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Got Root? - Initiation of the Embryonic Root Meristem

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ABSTRACT

Plant development relies on the activity of meristems, small groups of undifferentiated cells that produce all organs. The first meristems are formed in the embryo, and all subsequent development depends on their proper establishment, making embryonic meristem initiation a key step in plant life. The founder cells of the embryonic meristems are specified early in embryo development after the establishment of the body axis. Initiation of the root meristem in the early embryo is marked by the specification of a single cell, the hypophysis, and hence an attractive model to study meristem initiation. In this review, we will discuss the mechanisms that control embryo axis formation and root meristem initiation.

Keywords: Arabidopsis, cell communication, embryogenesis, pattern formation, stem cells

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INTRODUCTION

Plants are produced by their meristems, the plant equivalent of stem cell niches. These meristems are located at the growing tips of all higher plants, and generate leaves, shoots, flowers and roots. Even though the type of organ produced by each meristem is different, the underlying principle of meristem activity is a general one. On one hand, meristems must produce differentiated cells that are incorporated into the new organ, yet on the other hand, a population of undifferentiated stem cells must be maintained. To this end, meristems consist of two cell populations: a group of stem cells and a group of organizing cells that control the stem cells (Jürgens 2003; Stahl and Simon 2005). A critical question in plant biology is how such a meristem is set up. The first meristems are found in the seedling, and are made during embryogenesis. We will first describe embryogenesis in the model plant *Arabidopsis thaliana* and then discuss the mechanisms controlling the events leading to the initiation of the root meristem.

EMBRYOGENESIS IN *ARABIDOPSIS*

Embryo development in *Arabidopsis* can be divided into three major phases of development. Immediately after fertilization, the basic body plan is established and consists of an apicobasal axis combined with a radial pattern of different tissues. Following this initial phase of pattern formation (Jürgens and Mayer 1994), the embryo grows by cell division and elongation until it fills the seed at full maturity. Finally physiological processes of storage and desiccation prepare the seed for dormancy until it germinates.

The first microscopically observable event after fertilization is the rapid stretching of the zygote towards the central part of the endosperm (**Fig. 1A, 1B**). During this process, organelles of the zygote are relocated such that the nucleus is positioned in the apical part of the cell while the basal part holds the vacuole. A horizontal division in the upper half of the zygote yields two morphologically different daughter cells (**Fig. 1C**). The smaller cytoplasm-rich apical cell undergoes a relatively fast series of two vertical divisions and one horizontal division to form a spherical proembryo consisting of an upper and a lower cell tier of 4 cells each (**Fig. 1D, 1E**). This proembryo rests on top of an extraembryonic filamentous structure (suspensor) that is formed by descendants of the larger vacuolarized basal zygote daughter cell by a few rounds of horizontal divisions. Upon these initial cell divisions, the 8 cells of the proembryo undergo a round of periclinal divisions that divides the proembryo into an outer protodermal cell layer (epidermis precursors / protoderm) and an inner set of cells (ground tissue and vascular tissue precursors) (**Fig. 1F**). Around this stage, the uppermost suspensor cell bulges into the proembryo (**Fig. 1G**) and divides asymmetrically to yield a smaller apical and a larger basal cell (**Fig. 1H**).

At this developmental stage (dermatogen stage), the embryo consists of three different domains anchored in the ovule via the suspensor. While cells of the uppermost domain give rise to the shoot apical meristem and most of the cotyledons, abaxial (lower) parts of the cotyledons, the hypocotyl and most of the embryonic root meristem are generated by the lower domain. The quiescent center and columella root cap of the root apical meristem are derived from the hypophysis (**Fig. 1H, 1I**). Cells of this lineage divide

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Invited Mini-Review

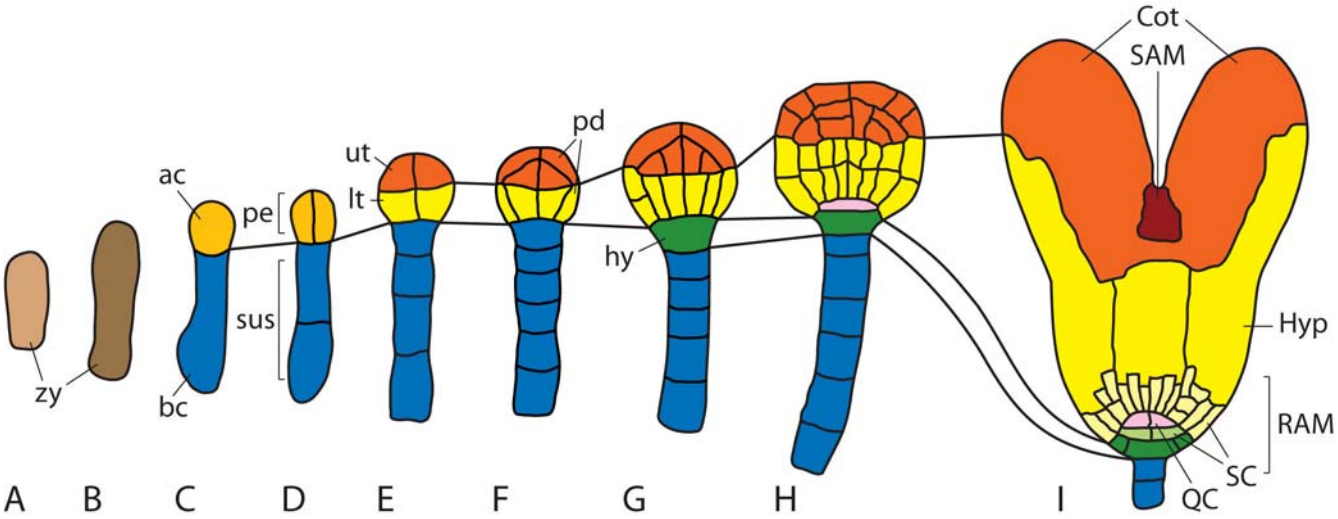

Fig. 1 Stages of *Arabidopsis* embryo development. From left to right, drawings represent successive stages of embryogenesis from the zygote (A) to the late heart stage (I). Clonally related regions are marked with distinct colors, and connected between embryos by lines. Upon fertilization, the zygote (zy, A, B) elongates, and divides asymmetrically to yield a smaller apical cell (ac, C; 1-cell stage) and a larger basal cell (bc, C). These cells then undergo different developmental programs, with the basal cell giving rise to the filamentous suspensor (sus, D; 4-cell stage), and the apical cell generating the proembryo (pe, D). The 4 cells of the proembryo divide horizontally to generate an upper tier (ut, E; 8-cell stage) and a lower tier (lt, E) of 4 cells each. All 8 proembryo cells then divide periclinally to set apart the protoderm (pd, F; dermatogen-stage), the epidermis precursor. Subsequently, in the early globular stage (F), the uppermost suspensor cell bulges into the proembryo, which marks its specification as hypophysis (hy), the root meristem founder cell. Next, the hypophysis divides asymmetrically to generate a lens-shaped apical daughter and a larger basal daughter cell (H; transition stage). The lens-shaped daughter will generate the quiescent center, the root meristem organizing center. At the same time, the lateral flanks of the proembryo apex flatten, marking the future cotyledon primordia. At the late heart stage (I), all seedling structures can be recognized, with two cotyledons (Cot) flanking the shoot apical meristem (SAM) at the embryo apex, the hypopcotyl (Hyp) in the center and the root apical meristem (RAM) at the embryo base. At this stage, the root meristem is already composed of a quiescent center (QC) that is surrounded by stem cells (SC) for all cell types in the root.

somewhat slower than the cells in the two other domains. A horizontal division at mid-globular stage (**Fig. 1H**), divides the hypophysis into a smaller apical lens-shaped cell and a larger basal cell. Two vertical divisions of the lens-shaped cell form the quiescent center, a small group of cells that control the surrounding stem cells of the root apical meristem (RAM). Descendants of the basal cell form stem cells and outer cell layers of the central root cap (columella) (**Fig. 1I**).

Since the formation of the RAM can be recognized somewhat earlier than the formation of the shoot apical meristem (SAM) and is not complicated by the formation of any attached organs we will focus in this review on the processes leading to the initiation of the RAM.

FORMATION OF THE EMBRYONIC ROOT MERISTEM

Obviously, the observed pattern of cell divisions and the establishment of different cell types are the outcome of an underlying genetically controlled program. While the embryo develops, certain sets of genes are switched on or off in the emerging cell types, thus marking differences in cell fate well before differentiation or division. In the following sections, we will discuss possible mechanisms of cell specification and pattern formation in the early *Arabidopsis* embryo that ultimately lead to the initiation of the root meristem.

Establishment of the embryo axis

Plant embryogenesis occurs in an extremely polar environment: the maternal tissues of the ovule are polar in that the egg apparatus is positioned at one end of the embryo sac, and the antipodes on the other. Nutrient supply from the mother plant to the developing seed occurs at this antipodal (chalazal) region, which again imposes polarity upon seed development. Furthermore, even before fertilization, the egg cell itself is highly polar, with the vacuole located at the basal and the nucleus at the apical end (Mansfield and Briarty 1991). Hence, it is questionable whether zygote polarity is autonomously established at all, or if this is through maternal control. No zygotic mutants have so far been identified where polarity of the zygote is clearly disrupted, and it might be envisioned that such mutants would be maternal effect mutations.

Several mechanisms could account for differential specification of apical and basal daughter cells during zygote division. The two most extreme are 1) that two equivalent cells are generated by the anatomically asymmetric division, that become instructed for apical and basal fates by extrinsic cues, derived for example from maternal tissues, or from endosperm; 2) that intrinsic factors within the zygote (organelles, cell wall or membrane determinants, proteins, mRNAs, etc.) are unequally partitioned in the two daughters, thereby creating two cells that are immediately different.

Although not excluding other models, the only study (Haecker *et al*. 2004), that addresses this problem, suggests the latter of the two mechanisms. While the expression of many genes is specifically induced in the apical or basal cell lineage after the division, the mRNAs of two *WUSCHEL-RELATED HOMEOBOX* (*WOX*) genes, namely *WOX2* and *WOX8*, are already present in the zygote and even in the egg cell (**Fig. 2A, 2B**). However, with zygote division, they become separated from each other. *WOX2* mRNA is restricted to the apical cell and its descendants, whereas *WOX8* mRNA is found only in the basal cell and its daughters (**Fig. 2C-E**). This pattern is also consistent with a scenario where the mRNAs are cleared before zygote division and asymmetrically reestablished after division, but the more probable explanation is that the mRNAs are differentially partitioned during zygote division. *wox8* mutants do not show defects in the early embryo, and it is therefore unclear what the relevance of its expression is for embryo polar axis formation. Redundancy of *WOX8* with its close homolog *WOX9* might explain the absence of a phenotype, particularly since *WOX9* is also expressed in the basal cell after zygote division (**Fig. 2C**). Interestingly, mutations in *WOX2* interfere with normal development of the proembryo, already at the 2-cell stage, suggesting that *WOX2* function is

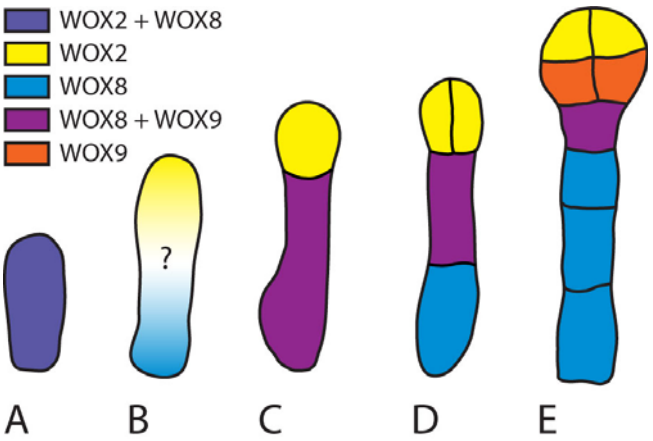

Fig. 2 Cell specification in the early *Arabidopsis* embryo as revealed by *WOX* gene expression. The mRNA accumulation of *WOX2*, *8* and *9*, and combinations thereof is depicted in different colors (see color legend), for early embryogenesis stages ranging from zygote (A) through 8-cell stage (E). Both *WOX2* and *WOX8* are expressed in the zygote (A, B), but accumulate differentially upon division (C). In the basal cell, *WOX8* is co-expressed with *WOX9* (C), but after the division of the basal cell, *WOX8* remains in the entire suspensor whereas *WOX9* is restricted to the upper suspensor cell (D, E). After formation of an upper and a lower tier in the 8-cell proembryo (E), *WOX* expression marks 4 domains, with *WOX2* being expressed in the upper tier, *WOX9* in the lower tier, *WOX8* and *9* in the uppermost suspensor cell and *WOX8* in all other suspensor cells. Drawing after Haecker *et al*. (2004).

required in those cells where its mRNA accumulates. This is also the case later in proembryo development. When the proembryo consists of 8 cells, it is composed of an upper and a lower tier of 4 cells each. These two tiers have distinct developmental fates as they will give rise to different parts of the seedling. This difference between upper and lower tier in the 8-cell proembryo is foreshadowed by domains of *WOX* gene expression.

When two tiers are formed, *WOX2* mRNA is expressed only in the upper tier (**Fig. 2E**). In *wox2* mutants, subsequent divisions in this upper tier are erroneous, suggesting that *WOX2* function is also required for normal patterning of this region.

At the same time, *WOX9* is activated in the lower tier in addition to its expression in the uppermost suspensor cell (**Fig. 2E**). Therefore, expression of *WOX2*, *8* and *9* marks a pattern along the apicobasal axis with four regions: An apical tier that expresses *WOX2*, a basal tier marked by *WOX9*, the uppermost suspensor cell that harbors both *WOX8* and *9* and the subtending suspensor cells where only *WOX8* is active.

Development and maintenance of the suspensor

WOX8 and *9* mRNA accumulation reveals that expression patterns can be dynamically controlled within the suspensor, suggesting functional specialization of suspensor cells. Very little is known about how suspensor development is controlled, but it was recently shown that a MAP Kinase cascade might be involved.

The mitogen activated protein kinase kinase kinase (MAPKKK) YODA (YDA) is required for proper suspensor development (Lukowitz *et al*. 2004). In *yda* mutants, the elongation of the zygote prior to division is impaired, allowing the generation of a normal sized apical daughter cell and only a shortened basal cell. While the apical cell develops normally until the 8-cell stage, the shortened basal cell fails to give rise to a proper suspensor. Cells of the basal lineage do not divide horizontally, but divide randomly, eventually leading to a loss of the clear boundary between suspensor and proembryo at the 8-cell stage. Whereas these results can be explained by YDA being a regulator of cell elongation rather than suspensor cell fate, one finding suggests that YDA function is intimately connected with the latter. Plants where YDA is constitutively active show the formation of a suspensor-like cell file at the expense of pro-embryo development. Thus activity of YDA is necessary to promote extra-embryonic cell fate in the basal lineage while enhanced activity in the apical lineage is sufficient to activate a suspensor-like developmental program. Recently, good candidates were identified for the kinases that act downstream of YDA in a canonical MAP kinase cascade. Post-embryonically, YDA controls stomatal versus non-stomatal cell fate (Bergmann *et al*. 2004). Mutations in both MAPK kinases MKK4 and MKK5 or in both MAP kinases MPK3 and MPK6 show phenotypes in stomatal development very similar to those in *yda* mutants (Wang *et al*. 2007). Furthermore, the *mpk3 mpk6* double mutant shows a *yda*-like embryo phenotype which suggests that both MAP kinases also act downstream of YDA during embryogenesis. Important remaining questions are what the input signals and output responses are. Two additional mutants with *yoda*-like early embryo defects, *grounded* (*grd*) and *short-suspensor* (*ssp*), have been isolated (Lukowitz *et al*. 2004). These genes might encode such in- or output components and therewith make the YDA-MKK4/5-MPK3/6 cascade specific for early embryogenesis.

Mutations in *yda* prevent suspensor formation at the earliest stages, and consequently, *yda* mutants have at best a rudimentary suspensor. In another class of *Arabidopsis* mutants, the suspensor is initially properly specified, but not maintained. Here, suspensor cells start to proliferate, and in cases behave much like embryo cells. These mutants can be divided into two categories. The first type of mutants includes loss-of-function mutations in the *SUSPENSOR1-3* (*SUS1-3*) (Schwartz *et al*. 1994) and *RASPBERRY1-3* (*RSY1-3*) (Yadegari *et al*. 1994; Apuya *et al*. 2002) genes, as well as a host of mutations in house-keeping genes (www.seedgenes.org). In these mutants, suspensor proliferation is preceded by visible defects in the proembryo at the globular stage. This initiates a transformation of suspensor cells towards embryonic cell fate but when suspensor-derived embryos reach the equivalent of the globular stage, also these arrest, so that no viable second embryo is formed.

This is different in mutants of the *TWIN* (*TWN*) type, where the secondary embryo developing from the suspensor does develop into a mature embryo. Of the *TWN* type mutants, there is one, *twn2*, where the proembryo arrests at very early stages prior to the formation of suspensor-derived embryos (Zhang and Somerville 1997). Two others, *twn1* and *amp1* do not affect the proembryo, but do show suspensor-derived embryos (Vernon and Meinke 1994; Vernon *et al*. 2001).

It has also been found that experimental interference with proembryo development, for example by acid treatment, X-ray irradiation (for review see Yeung and Meinke 1993) or toxin expression (Weijers *et al*. 2003), also leads to proliferation of suspensor cells. Therefore, it is likely that the proembryo is a source of signals that actively repress embryogenesis in suspensor cells.

Mutants of the first category (including *SUS* and *RSY*) affect the suspensor indirectly through impairment of general functions in the proembryo, very much like for example X-ray irradiation or toxin expression. The *TWN* class mutations however, might affect the suspensor more directly by interfering with embryo-dependent repression. This interpretation is supported by the isolation of only a handful of this type of mutants. *TWN2* encodes a valy-tRNA synthetase (Zhang and Somerville 1997), *AMP1* encodes a glutamate carboxypeptidase (Helliwell *et al*. 2001), and the *TWN1* gene has not yet been identified. Although AMP1 might be involved in generating a presently unknown signaling molecule, the anonymous character of TWN1 and the supposed housekeeping function of TWN2 do not allow a mechanistic model to be drawn. In addition, it should be noted that for both *twn1* and *twn2* mutations, only a single allele has been reported, and it is therefore unclear whether or not these might be neomorphic.

Specification of hypophysis cell fate

During normal embryogenesis, the uppermost suspensor cell behaves rather differently from the other suspensor cells. This is observed at the anatomic level by its asymmetric division, but also by the activity of several genes. For example, the *WOX* genes are dynamically regulated during hypophysis establishment, with *WOX8* mRNA being lost specifically from the upper suspensor cell after hypophysis specification, and another member of the *WOX* family, *WOX5*, being switched on in this cell immediately upon its specification (Haecker *et al*. 2004). Several other genes are specifically activated in the hypophysis, which suggests significant transcriptional reprogramming. Since at least one other suspensor-specific gene is not downregulated in the hypophysis (E. Rademacher and D. Weijers, unpublished), hypophysis fate does not seem to be specified at the full expense of suspensor fate, but rather in addition to it.

Specification of the upper suspensor cell as hypophysis not only marks the initiation of the root meristem, it is also of paramount importance for root formation. This is highlighted by the finding that the earliest defect in all rootless mutants is during hypophysis division. The two most well-studied rootless mutants are *monopteros* (*mp*) and *bodenlos* (*bdl*) (Berleth and Jürgens 1993; Hamann *et al*. 1999). Both mutations affect hypophysis-specific gene expression prior to division (Haecker *et al*. 2004; D. Weijers, unpublished). Since *mp* and *bdl* mutants show almost identical embryo phenotypes, it has been suggested that MP and BDL act in a common pathway (Hamann *et al*. 1999). This indeed appears to be the case, since map-based cloning has shown them to be an antagonistic pair of transcription factors that regulate gene expression in response to the plant hormone auxin (Hardtke and Berleth 1998; Hamann *et al*. 2002). *MP* encodes AUXIN RESPONSE FACTOR 5 (ARF5), which activates auxin-responsive genes. *BDL* encodes Aux/IAA12, a protein that binds MP and prevents activation of MP-dependent genes (Hamann *et al*. 2002; Weijers *et al*. 2005, 2006). Auxin activates MP by promoting the ubiquitin-proteasome-dependent degradation of BDL (Dharmasiri *et al*. 2005). *mp* mutants are loss-of-function alleles whereas the *bdl* mutation prevents its auxin-dependent degradation, leading to constitutive inhibition of MP. Likewise, mutations in the ubiquitin pathway that prevent BDL degradation also impair hypophysis division and root formation (Dharmasiri *et al*. 2003, 2005).

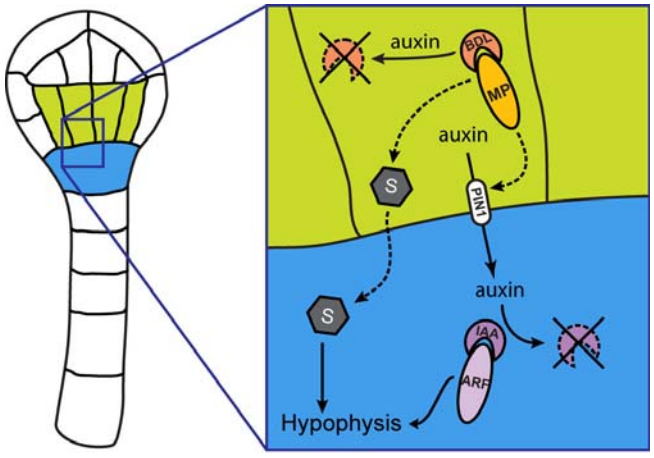

Fig. 3 A model for auxin-dependent cell communication in hypophysis specification. The future hypophysis (blue) is specified in response to signals from the adjacent central inner cells of the proembryo (green). In proembryo cells, auxin promotes degradation of BDL, thereby releasing the MP transcription factor. MP promotes transport of auxin to the adjacent suspensor cell through the membrane-localized PIN1 auxin transporter. Within this neighboring cell, auxin triggers the degradation of another Aux/IAA inhibitor (IAA), which sets free another ARF transcription factor. In parallel, MP promotes signaling to the future hypophysis through a second signal (S). Within this cell, ARF activity and the second signal (S) converge to specify its fate as hypophysis. Model after Weijers *et al*. (2006).

Interestingly, MP does not act in the future hypophysis itself to specify its fate. Rather, MP and BDL act in the 8 inner cells of the lower tier of the 32-cell embryo (**Fig. 3**) to control hypophysis specification in the adjacent suspensor cell (Weijers *et al*. 2006). Hence, MP promotes signaling between a small group of proembryo cells and the neighboring suspensor cell. The non-autonomous control of hypophysis specification by factors in adjacent proembryo cells provides a very plausible explanation for why it is the uppermost suspensor cell that is specified and not any of the other cells.

This cell-cell communication could involve any possible signal, including hormones, mobile mRNA, secreted peptides or proteins. Several lines of indirect evidence suggest that auxin might be such a signal (Weijers *et al*. 2006). An auxin-dependent reporter gene that is activated by ARF transcription factors (*DR5-GFP*) is switched on in the hypophysis at the time of its specification. Furthermore, the PIN1 auxin transporter is localized at the basal cell membranes of the inner 8 cells of the lower tier of the proembryo. Both the PIN1 protein in these 8 cells and the *DR5-GFP* activity in the hypophysis are lacking in *bdl* and *mp* mutant embryos, implying that this auxin transport is downstream of MP activity. There are however two observations that suggest auxin accumulation alone not to be sufficient for hypophysis specification. First, feeding developing *mp* or *bdl* embryos with the strong synthetic auxin 2,4-dichlorophenoxy acetic acid (2,4-D) does not overcome the hypophysis division defect. Second, *DR5-GFP* is expressed in more than one suspensor cell, yet only the uppermost cell is specified as hypophysis. Therefore, we proposed the existence of a second embryo-derived signal involved in proembryo-hypophysis signaling (**Fig. 3**) (Weijers *et al*. 2006).

As the identity of this second signal is unknown, it is presently not clear how a double-input signaling system would outperform a system where only one signal is used to specify the adjacent cell. However, one could imagine a second signal that would promote the competence of the adjacent cell towards hypophysis fate and auxin to be a trigger that defines the precise timing of specification. Identification of the genes that are activated by MP prior to hypophysis specification, as well as dissection of the auxin response machinery within the hypophysis will be required to gain comprehensive understanding of this process, which is at the basis of root meristem formation. Naturally, root meristem formation is not complete with hypophysis specification, but requires further elaboration, definition of stem cells and differentiation of various cell types. For the sake of brevity, we have discussed here only the events leading to the establishment of the hypophysis, and therefore the initiation of the root meristem. Excellent reviews discuss those events that are required later to set up a functional root meristem (Nakajima and Benfey 2002; Willemsen and Scheres 2004).

OUTLOOK

Molecular genetic studies in *Arabidopsis* have given a fairly detailed view of the processes leading to the initiation of the first meristem. We have discussed those events required for, and preceding specification of the uppermost suspensor cell as hypophysis, the founder cell for the root meristem. Intriguingly, this cell is specified in response to signals derived from adjacent cells in the proembryo. A very important question is to what extent this cell-communication based mechanism for root meristem initiation is universal in higher plants. *Arabidopsis* embryos have exceptionally regular cell divisions, and consist of much fewer cells than embryos of many other plant species (Johri *et al*. 1992). Nonetheless, embryos of all higher plants do consist of proembryo and suspensor, and the root meristem is initiated at the junction of the two (Johri *et al*. 1992). Cell communi-

cation-based hypophysis specification minimally requires that two regions with different fate (proembryo and suspensor) are present, and that these exchange signals. This does not require precise relative sizes of the regions, nor does it require regular cell divisions, and could hence be operational in most if not all higher plants. Interestingly, a rice homolog of the hypophysis-specific *Arabidopsis WOX5* gene has been isolated, and its mRNA accumulates at about the position that is expected to be the rice hypophysis equivalent (Kamiya *et al*. 2003). Several other of the *Arabidopsis* "patterning genes" have homologs with matching expression patterns in other species (Lim *et al*. 2000; Nardmann and Werr 2006; Prigge and Clark 2006), which does suggest conservation of patterning mechanisms across species.

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