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The Role of Auxin in Apical-Basal Pattern Formation During *Arabidopsis* Embryogenesis

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Abstract

Pattern formation during plant development has been a focus of scientific attention for a very long time. The results showed that only an approach integrating different scientific disciplines will allow us to understand the mechanisms regulating pattern formation. The purpose of this review is to bring together results from physiological, morphological, and genetic approaches in order to provide a conceptual overview of our current understanding of apical-basal patterning processes during plant development. For the sake of clarity and because most of the recent results derive from *Arabdiposis,* the focus of the review will be on this model plant. To provide a basis for further discussions, a cursory overview of auxin biology will be given followed by a morphological description of *Arabipdosis* embryogenesis. Apical-basal axis formation and formation of the primary root will be discussed in addition to the establishment of bilateral symmetry during embryogenesis.

Key words: *Arabidopsis;* Auxin; Embryogenesis; Pattern formation

Auxin Biology

Auxin has been implicated in many aspects of plant development. One of which is pattern formation during embryogenesis (Jürgens 2001). During the last years, some progress has been made towards an understanding of the function of auxin in these patterning events. Several genes involved in patterning processes and/or auxin signal transduction have been isolated, providing starting points for a molecular analysis of underlying mechanisms (Busch and others 1996; Hardtke and Berleth 1998; Shevell and others, 1994). Results from approaches using morphological or physiological methods suggest that embryogenesis does not simply position meristems at both ends of the axis but instead seems to provide the "blue print" for the postembryonic part of the plant's life cycle. The following paragraph briefly summarizes our current knowledge of the molecular network of auxin biology and provides reference points for the discussion of the molecular mechanisms of auxin signaling involved in embryonic pattern formation.

In *Arabidopsis* a putative receptor has been identified with the *AUXIN BINDING PROTEIN 1* (*ABP 1*) but its mode of action remains to be clarified (Chen and others 2001). Members of the AUX/IAA gene family of transcriptional regulators could be potential targets, because their transcription is controlled by auxin (Abel and others 1995; Ulmasov and others 1997c). The second important group of genes in this context is the ARF family of auxin response factors that can bind to auxin-responsive elements in the

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promotor region of auxin-regulated genes (Guilfoyle and others 1998; Ulmasov and others 1997a; Ulmasov and others 1997b). Members of both families can homo- and heterodimerize forming complexes of different AUX/IAA and ARF proteins apparently able to mediate auxin response during plant development (Hardtke and Berleth 1998; Ulmasov and others 1999; Ulmasov and coworkers 1999). Another class of genes was originally isolated because their loss of function leads to resistance against auxin or auxin transport inhibitors at the seedling level (*AXR 1/TIR 1*) (Lincoln and others 1990; Ruegger and others 1998). Based on molecular data and homology to genes involved in cell-cycle regulation in *S. cerevisiae,* both AXR 1 and TIR 1 have been implicated in control of the plant cell cycle via degradation of nuclear proteins (Gray 1999; del Pozo 1998; Ruegger and others 1998). Candidate target proteins for this degradation are the IAA genes (Worley and others 2000). Probably the most studied aspect of auxin biology is auxin transport. Several mutants apparently affecting different aspects of auxin transport have been isolated (Carland and McHale 1996; Müller and others 1998). Mutations in the AUX 1 gene conferred resistance to auxin and ethylene (Bennett and others 1996). An experiment with different auxins showed that the loss of AUX 1 gene activity apparently knocks out the auxin import carrier (Marchant and others 1999). By contrast, mutations in the PIN FORMED 1 (PIN 1) gene are supposed/believed to affect the putative auxin efflux carrier. The *PIN 1* protein localizes to the basal end of the xylem parenchyme cells and mutations in this gene lead to defects in auxin transport (Gälweiler and others 1998). These observations support the hypothesis that PIN1 is the auxin efflux carrier postulated in various models.

Embryo Development

Development of plant embryos has been studied for quite some time using morphological and genetic methods. For two main reasons most of the recent work has been done on *Arabidopsis thaliana.* The first is the very regular cell division pattern that allows tracing of seedling structures back to their origin during the early stages of embryogenesis (Jürgens and Mayer 1994). The second is the ability to use genetics to identify genes involved in pattern formation during plant embryogenesis (Mayer and others 1991; Patton and others 1991). Because of the close evolutionary relationships among flowering plant species it is likely that the principal mechanisms of pattern formation are similar, although there are considerable morphological differences among plant families during embryogenesis (Jurgens and others 1994).

It is possible to position the different seedling structures along the main axis (apical-basal) of a plant seedling (Wolpert 1998). These are from top to bottom: the shoot meristem, one or two cotyledons (embryonic leaves), the hypocotyl (embryonic shoot), the radicle (embryonic root), and the root meristem.

The first event in *Arabidopsis* embryogenesis after fertilization of the oocyte is the approximately threefold elongation of the zygote. The zygote then divides asymmetrically giving rise to a small apical and a larger basal cell (Figure 1a). This asymmetric cell division marks a segregation of cell fates because the appearance of the cytoplasm and the cell division behavior of the apical cell differs from that of the basal cell afterwards. The apical cell has a dense cytoplasm and divides twice vertically before it divides horizontally forming the proembryo (Figures 1b, c). By contrast, the basal cell exhibits a lighter cytoplasm with a large vacuole and divides repeatedly horizontally, thus giving rise to the suspensor. This structure probably functions both as an anchor and a conduit for nutrients and other substances. The uppermost cell of this cell file becomes the so-called hypophysis, which will contribute to the seedling and give rise to the quiescent center and the central root cap of the primary root. Besides exhibiting differences on the morphological level there are also differences in gene expression, reflected by the expression of the *Arabidopsis thaliana* MERISTEM LAYER 1 (AtML1) gene. This gene is initially transcribed in the apical cell of the embryo; later on the expression becomes restricted to the epidermal cell layer (Figures 1a–1f) (Lu and others 1996).

After the first three rounds of cell divisions the proembryo consists of eight cells that are arranged in two tiers, the upper and the lower tier (Figure 1c). The upper tier (ut) will give rise to the shoot meristem and most of the cotyledon primordia (the so-called apical region) while the descendents of the lower tier (lt) will form the remaining parts of the cotyledons, the hypocotyl, root meristem initials for the different tissue layers, and the lateral root cap of the primary root (the so-called central region). The division of the embryo in an apical, central, and basal region can be based on both morphological and gene expression data. The cells of the apical region expand without preferential orientation and exhibit expression of the WUSCHEL(WUS) gene in the inner cells at the dermatogen stage (Figure 1d) (Mayer and others 1998). By contrast, central cells of the lower tier form cell files that exhibit polar

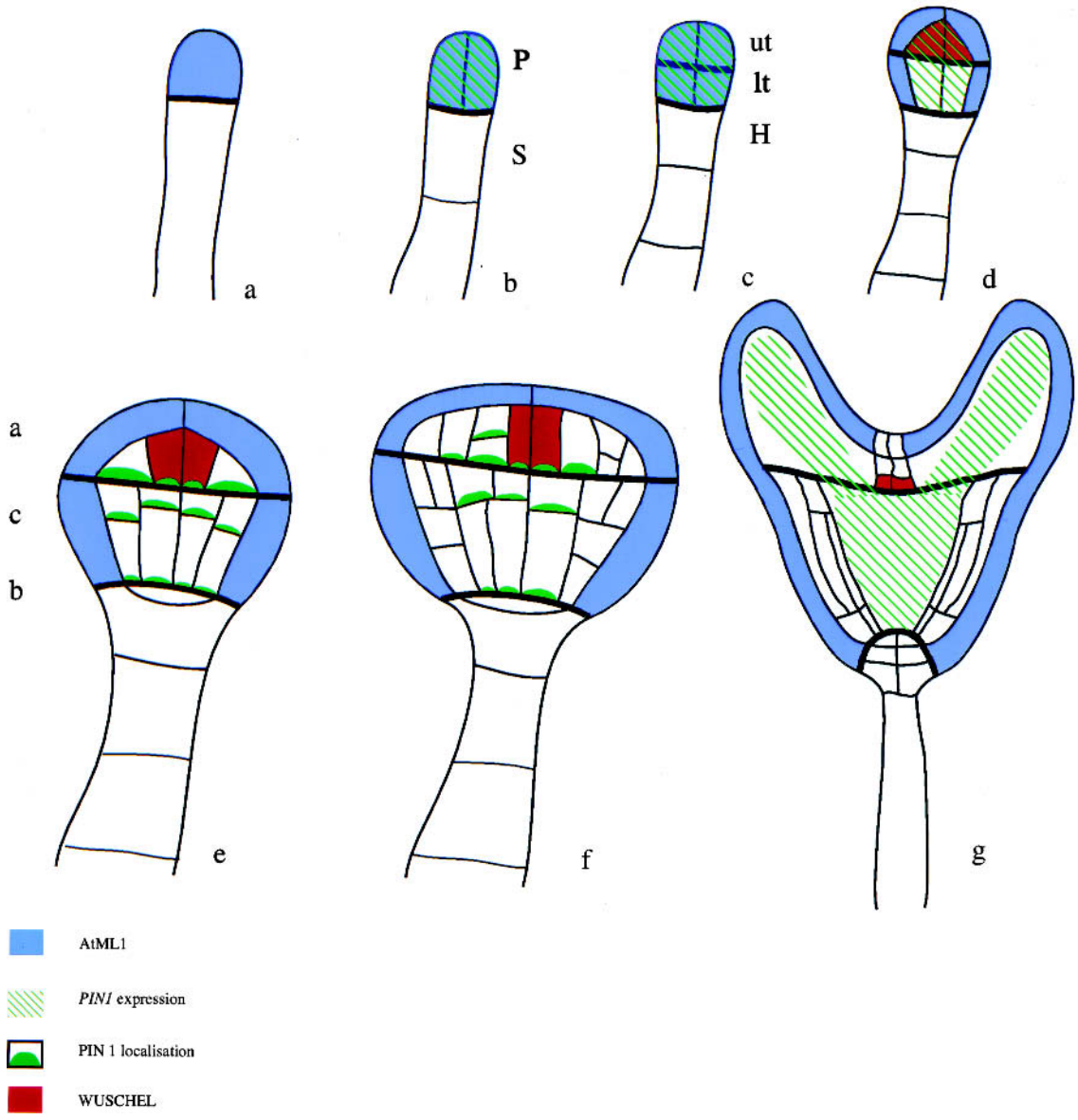

Figure 1. Schematic presentation of *Arabidopsis* embryogenesis; a=2-cell stage; b=4-cell stage of the proembryo P= proembryo, S= suspensor; c=octant stage, ut= upper tier, lt= lower tier, H= Hypophysis; d dermatogen stage; e= globular stage, a= apical region, c= central region, b= basal region; f= triangular stage; g= heart stage.

localization of PIN 1 from the early globular stage on (Steinmann and others 1999) (Figure 1e–1f). At this stage, the basal region consists only of the hypophysis (the uppermost derivative of the basal daughter cell of the zygote). This cell will divide asymmetrically at the globular stage and give rise to a small lens-shaped and a larger basal cell. The descendents of the small lens-shaped cell will form the quiescent center. The initials of the central root cap (of the primary root primordium visible at the late heart stage, Figure 1g) derive from the larger basal cell. The description of plant embryogenesis (above) shows the importance of cell-cell communication during development. In the case of the primary root meristem, the necessity of cell-cell communication is particularly obvious, because cells from two clonally distinct origins are involved in the formation of this seedling organ (see below).

Establishment of the Apical-Basal Axis

We have only little evidence of the mechanisms responsible for the initial establishment of the apical-

basal axis. What we do know is that the axis has been established by the early globular stage because at that point it is possible to distinguish among the three embryonic regions. It is conceivable that some form of maternal influence is involved in the initial establishment of polarity but the available evidence is scarce and has already been thoroughly reviewed elsewhere (Jurgens and others 1997).

The next question is how the initial polarity of the zygote is translated during early embryogenesis to form an apical-basal axis. In this context, the results of several groups are of interest. Different groups have examined the effect of auxins on plant embryos in different species such as *Zea mays* and *Brassica juncea* (Fischer and others 1996; Fischer and others 1997; Hadfi and others 1998). They were able to alter the establishment of the apical basal axis itself, the establishment of primordia along the axis, or the establishment of bilateral symmetry, thus implicating auxin in establishment or maintenance of apical-basal patterning processes. Two mutants (*abp 1* and *gnom*) have been isolated in *Arabdiopsis* that are apparently involved in auxin perception or transport and exhibit at the same time an embryonic phenotype. Chen and others (2001) have isolated an insertion line for the ABP1 gene of *Arabidopsis.* The insertion causes a developmental arrest and subsequent lethality at the globular stage of embryogenesis, whereas at this stage in wildtype embryos the elongation of vascular primordia begins and the number of cell divisions increases dramatically. Based on data from morphological analysis of embryos and experiments with tissue cultures, the authors suggest that ABP1 is required for cell elongation and might also regulate cell division.

GNOM mutant embryos/seedlings exhibit phenotypes similar to the ones observed with *Brassica juncea* embryos upon treatment with inhibitors of polar auxin transport, both in terms of variability of the phenotype and defects (such as loss of primary root, fusion of cotyledons, or formation of ball-shaped seedlings) (Hadfi and others 1998; Mayer and others 1993; Vroemen and others 1996). These similarities are of interest because they suggest that defects in polar auxin transport may cause the phenotypes observed in *gnom* embryos and seedlings. Studies of PIN 1 expression in both wildtype and *gn* mutant embryos have added further support to this hypothesis. Stainings of wildtype embryos with *PIN 1* antibodies showed that the protein is localized in a polar fashion from the early globular stage onwards in an initially broad domain that becomes gradually restricted to vascular primordia cells and increasingly polarized within these cells (compare Figures 1e and f). This localization pattern suggests that polar auxin transport occurs from the apical region of the embryo towards the basal region. *PIN 1* stainings done with *gnom* mutant embryos lead to a different result. The protein was only occasionally polarly localized in single cells, but never in the coordinated fashion observed in wildtype embryos. These observations suggest that coordinated polar auxin transport is severely compromised in *gnom* embryos which might cause the observed embryonic and seedling phenotypes. If this hypothesis is true, *gnom* could be the most severe auxin transport mutant isolated to date because it might affect not only the polar localization of *PIN 1* but also that of other auxin transport proteins.

The *GNOM* (*GN*) gene has been cloned and shown to encode a brefeldin A (BFA) sensitive guanine-nucleotide exchange factor (GEF) for small GTP binding proteins of the ARF family (Steinmann and others 1999). These ARF-GEFs are important regulators of vesicle transport processes. BFA has been described as an inhibitor of vesicle transport that acts by blocking ARF/GEF complexes. It is of particular interest to note that BFA also affects auxin transport and localization of PIN 1 (Delbarre and others 1998; Morris and Robinson 1998; Steinmann and others 1999).

The question that arises naturally in this context is how on one side single cells can polarize occasionally in *gnom* embryos whereas the coordinate localization of PIN 1 on the multicellular level does not take place. A possible answer could be based on the concepts developed by Sachs (2000). Sachs described the establishment of polar auxin transport as a process in which a cell becomes polarized and competent to transport auxin. This cell induces polarization in neighboring cells, making these cells also competent for auxin transport, thus creating auxin transport channels (exemplified by *Pin 1* expression in Figure 1g). In cellular terms, this means that a cell must be able to perceive signals and translate them into polar localization of efflux carriers (Pin 1 localization in Figures 1e and f). A prerequisite for this event to take place is polar vesicle transport, which would be disrupted in *gnom* embryos thus making coordinate polarization impossible.

Establishment of the Primary Root

As described above the primordium of the primary root derives from two clonally distinct origins. The quiescent center (QC) and the central root cap derive from the hypophysis; the initials for the lateral root cap and the different tissue layers derive from the lower tier of the proembryo. Several mutants

have been isolated, affecting primary root formation and implicating auxin signaling in this process. These mutants are *bodenlos (bdl), monopteros (mp),* and *auxin resistant 6 (axr 6)* which do not form an embryonic root but can form roots postembryonically (the resulting homozygous plants exhibit auxin-related phenotypes) (Hamann and others 1999; Hobbie 2000; Przemeck and others 1996). The first two mutants were isolated based on their seedling phenotypes whereas the *axr 6* mutant was isolated based on its resistance to 2,4-D as a heterozygote (Hobbie and others 2000). The *axr6* mutants lack the primary root and hypocotyl and exhibit severe vascular pattern defects in their cotyledons (one of them can also be missing). It is not yet clear if the first deviation from wildtype occurs in the proembryo or the suspensor. In the case of the *BODENLOS* (*bdl*) mutant the seedlings show a variable deletion of the basal structures (for example, primary root and hypocotyl) (Hamann and others 1999). Surprisingly, the first deviation during embryogenesis from wildtype occurs at the 4-cell stage in the proembryo. The mutated gene has been isolated meanwhile and it encodes a member of the IAA gene family (T. Hamann and others unpubl. res.). In *mp* mutant seedlings the primary root is always missing and most of the hypocotyl is also deleted. The cotyledons are either normal in spacing and shape or only one somewhat enlarged cotyledon is formed (Berleth and Jürgens 1993). Other defects caused by mutating MP are aberrations in the differentiation of the vasculature and the reduction of auxin transport (Przemeck and others 1996). Morphological analysis of *mp* embryogenesis showed that the first deviation from wildtype development was also at the 4-cell stage in the apical part of the proembryo (Hamann and others 1999). The *MP* gene encodes *ARF 5* (Hardtke and Berleth 1998).

Based on morphological and cell ablation data (from postembryonic roots), a model postulating two signaling events during embryonic root development has been formulated (Hamann and others 1999; van den Berg 1995, 1997). During early embryogenesis a signal is apparently required for the uppermost suspensor cell to form the hypophysis, competent for QC formation. This conclusion is based on the morphological analysis of the *bdl* and *mp* mutant, where the first defect occurs in the apical part of the embryo whereas on the seedling level organs that derive from the basal region are affected (Hamann and others 1999). Additional support comes from the expression analyses of the *BDL* and *MP* genes showing that in in situ experiments both genes are transcribed initially in the apical region of the embryo. Transcription of these genes can be detected in the basal region of the embryo only after the division of the hypophysis in lens-shaped and larger basal cell (T.Hamann and others unpublished), suggesting that *MP* and *BDL* are involved in communication between the apical and basal part of the embryo.

The second signaling event takes place later on, when the QC apparently recruits adjacent cells that are being kept in an actively dividing state as meristematic initials. This second part of the model is supported by results from the morphological analysis of the *bdl* phenotype and data from ablation studies in the postembryonic root (Hamann and others 1999; van den Berg 1995, 1997). Sections of *bdl* mutant embryos occasionally showed a single division of the initial for the epidermis and the lateral root cap. These observations suggest that the initials for the different tissue layers are being formed but not kept in an actively dividing state, thus preventing primary root formation. Ablation of quiescent center cells bordering a cortex initial in the postembryonic root leads to differentiation of the initial (van den Berg C 1995, 1997). Taken together, these data implicate cell-cell communication in two different contexts: initiation of the root primordium on one hand and preservation of actively dividing meristem initials on the other.

The next point concerns the identity of the signal employed during the two inductive cell-cell signaling events. A strong candidate for the signal is auxin. This is supported by data from MP and BDL because both exhibit primary root defects and are involved in auxin signal transduction. In the case of postembryonic root growth, auxin can apparently provide the necessary positional information for organization of the distal primary root tip, but it remains to be seen if that is also the case during embryogenesis (Sabatini and others 1999). There are many different possibilities for how positional information could be provided, two of which are described here. Auxin could either function similarly to the postembryonic situation as a putative morphogen providing positional information for the initial formation of the QC, or it could be required for an auxin-dependent process in the apical part (for example, axialization of the vascular primordia via MP/BDL) influencing primary root organization in an indirect fashion. Unfortunately, it is not yet possible to resolve this question.

For the postulated second signaling event required to prevent the initials from terminally differentiating, the situation could be somewhat different. The available data only indirectly support the notion that there is a signal from the QC keeping the meristem initials in an actively dividing state, because

the functional data derive from experiments with postembryonic roots. Consequently, the mechanism and the nature of the signal remain to be elucidated.

Organization of the Apical Region of the Embryo

The available data also implicate auxin in the organization of the apical part of the embryo. During organization of the apical region of the embryo two different processes take place. Initially, the central part containing the prospective shoot meristem is separated from the outer ring that is competent to give rise to cotyledon primordia. Since this process has already been thoroughly discussed recently in a review by Jürgens, (2001) and no direct involvement of auxin has been shown yet, I will focus during the remaining part of this review on the establishment of bilateral symmetry and how auxin can influence the process (Hadfi and others 1998; Liu and others 1993).

Several lines of evidence are of interest in this context. Woodrick and others (2000) used the *chloroplast mutator 1-2* mutation as a tool to create an embryonic shoot fate map of *Arabidopsis.* Their results show that one of the two cotyledons is initiated earlier in development than the other, suggesting that initially, one starting point for primordia formation is defined and then the second primordium is apparently initiated on the opposite side of the embryo.

Several different groups employed tissue culture methods to test the effects of exogenously applied auxins on the establishment of the apical-basal axis and bilateral symmetry in different plant species (Fischer 1996; Hadfi and others 1998; Liu and others 1993). Depending on the amount of exogenous auxin used, time of application, and type of transport inhibitor, the organization of the apical region was disturbed to a variable extent.

Several mutants have been isolated that are involved in auxin biology and exhibit defects in the establishment of bilateral symmetry. *pin I* mutant seedlings exhibit a rather low frequency of defects in apical patterning where either a complete or a partial fusion of the cotyledons is observed (Liu and others 1993). Another mutant worth mentioning in this context is *gn* because one of its obvious deviations from wildtype development is the formation of fused cotyledons (Mayer and others 1993). *mp* seedlings exhibit a somewhat variable phenotype (Berleth and Jürgens 1993) ranging from two quite normal cotyledons with defects in vascular differentiation to seedlings that form only a single cotyledon. *Bdl* mutant seedlings only occasionally form a single cotyledon and show only minor vascular defects in the cotyledons (Hamann and others 1999). The *bdl mp* double mutant exhibits a reduced penetrance and shows a significantly enhanced apical patterning defect (Hamann and others 1999). Results from *in situ* hybridization analysis with probes for the *AINTEGUMENTA (ANT)* and the PIN 1 gene in double mutant embryos suggest that no bilateral symmetry is established in those embryos (T Hamann and others unpublished). These results are reminiscent of those from Vernoux and colleagues (2000) who did *in situ* hybridization experiments on the pin-like inflorescences of *pin 1* mutant plants with probes for LEAFY (LFY), *ANT,* and CUP-SHAPED-COTYLEDON 2 *(CUC 2).* They showed that markers for organ initiation such as *LFY* and *ANT* are not expressed in distinct organ primordia but instead, in a ring-like domain around the shoot meristem. The *CUC 2* gene (used here as a boundary marker) seems to be expressed in the same domain. Their conclusion from these results is that PIN 1 is involved in organ separation and positioning of the primordia by regulating the local distribution of auxin. Thus, auxin is apparently involved in establishment of bilateral symmetry and phylotaxis. This conclusion is in accordance with results from Kuhlemaier and coworkers (Reinhardt and others 2000). Their results show that auxin is able to induce primordium formation and is required for outgrowth. Then, after establishment of the first primordium, some kind of lateral inhibition mechanism could be active that determines the growth position of the next primordium.

Taken together, the results presented above suggest the following chain of events during establishment of bilateral symmetry. Initially, one point is somehow determined as the site of initiation and outgrowth of the first cotyledon primordium. Then, perhaps by some kind of lateral inhibition, the position of the second cotyledon is determined on the opposite side of the embryo. Auxin could be involved in both events. In the next stage, auxin has to be differentially removed from the apical region, allowing outgrowth of the primordia. If auxin is not differentially removed, either some kind of collar cotyledon or no organ at all is formed, as observed by Neuhaus and others in *Brassica juncea* embryos and by Kuhlemaier and others. Mutations in *bdl, gn, mp, pin 1* could influence the removal of auxin from the apical region of the embryo by affecting polar auxin transport and in turn cause the observed defects in bilateral symmetry.

The last question, but as yet unanswerable: Is the position of the first cotyledon primordium deter-

mined by a specific mechanism or is it established arbitrarily? It may be possible to address the role of auxin in this context by manipulating auxin levels in the apical region through expression of genes that influence local auxin concentrations and are under control of region-specific promoters such as *WUS* or *ANT* (Long and Barton 1998; Mayer and others 1998).

Concluding Remarks

The purpose of this review was to summarize recent advances in our understanding of the function of auxin during plant embryogenesis and to provide some "food for thought." Obviously auxin is involved in different aspects of apical-basal pattern formation. We do not know yet if it is required for establishment of polarity itself but we do know that polar auxin transport is required for formation of the embryo axis. Vesicle trafficking in turn is apparently required for polar auxin transport, because a mutation disrupting vesicle transport also disrupts coordinate localization of a putative auxin transporter and creates embryonic phenotypes that are phenocopies of embryos treated with inhibitors of polar auxin transport.

Initiation and organization of primordia along the apical-basal axis is the other aspect auxin is apparently required for. In the case of the primary root, we can only conclude that it is probably required for initiation, but we cannot yet determine if it is acting as a morphogen providing positional information or if it is acting in an indirect way by affecting cell morphogenesis. During the establishment of bilateral symmetry in the apical region of the embryo, auxin apparently determines the radial position of primordia. Data supporting this notion derives from experiments in which embryos or postembryonic plants were treated with auxins, causing defects in radial pattern. This hypothesis is additionally supported by morphological and molecular data from different mutants. Using these mutants (*MP*, *BDL*, and *GN*) as starting points for a molecular analysis should allow to unravel the mechanisms regulating apical-basal pattern formation during embryogenesis and to clarify the role of auxin in this context.

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