Auxin Regulation of Embryonic Root Formation

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The plant hormone auxin was initially identified as the bioactive substance that induces roots in plant tissue culture. In the past decades, mechanisms for auxin action, including its transport and response, have been described in detail. However, a molecular and cellular description of its role in root initiation is far from complete. In this review, we discuss recent advances in our understanding of auxin-dependent embryonic root formation. During this process, a root meristem is initiated in a precise and predictable position, and at a stage when the organism consists of relatively few cells. Recent studies have revealed mechanisms for local control of auxin transport, for cellular differences in auxin response components and cell type-specific chromatin regulation. The recent identification of biologically relevant target genes for auxin regulation during embryonic root initiation now also allows dissection of auxin-activated cellular processes. Finally, we discuss the potential for hormonal cross-regulation in embryonic root formation.

Keywords: Auxin.

Abbreviations: AHP, ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN; ARF, auxin response factor; ARR, ARABIDOPSIS RESPONSE REGULATOR; GEF, guanine exchange factor; GFP, green fluorescent protein; HAN, HANABA TARANU; JLO, JAGGED LATERAL ORGANS; MAB, MACCHI BOU; MP, MONOPTEROS; OBE, OBERON; PLT, PLETHORA; QC, quiescent center; TMO, TARGET OF MP; TTA, TITANIA.

The two primary meristems of the seedling are established during embryogenesis, and contain the cells to support further growth of the organisms throughout its lifetime. A failure to initiate the embryonic shoot or root meristem often leads to arrest of seedling growth (Berleth and Jurgens 1993, Aida et al. 1997). In addition, based on genetic evidence, mechanisms that underlie formation of the embryonic meristems are redeployed later in plant development, e.g. in lateral root formation or shoot branching (Endrizzi et al. 1996, De Smet et al. 2010). In particular, since the early embryo, at the time when meristems are established, is relatively simple and consists of few cells, embryonic meristem formation is a good starting point to dissect mechanisms of plant development.

We focus this review on the regulation of embryonic root meristem formation. Since the initiation of the root meristem occurs relatively early during embryogenesis, and is distinctly marked by several molecular and cellular events (Scheres et al. 1994, Haecker et al. 2004), this process has been dissected in somewhat more detail than the formation of the embryonic shoot meristem. Importantly, many mutations that interfere with normal activity of the plant hormone auxin affect embryonic root meristem formation (reviewed in Möller and Weijers 2009), hence auxin has surfaced as a key regulator of this process. This finding is in line with the ‘classical’ physiological activity of auxin as a rooting factor in tissue explants (Thimann and Went 1934). In this review, we will first describe the key events in embryonic root meristem formation, and next review recent studies that shed light on how auxin controls this process. Unless otherwise indicated, this review focuses on insights gathered in the plant Arabidopsis thaliana.

Hallmarks of Embryonic Root Formation

In Arabidopsis (Capron et al. 2009), as in many other plants, the first division of the zygote creates two different cells that follow distinct developmental trajectories. The smaller upper (apical) cell (Fig. 1A) switches cell division plane several times (Fig. 1B, C) to form a spherical structure (pro-embryo), on whose upper flanks the cotyledons are later initiated. The larger lower (basal) cell only divides horizontally and forms an extra-embryonic structure termed a suspensor. As such, the apical cell produces the majority of the mature embryo. However, the basal cell lineage contributes a very significant part to the embryo. When the pro-embryo reaches a size of about 50 cells, the uppermost suspensor cell is ‘recruited’ into the pro-embryo (Fig. 1D). It is re-specified as the ‘hypophysis’ (Hamann et al. 1999) and, following an asymmetric division (Fig. 1E), it generates the precursors for the quiescent center (QC), the stem cell organizer of the post-embryonic root meristem (van den Berg et al. 1997). The specification of the hypophysis and its subsequent asymmetric division (Fig. 1D, E) are considered as one of the first and key steps in meristem initiation. At about the same time, adjacent cells in the pro-embryo are specified as precursors for the stem cells of vascular, ground and epidermal tissues (Fig. 1D, E). Hence, by this time in development, despite the
small number of cells, a primordial version of the root meristem (Fig. 1F) is already established. As these specification events can be visualized using molecular markers (Fig. 1; e.g. Haecker et al. 2004), and by characteristic cell division patterns (Fig. 1), the effect of mutations on precise events in root meristem formation can be determined in some detail. In the following, we will discuss the role of auxin activity and its regulation in these events underlying root meristem formation.

**Auxin Transport and its Regulation in Embryonic Root Formation**

Auxin is directionally transported through the activity of a family of integral membrane proteins, the PIN family (Grunewald and Friml 2010), whose subcellular polarity determines the direction of auxin flux (Wisniewska et al. 2006). Of the eight Arabidopsis PIN proteins, five have all the hallmarks of plasma membrane-localized auxin transporters (Mravec et al. 2009), and four of these are redundantly required for embryo development (Friml et al. 2003, Blilou et al. 2005). However, the transcriptional regulation and precise spatial requirements of these efflux facilitators or the mechanisms underlying their polar subcellular localization are still largely unknown. Recent studies have illuminated some of these problems.

The redundancy among PIN proteins complicates the analysis of the spatial requirements for these proteins in different developmental processes in the embryo. Recently, Wolters et al. (2011) have used a more generic tool to address this problem. The GNOM protein encodes an ADP ribosylation factor (ARF)-guanine exchange factor (GEF) that regulates recycling and polar localization of PIN1 protein (Steinmann et al. 1999, Geldner et al. 2003). However, the transcriptional regulation and precise spatial requirements of these efflux facilitators or the mechanisms underlying their polar subcellular localization are still largely unknown. Recent studies have illuminated some of these problems.

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PIN1 is polarized toward the future hypophysis at around the time of its specification (Steinmann et al. 1999, Friml et al. 2003). Indeed, when GNOM is expressed from vasculature-specific promoters in the gnom mutant background, formation of the primary root is rescued (Wolters et al. 2011). In these embryos, polarized PIN1 signal is recovered in the pro-vasculature and even in apical cells. By using a DR5–green fluorescent protein (GFP) reporter, it was shown that the otherwise excessive auxin accumulation in the apical domain of the mutant is also reduced. Interestingly, when GNOM is expressed in the hypophysis, a significant proportion of gnom embryos formed a primary root. This suggests that GNOM-dependent PIN1 polarization can act either in ‘source’ or in ‘sink’ cells, which demonstrates a cell-non-autonomous developmental function of GNOM. This study also clearly showed that apical and basal functions of GNOM are separable; expression of GNOM in the cotyledon domain of mutant embryos restored the cotyledon fusion defects but not the root initiation defect (Wolters et al. 2011).

Another recent study highlighted the importance of local PIN protein activity. Nawy and co-workers (2010) identified the GATA transcription factor HANABA TARANU (HAN) as a spatial regulator of PIN gene expression during embryogenesis. HAN is already expressed in the zygote, and subsequently detected in embryonic cells until the 16-cell stage. The expression is then restricted to the pro-vasculature from the globular stage (Zhao et al. 2004, Nawy et al. 2010). hAN mutant embryos...
display abnormal embryonic cell division by the 16-cell stage. From the 16-cell stage on, the basal cells of *han* embryos appear similar to suspensor cells both by morphology and by gene expression. Expression of suspensor and hypophysis marker genes such as *SUCROSE TRANSPORTER 3* (SUC3) and *WUSCHEL-RELATED HOMEBOX 5* (WOXS) is expanded to the basal embryo cells (Nawy et al. 2010). Correspondingly, HAN also affected auxin distribution, as the DRS–GFP reporter is also expressed in the lower embryo tier. Based on these changes, it can be concluded that the embryo–suspensor boundary is shifted apically in *han*. Interestingly, expression and localization of PIN1 and PIN7, that normally mark the pro-embryo and suspensor, respectively, are also shifted apically. These results are consistent with HAN regulating transcription in the basal region of the embryo by controlling PIN-dependent establishment of an auxin maximum.

As the auxin-dependent transcription factor AUXIN RESPONSE FACTOR 5 (ARFS)/MONOPTEROS (MP) is a key effector of auxin activity in the embryo (Hardtke and Berleth 1993, Weijers et al. 2006), the interconnections between HAN and MP were tested. Interestingly, while MP mutants are rootless (Berleth and Jurgens 1993), *mp* double mutant embryos resemble the phenotype of *han* embryos since seedlings had a functional root meristem (Nawy et al. 2010). This result is consistent with the observation that in the *han* mutant, by shifting the pro-embryo–suspensor boundary, the root initiates from cells that do not express MP. As direct targets of HAN are not yet known, it is unclear how direct the control of PIN gene expression is, and how its activity relates to auxin response pathways. Other factors have recently been shown to affect PIN gene expression in the embryo, including the JAGGED LATERAL ORGANS (JLO) transcription factor (Borghi et al. 2007, Bureau et al. 2010). However, while mutant phenotypes and expression patterns are largely consistent with a role for JLO in regulating PIN gene expression, direct evidence is lacking. Therefore, the outlines for a regulatory network are developing, but establishment of direct connections will be required to understand how the PIN expression patterns are determined.

**Mechanisms of Transcriptional Regulation by Auxin**

Once auxin accumulates in cells, through biosynthesis and transport, the signal elicits a transcriptional response. The mechanism of this transcriptional response has been elucidated (reviewed in Chapman and Estelle 2009, Hayashi 2012), and involves binding of auxin to both a ubiquitin ligase (SCF-TIR/AFB) and its substrates, the Aux/IAA transcriptional regulators. This interaction promotes ubiquitination of the Aux/IAA protein (Maraschin et al. 2009), which marks these for degradation. When not degraded, Aux/IAA proteins bind to and inhibit DNA-binding ARF transcription factors (Tiwari et al. 2003). While this system is understood in generic terms, an important unanswered question is how such a simple signal transduction pathway can create the observed diversity in auxin-dependent processes. Recently, work on early embryos has addressed this problem. One explanation for the diversity in auxin responses is that the individual components have diversified in function. Indeed, there are six SCF-TIR/AFBs, 23 ARFs and 29 Aux/IAAs in Arabidopsis (Remington et al. 2004). It was shown that each SCF-TIR/AFB protein has substrate preferences. Different pairs of SCF-TIR/AFB–AUX/IAA co-receptor have different binding affinity for auxin (Calderón Villalobos et al. 2012). Therefore, the variation in combination of SCF-TIR/AFB and its substrates Aux/IAA results in different auxin sensitivity.

Rademacher and colleagues (2011) showed that the 23 ARFs are expressed in very different spatio-temporal patterns throughout embryo development (Fig. 2A). The interesting consequence is that most cell types appear to have different, unique sets of ARF transcription factors. While some, even unrelated ARFs appear to act redundantly, others have different functions as suggested by promoter-swap experiments. Hence, the transcriptional regulation of ARFs seems to set up a pre-pattern of distinct auxin-dependent competences in different cells in the embryo. In part, this pre-pattern is itself an output of auxin activity, as it was recently shown that MP/ARFs activates its own expression (Lau et al. 2011).

Another example that underlines the distinct auxin-dependent processes in early embryogenesis and root meristem formation is the identification of the *IAA10* gene (Rademacher et al. 2012). *IAA10* is expressed specifically in the suspensor. The specification of the hypophysis is accompanied by activation of the DRS–GFP reporter (Weijers et al. 2006). So far, however, auxin response components that cell-autonomously regulate auxin response in the hypophysis had not been identified. A mutation that prevents auxin-dependent degradation of *IAA10* interferes with the activation of auxin-dependent gene expression in the hypophysis, and also with normal division of this cell (Rademacher et al. 2012). What this finding shows is that indeed, a local auxin response in the hypophysis is required for its normal division and for root formation. This auxin response relies on a specific set of ARFs, distinct from those that regulate auxin response in the pro-embryo (e.g. ARFs/MP). Hence, hard-wired differences in local auxin response competence underlie root initiation.

A key question is what mechanisms allow local differences in auxin-dependent gene expression. In part, such differences will perhaps depend on the unique cofactors that different ARFs recruit. On the other hand, components of chromatin regulation and the general transcriptional machinery have recently emerged as potential contributors to local auxin activity. Recent findings demonstrated that plant homeodomain (PHD) finger proteins OBERON1 (OBE1), OBE2, TITANIA1 (TTA1) and TTA2 act in MP-dependent root initiation. Double mutations in the closely related OBE1 and OBE2 or in TTA1 and TTA2 genes lead to rootless defects that are very similar to those observed in *mp* mutants (Saiga et al. 2008, Thomas et al. 2009, Saiga et al. 2012). While MP expression is unaffected, the expression of the direct MP target gene **TARGET**
OF MP 7 (TMO7) (Schlereth et al. 2010) is lost in obe1 obe2 embryos, suggesting that OBE proteins mediate the MP-dependent activation of TMO7 gene expression. During root initiation, MP has been shown to act in a small domain of pro-embryo cells adjacent to the future hypophysis (Weijers et al. 2006). While OBE1, OBE2, TTA1 and TTA2 proteins accumulate ubiquitously in embryos, OBE1 activity in hypophysis-adjacent cells is sufficient for restoring root formation defects in the obe1 obe2 mutant. Furthermore, OBE1 associates with the promoter region of the TMO7 gene (Saiga et al. 2012), at locations that correspond to the approximate MP-binding sites (Schlereth et al. 2010). While direct binding of MP to OBE1 has not been demonstrated, these data strongly support a model in which OBE1 is required for local activation of MP targets in the lower embryo tier (Fig. 2B). Indeed, OBE1 action appears to be highly regional; the MP target gene TMOS is normally expressed in both the upper and lower embryo tier, and expression in both tiers is lost in the mp mutant (Schlereth et al. 2010). Strikingly, in the obe1 obe2 mutant, TMOS expression is absent from the lower tier, yet maintained in the upper tier (Saiga et al. 2012). This suggests that, while OBE1/2 activity is required for activating MP targets in the lower tier, it is dispensable for activating the same genes in the upper tier.

The PHD finger domain has been shown to bind specifically to H3K4me3 (Li et al. 2006), which is generally associated with nucleosomes near the promoter and 5′ end of highly transcribed genes (Zhang et al. 2009). PHD fingers are present in a variety of proteins with diverse functions, but uniformly recognize H3K4me3. Specificities of PHD finger proteins that differentiate their functions are determined by various mechanisms. The majority of PHD finger proteins are resident in protein complexes that contain other proteins with different histone-binding modules. The interplay between effectors present in the different subunits within a complex generates a multifaceted network of intertwined contacts that can ensure the recruitment of a particular complex to a specific genomic

**Fig. 2** Auxin-dependent gene activation and auxin–cytokinin interactions. (A) Schematic representation of ARF gene expression patterns at the early globular stage. Each unique combination of ARF genes is highlighted by a different color code. (B) At the early globular stage, MP activates the expression of TMO7 in the basal part of the pro-embryo (green). The OBE protein complex, in which TTA proteins are also included, mediates MP-dependent TMO7 expression, presumably by alteration of the chromatin property at the TMO7 locus. TMO7 protein is translated in the basal part of the pro-embryo, and then moves (gray dotted arrow) into the future hypophysis (yellow). The accumulation of TMO7 with auxin in the uppermost suspensor cell specifies it as hypophysis. MP also activates the TMOS expression in both the apical (blue) and basal (green) part of the pro-embryo. OBE protein complex mediates its expression in the basal but not in the apical part of the pro-embryo. (C) Patterns of auxin activity (DR5 expression, upper row, shades of red), cytokinin signaling inhibitors ARR7 and ARR15 (middle row, shades of yellow) and cytokinin activity (TCS expression, bottom row, shades of blue) in pre-globular (left cartoon) and globular (right cartoon) embryos. Note that the pattern of cytokinin response in hypophysis descendants is opposite to that of the ARR7 and ARR15 genes, which are activated by auxin, and mirror DR5 activity. (D) Auxin–cytokinin interactions in root vascular tissue patterning. The vascular bundle (cartoon shows cross-section) comprises distinct zones of auxin (red) and cytokinin (blue) activity. These zones are maintained by auxin-dependent AHP6 expression in xylem, and cytokinin-dependent PIN protein localization in cambium cells. In addition, cytokinin prevents protoxylem differentiation. Cartoons were redrawn from those in the original papers (A, Rademacher et al. 2011; C, Muller and Sheen 2008; D, Bishopp et al. 2011).
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Downstream Effectors of Auxin Activity

Auxin has several distinct effects on cells. First, auxin can induce a rapid physiological response leading to membrane hyperpolarization, and swelling (Phillippar et al. 1999). In addition, auxin elicits changes in the expression of many genes (Chapman and Estelle 2009). Lastly, auxin was shown to control endocytosis (Paciorek et al. 2005). Most of the activity of auxin in controlling development in general, and embryonic root formation in particular, appears to be mediated by its effect on gene expression. For example, mutations in the TIR1/AFB receptors for auxin-dependent gene expression produce defects in embryonic root formation (Dharmasiri et al. 2005) that are almost indistinguishable from those caused by mutations in auxin biosynthesis components (Cheng et al. 2007, Stepanova et al. 2008). Hence, an important yet mostly unresolved question is which genes are controlled by auxin to promote embryonic root formation. As indicated, the main auxin-dependent (ARF) transcription factor in root formation is MP (Hardtke and Berleth 1998). Eliminating MP activity completely inhibits embryonic root formation (Berleth and Jurgens 1993). Key regulators of root formation, the PLETHORA (PLT) transcription factors, were shown to be downstream of auxin activity, as expression was mostly lost in mp arf7/nph4 double mutant embryos (Aida et al. 2004). Since PLT2 is capable of inducing root identity ectopically, and because higher order plt mutants are not able to make an embryonic root (Galinha et al. 2007), PLT proteins may be important effectors of auxin and MP activity in embryonic root formation. However, the kinetics of auxin-induced expression suggest that these genes are not directly activated by MP or other ARFs (Aida et al. 2004).

Recently, the first genes were identified that are directly controlled by MP. Several of these (ATHB8, Donner et al. 2009; ARR7/15, Zhao et al. 2010; DRN, Cole et al. 2009) do not mediate the role of MP in embryonic root formation. Yet, a small set of TMO genes does appear to mediate MP-dependent root formation (Schlereth et al. 2010). TMO genes were identified in a transcriptome profiling study in which MP was either eliminated (mp mutant), or briefly inhibited through induction of its inhibitor BODENLOS (bdl-GR) (Hamann et al. 2002, Weijers et al. 2006). TMO genes were identified because the genes cannot be induced by auxin in either of the two cases. The four TMO genes studied in detail encode transcription factors that are expressed in the domain of the embryo in which MP acts to promote root initiation (Fig. 2B) (Weijers et al. 2006, Schlereth et al. 2010). At least three TMO genes (TMO3, 5 and 7) are direct MP target genes, and three are able to suppress the root initiation defect in a weak mp mutant when expression is driven from the MP promoter. Interestingly, one of these genes, TMO7, appears to be functionally required for root initiation, as reduction of its transcript level causes defects similar to the mp mutant. Finally, TMO7 appears to act as a signal in intercellular communication: the gene is expressed in the pro-embryo, yet the protein also accumulates in the hypophysis (Fig. 2B) (Schlereth et al. 2010). Taken together, a first set of transcriptional targets of the main auxin effector in the embryo (MP) have been identified, and these mediate root initiation. Probably, expanding the repertoire of MP target genes will reveal the cellular mechanisms that operate in auxin-dependent root formation.

Hormonal Interactions in Embryonic Root Formation

Recent studies have shown extensive interactions of various hormones during growth and development (Mouchel et al. 2006, Dello Ioio et al. 2008b, Zhao et al. 2010, Marhavy et al. 2011). Among these, in particular the cross-talk between auxin and cytokinin has been dissected in some detail. Interestingly, depending on the context, these two hormones can act either synergistically or antagonistically. An example of the former is the shoot meristem, where MP represses the cytokinin response inhibitors ARABIDOPSIS RESPONSE REGULATOR 7 (ARR7) and ARR15 (Zhao et al. 2010). In contrast, in the root meristem transition zone, cytokinin promotes the expression of the auxin signaling inhibitor SHY2/Aux/IAA3, while auxin promotes the
degradation of the same protein (Dello Ioio et al. 2008a). These examples highlight the absence of a coherent generalizable framework for hormonal interactions. However, as cross-talk of this kind is likely to be found in embryonic root formation, we review some recent data that link auxin and cytokinin in patterning the embryo or vascular tissue.

Auxin and cytokinin also interact antagonistically in root vasculature patterning. It was recently shown that the ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6) gene is activated by auxin (Bishopp et al. 2011). AHP6 is a member of a small family of histidine phosphotransfer proteins, whose role is to transduce the cytokinin signal from the receptor to downstream proteins through phosphotransfer (Mahonen et al. 2006). AHP6 lacks an important conserved histidine residue and is considered a pseudo-AHP. Indeed, AHP6 negatively regulates cytokinin signaling to promote protoxytem specification (Mahonen et al. 2006). Thus, auxin activates the expression of a cytokinin signaling inhibitor. Recently, a further addition to this regulatory interaction was revealed. It was observed that in root vasculature, markers for cytokinin and auxin activity are mutually exclusive: cytokinin signaling markers TCS (Mulder and Sheen 2008) and ARRs are expressed in procambial cells adjacent to the xylem, while auxin signaling markers such as DR5 and IAA2 are expressed in the xylem (Bishopp et al. 2011). Cytokinin treatment further restricts auxin-dependent gene expression, while impaired cytokinin activity leads to expansion of the auxin activity domain. Furthermore, the xylem-localized auxin response requires PIN activity in the adjacent cambial cells, and in these cells cytokinin activity promotes PIN polarity towards the xylem axis. Hence, two interlinked regulatory events ensure separation of the vascular tissue in two stable domains that have either auxin or cytokinin activity (Fig. 2D).

Expression analysis of AHP6 showed that these regulatory connections may already be established during embryogenesis. AHP6 is first activated in a domain of high auxin activity in cotyledons (as marked by DR5; Friml et al. 2003), and is already restricted to the narrow xylem domain at a slightly later stage. Furthermore, AHP6 expression is lost in mp seedlings, and repressed in bdl-GR lines (Schlereth et al. 2010, Bishopp et al. 2011). Therefore, activation of AHP6 probably involves MP activity, and it will be interesting to see if AHP6 regulation is an important biological output of MP activity in the embryo.

One of the main accelerators in studying developmental roles of auxin has been the construction of the DR5–GFP reporter that helps visualize sites of action (Ottenschlager et al. 2003). Until recently, no such reporter was available for cytokinin, and hence genetic studies provided the only information on when and where this hormone acts. The lack of well-described embryo defects in cytokinin receptor mutants (Mahonen et al. 2000, Higuchi et al. 2004) could suggest that this hormone is not involved in embryogenesis. However, the absence of mutant phenotypes cannot be taken as ultimate evidence that cytokinin is not involved in a developmental process. With the recent development of a reporter for cytokinin activity (TCS–GFP; Muller and Sheen 2008), there are now first indications that cytokinin is actually involved in embryonic root formation. This reporter consists of a concatamer of binding sites for the cytokinin-dependent ARR transcription factors, and can be considered a generic sensor for output of the cytokinin pathway. Activity of this marker is first detected in the hypophysis and suspensor at the 16-cell stage (Muller and Sheen 2008). After the division of the hypophysis, TCS expression is only maintained in the apical cell (Fig. 2C), suggesting the existence of a mechanism to suppress cytokinin signaling in the basal cell. In contrast, the negative regulators of cytokinin signaling, ARR7 and ARR15, are up-regulated in the basal cell (Fig. 2C). The expression of these ARRs is promoted by auxin. Therefore, auxin probably promotes transcription of these ARRs to suppress cytokinin signaling in the basal cell. Reducing mRNA levels of ARR7 and ARR15 causes ectopic TCS expression in the basal hypophysis descendant, and induces defects in root patterning. Hence, auxin-dependent suppression of cytokinin response appears to be required for normal root formation. This formally positions cytokinin downstream of the auxin pathway in embryogenesis, and it will be interesting to see how direct this regulation is, how cytokinin connects to other regulators and what cellular functions depend on cytokinin activity.

Concluding Remarks

In the past decade, the key players in auxin transport and response have been identified, and generic mechanisms for these processes have been described. How these mechanisms operate to control specific developmental events is a question that has largely been unanswered. In this review, we have discussed several important studies that provide insight into how the developmental functions of this hormone are controlled. A picture emerges where local regulation of auxin transport directs accumulation of auxin in specific cells, that then respond in unique ways depending on which auxin-dependent transcription factors are expressed. The identification of genes controlled by auxin during embryonic root formation now allows the dissection of the cellular events underlying this developmental process. In addition to providing the first insight into auxin control of embryonic root formation, the studies discussed here also allow new questions to be addressed. In particular, future studies should help in defining whether the Arabidopsis-centric view reflects reality in other plants as well. Finally, as the paths of different plant hormones tend to cross in various contexts, an important future question will be to what extent hormone interactions direct embryonic root formation.

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