REVIEW ARTICLE

Polarity and signalling in plant embryogenesis

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Abstract

The establishment of the apical–basal axis is a critical event in plant embryogenesis, evident from the earliest stages onwards. Polarity is evident in the embryo sac, egg cell, zygote, and embryo–suspensor complex. In the embryo-proper, two functionally distinct meristems form at each pole, through the localized expression of key genes. A number of mutants, notably of the model genetic organism Arabidopsis thaliana, have revealed new gene functions that are required for patterning of the apical–basal axis. There is now increasing evidence that two particular modes of signalling, via auxin and cell wall components, play important roles in co-ordinating the gene expression programmes that define determinative roles in the establishment of polarity.

Key words: Embryogenesis, polarity, intercellular signalling, auxin, cell wall components, Arabidopsis thaliana.

Introduction

The growth and development of higher plants can be considered to be characterized by the execution of cell division, expansion and differentiation along two axes: the apical-basal axis and the radial axis. The radial axis is most clearly evident in dicotyledonous species as the concentric rings of cell layers in the seedling stem, hypocotyl and root, and an increase in size across this axis can arise from the generation of new cell layers following divisions in the vascular cambium in the older plant. The apical-basal axis can be defined by the patterning of functionally distinct structures, rather than cell layers, from the shoot apical meristem, to the hypocotyl and stem, to the root apical meristem. In evolutionary terms, the apical-basal axis of development can be considered to have a strong selective advantage based upon plant competition for light, water and nutrients. Such competition is severe, since reproductive success depends absolutely upon the ability of individual plants to acquire these. The germination of seeds beneath the soil elicits a complexity of phytochrome-dependent and COP/DET gene-dependent signalling pathways to ensure rapid cell expansion along the apical-basal axis, to reach the light (Deng, 1994; Chory, 1997). Post-germinative growth is most successful for those individuals able to out-compete their neighbours for available light through the shade avoidance response (Ballaré, 1999), leading to rapid cell division and expansion in the hypocotyl and stem. The acquisition of water and nutrients from the soil requires the modulation of root meristem activity and cell expansion in the opposite direction, that is, downwards. Taller stems also facilitate spore and seed dispersal, promoting reproductive success through the exploitation of more distant ecosystems. The seedling can therefore be viewed as a polar structure, with each pole exhibiting different activities; both of which must have been critical for the early success of the higher land plants.

The focus of this review is to examine the cellular and molecular basis of axialization in higher plants, with an emphasis on studies in dicotyledonous species, using Arabidopsis as a model. In particular, the origins of apical-basal polarity in the embryo, its genetic control, and the signalling systems that regulate the expression of relevant genes will be examined. The first part of the article will focus on how polarity is established and then fixed, whilst the second part will look at the different signalling systems involved in maintaining this polarity and using it to enable the correct elaboration of the apical-basal pattern.

Polarity originates early in development

In Arabidopsis, and other species such as Capsella bursapastoris that have been studied in much detail, it is clear that apical-basal polarity is evident even before the first

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zygotic division within the egg cell itself (Schulz and Jensen, 1968; Mansfield and Briarty, 1991). It is, furthermore, the case that the embryo sac itself also exhibits polar organization, with the egg cell and synergids adjacent to the micropyle, while the antipodal cells are found at the opposite chalazal end. Polarity in the egg cell is seen anatomically as the location of a large vacuole at its micropylar end, while the chalazal end is relatively cytoplasmic (Fig. 1). In some species, polarity in the egg cell and, subsequently, the zygote is exaggerated by a reorganization of cytoplasmic components (Natesh and Rau, 1984; Schulz and Jensen, 1968). However, the molecular mechanisms that generate this polarity are still obscure, and fall far behind current understanding of polarization within, for example, the Drosophila egg (Gonzales-Reyes et al., 1997).

The brown alga Fucus offers some experimental features that greatly facilitate the study of early events of zygote polarization. Free-living egg cells and zygotes can be harvested, manipulated and observed under the microscope, and some elegant recent work has provided new insight into polarity generation early in plant development. In the fucoid zygote, polarization events can be triggered by a range of stimuli, including unidirectional light and fertilization (Hable and Kropf, 2000). For a short period after the induction of polarization axis formation is reversible, but subsequently irreversible (Quatrano and Shaw, 1997). Associated with axis formation there is an observed localization or redistribution of plasma membrane components, including ion channels; a redistribution of calcium to the basal shaded end; a localization of F-actin at the rhizodermis; an asymmetric distribution of RNA molecules in the zygote (though actin mRNA interestingly accumulates at the opposite pole to F-actin protein; Bouget et al., 1996); and a polarized secretion of Golgi-derived cell wall components towards the ‘basal’ region from which the rhizoid cell will develop. Experimental disruption of this secretion by brefeldin A disrupts axis fixation and polarized growth (Shaw and Quatrano, 1996).

**Cell fate decisions: embryo-proper versus suspensor**

The observed apical-basal polarity in the zygote of Arabidopsis and Fucus presages polar development during embryogenesis. In each species, the zygote undergoes an asymmetric transverse division to generate two daughter cells that are of unequal size and follow distinct developmental pathways. In Arabidopsis one cell, the basal cell which is the larger of the two, derives from the vacuolar region of the zygote, while the smaller upper cell derives from the cytoplasmic region (Fig. 1). The upper cell then divides to form the embryo proper, while the basal cell forms a single file of typically six to nine cells, the suspensor. Only the uppermost cell of the suspensor, the hypophysis, contributes to the embryo proper as part of the root meristem (Dolan et al., 1993; Scheres et al., 1994). The suspensor appears to have a number of different functions: it physically projects the embryo into the endosperm, and provides both a conduit and a source of hormones and nutrients for the developing embryo. Perhaps the most clear difference in fate between the embryo-proper and suspensor is seen as the programmed cell death of the suspensor when the embryo reaches the torpedo-stage of development (Yeung and Meinke, 1993).

There is also increasing evidence that the embryo and suspensor express distinct gene expression programmes. While a number of embryonic mutations, such as knolle (Lukowitz et al., 1996), fass (Berleth and Jürgens, 1993), gnom/emb30 (Mayer et al., 1993), and hobbit (Willemsen et al., 1998) affect the cellular organization and/or division activity of the embryo, hypophysis and suspensor, other mutants, such as hydral, show embryo-specific defects (Topping et al., 1997), suggesting that the HYDRA1 gene is expressed in the embryo, but not in the suspensor. Direct evidence for different gene expression profiles in embryo and suspensor comes from promoter trap analysis in Arabidopsis, which has led to the identification of genes that are specific to the embryo-proper (Topping et al., 1994; Topping and Lindsey, 1997) and to the suspensor (P Gallois, unpublished results). Differences in gene expression between the apical and basal cell following the first zygotic division have also been identified. For example, the apical cell has been shown to accumulate the ARABIDOPSIS THALIANA MERISTEM LAYER
A role for cell wall components

Of particular interest is the nature of the molecular mechanisms that regulate cell fate determination and the associated gene expression programmes. In pollen development, the formation of the structurally and functionally distinct vegetative and generative cells, and the expression of genes within those cells, has been shown by in vitro techniques to depend on the asymmetry of the generative cell division, pollen mitosis I (Eady et al., 1995). More recently, van den Berg et al. have used laser ablation techniques to demonstrate the role of short-range signaling between cells to direct their fates (van den Berg et al., 1995, 1997). The most productive approach to date in addressing such questions in embryogenesis is the genetic approach, which involves screening for mutants in which cell fate control is defective.

In Fucus, the cell differentiation event leading to the generation of the thallus and rhizoid cells, respectively, is preceded by an asymmetric cell division, with the larger upper cell forming the thallus cell, which in turn forms the laminate thallus structures of the mature alga. The smaller basal cell forms the rhizoid that undergoes polarized growth. Genestain, an inhibitor of tyrosine phosphorylation, inhibits axis formation in the dark and in light-grown zygotes if applied early. Compelling evidence has also been found to demonstrate a role for the differential secretion of cell wall components in determining the subsequent identities of the rhizoid and basal cells. Here, wall fragments from thallus and rhizoid cells, respectively, can direct the fate of protoplasts of either cell (Berger et al., 1994), and a system of intercellular communication defines positional information to regulate cell fate (Bouget et al., 1998). Candidate regulatory molecules within the cell wall of Fucus are sulfonated polysaccharides; interestingly, their secretion is inhibited by genestain (Corellou et al., 2000). Gradually, then, evidence is emerging for the molecular basis of polarity generation in the Fucus zygote.

How far can there be extrapolation from the Fucus studies to developmental mechanisms in higher plants? A prerequisite for a model in which cell wall components carry positional information would be that there are detectable differences in such components between cells. What is the evidence that such differences exist? Much of the evidence for cell wall differences that are cell type- or tissue-specific comes from work in which monoclonal antibodies have been raised in response to immunizations with complex mixtures of plant cell material. By labelling these antibodies and localizing their binding sites in plants, a series of probes has been generated that each recognize cell surface polysaccharide epitopes associated with particular cell types (Knox et al., 1991; Pennell et al., 1991, 1995). The antibodies recognize components of the pectin matrix of the wall, specifically arabinogalactan moieties attached to proteins in the plasma membrane, the so-called arabinogalactan proteins (AGPs). Interestingly, there are differences in AGP localization during brassica embryogenesis. For example, the JIM8 antibody reveals cell differences between embryo-proper and suspensor, binding only to the cells whose future fate is as the suspensor (Pennell et al., 1991).

Not only have AGPs been identified that are differentially expressed during zygotic embryogenesis, but they are also differentially expressed during somatic embryogenesis. Somatic embryos develop, not from fertilized egg cells, but from somatic (non-reproductive) cells that have been tissue-cultured. These cells are induced to become structurally disorganized, and lose the characteristics of the differentiated state of the tissue from which they derive. However, they can reorganize if given appropriate hormonal signals (usually a removal of auxin from the culture medium). Despite the fact that they are not in contact with the maternal influences of the seed, they are able to develop in a polar way, to generate embryoidal structures that are similar to zygotic embryos, and indeed can go on to ‘germinate’ into plants.

The classical system to study somatic embryogenesis is in cultured cells of carrot. In this system, meristematically undifferentiated cells are grown in liquid medium in the presence of auxin as globular cell clusters: these have been designated proembryonic masses (PEMs). These probably represent preglobular-stage embryos, arrested in their further development by the presence of auxin. But when transferred to an auxin-free medium, cells of the PEMs become organized to form adventitious embryos (Krikorian and Smith, 1992). It is also possible to induce single cells of carrot to form embryos directly by manipulating auxin–cytokinin concentrations in the culture medium (Nomura and Komamine, 1985; Pennell et al., 1995).

In relation to the question of a role for AGPs in polarity, the single cell embryogenic system is of interest. The single cells divide, and the products of the division have separate fates: one cell becomes an embryonic initial, which undergoes further divisions to form an embryo; while the other cell fails to divide further. The original single cell expresses one particular AGP epitope, recognized by JIM8: and this is indicative of a cell with embryonic potential, shown by video tracking (McCabe et al., 1997). When this cell divides, the cell that becomes the ‘embryo initial’ switches off the JIM8 epitope, while the second cell (the ‘nurse cell’) continues to express that protein. This is reminiscent of the suspensor cell expression pattern of JIM8 in the zygotic embryo (Pennell et al., 1991), and the two division products of the single cell are analogous to the zygotic apical and basal cell.
But is there evidence that the JIM8 target actually regulates cell fate? To investigate this, McCabe et al. purified JIM8-positive or JIM8-negative cells, and collected cell wall components released from the walls of each. JIM8-negative cell wall components, lacking that epitope, were found not to continue to divide and form embryos (McCabe et al., 1997). However, if the JIM8 epitope, collected from the ‘nurse’ cells is added to the ‘initial’ cells, they will go on to form embryos; however, they require JIM8-positive cell-conditioned medium in order to do so.

This indicates that the JIM8 epitope can be used to identify cells which have a role in cell–cell communication and early cell fate specification in carrot somatic embryogenesis. Indeed, the JIM8 epitope may itself be involved in early events of determination of cell fate in carrot somatic embryogenesis, and also in maintaining activity of division of the embryo: i.e. it may signal to the initial cells to keep dividing. Further support for an inductive effect of AGPs in somatic embryogenesis comes from some earlier work (Kreuger and van Holst, 1993, 1995). These authors found that the addition of AGPs from an embryogenic carrot cell line to a non-embryogenic line caused an induction of embryogenic capacity of those cells. A functional role for AGPs has been further supported (Willats and Knox, 1996). By treating seedlings of Arabidopsis with Yariv reagent, which binds specifically with AGPs, they observed a reduced overall growth of shoot and root. In roots, this correlated with a reduced longitudinal cell expansion and increased radial expansion. These data suggest that Yariv binding to AGPs inhibits their biological activity, which may include a role in the control of cell expansion and organogenesis.

Yet further evidence for the importance of cell wall components in development comes from work with the carrot somatic embryogenesis system. One mutant cell line, ts11, has been identified that fails to undergo embryogenesis when grown at an elevated temperature, even under conditions which are inductive for non-mutant lines (i.e. auxin-free). At elevated temperatures (32°C), ts11 embryos arrest at the globular stage. However, it was found that developmental arrest at elevated temperatures could be bypassed by the addition of culture medium in which fully embryogenic lines had been grown. The secreted molecule was identified as a 32 kDa protein with homology to an endochitinase (de Jong et al., 1992). In search of a substrate for this enzyme, a range of molecules containing N-acetylglucosamine moieties were added to ts11 cells to find compounds which also rescue the mutant and so might represent natural substrates or products of the chitinase. Interestingly, it was found that the mutant could be rescued by the application of lipo-oligosaccharides to the culture (de Jong et al., 1993). This class of molecule consists of an oligosaccharide backbone of 4 or 5 β-1,4-linked N-acetyl-glucosamine residues with a C16 or C18 fatty acid group attached to the non-reducing end. They are known to act as important signals in the nodule formation following Rhizobium interaction with legume roots, and have been designated Nod factors (Schultz and Kondorosi, 1996). Purified Nod factors have a wide range of effects on the roots of legumes: some effects are very rapid, some over a period of days or weeks.

The most rapid response is transient depolarization of the plasma membrane, occurring within 15 s. This leads to an increase in intracellular pH, and a spiked oscillation in intracellular calcium levels (reviewed by Schultz and Kondorosi, 1996). This may represent an activation of an intracellular signal transduction pathway, but a causal relationship has not yet been demonstrated. Synthetic Nod factors can also induce division in tobacco protoplasts in the absence of auxins and cytokinins and the fatty acid structure has been shown to be important in this activity (Röhrig et al., 1995). So a common role for lipo-oligosaccharides in somatic embryogenesis and root nodule formation may be as stimulators of cell division, and at concentrations as low as 10^{-15} M.

One speculative view of the molecular mechanisms of targeted secretion of wall components, and subsequent role in higher plant embryogenesis, derives from the observation that the GNOM (GN) protein of Arabidopsis, which is believed to play a role in Golgi vesicle transport/tidbitting protein, is susceptible to brefeldin A inhibition (Steinmann et al., 1999). It was seen earlier how brefeldin A can inhibit targeted wall secretion and polar axis fixation in Fucus (Shaw and Quatrano, 1996), and, similarly, gn mutants, defective in GN protein function, are also defective in establishing the asymmetry of the first zygotic division and subsequent apical-basal patterning. Golgi vesicle transport proteins such as Sec7 of yeast, which has similarities to GN, have roles in cell wall elongation and in cell division, delivering important precursors for both the plasma membrane and the cell wall, as well as other proteins that require directional delivery to the cell membrane or wall (Shevell et al., 1994). Such processes require directed and precise delivery of the vesicle. The work of Pennell et al. (Pennell et al., 1991) demonstrates the differential distribution of the JIM8 epitope along the apical-basal axis of the brassica embryo-suspensor complex, and the results of McCabe et al. show similarly its targeted and polar distribution in the bicellular embryo–nurse cell complex in the carrot system (McCabe et al., 1997). It is therefore possible that cell wall components such as the JIM8 epitope are crucial for imparting positional information at the earliest stages of apical-basal axis formation, and require GNOM protein function for their correct spatial distribution.

These results therefore suggest a role for cell wall-related molecules in regulating important aspects of embryogenesis and polarity. Whether fertilization induces...
targeted secretion of wall-localized regulatory molecules in higher plants is still unknown, but is an intriguing possibility. There will be a return to the relationship between targeted secretion, hormonal signalling and polarity later.

**Genetic control of embryo-suspensor cell fate determination**

The fates of the apical and basal cells, following zygotic division in *Arabidopsis*, are clearly distinct. Direct evidence for a genetic control of suspensor cell identity derives from studies of mutants in which the suspensor undergoes abnormal patterns of cell division, most commonly ectopic division. In the abnormal suspensor (Schwartz et al., 1994) and raspberry (Yadegari et al., 1994) mutants of *Arabidopsis*, the embryo-proper arrests and the suspensor subsequently enters into a series of inappropriate divisions. Significantly, the modified suspensor takes on a variety of characteristics of the embryo-proper. Ultrastructural analysis has revealed that, in the case of the sus mutants, for example, accumulation of storage protein bodies, lipid bodies and starch grains occurs in both the embryo-proper and, unusually, the suspensor (Schwartz et al., 1994). It has also been observed that *AtLTP*, which encodes an *Arabidopsis* homologue of the carrot *EP2* lipid transfer protein (Sterk et al., 1991; Thoma et al., 1994), is strongly expressed in the protoderm/epidermis of embryos and seedlings but is not expressed in the wild-type suspensor. However, it is expressed in the peripheral cells of the *raspberry* embryo-proper and suspensor (Yadegari et al., 1994). Even more spectacular is the re-differentiation of suspensor cells in the *twin* (*twn*) mutants. Here, the suspensor cells reorganize into secondary embryos, following arrest of the embryo-proper (Vernon and Meinke, 1994). The *TWN2* gene has now been cloned, and encodes a valyl-tRNA-synthase, though its mode of action remains unclear (Zhang and Sommerville, 1997).

It has been suggested that the wild-type embryo-proper signals to the suspensor to maintain its differentiated state, and in the case of the *sus* and *raspberry* mutants, this signal is blocked or not produced, and the suspensor embarks on a default pathway of embryonic development (Schwartz et al., 1994). In this laboratory a novel mutant of *Arabidopsis*, designated *asf1* (for *altered suspensor fate 1*) that exhibits a novel pattern of inappropriate cell division in the suspensor, and exhibits a reprogramming of gene expression and cell differentiation (Fig. 2) has been identified. Activation of auxin-inducible genes in the modified suspensor leads us to propose a model in which the mutant phenotype is mediated by the de-regulated partitioning of auxin between embryo-proper and suspensor, to activate the observed ectopic cell division (Horne, 1998; Horne and Lindsey, in preparation). The recently described *axr6* mutant, which is auxin-resistant and shows supernumerary suspensor cells (Hobbie et al., 2000), also lends support to this model. The likely role of auxin in embryonic patterning will be discussed later.

**Apical-basal patterning: the embryo-proper and seedling**

The apical-basal pattern is defined by the positioning of the shoot meristem and cotyledons, the hypocotyl and the root and root meristem. The study of mutants has led to the theory that the embryonic axis is therefore partitioned into three main regions; apical, central and basal (Mayer et al., 1991). The shoot meristem and the majority of the cotyledons originate in the apical region, while the central region contributes to the majority of the rest of the axis, namely the shoulder of the cotyledons, the hypocotyl, the embryonic root, and the vascular, cortex and endodermal root initials of the root meristem. It is only the quiescent centre, the columella initials and the central root cap that arise from the clonally separate hypophyseal cell, the uppermost suspensor cell, whilst the rest of the pattern is derived from the embryo-proper (Scheres et al., 1994; Mayer and Jürgens, 1998). Despite the temptation to consider the formation of each of the three regions as independently regulated events, it will become clear that interactions between tissues in each region are essential for the correct integrated patterning of the whole seedling. For convenience, however, relevant features of each of the three regions, respectively, will be examined.

Each region follows its own programme of cell divisions once they have been established, all three being present by the octant stage. The formation of the O° boundary at the quadrant stage creates the upper and lower tiers, corresponding to the apical and central regions, respectively, whilst the hypophyseal cell is formed by divisions in the suspensor. The apical region divides without preferential orientation, while divisions that are perpendicular to the axis create the cell files that characterize the central region. Within the basal region a more stereotyped set of divisions is required to create the root meristem and central root cap, such that the fate of any cell in that region can be predicted with high probability (Scheres et al., 1994).

Through studying the development of each of these regions in both wild-type and mutant backgrounds, the different signalling mechanisms involved are becoming clearer. Much progress has come from the application of a strategy of mutagenesis and the progressive isolation and characterization of genes that are specifically involved in embryonic pattern formation. It is worthwhile to note that, as the embryonic pattern is reiterated through the meristems during post-embryonic development, many defects that originate in the embryo are often identifiable in seedling mutant screens.
What then are the mechanisms that generate positional information to promote region-specific gene expression patterns? In the remainder of this review article the genes that specify cell fate within the Arabidopsis apical-basal axis will be examined and evidence for the signalling events involved considered.

The apical region of the embryo

The apical region forms the self-perpetuating shoot meristem. A number of genes have been isolated which affect the establishment and characteristics of the shoot meristem (Laux and Mayer, 1998). The four inner apical cells at the 16-cell stage Arabidopsis embryo start to express the WUSCHEL (WUS) gene, which is an early marker of the shoot meristem cell fate (Mayer et al., 1998). WUS is expressed through a number of asymmetric divisions which also produce the future cotyledonary primordia, though expression now becomes restricted to the group of cells at the apex of the embryo which will become the shoot meristem (Laux et al., 1996). The WUS gene has been shown to encode a novel homeodomain protein (Mayer et al., 1998). A possible role for WUS is in maintaining the pluripotent capacity of the shoot meristem precursor cells (Lenhard and Laux, 1999). The GURKE gene of Arabidopsis is also required for the correct organization of the shoot apical region (Torres-Ruiz et al., 1996). Strong mutant alleles are unable to construct the entire apical region, and even part of the hypocotyl, while weaker alleles produce abnormally shaped leaves and flowers. The root and radial patterning is apparently unaffected, even in strong mutant alleles. The defect can be traced back to the transition-stage embryo.

SHOOT MERISTEMLESS (STM) expression is initiated at the late globular stage in the central region of the embryo apex (Long et al., 1996), and is independent of WUS action (Mayer et al., 1998). sim mutants have fused organs originating from the shoot meristem, which indicates a role for STM in restricting cells with a shoot meristem fate from participating in organ formation (Long et al., 1996; Long and Barton, 1998; Endrizzi et al., 1996). STM is expressed by only a specific set of cells within the apex of the embryo, and has been shown to be a member of the KNOTTED homeodomain proteins (Long et al., 1996). AINTEGUMENTA (ANT) meanwhile is expressed by the two cell groups which flank the shoot meristem, and which will eventually form the cotyledons (Elliott et al., 1996).
**CLAVATA1 (CLV1)** is also expressed in the embryonic shoot apex, from the heart stage onwards. **CLV1** has been cloned and shown to encode a predicted membrane-bound kinase receptor (Clark et al., 1996), which suggests a role in signalling. **CLV1** acts independently of **STM** (Long and Barton, 1998), although it is thought that they act competitively between each other to regulate the balance between undifferentiated cells and organ formation in response to positional information (Clark et al., 1996; Laux and Schoof, 1997). **clv1** mutants have enlarged meristems in post-embryonic development. **PRIMORDIA TIMING (PT)** also causes an enlargement of the shoot meristem, though it acts from the globular stage onwards. From analysis of **pt clv1** double mutants, it is clear that these two genes work in different pathways despite their apparently similar roles. **CLV1** is therefore probably not involved, like **PT**, in early meristem formation processes, which is supported by the temporal differences in their phenotypes (Mordhorst et al., 1998).

One gene which does interact with **STM** is **ZWILLE (ZLL)**, Moussian et al., 1998). The **zll** mutant shoot meristem is initiated correctly, but **STM** expression is either restricted or down-regulated, resulting in cells which follow other development fates, possibly due to the influence of other spatial cues. **ZLL** is therefore required to maintain meristem cell identity within the apex, possibly through acting as a translational control. The **ZLL** gene is expressed in the vascular precursor cells, situated just below the meristem primordia, from early stages until leaf primordia are established, when presumably the meristem can maintain itself.

The analysis of these genes has shown that position-dependent cell fate specification is achieved from the late globular stage onwards. It appears that meristem formation occurs through the activation of genes which specify cell fate in a spatially precise manner. A key area of research has been to identify possible signals that may activate and regulate the expression of the genes described above. One signal molecule which has proven particularly interesting is auxin.

Auxin has been proposed as a key signal molecule in providing positional information within the apical region of the embryo, particularly during the transition period from globular to heart stage. Liu et al. first reported the use of auxin transport inhibitors to study development in cultured zygotic embryos of *Brassica juncea* (Liu et al., 1993). They showed that inhibition of auxin transport at the globular stage leads to the formation of embryos which lack bilateral symmetry at the heart stage. Bilateral symmetry is established when the two cotyledons form either side of the shoot meristem region. Instead of two cotyledons, embryos developed with fused and collar-like cotyledons, which interestingly phenocopied known auxin transport-defective mutants **pin1** (Okada et al., 1991) and **gnom** (Steinmann et al., 1999). Hadfi et al. used this same *B. juncea* culture system to look at the effects of auxin (**IAA**), an anti-auxin (**PCIB**), and an auxin transport inhibitor (**NPA**) (Hadfi et al., 1998). When auxin was supplied, bulb-shaped or cucumber-shaped embryos resulted, possibly because the embryo, flooded with exogenous auxin, is unable to establish the auxin gradients which are essential for morphogenesis. The anti-auxin **PCIB** inhibited cotyledon growth so that either only one or no cotyledons developed. Correct hypocotyl and radicle growth was also found to require auxin action and movement. Furthermore, when globular-stage embryos were treated with exogenous **NPA**, axis duplication was seen, whilst a later application produced split-collar or collar-like cotyledons. These results confirm the findings of Liu et al. (Liu et al., 1993), and help clarify the model of auxin movement which they first proposed: continuous auxin transport removes auxin from the area between the two emerging cotyledons, and supplies the auxin back to the cotyledonary primordia. Auxin removal starts in the central apical region of the globular or early transition embryo, and continues asymmetrically across the apex of the embryo.

Inhibition of auxin transport therefore blurs the positional information that is created by its normally precise redistribution, resulting in increased cell division throughout the shoot apex. These findings indicate that auxin translocation is a prerequisite for the radial globular embryo to progress to the bilaterally symmetrical heart stage embryo. Similar results were found by Fischer et al. for morphogenesis of the embryo of the monocot wheat (Fischer et al., 1997).

**The central and basal regions of the embryo**

The central part of the embryo produces the majority of the embryonic axis, and a number of mutants have been found that are defective not only in the generation of hypocotyl and root, but also the radial axis within this region. Indeed, the radial organization of the seedling is established during embryogenesis, to define the cellular patterning that runs throughout the hypocotyl and the root (Scheres et al., 1995).

The **MONOPTEROS (MP)** gene is required for the formation of the hypocotyl, root, root meristem, and root cap; products of the central and basal regions of the embryo (Berleth and Jürgens, 1993). The **MP** gene is also required for correct cell axialization and development of aligned vascular strands (Przemeck et al., 1996). The **MP** gene has been cloned and found to encode a transcription factor with nuclear localization sequences and a DNA binding domain which is highly similar to a domain which binds auxin-inducible promoters. In fact **MP** has the same binding specificity as **AUXIN RESPONSE FACTOR 1 (ARF1)** (Ulmasov et al., 1997a), which is a transcription factor that binds to auxin response elements.
(AREs) within promoters of auxin-inducible genes. Expression of MP is initially in broad domains in the embryo, becoming eventually confined to the procambial tissues (Hardtke and Berleth, 1998). This is similar to PIN1 expression, although PIN1 has been shown not to require MP gene function (Steinmann et al., 1999; Palme and Gälweiler, 1999). MP is therefore required for correct cell axialization in the early embryo, and for correct vascular development in the later stages of embryogenesis and during post-embryogenic development, through its likely role in regulating the transcription of auxin-responsive genes.

Whether the central region of the mp mutant fails to recover from its altered axialization and, therefore, cannot recover hypocotyl and root formation, or if the basal region’s failure to generate the root meristem is because of a lack of aligned vascular primordia, is not known. There is a large amount of evidence to indicate that auxin is required for root formation (Boerjan et al., 1995; Celenza et al., 1995; Reed et al., 1998). However, if the central section of the embryo does not develop correctly, then the corollary of this for the basal region must be considered. The MP gene is required for correct alignment of the vascular tissue, and cell axialization within the hypocotyl (Przemeck et al., 1996). It is therefore open to suggestion that the defective polar auxin transport system may cause downstream effects on root development in the mp mutant.

Within the radially swollen fass and hydra mutants, multinumerary cotyledons and apical meristem regions develop (Torres-Ruiz and Jurgens, 1994; Topping et al., 1997). hydra mutants also exhibit a form of axis duplication through their hypocotyl region, which is radially swollen and highlighted by separated vascular strands running through the tissue (Topping et al., 1997). These phenotypes may result as secondary effects from impaired auxin transport and/or auxin action within these tissues. Hormonal studies of fass show that it has an average of 2.5 times more free auxin than wild-type plants (Fisher et al., 1996). It is possible that the high level of auxin may trigger higher levels of ethylene—it has been demonstrated that transcripts encoding pea ACC synthase isoenzymes, for example, are rapidly induced by exogenous IAA (Peck and Kende, 1998). Interestingly fass roots elongate 2.5-fold more when removed from the plant and cultured than when left intact on the plant. This suggests that a signalling from the upper part of the plant inhibits fass root length. A shorter root phenotype is a common response to exogenous ethylene. Like fass, hydra also has a short root phenotype, which is rescued by treatment with silver ions, inhibitors of ethylene action (M Souter and K Lindsey, unpublished data). Clearly then, these two mutants have hormonal imbalances which have led to alterations in the number and size of pattern components. The radially swollen apical and central regions may cause either a break in the auxin transport system, or a diffusion of the auxin gradients and short-range signals which maintain the correct gene expression patterns.

Does the central region signal to the basal region to enable the correct development of the latter? There is growing evidence that signalling between embryonic domains establishes the positional information that allows cells to activate fate-determining gene expression programmes.

The BODENLOS (BDL) gene of Arabidopsis has been implicated in auxin-mediated apical-basal patterning processes (Hamann et al., 1999). Development in bdl mutants is disrupted at the two-cell stage, when the apical cell divides horizontally rather than vertically. Hypophyseal development is subsequently compromised, leading to mutants that lack an embryonic root (quiescent centre and central root cap). Hypocotyl development is also affected in some mutant individuals. Interestingly, bdl mutants show insensitivity to the synthetic auxin 2,4-D within the same range as axr1 seedlings, which suggests that auxin-mediated signalling is required to specify the fate of the basal region of the embryo. Furthermore, the BDL gene only affects the embryonic root, since bdl seedlings can still form lateral root meristems. The model put forward for the action of BDL suggests that auxin is involved in determining hypophyseal cell fate at the octant stage. Later, at the heart stage, the quiescent centre signals to the cells above it to block differentiation, conferring the fate of root meristem initials (Hamann et al., 1999). Studies show that ablation of the quiescent centre in seedlings results in the differentiation of the adjacent initial cells (van den Berg et al., 1997).

auxin resistant6 (AXR6) mutant seedlings are arrested in their development soon after germination, and lack a root and hypocotyl (Hobbie et al., 2000). The stronger axr6–1 allele has more severe vascular defects than the weaker axr6–2, and tends to produce only one cotyledon. Mutants are also more resistant to auxin, undergoing irregularly timed and oriented cell divisions, which are first observed in the early embryo. Principally the suspensor is disrupted by cell divisions which create radial layers rather than the characteristic single file of seven to nine cells. As a result, the hypophyseal cell does not form correctly, and the distinction between the embryo proper and the suspensor is lost. Within the central region the vascular precursor cells fail to establish during the globular stage, a defect which is also seen in monopteros (Przemeck et al., 1996). AXR6 therefore represents a novel gene which causes defects in cell division patterns within the embryo and the suspensor. It is feasible that the aberrant cell divisions occur because there are problems in auxin-mediated positional or cell-fate signalling. Indeed, the similarities between the phenotypes of the mp, bdl and axr6 mutants suggests that they may function in similar pathways (Hobbie et al., 2000).
The **HOBBIT (HBT)** gene is required for correct hypophyseal cell formation (Willemse *et al*., 1998). **hbt** embryos have incorrect hypophyseal cell development from the quadrant stage onwards, so that by the heart stage activation and formation of the lateral root cap layer has not occurred. Mature embryos lack a quiescent centre and columella root cap. Root meristem formation is not only defective in the embryonic root, but also in the seedling, where secondary roots fail to form, even when cultured. **HBT**, unlike **BDL**, is therefore required for root meristem formation both embryonically and post-embryonically. It is unclear at present whether the exact role of the **HBT** gene is to specify the basal region or if it is required for the correct division programme that the hypophysis must go through to produce the root meristem and root cap.

The correct patterning of the root therefore would appear to depend on signalling between the central and basal regions of the embryo, as well as the cell-cell communication which is established once the root meristem becomes active.

**A synthesis: auxin as a positional and a patterning signal molecule**

Clearly the results presented so far implicate auxin as playing a major role in embryogenesis, providing positional information for the co-ordination of correct cellular patterning from the globular stage onwards. Auxin has proved a difficult molecule to localize in tissues, being highly diffusible and occurring in both active and inactive (conjugated) forms (Normanly and Bartel, 1999). Shoot meristems and leaf primordia are regarded as the main sites of synthesis, with the polar auxin transport system holding the key to many responses. Vascular tissue formation follows the flow of auxin (Aloni, 1987; Mattsson *et al*., 1999), which is canalized into files of cells so that connected vascular strands form (Sachs, 1991). Auxin controls much of post-embryonic development, especially plant architecture, through the modulation of meristem activity and cell expansion in response to environmental factors (Hobbie, 1998).

Auxin transport therefore holds a key to our understanding of much of auxin’s role within the plant. The chemiosmotic theory proposes that auxin requires an influx and efflux carrier in order to move through cells and tissues. This requires anion symport (influx) and efflux carrier proteins. **AUX1** is a candidate for the influx carrier (Bennett *et al*., 1996), whilst the **PIN** gene family constitutes the putative transport protein of the efflux carrier complex. For a comprehensive review of auxin transport the reader is referred to Lomax *et al.* (Lomax *et al*., 1995) and Palme and Gälweiler (Palme and Gälweiler, 1999). Here the focus will be on the efflux carrier, whose cellular localization needs to be precise as it might be expected to determine the course of auxin flow.

To date, seven **PIN** genes have been identified, whilst more than ten different **PIN** homologues have been found in Arabidopsis. **PIN** genes have also been identified in maize, rice and poplar, with high conservation between monocot and dicot species indicating a conserved function for **PIN** proteins throughout the plant kingdom (K Palme, personal communication). In Arabidopsis members of this family of transporters have different expression patterns within time and space, and so offer the plant a means by which auxin can be transported precisely. **PIN1** has shown to be linked to the development of vascular tissue, which follows Sach’s canalization hypothesis (Sachs, 1991). **PIN1** is located at the basal end of cells within the vascular stele (Gälweiler *et al*., 1998). During embryogenesis, **PIN1** becomes polarized in its expression pattern at the mid-globular stage, before the two cotyledons have started to develop. By the heart stage the pattern very much resembles the pattern it takes throughout the rest of the plant’s post-embryonic development, forming a characteristic Y shape from the two cotyledons to the basal end of the embryo (Steinmann *et al*., 1999). **PIN1** expression in MP is not affected, which suggests that its targeting to the basal membrane does not require the **MP** **ARF**: although correct axialization of vascular strands does. In contrast, **PIN1** localization in the gnom background is severely affected, indicating that directed vesicle secretion is required, as indicated above (Steinmann *et al*., 1999).

Recent direct evidence for the existence of auxin gradients that correlate with a physiological response is described by Uggla *et al.* (Uggla *et al*., 1996, 1998). These authors used the highly sensitive technique of GC-MS to show the presence of a steep radial gradient of auxin across the vascular cambium in *Pinus sylvestris* (L.). This lateral meristem contributes to the secondary growth of the plant which is activated at the start of each new growing season. The gradient of auxin across the tissue appears to provide positional information for the developing tissue, with possibly other morphogen gradients or cell–cell communication systems determining the precise cell division patterns and cell fates required to produce the specific cell types that exist within this tissue. The significance of this work lies in the fact that auxin appears to be providing positional information to a developing and patterning tissue.

Studies on the **POLARIS** gene of Arabidopsis provide further information on the role of auxin in defining position and cell activities during embryonic and seedling root development. This gene was identified by promoter trapping, leading to the activation of GUS expression in the basal region of the embryo, from heart-stage onwards; and subsequently in the seedling root tip (Topping *et al*., 1994). It encodes a very short transcript that appears to
regulate root sensitivity to ethylene, to modulate root growth (S Casson, P Chilley, K Lindsey, unpublished data). Although this GUS fusion gene was originally considered to be a root-meristem marker, it was found to be expressed in the appropriate position, i.e. in a polarized pattern, even in mutants such as gnom, hydra and hobbit that either lack root meristems or have defective root meristem patterning (Topping and Lindsey, 1997; Willemsen et al., 1998). The POLARIS gene promoter is up-regulated by auxin very rapidly, within minutes, and its spatial expression pattern represents a useful marker of auxin localization in the root (Topping and Lindsey, 1997, and unpublished data). Interestingly, correct spatial patterning of POLARIS expression is disrupted significantly only in the most severe, ball-shaped gnom seedlings, suggesting that these individuals, but not the more conical-shaped gnom seedlings, are defective in polar auxin transport (Topping and Lindsey, 1997). This is consistent with the observed defective PIN1 localization in gnom embryos (Steinmann et al. 1999), and suggests that auxin provides a chemical framework for the patterning of apical-basal gene expression and cellular activity in both embryo and seedling.

Kerk and Feldman have proposed a biochemical model for auxin’s role in initiating and maintaining the quiescent centre of the maize root meristem (Kerk and Feldman, 1995). The quiescent centre is located at the distal part of the root, and is also the most distant tissue from the path of polar auxin transport. Ascorbic acid is a compound which is necessary for the transition from G1 to S phase in the cell cycle, and which is broken down by ascorbic acid oxidase (AAO). AAO mRNA is increased in response to auxin, which was shown to have higher levels in the quiescent centre than surrounding cells, determined by immunolocalization of auxin in the root tip. These results suggest that auxin is influencing AAO levels within the root meristem, and that this ensures the continued stem cell ability of the quiescent centre.

The influence of auxin on the activity of the root meristem is also elegantly demonstrated through studies by Sabatini et al. (Sabatini et al., 1999). The authors utilized a synthetic auxin-responsive promoter construct, termed DR5, which consists of seven tandem repeats of a auxin-responsive element fused to the β-glucuronidase (GUS) reporter gene (Ullmanov et al., 1997b). The DR5 reporter is activated rapidly by auxins within the $10^{-9}$–$10^{-4}$ M range. Expression of this gene fusion shows a ‘maximum’ in the distal root meristem region, in the columella initials of wild-type seedlings. By studying the effect of known mutations on the position of the auxin maximum, they suggest that pattern and polarity in the Arabidopsis root is mediated by an auxin-dependent organizer, which is established by the auxin maximum located distal to the vascular tissue boundary. Therefore, ascorbic acid oxidase may help to maintain the meristem’s identity, whilst the auxin maximum allows the maintenance of the meristem itself, which is the source of the pattern in the root. Directional signals are responsible for the cell fate specification within the root, with the more differentiated cells within a cell file signalling to the daughters of the meristem initials to initiate cell fate processes (van den Berg et al., 1995).

**Conclusions**

Both intrinsic and extrinsic signals help to establish polarity in the early plant embryo. The asymmetric zygotic division fixes polarity, which may rely on the asymmetric delivery of cell wall components, possibly AGPs, and which requires GN in order to execute it. The fate of the basal cell is now established, and is marked in species as diverse as Brassica napus and carrot, by the expression of JIM8-binding AGPs, which may provide cell fate information to the suspensor.

Once the Arabidopsis embryo has reached the globular stage, containing roughly 100 cells, the auxin transport mediator PIN1 becomes polarized in its expression. Again, directional vesicle transport, via GN, is required for the correct localization of the protein within the cell membrane, which is expressed in a polar pattern at the basal end of the cell. The establishment of the auxin transport system is a prerequisite for patterning events in the apical region of the embryo at the beginning of the transition from globular to heart stage embryo. Later in development it is required for hypocotyl and root formation and maintenance, with auxin responsiveness essential in order for the positional information provided by the polar transport of auxin to be interpreted into pattern elements. Short-range cell–cell communication is required for many of the cell fate decisions, but these clearly depend on the presence of information indicating their position within the apical-basal axis. Regional signalling, involving genes such as BDL and other auxin response pathways such as AXR6 and MP, is also crucial to the correct cell division patterns and cell fate decisions which need to occur in the central and basal regions. Analysis of mutants such as asf1 and axr6 suggests strongly that auxin signalling is required for the correct cell divisions and cell fate of the suspensor to be established.

Once the meristems in the root and shoot have been established, their self-maintaining ability is determined by the expression of a number of recently discovered genes, although the signalling systems that regulate their expression are far from fully understood. Germination activates the meristems to reiterate the programmes of patterning initiated in the embryo, programmes which can be altered by the inhibition or antagonism of auxin. There are some differences in the gene expression programmes that specify embryonic and post-embryonic patterning, as the different
temporal patterns of CLAVATA1 and PRIMORDIA TIMING clearly highlight.

A number of studies of the molecular mechanism of auxin in the seedling have been highlighted. However, it is important to note that these mechanisms are established in the embryo, and their interruption or disturbance at this early stage cannot always be corrected during post-embryonic development. Continued study of the mechanisms that control the movement and action of auxin, and its possible relationship with cell wall contraction and composition, can be expected to lead to the discovery of more upstream events and downstream targets which are required for patterning in plant embryogenesis.

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Polarity and signalling in plant embryogenesis 981


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Polarity and signalling in plant embryogenesis


