryo? Obviously, lateral inhibition could also account for opposed cotyledon positions, if these were specified sequentially rather than simultaneously. The first primordium could be positioned arbitrarily in the periphery of the apical domain and the second at the position of least inhibition, opposite the first (Figure 2C). While attractive, this interpretation has been considered in conflict with the apparent simultaneous emergence of the two cotyledons. However, recent fate mapping of the embryonic shoot indicates that the two cotyledon primordia are in fact initiated sequentially (Woodrick et al., 2000). Therefore, current evidence is not in conflict with an integrated lateral inhibition mechanism regulating the position of shoot organs throughout development, after initiation at a single reference point in the early embryo (Figure 2C).

Embryo mutants and recent experiments also provide some insights into the molecular mechanisms underlying the spacing of shoot organs. Genes involved in the separation of cotyledon primordia fall into two broad categories. The first category of genes implicates auxin perception and auxin transport in the positioning and separation of cotyledon primordia. Defects in the aforemen-

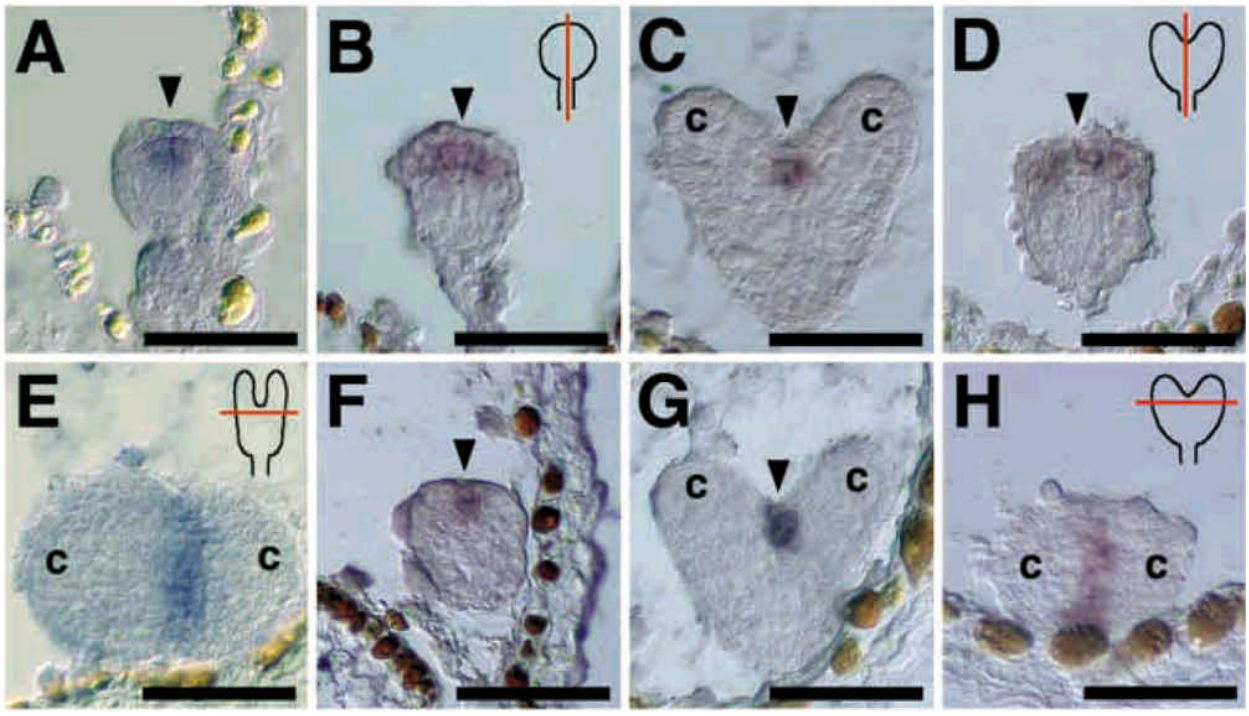

Figure 10: Embryonic expression of *CUC2* and *STM*
A-E: *CUC2* expression in globular (**A** and **B**), early heart (**C** and **D**) and torpedo (**E**) stage embryos. Red line in diagrams marks the position of the section, except for frontal sections in **A, C, F** and **G**. Note that the *CUC2* expression domain is elongated (central in **A** versus extended in **B**) already in the globular embryo, documenting bilateral symmetry in embryos of this stage. **F-H**: *STM* expression in globular (**F**) and heart-stage (**G** and **H**) embryos. The expression domains overlap except for the protoderm, where only *STM* is expressed (arrowheads in **A-C** and **F, G**). c, cotyledons. Bars = 40µm, from Aida et al., 1999.

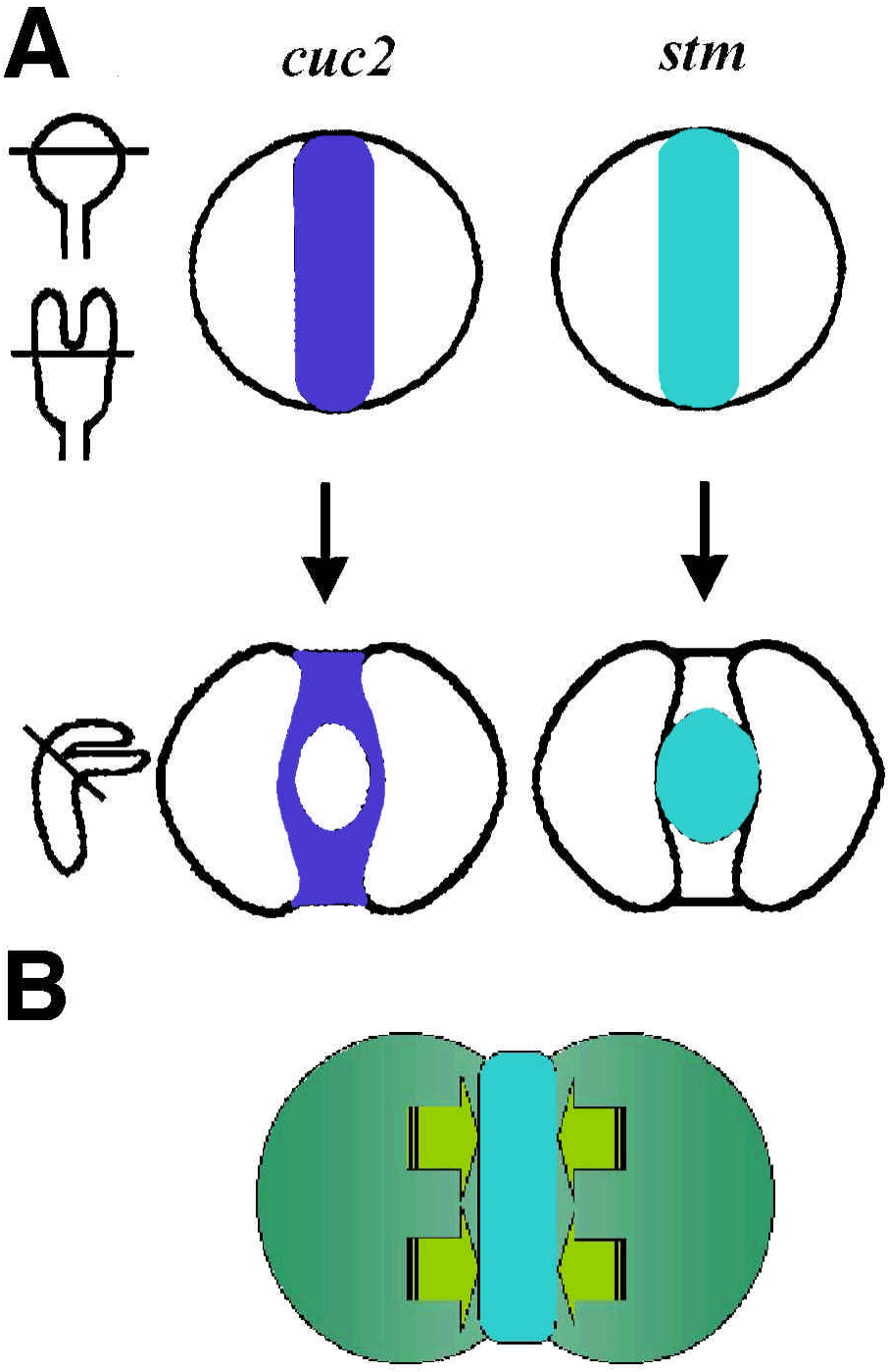

Figure 11: Positive and negative regulators of STM expression
A: In the early embryo, the functionally redundant genes *CUC 1* and *2* (*CUC 2* expression, dark blue) are required for separation of the cotyledons and for *STM* expression (light blue). Expression of *STM* across the entire diameter of the embryo is likely to promote cotyledon separation by suppressing founder cell identity in the periphery. After heart-stage, *CUC* genes do not seem to be required for *STM* expression as the expression domains no longer overlap. **B**: *STM* is permanently expressed and required in the SAM to suppress *AS1* activity, which confers founder cell identity to cells in lateral shoot organs (green). **A**: from Aida et al., 1999.

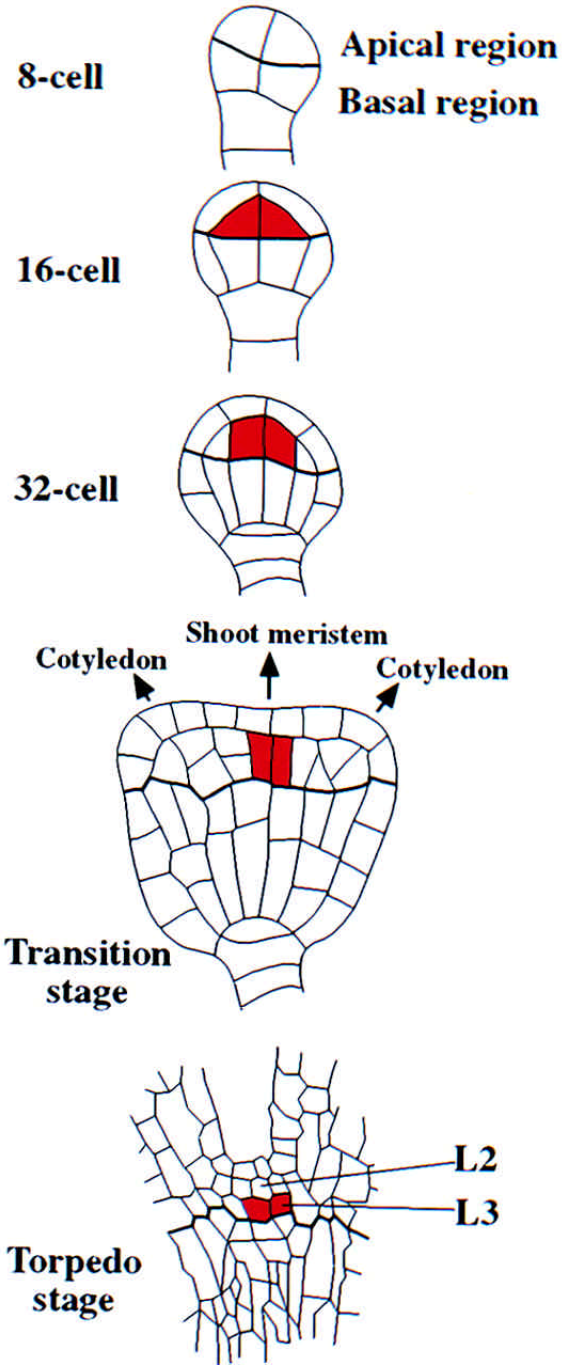

Figure 12: Embryonic expression of *WUS*
WUS transcripts are first detected in 16-cell dermatogen-stage embryos (red). Initially expressed in all sub-epidermal cells of the apical domain, *WUS* transcripts become gradually restricted to more central positions and deeper layers at the base of the shoot meristem. No functions have been assigned to WUS expression prior to the heart-stage and the molecular basis of *WUS* regulation is unclear. Precise regulation of *WUS* expression is critical to the formation of the apical pattern, since ectopic expression of *WUS* seems to confer stem cell identity at inappropriate sites. Modified from Mayer et al., 1998.

tioned auxin signal transduction genes *MP*, *BDL*, *AXR6* (Hardtke and Berleth, 1998; Hamann et al., 1999; Hobbie et al., 2000) and in the presumptive auxin efflux carrier gene *PIN FORMED 1* (*PIN 1*) (Galweiler et al., 1998) result in cotyledon fusions. Further, the exposure of cultured *Brassica juncea* embryos to chemical inhibitors of auxin transport results in similar defects (Liu, 1993; Figure 13). What kind of role could auxin have in positioning shoot organs? Local auxin application has been shown to induce shoot organ formation at the site of application (Sachs 1993; Reinhardt et al., 2000). The response is restricted to the peripheral zones of meristems, but within this zone it is the location and quantity of applied auxin that defines the position and width of the emerging lateral organ (Reinhardt et al., 2000; Figure 14). Most interestingly, application of excessive quantities of auxin result in

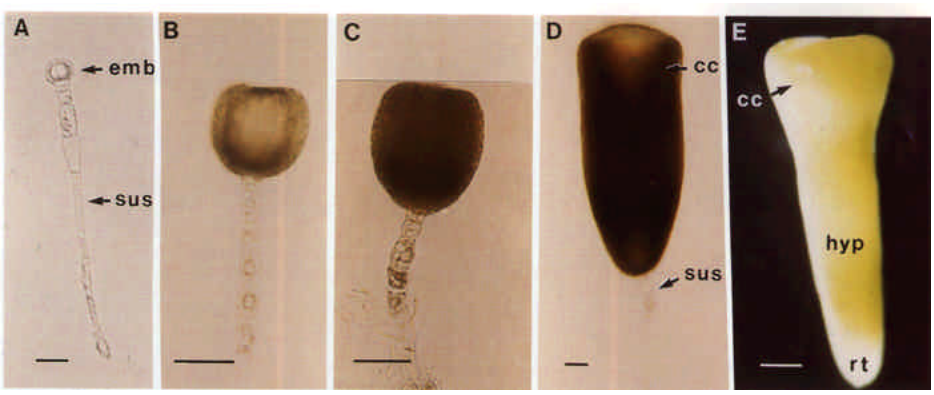

Figure 13: Auxin transport and cotyledon position
Culture of early embryos of *Brassica juncea* in the presence of auxin transport inhibitor substances results in high frequencies of seedlings with fused cotyledons. **A**-**E**: *Brassica juncea* embryos cultured *in vitro* in the presence of 2,3,5-triiodobenzoic acid (TIBA), an auxin transport inhibitor, for 0, 1, 2, 4, and 7 days respectively. From Liu et al., 1993.

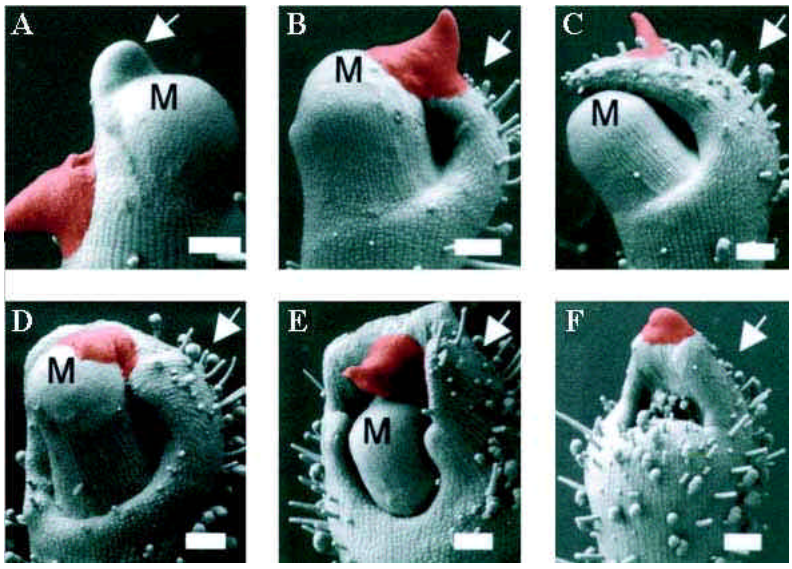

Figure 14: Induction of leaf formation by local application of IAA
Tomato shoot meristems exposed to the auxin transport inhibitor NPA grow as an apical dome without lateral leaf primordia. When IAA is locally applied to the flank of this dome, a leaf primordium is induced at the site of application. Increasing amounts of IAA result in primordia with enlarged width around the circumference/perimeter of the meristem. **A-F**: Leaf primordia one week after IAA has been applied to a site marked by red coloration at concentrations of 0.1, 0.3, 1, 3, 10 and 30mM, respectively. White arrows point to leaf primordia. Bar = 100µm. From Reinhardt et al., 2000.

collar-shaped primordia encircling the entire apex. Therefore, normal organ formation requires a highly regulated, local auxin signal, which is probably dependent on a precise balance between local auxin synthesis and auxin removal through transport or metabolism. Various scenarios can be envisaged as to how such a mechanism could effectively result in lateral inhibition around a growing primordium (Figure 15).

The second class of genes involved in the separation of the cotyledon primordia comprises *STM* and the *CUP-SHAPED COTYLEDON 1* and *2* (*CUC1* and *2*) genes. These genes are expressed in a domain across the apex until the heart stage and seem to define the zone that does not contribute to cotyledon primordia (Aida et al., 1999; Takada et al., 2001; Figures 10 and 11). *CUC1* and *2* encode highly related, functionally redundant NAC-domain proteins. The biochemical function of the CUC gene products is unknown, but they seem to exert their early influence on the cotyledon pattern partly through *STM*. *STM* mRNA is not detectable in early *cuc1*:*cuc2* double mutants and *stm* mutants show some degree of cotyledon fusion. Although the *CUC* genes and *STM* do

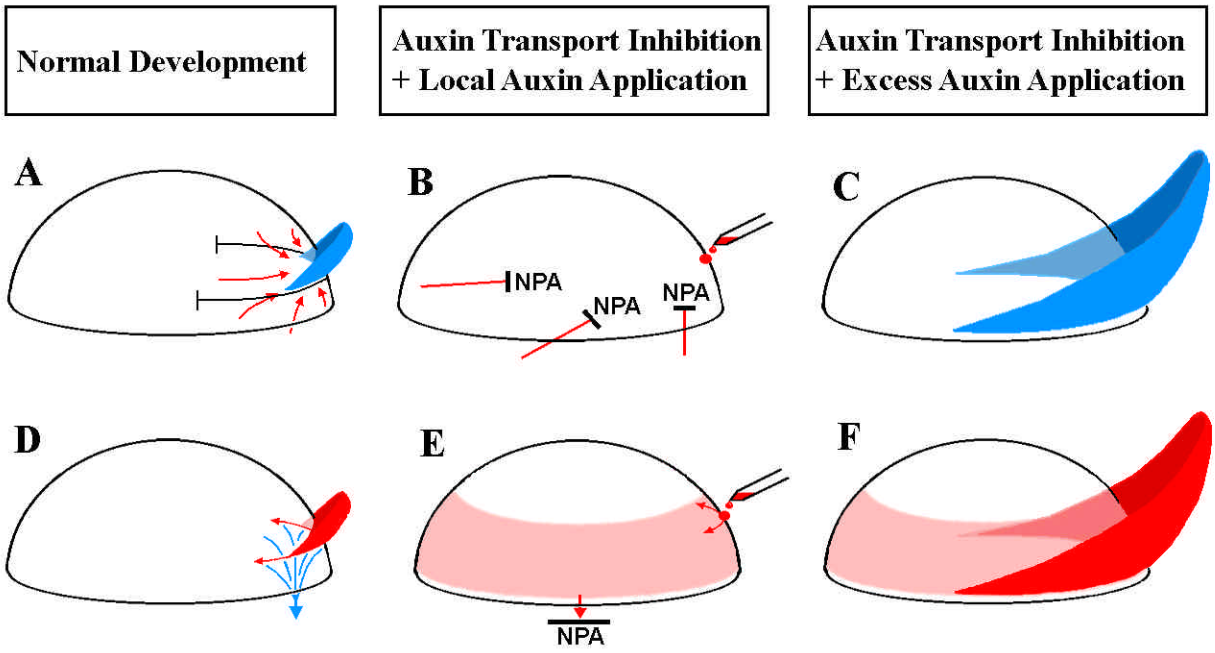

Figure 15: Possible roles of auxin in lateral organ induction
Inhibition of auxin transport interferes with lateral organ formation in shoot meristems and supply of auxin to these meristems can restore lateral organ formation (compare Figure 14). These observations suggest that lateral organs are formed at local auxin maxima, whose formation could be prevented by auxin transport inhibition in at least two ways.
A-C, sink model. A: Auxin required for lateral organ formation would normally be transported from diverse sources. Therefore, at least initially, the organ primordium would constitute an auxin sink (blue). Lateral inhibition could be due to a specific inhibitor, to biophysical strain (Green, 1996) or to auxin depletion in flanking regions. **B**: If auxin is not transported due to application of NPA, it cannot accumulate at particular sites and organ formation becomes dependent on external auxin application. **C**: Excess amounts of auxin, passively distributed over larger portions of the peripheral zone, induce oversized primordia.
D-F, source model. Alternatively, young primordia could act initially as auxin sources (red). **D**: After stochastic initiation, the position of a primordium would be stabilized by a coupled stimulation of auxin synthesis and auxin drainage. Irreversible differentiation of auxin efflux routes could anchor the position of the developing primordium. While in the center of the primordium the positive effect on auxin synthesis prevails, a larger surrounding area is affected by the associated drainage, thereby limiting the size of the primordium (compare **F**) and restricting further primordium initiation to distal positions. **E**: Because of the feed-back coupling of auxin synthesis and auxin drainage, inhibition of auxin transport below a certain threshold prevents localized auxin synthesis and primordium growth. This inhibition can be overcome by the local application of auxin. **F**: As in **C**, excess amounts of auxin could spread independent of polar auxin transport and induce lateral organ formation in the peripheral zone (right).
Note that both hypothetical mechanisms would result in the inhibition of organ formation next to an existing primordium (lateral inhibition), through the formation of an auxin sink. In **A**, the primordium itself would consume auxin from the vicinity; in **B**, it could stimulate basal auxin drainage in a larger area.

not act in a simple linear pathway, and their expression domains no longer overlap beyond the heart-stage, *CUC* gene input seems to help define the initial *STM* domain. This initial *STM* expression domain is extended and spans the entire diameter of the embryo (Figure 10 and 11). As indicated by cotyledon fusions in *stm* mutants, *STM* expression in the periphery is likely to contribute to local suppression of founder cell fate in two opposed peripheral regions and thereby to the separation of the cotyledon primordia. Once properly separated, the two cotyledons could serve as references in the positioning of subsequent shoot organs.

4.2.3. Adaxial-abaxial patterning

The development of laminar lateral organs involves an additional patterning step, the specification of adaxial and abaxial cell identities (Figure 2B). Histological and gene expression studies in vegetative leaves suggest that leaf anlagen are initially uniform, but become polarized in the adaxial-abaxial (or dorso-ventral) dimension by the time that the primordium becomes morphologically distinct (reviewed in Bowman, 2000). Surgical and genetic data indicate the existence of extrinsic signals from the meristem center as well as of intrinsic signaling within the primordium. An increasing number of regulatory genes is being implicated in dorso-ventral patterning and their roles have been recently reviewed (Hudson and Waites, 1998; Sessions and Yanofsky, 1999; Bowman, 2000)

Although cotyledons are adaxial-abaxially polarized and adaxial-abaxial patterning is therefore an integral part of embryonic patterning, available experimental data are mainly derived from post-embryonic studies and therefore more appropriately covered in the chapter on shoot development. However, there are two observations, which are important for the discussion of patterning in the embryo (Figure 2B). First, it has long been recognized that adaxial-abaxial patterning uses the meristem center as a reference, evidenced by the fact that lateral organs fail to differentiate adaxial-abaxial polarity, if insulated from apical signals through vertical incisions in the meristem (Sussex, 1954). Secondly, adaxial-abaxial patterning seems to feedback on SAM formation (Figure 2B). Mutations causing an abaxialization of the lateral primordium negatively affect SAM development, while adaxialization of the primordium promotes SAM development (McConnell and Barton, 1998; Siegfried et al., 1999). A recent study implicates the GARP transcription factor *KANADI* (*KAN*) in both adaxial-abaxial and radial (central peripheral) polarity (Kerstetter et al., 2001). Loss-of-function mutations in *KAN* cause adaxialization of lateral organs, suggesting that *KAN* is required for abaxial identity. *KAN* is already expressed in peripheral positions in the early embryo, and interestingly it has been shown that ubiquitous expression of *KAN* causes not only the abaxialization of lateral organs, but also frequently leads to a reduction in vascular tissue in the hypocotyl. These observations and the known embryonic expression patterns of other genes involved in adaxial-abaxial patterning (McConnell et al., 2001) together suggest that adaxial-abaxial polarity may be established with reference to radial polarity.

In summary, a complex network of interdependent processes regulates patterning in the apical domain. Early partitioning events in the apical domain effectively set up systems of balanced controls that ensure the stable maintenance of a small central stem cell pool and the recruitment of cells exiting this pool into lateral organs. The phyllotactic positioning of these lateral organs in the periphery of the apex is not understood in molecular detail, but seems to be regulated by a largely autonomous mechanism that may operate throughout development. Finally, there seems to be a mutually promoting influence exchanged between cells at the adaxial side of lateral organs and the shoot meristem.

4.3. Apical-basal polarity

Plant cells have a strong tendency to integrate their apical-basal polarity under a variety of conditions and this general propensity may be sufficient to maintain cell polarity over generations. Even in embryos developing abnormally in an inverted orientation (Figure 3C) the polarity of the constituent cells is still integrated correctly and it may be that this accomplished through general cellular mechanisms. Alternatively, there could be specific signals in the gametophyte or early embryo, which have yet to be identified genetically. So far genetic analysis has implicated only one gene, *EMBRYO DEFECTIVE 30/GNOM* (*EMB30/GN*), in the stable establishment of the apical-basal axis in the embryo. However, it was found that *EMB30/GN* is also required for stable maintenance of plant cell polarity in general. Seedlings mutant for *EMB30/GN* may be ball-shaped with fully differentiated, but randomly oriented, vascular cells at the center, indicative of a selective loss of apical-basal polarity (Mayer et al., 1993). In culture, mutant cells seem to be defective in acquiring common polarity and are incapable of organized growth. Interestingly, many aspects of the *emb30/gn* phenotype are mimicked in *Brassica juncea* embryos exposed to high concentrations of auxin trans-

port inhibitors (Hadfi et al., 1998). While this observation suggests a central role for *EMB30/GN* in polar auxin transport, the *EMB30/GN* product does not seem to be an integral part of the auxin import or export machinery (Shevell et al., 1994; Busch et al. 1996). Presumptive auxin influx and efflux carriers have been identified in Arabidopsis as products of the *AUX* and *PIN* gene families (Bennett et al., 1996; Galweiler et al., 1998; reviewed in Bennett et al., 1998; Palme and Galweiler, 1999), but functional overlap within these families seems to preclude strong, non-polarized phenotypes. Recent evidence suggests that *EMB30/GN* could be critical for auxin transport and cell polarity through a role in positioning of multiple auxin transport membrane proteins. The *EMB30/GN* product is highly homologous to the yeast guanine exchange factor GEA1, which acts on GTP-binding proteins of the ADB ribosylation factor family involved in vesicle trafficking (Steinmann et al., 1999). Consistent with this interpretation, mutations in *EMB30/GN* were shown to interfere with the coordinated polar localization of the presumptive auxin efflux protein AtPIN1 (Figure 16).

In conclusion, recent genetic and experimental evidence reemphasizes the importance of polar auxin transport in the integration and maintenance of plant cell polarity. It is possible that polarized auxin transport is also sufficient to account for early embryo patterning steps, such as the oriented divisions of sub-epidermal cells and the delimitation of the central procambium (Figure 7; Section 5.2.). Alternatively, there could be unknown early embryonic cues to facilitate the establishment of initial polarities and ensuing patterning steps.

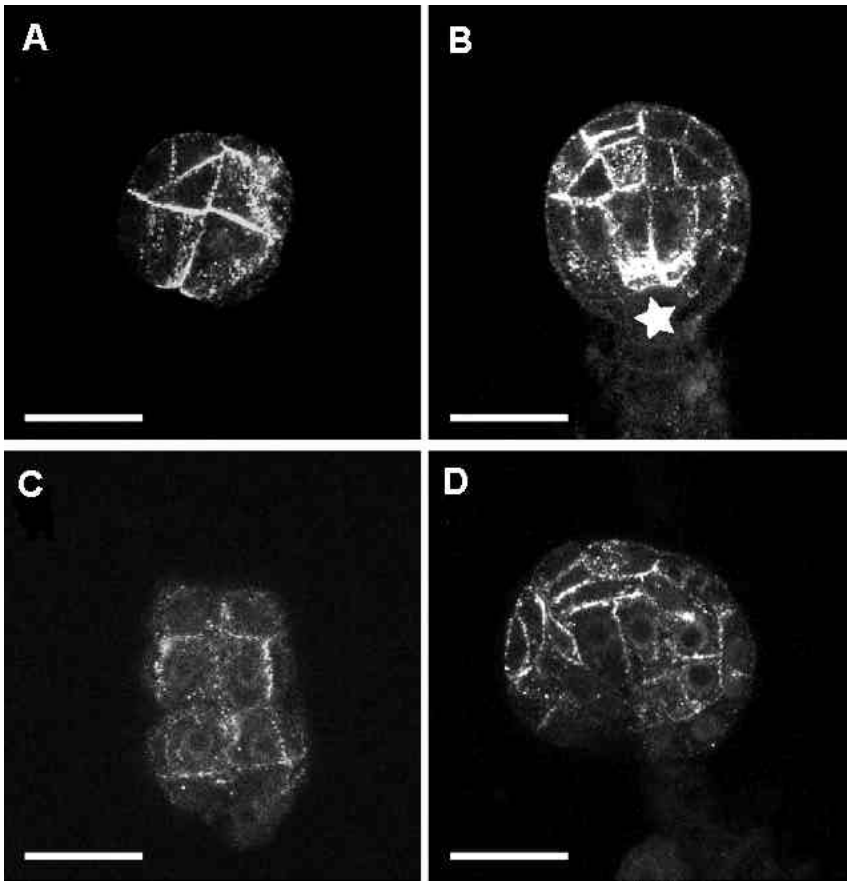

Figure 16: AtPIN1 localization and cell polarity
Immunolocalization of AtPIN1 in embryos of wild-type (**A** and **B**) and the *emb30/gnom* (**C** and **D**) mutant embryos, at dermatogen (**A** and **C**) and early globular (**B** and **D**) stage. The presumptive auxin efflux protein AtPIN1 is normally localized to cell membrane on the basal side of immature vascular cells. In the *emb30/gn* mutant, there is a correlated loss of coordinated AtPIN1 localization and of overall apical-basal cell polarity. Bars = 20 mM. From Steinmann et al., 1999.

5. The radial pattern

Radial patterning diversifies cell identities in the radial dimension. The first radial patterning event at the dermatogen-stage separates a surface layer and inner cells of a sphere, while subsequent radial patterning occurs within the radial symmetry of a cylinder (Figure 17). Therefore, it is possible that the separation of the epidermis and subsequent radial patterning are mechanistically unrelated.

5.1. Separating epidermal and inner cells

The mechanism responsible for differential cell fate acquisition by outer and inner cells at the dermatogen stage is not clear, and no mutation has been identified that completely abolishes this first step in radial patterning. Several models can be envisaged. For example, a concentration gradient of an unknown signal molecule could function as a morphogen (Wolpert, 1989; Gurdon and Bourillot, 2001) to specify cell fates in the radial dimension. Even production of the signal substance throughout the globular embryo could result in a central concentration maximum and cells could acquire distinct cell identities as a function of their distance from the center (Figure 17A). Alternatively, for example, an external signal from the endosperm could specify epidermal fate (Figure 17B). A corollary of such an 'outside-in' signaling mechanism is that the acquisition of inner cell fate could be dependent on proper tangential cell divisions to insulate inner cells from the external signal. This would also be true of a variation on an 'outside-in' model, in which epidermal fate information is deposited within the cell wall of the zygote. In this model the epidermal cells remain in contact with the outer cell wall, whereas the inner cells become separated with the first tangential division.

Since the molecular signal is unknown, none of the above models can be systematically explored. However, there is some circumstantial evidence for 'outside-in' signaling. Certain marker genes, such as the homeobox gene *ATML1*, are expressed in the apical daughter cell of the zygote, but are turned off as soon as divisions separate cells from the embryo surface (Lu et al., 1996; Sessions et al., 1999). Moreover, mutations in at least two genes,

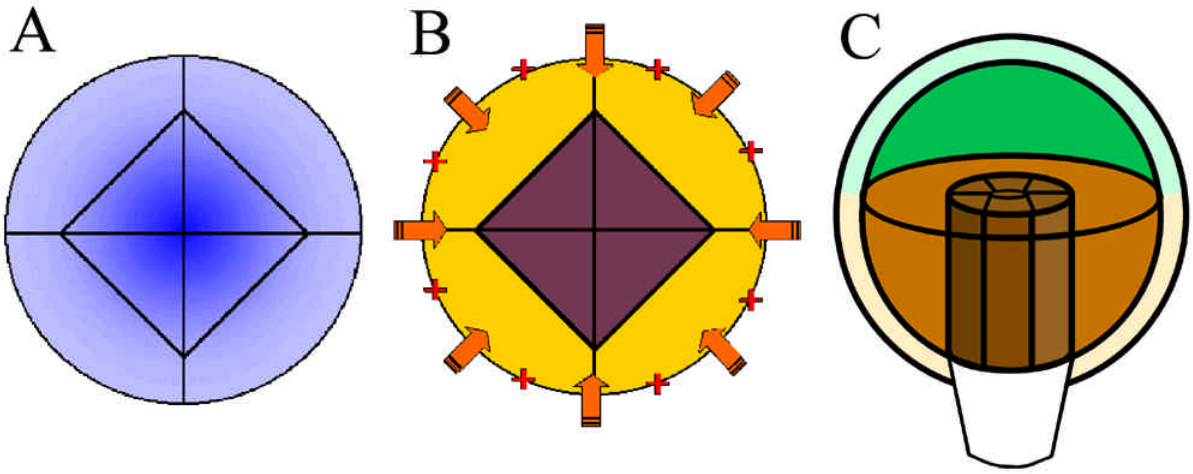

Figure 17: Hypothetical mechanisms to separate epidermis and inner cell fate
A: A stable morphogen gradient (blue) could specify radial cell fates. This mechanism would allow for the concentration dependent specification of several cell identities, but only two fates, outer (epidermal) and inner cells, are specified at this stage. **B**: Alternatively, epidermal cell fate (yellow) could depend on signals from outside. External signals could originate from the surrounding milieu (orange arrows) or could be stored in the outer cell wall (red crosses) inherited from the zygote. Cells excluded from the external signal would switch to an inner cell ground state (purple). **C**: Subsequent radial patterning occurs along the axis of an elongating cylinder and may therefore be specified by a separate mechanism. This process is initiated with the formation of procambial tissue in the basal domain (brown, versus apical domain green).

KNOLLE (*KN*) and *KEULE* (*KEU*), are associated with incomplete cell wall formation during cytokinesis (Lukowitz et al., 1996; Assaad 1996) and lead to the expression of epidermal markers in sub-epidermal tissues (Vroemen et al., 1996). Overall then, although the mechanism separating epidermal and inner cell identity remains elusive, its dependence on cytoplasmic discontinuity points towards some type of outward-in signaling.

5.2. Separating vascular and ground tissue

Immediately after the dermatogen-stage, divisions of inner cells become strictly oriented along the apical-basal axis, thereby establishing the external and internal cell layers of a cylinder (Figures 4D and 17C). The first procambial cells are generated through periclinal divisions in the center of the *l.t.* and the only known mutations that selectively abolish this process, are those of the aforementioned auxin perception class, comprising *mp*, *bdl* and *axr6* (Hardtke and Berleth, 1998; Hamann et al., 1999; Hobbie et al., 2000) (Figure 6). The defect is consistent with the postulated role of auxin in promoting oriented vascular differentiation (Sachs, 1981; Sachs, 1991), indicating that polar auxin signaling is involved in both the establishment of the apical-basal axis and in radial patterning among the inner cells. Which cell differentiation events are precisely dependent on the function of the *MP*; *BDL*; *AXR6* genes is less clear. They could constitute part of an auxin-flow-cell-polarization feedback mechanism (Figure 7), thereby indirectly promoting vascular differentiation. Alternatively, their function may be restricted to the formation of a vascular cylinder, which could in turn serve as positional reference for patterning of surrounding cells (compare 5.3.). Available evidence supports both interpretations. The *MP*;*BDL*;*AXR6* genes seem to possess patterning functions beyond the vascular system. Patterning defects in all three mutants precede vascular differentiation and include many aspects of auxin-mediated patterning, such as positioning of cotyledons and a variety of post-embryonic features (Berleth and Jürgens, 1993; Przemeck et al., 1996; Hamann et al., 1999; Hobbie et al., 2000). Consistent with a function beyond vascular tissues, *MP* mRNA distribution is correlated with oriented cell differentiation and becomes largely restricted to procambial tissues only after the embryo has acquired an anatomically distinct apical-basal axis. Simultaneously however, vascular tissues do appear to control patterning in other tissues through direct, auxin-independent signaling. Examples of cell differentiation correlated to vascular patterns have been found in many species (Nelson and Dengler, 1997) and a direct influence on the pattern of the overlying ground tissue has recently been discerned in the Arabidopsis embryo (Section 5.3.).

More recently, cytokinins have been implicated in cell division and cell differentiation within the vascular cylinder. Loss-of-function mutations in the *CYTOKININ RESPONSE1/WOODEN LEG* (*CRE1/WOL*) gene result in reduced cell divisions in the center of the root meristem and the formation of phloem is reduced or absent in the mutant hypocotyls and roots (Scheres et al., 1994). The *CRE1/WOL* gene encodes a membrane associated two-component histidine kinase (Mähönen et al., 2000) and has been identified as a cytokinin receptor by conferring cytokinin responsiveness to yeast cells, when inserted into an appropriate yeast signal transduction pathway (Inoue et al., 2001). Therefore, control of cell division through cytokinin, appears to be essential for proper cell numbers and phloem formation in the vascular cylinder.

5.3. Radial patterning in the ground tissue

At around torpedo stage, the inner of two ground tissue layers is split by an asymmetric, periclinal division and the resulting two layers acquire distinguishable identities as endodermis and cortical parenchyma. In the root, separation of endodermal and cortical cell fates is ongoing, since both tissues are derived from a common initial in the RAM (Figure 18A). Two genes, *SHORT ROOT* (*SHR*) and *SCARECROW* (*SCR*), both of which encode GRAS domain transcription factors, were found to be required for both the critical asymmetric cell division and for the acquisition of separate cell identities by its products (Scheres et al., 1995; Di Laurenzio et al., 1996; Helariutta et al., 2000). *SHR* seems to act upstream of *SCR*, because its activity is required for the expression of endodermal marker genes, including *SCR* (Helariutta et al., 2000). When switched on by *SHR*, *SCR* seems to promote the periclinal division of the ground tissue, associated with the suppression of cortical markers in the inner layer. This suggests that *SHR* acts at the top of a hierarchy specifying differential cell identities in the ground tissue. If this is so, how is *SHR* activity polarized to induce endodermal fate only in the inner ground tissue layer? Interestingly, *SHR* mRNA is expressed exclusively in the stele, where it does not appear to fulfil any function (Figure 18B and insert in 18C). Further, recent evidence indicates that the non-cell autonomous action of *SHR* is due to short range transport of its protein product to the adjacent ground tissue layer, but no further (Figure 18 C;

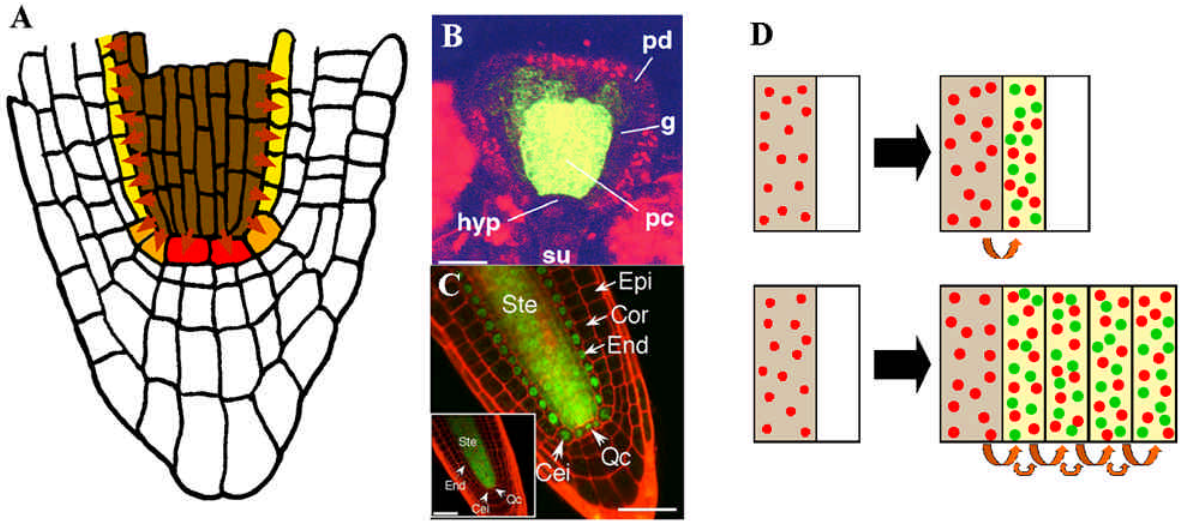

Figure 18: Radial patterning in the ground tissue
A: The *SHR* gene is expressed in the vascular cylinder (brown), but its activity is required for radial patterning in the adjacent cortex-endodermal initials (orange) and in the endodermis (yellow). SHR protein moves (arrows) to cells surrounding the vascular cylinder, where it induces the expression of *SCR* and, with the exception of the QC (red), other endodermis specific genes. *SCR* acts downstream of *SHR* and is required for the periclinal divisions of ground tissue initials which yield the two layers of ground tissue and possibly, for maintaining endodermis identity in the inner layer. **B**: *SHR* expression in the embryo. A green fluorescent protein reporter gene under control of the *SHR* promoter is expressed exclusively in the central procambium of a triangular stage embryo. Expression remains associated with vascular tissues throughout development. **C**: SHR::GFP translational fusion protein is present in the stele, but selectively accumulates in the nuclei of endodermal cells (compare transcriptional fusion in insert). **D**: Ectopic expression of *SHR* driven by the *SCR* promoter results in extra layers expressing endodermal markers. SHR protein (red) can move one layer beyond its site of production. In normal development (upper panel), it is produced in the vascular tissues (brown) and triggers *SCR* expression (green) and differentiation events in the overlying ground tissue. *SCR* is required for periclinal cell divisions subdividing the ground tissue and for proper differentiation of the endodermis (yellow). Expression of *SHR* in the in the *SCR* domain leads to a reiteration of the layer-amplification process and eventually to supernumerary endodermal layers.**A**: modified after Helariutta et al., 2000; **B**: from Helariutta et al., 2000; g, ground tissue; hyp, hypophysis; pc, procambium; pd, protoderm; su, suspensor. Bar = 25 mm. **C**: from Nakajima et al., 2001. Abbreviations: Cei, cortex/endodermis initial. Cor, cortex. End, endodermis. Epi, epidermis. Ste, stele. Qc, quiescent center. Bar = 50 mm.

Nakajima et al., 2001). Finally, misexpression of *SHR* in the *SCR* domain results in extra layers of endodermis, probably by reiterating the emission of a positional signal, which in normal development is communicated only from the vascular cylinder to just one cell layer above (Figure 18D). Expression of *SHR* under control of the promoter of its downstream target *SCR* is sufficient to convert this layer into a signal source and to reinitiate the entire ground tissue patterning process. Therefore, the *SHR* product seems to be the key signal in a short range induction mechanism and in moving just one layer triggers endodermal differentiation from a cortical ground state right next to its vascular origin.

6. Conclusions and prospects

The still incomplete list of mechanisms coordinating cell behavior in the embryo is already substantial and includes differential cell fate acquisition after asymmetric cell division, long-range signaling through low-molecular weight substances, short-range signaling via transcription factor transfer, unknown tissue-identity maintenance signals and possibly signaling through extra-embryonic and/or cell wall associated determinants. The cell pattern established by these signals constitutes a foundation for

Appendix 1. Summary table of all genes referred to in this chapter.

Symbol	Gene Name	Accession Number
ABP1	*AUXIN BINDING PROTEIN 1*	X69901
ARF1	*AUXIN RESPONSE FACTOR 1*	U83245
AS1	*ASYMMETRIC LEAVES 1*	At2g37630
ATML1	*MERISTEM LAYER1*	U37589
AXR6	*AUXIN RESISITANT 6 (AtCUL1)*	
BDL	*BODENLOS (IAA12)*	
CRE1/WOL	*CYTOKININ RESPONSE 1/WOODEN LEG*	At2g01830
CUC1	*CUP-SHAPED COTYLEDON 1*	AB049069
CUC2	*CUP-SHAPED COTYLEDON 2*	AB002560
EMB 30/GN	*EMBRYO DEFECTIVE 30/GNOM*	U36433
FS	*FASS (TON2)*	AF290025
FK	*FACKEL*	AF256536
GEA1	*GUANINE EXCHANGE FACTOR 1*	Z49531
GK	*GURKE*	
HBT	*HOBBIT*	AJ487669
KAN	*KANADI*	AY030192
KEU	*KEULE*	AF331066
KN	*KNOLLE*	U39451
MP	*MONOPTEROS (ARF5)*	AF037228
PAS	*PASTICCINO*	U77366
PHB	*PHABULOSA (AtHB14)*	Y11122
PHV	*PHAVOLUTA (AtHB9)*	Y10922
PIN1	*PINFORMED*	AF089085
PNH	*PINHEAD*	AF154272
SCR	*SCARECROW*	U62798
SHR	*SHORTROOT*	AF233752
STM	*SHOOT MERISTEMLESS*	U32344
TWN2	*TWIN 2*	U89986
WUS	*WUSCHEL*	AJ012310

further, equally complex interactions establishing and maintaining the organization of the apical meristems and regulating late-embryonic programs. Together therefore, the tight overlap of developmental programs operating in the embryo, has made the genetic analysis of this developmental phase a rich source of insight into plant cell signaling in general.

The preliminary nature of all presented interpretations should be emphasized and for the most part it is fair to say that far stronger molecular underpinning is needed before many can be considered more than constructive working hypotheses. Recent results from plant genomics research highlights the enormous proportion of ‘dark matter’ in the genome. Even in Arabidopsis, no loss-of-function phenotype has been found for more than 90% of genes (Somerville and Dangl, 2000) and, as in other

organisms, widespread functional overlap is likely to mask important players in many processes (Miklos and Rubin, 1996). Large-scale enterprises are underway to elucidate more of the dark matter through gene-trap expression patterns, large-scale expression profiling and by determination of reverse genetics loss-of-function and misexpression gain-of-function phenotypes. These approaches may prove particularly informative in investigating embryonic development, where numerous early lethal mutant phenotypes cannot be readily interpreted. Recent progress does suggest that more comprehensive understanding and manipulation of embryonic patterning in important angiosperm species is attainable within the next five years. Knowledge of the molecular mechanisms regulating early development in the Arabidopsis embryo will likely constitute the backbone of these enterprises.

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