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Review article

Going mainstream: How is the body axis of plants first initiated in the embryo?

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ABSTRACT

Vascular plants have an open body plan and continuously generate new axes of growth, such as shoot or root branches. Apical-to-basal transport of the hormone auxin is a hallmark of every axis, and the resulting pattern of auxin distribution affects plant development across scales, from overall architecture to cellular differentiation. How the first axis is initiated in the early embryo is a long-standing question. While our knowledge is still sparse, some of the key players of axialization have emerged, and recent work points to specific models for connecting cellular polarity to the asymmetric division of the zygote and domain specific gene expression to the organization of basipetal auxin flux.

The embryos of vascular plants develop in a controlled environment (the immature seed or the free-living female gametophyte), where the position of the egg within the surrounding tissue as well as the site of sperm entry is fixed. All available evidence indicates that apical-to-basal transport of the hormone auxin, a defining feature of vascular plants, is established within the first few rounds of cell divisions after fertilization and signals the establishment of the main body axis. Here, we discuss our current understanding of how positional information is processed from fertilization to axis initiation with an emphasis on Arabidopsis thaliana, the most widely used model for studying plant embryos (for more comprehensive reviews on embryogenesis that also cover subsequent patterning of the apical-basal axis, including the organization stem cell niches and the establishment of root and shoot fates, see: Lau et al., 2012; Ten Hove et al., 2015).

Early development of Arabidopsis follows a predictable pattern, and new domains, marked by the expression of specific regulatory genes, are produced with nearly every round of cell divisions (Fig. 1). Division of the zygote gives rise to the progenitors of the suspensor and proembryo, the first two apical-basal domains to be established; the proembryo becomes further subdivided into an upper and lower tier, roughly corresponding to the future shoot and root domain (8-cell stage); a series of divisions in tangential planes then produce the main tissue types, the epidermis (16-cell stage) as well as the ground and vascular tissue (32-cell stage). At about the same time, polar localization of the auxin efflux carrier PIN1 to the basal plasmamembrane establishes basipetal auxin transport across the proembryo.

A recent quantitative analysis of cellular geometries by segmentation of high-resolution confocal image stacks reveals a striking level of precision in these early divisions (Yoshida et al., 2014). For example, the longitudinal cell wall of the 2-cell proembryo is preferentially positioned at a right angle to the

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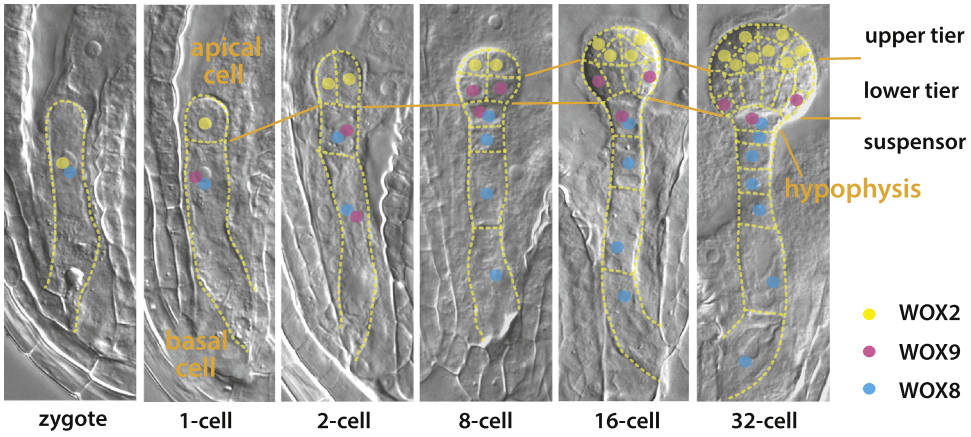

Fig. 1. Developmental progression of early Arabidopsis embryos. Images of whole-mount cleared seed from the zygote to the 32-cell stage; the expression of three WOX genes is indicated by colored dots.

median plane of the seed; and the transverse divisions giving rise to the 8-cell proembryo are not entirely equal, as the volume of upper tier cells is slightly but consistently smaller than the volume of lower tier cells. How is this regular pattern controlled?

1. An asymmetric first division

The future axis of the embryo is aligned with the long axis of the ovule and the polar axis of the egg cell. Large organelles are positioned asymmetrically in the Arabidopsis egg, with the nucleus at the apex (the site of sperm entry) and a vacuole at the base (Mansfield et al., 1991). Upon fertilization, the basal vacuole becomes fragmented and the nucleus retracted from the apex (Faure et al., 2002), resulting in a "transient symmetric stage" (Ueda et al., 2011). The zygote then elongates two- to three-fold, repositions the nucleus close to the apex and re-assembles a large vacuole at the base. It is unknown whether polarity marks present in the egg cell are maintained throughout this process or whether cellular polarity is established anew. Division of the zygote is asymmetric, producing daughters of different fates: the small apical cell assumes an isodiametric mode of growth to produce the spherical proembryo; the basal daughter continues to elongate and to divide transversely, forming the filamentous suspensor.

A plethora of anatomical studies suggest that asymmetric first divisions are pervasive among land plants and likely to represent an ancestral trait. For example, zygotes of the moss Physcomitrella patens swell by increasing the volume of their vacuoles before dividing into the apically positioned, two-faced stem cell of the sporophyte and a basal daughter that contributes to the foot, a support structure with similarity to the suspensor of flowering plants (Kofuji et al., 2009; genetic control of sporophyte development in Physcomitrella is reviewed in Kofuji and Hasabe, 2014). Cellular growth is more isodiametric than in Arabidopsis, and the two daughter cells are of more similar size – but in both species the first division is perpendicular to the future main axis and produces daughter cells that follow fundamentally different trajectories.

How important is the asymmetric first division for subsequent development? Two recent studies have pioneered direct manipulations of embryos contained in cultured immature seeds to address this question. Using optimized synthetic media and a custom-built device for immobilizing immature seed, Gooh et al. (2015) were able to follow the development of live embryos from the zygote stage to maturity by 2-photon microscopy. They then inactivated specific cells of the early embryo using laser pulses and monitored the effect on the patterning process with cell fate reporters. Upon inactivation of the apical cell, the basal daughter of the zygote appeared to reiterate the first division: it divided transversely to generate an apical daughter that lost expression of a basal marker gene, WOX8 (Haecker et al., 2004), and initiated expression of an apical marker gene, DRN (Chandler et al., 2007). The new apical cell then divided longitudinally to form a proembryo. Upon ablation of the basal cell, the isolated apical cell produced a relatively normal proembryo of 4–8 cells; further growth, however, was slow and aberrant, perhaps because nutrient flow to the proembryo had been disrupted.

Liu et al. (2015) obtained similar results after severing the suspensor at various positions and stages of embryonic development by targeted irradiation. Severed proembryos were able to form a normal root as long as at least one suspensor cell remained attached to them, but showed root and axis defects otherwise. These findings support the idea that the uppermost suspensor cell, the hypophysis, anchors axialization. Furthermore, the suspensors were able to regenerate a complete proembryo if severing occurred before the globular stage. Older suspensors failed to initiate cell divisions in response to severing and degenerated. Both studies directly demonstrate that the basal daughter of the zygote and the cells of young suspensors remain omnipotent, arguing against a mosaic mode of fate specification. Primary and regenerated embryos showed the same polarity and even followed a similar developmental sequence, suggesting that positional information for either organizing an asymmetric division or for polarizing the regenerating embryo persist in the absence of an apical cell or proembryo.

2. Polarity factors in guard cell development

How is cellular polarity regulated in the zygote? As in animals and fungi, the RHO GTPases OF PLANTS (ROPs) play a key role in polarizing the actin cytoskeleton and marking polar domains in the plasmamembrane (Yang and Lavagi, 2012). Arabidopsis ROP3, in particular, is required for positioning the plane of cell divisions throughout embryonic development (Huang et al., 2014). In the context of the first division, loss of ROP3 or over-expression of a dominant-negative form results in apical and basal cells with similar size, suggesting a more equal partitioning of the zygote. However, the effect is not penetrant (about 10–15% of the mutant embryos show this phenotype), perhaps because other ROPs provide redundant function.

Important cues can also be inferred form a plant-specific polarity factor regulating asymmetric divisions in the leaf epidermis, BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL;

Dong et al., 2009). BASL accumulates in the precursors of guard cells and localizes to the nucleus as well as a polar crescent-shaped domain at the cell cortex. The plane of division is organized distal to this mark, creating a large daughter that inherits the BASL crescent and a small daughter that does not. While the large daughter eventually differentiates into a pavement cell, the small daughter can either repeat the asymmetric division or enter a differentiation program to form a pair of guard cells (reviewed in Torii, 2015). A potential mechanistic basis for the polar distribution of BASL at the cell cortex was recently uncovered in the direct interaction of BASL with the MAPKK kinase YODA (YDA), a negative regulator of the stomatal lineage (Zhang et al., 2015). In the absence of YDA activity, nearly all epidermal cells differentiate into stomates, resulting in massive clustering; conversely, hyper-activation of YDA nearly eliminates guard cells from the leaf epidermis (Bergmann et al., 2004). BASL was found to act as a scaffold for YDA and the downstream MAP kinases MPK3/MPK6. YDA-dependent phosphorylation, in turn, is required for BASL function and polar localization (Zhang et al., 2015). Mutually positive interactions between BASL and the MAP kinase cascade may be sufficient to trigger symmetry breaking, as co-expression of YDA, MPK6, and BASL in tobacco resulted in polar distribution of all three proteins at the cell cortex. BASL expression is tightly correlated with transcripts specific to the stomata lineage (Dong et al., 2009) and has not been detected in transcriptional profiles of the early embryo (Autran et al., 2011; Nodine et al., 2012); but the YDA MAP kinase pathway dramatically affects the asymmetric first division.

3. Zygote polarity – pieces of the puzzle

Loss of YDA (Lukowitz et al., 2004) or MPK3/MPK6 (Wang et al., 2007; the two proteins provide largely equivalent activity in the embryo) almost completely blocks zygote elongation. As a consequence, the division produces a basal cell that is only marginally larger than the apical cell. Subsequent divisions of the basal cell and its descendants are also aberrant, such that mutant embryos either show a malformed, rudimentary suspensor or no recognizable suspensor at all. Hyper-activation of YDA produces embryos with exaggerated suspensors and often inhibits development of the proembryo, suggesting that the pathway is used to enforce the segregation of apical and basal fates. In analogy to the leaf epidermis, polar localization of YDA in the zygote may provide a mechanism for ensuring higher activity of the MAP kinase cascade in the basal daughter. According to this speculative model (Fig. 2), YDA may be sequestered to the base of the zygote by unknown polarity factors, perhaps effector proteins of ROP3; an asymmetric distribution of the YDA MAP kinase cascade could then cause differential phosphorylation of target proteins in the apical and basal cell.

Activation of the YDA MAP kinase cascade in the zygote is tied to fertilization by an unusual parent-of-origin effect involving the cytoplasmic receptor-like kinase SHORT SUSPENSOR (SSP; Fig. 2; Bayer et al., 2009). SSP transcripts are produced only in sperm cells but remain un-translated; instead, they are delivered to the zygote, where SSP protein transiently accumulates. Ectopic SSP expression in the leaf epidermis blocks the formation of guard cells in a YDA-dependent manner, suggesting that SSP acts upstream of YDA and that SSP protein may be sufficient to trigger activation of the MAP kinase cascade (through an as yet unknown mechanism). Since SSP is not transcribed after fertilization, the transient accumulation of SSP protein translated from sperm-derived mRNA has been proposed to set a temporal window for YDA activation in the zygote (Bayer et al., 2009). However, other activators have to exist, as loss of SSP function is associated with significantly weaker phenotypes than observed with loss of YDA or MPK3/MPK6.

SSP is an evolutionary recent addition to the BSK genes (Tang et al., 2008; Liu and Adams, 2010), a family of pseudokinases targeted to the plasmamembrane by fatty acid modification and likely serving scaffolding functions in cell surface receptor complexes (Grütter et al., 2013). This evolutionary link raises the possibility that extracellular signals may contribute to activate the YDA MAP kinase cascade. Circumstantial support for this idea comes from recent reports on the effect of two secreted signaling molecules on early embryonic development, the CLAVATA3-like peptide CLE8 (Fiume and Fletcher, 2012) and a group of tree closely related members of the cysteine-rich peptide superfamily called EMBRYO SURROUNDING FACTOR 1 (ESF1.1 through 1.3; Costa et al., 2014; see Ingram and Gutierrez-Marcos, 2015, for a review on cysteine-rich peptides). CLE8 is expressed broadly in the early embryo and endosperm, and a mutant allele causes variable

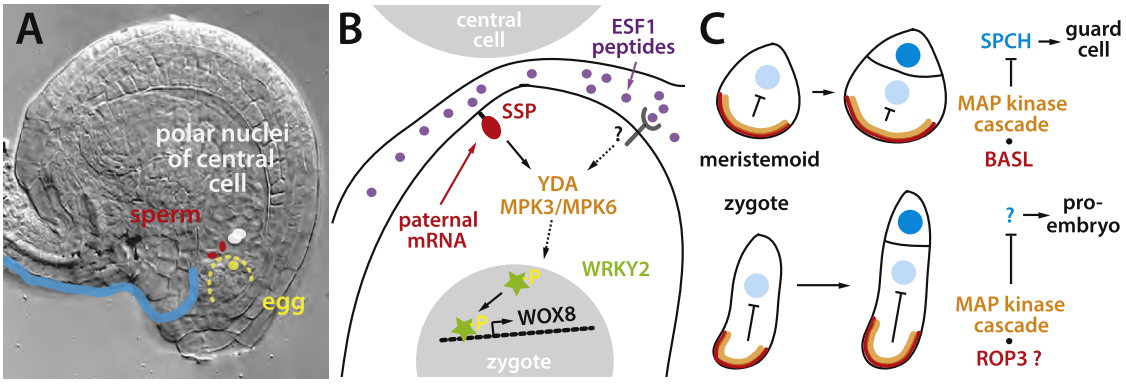

Fig. 2. Regulation of the asymmetric first division. (A) Regulation of YDA activity in the context of double fertilization: the pollen tube (blue) releases the two sperm nuclei (red ovals) between the egg cell and the central cell; SSP transcripts are contained in the sperm cells but not ranslated; the central cell (two polar nuclei shown as light grey dots) expresses ESF1. (B) Potential activators and targets of the YDA MAP kinase cascade in the zygote: sperm-derived transcripts of the SSP gene become translated after fertilization resulting in accumulation of SSP protein at the plasma membrane (red oval; it is not known whether SSP is distributed uniformly or in a polar fashion) to promote YDA activity; the central cell and the embryo-surrounding endosperm secrete ESF1 peptides (purple dots), which may also contribute to the activation of the YDA MAP kinase cascade through an unknown receptor. The WRKY2 transcription factor (green star) is a possible target of YDA-dependent phosphorylation and activates transcription of WOX8 in the zygote and suspensor. (C) Speculative model for unequal partitioning of YDA MAP kinase activity: asymmetric divisions of merestoids in the stomatal lineage (top row) are regulated by the polarity factor BASL (red), which sequesters the MAP kinase cascade (orange) in a crescent-shaped domain at the cortex; the SPCH transcription factor (blue) is released from MAP-kinase-dependent inhibition in the smaller daughter cell and promotes guard cell differentiation. By analogy, division of the zygote (bottom row) may be regulated by polarity factors such as ROP3 (red) that may localize the MAP kinase cascade (orange) to the base of the zygote; MAP-kinase targets promoting development of the proemrbyo have not yet been described (blue question mark).

defects, including shorter suspensors and aberrant divisions at the base of the proembryo. However, other abnormalities observed in mutant plants seem unrelated to the effects of yda mutations, confounding the comparison. The three ESF1 peptide genes are primarily expressed in the central cell of the female gametophyte and, after fertilization, in the endosperm (Fig. 2; Costa et al., 2014). Block of ESF1 transcription by RNA-interference is associated with suspensor defects: the suspensors are generally shorter, and aberrant tangential divisions are frequently observed at the junction between suspensor and proembryo; furthermore, expression of a marker gene for suspensor fate, WOX8 (Haecker et al., 2004), is reduced. Culture of immature seed in the presence of ESF1 peptides causes formation of longer suspensors, reminiscent of the effect observed with YDA hyper-activation. Furthermore, the phenotype of ssp mutants is enhanced by ESP1 knock-down, suggesting that SSP and ESP1 may both contribute to YDA activation. Although the supporting evidence still is indirect, this idea is rather intriguing and implies that the YDA pathway is under the control of both paternal and maternal effect regulators. Since the suspensor likely functions in nutrient transport, it seems possible that these parent-of-origin effects have evolved from a parental conflict over resource allocation (discussed in Ingram and Gutierrez-Marcos (2015)).

The targets of the YDA MAP kinase cascade in the zygote remain open. In the leaf epidermis, MPK3/MPK6 phosphorylate the bHLH transcription factor SPEECHLESS (SPCH), promoting its degradation (Fig. 2; Lampard et al., 2008); however, there is no evidence that SPCH or the other two bHLH genes directing guard cell differentiation, MUTE and FAMA (Torii et al., 2015), play a significant role in the embryo. In contrast, the effect of yda mutations on embryonic development are closely mimicked by loss of the GROUNDED gene (GRD, also known as RKD4; Jeong et al., 2011; Waki et al., 2011). GRD is a member of the plant-specific RWP-RK proteins, a family that, on the basis of structural similarity to helix-turn-helix proteins, is thought to act in transcriptional regulation. GRD is transcribed broadly in the egg apparatus and early embryo. A mutational analysis of potential phosphorylation sites implies that GRD activity is not directly regulated by the YDA MAP kinase cascade (Jeong et al., 2011); instead, GRD may act in concert with a co-factor that is subject to phosphorylation by MPK3/MPK6 or promote the expression of MAP kinase targets in the egg cell and zygote.

4. WRKY2 – from cellular polarity to differential gene expression

A direct link between zygote polarity and differential gene transcription in the apical and basal daughter of the zygote is revealed by the transcription factor WRKY2 (Ueda et al., 2011). Loss of WRKY2 has a striking and specific effect on the first division: mutant zygotes elongate to about the same size as wild type, but fail to reassemble a large basal vacuole and to move the nucleus from the center of the cell toward the apex. Thus, they seem unable to transition from the “transient symmetric” arrangement of organelles to a polar distribution. The first division is often equal, generating apical and basal daughters of similar size; subsequently, aberrant longitudinal divisions are prominent in the upper suspensor cells. These later defects are reminiscent of weak yda phenotypes and suggest a failure to fully establish or maintain basal fates. Expression of WRKY2 is consistent with this view: WRKY2 transcripts accumulate in the egg apparatus, the zygote and, after the first division, the cells of the suspensor.

Transcription factors of the WRKY family are often regulated by MAP kinase-dependent phosphorylation (Ishihama and Yoshika, 2012), and WRKY34, the sister gene of WRKY2, was recently shown to be phosphorylated by MAPK3/MPK6 in early pollen development (Guan et al., 2014). Could WRKY2 be a target of the YDA MAP kinase cascade? If so, loss of YDA signaling should also affect the plane of the first division. In the case of yda zygotes, which are very small, it seems difficult to determine whether the first division is equal or unequal (Lukowitz et al., 2004); but in the weaker ssp mutants, equal first divisions are observed only rarely (Bayer et al., 2009). Perhaps zygote elongation is more sensitive to loss of YDA function than positioning of the division plane; alternatively, the two processes may be regulated independently (Ueda and Laux, 2012).

Beginning with the first division, the embryo becomes progressively partitioned into distinct transcriptional domains, a process closely mirrored by the dynamic expression of WUSCHEL-RELATED HOMEOBOX (WOX) genes (Fig. 1; Haecker et al., 2004). WRKY2 directly impacts gene expression in the basal cell. Indeed, the protein was first identified as a transcriptional activator binding to a canonical W-box in the promoter of WOX8 (Ueda et al., 2011). WOX8 transcripts can be detected in the zygote; later, WOX8 and its sister gene WOX9 (also known as STIMPY) are predominantly transcribed in basal domains of the embryo (basal cell, suspensor, lower tier; Fig. 1). WRKY2 is required for maintaining normal levels of WOX8 expression after the first division (Ueda et al., 2011). However, WOX8 expression is not completely abolished in wrky2 mutants, implying that WRKY2 is not the only activator of WOX8.

5. Partitioning of apical-basal domains

The complex functional relationship between WRKY2 and WOX8 is further illustrated by their rather different mutant phenotypes. Loss of both WOX8 and WOX9 has no apparent effect on zygote development but disrupts patterning of the proemrbyo and prevents axis formation (Wu et al., 2007; Breuninger et al., 2008): mutant zygotes and young suspensors seem normal, but divisions in the proembryo domain are aberrant; depending on the allelic combination, mutants arrest before the globular stage (Wu et al., 2007) or grow into “finger-like” structures containing large, vacuolated cells (Breuninger et al., 2008). An analysis of molecular markers reveals that apical-basal auxin transport is never established: wox8/wox9 double fail to initiate transcription of the auxin efflux carrier PIN1; and expression of DR5, a reporter of auxin-induced gene transcription normally marking the incipient root at the base of the embryo, is uniform within the proembryo (Breuninger et al., 2008).

This dramatic phenotype appears to be due to a non cell-autonomous effect, as WOX8/WOX9 are not transcribed in the proembryo. Two other WOX genes, the founding member of the family WUSCHEL (WUS) and WOX5, show similar non cell-autonomous effects in the shoot and root apical meristem, respectively; both proteins move, presumably through plasmodesamta, from the cells expressing them to adjacent cells to regulate the transcription of direct target genes (Yadav et al., 2011; Daum et al., 2014; Pi et al., 2015). It is unknown whether WOX8/WOX9 too are mobile, but expression of WOX2 in the proembryo has been shown to depend on WOX8/WOX9 (Breuninger et al., 2008). WOX2 is coexpressed with WOX8 in the zygote; after the first division, however, WOX2 transcripts become confined to apical domains of the developing embryo (apical cell, early proembryo, upper tier; Fig. 1). Mutations in WOX2 cause relatively weak, transient defects in the upper tier of globular embryos; concomitant removal of other WOX genes expressed in the proembryo (WOX1, WOX3, WOX5) enhances these defects, eventually blocking the formation apical structures, such as cotyledons and a shoot apical meristem. None of the combinations reported to date result

in a complete breakdown of the patterning process, suggesting that WOX8/WOX9 have other important targets in the proembryo.

In addition, a potential role for WOX8/WOX9 in the zygote was uncovered by their strong, synthetic interaction with GRD: grd/wox8/wox9 triple mutants arrest as very small zygotes or seemingly symmetric 1-cell embryos (Jeong et al., 2011). Loss of GRD does not affect WOX8 expression in the zygote and 1-cell embryo, suggesting that the genes act independently at this stage. The reason for this early arrest of triple mutants is not clear. It has been suggested that it reflects a complete loss of embryonic polarity. However, many WOX transcription factors, including Wuschel (WUS; Laux et al., 1996), WOX5 (Sarkar et al., 2007) and WOX9 (Wu et al., 2005), promote maintenance of cell divisions in the stem cell niches of the apical meristems, such that an alternative explanation may be that the mutations primarily interfere with proliferation.

Gene expression patterns at the boundary between suspensor and proembryo, the two domains created with the first division, are regulated by the GATA factor HANABA TARANU (HAN; Nawy et al., 2010). In han mutants, the transcription of several genes changes coordinately, suggesting an apical shift in the fate map. For example, the auxin efflux carriers PIN7 and PIN1 are normally expressed in the suspensor and the proembryo, respectively (see below). These expression domains are both positioned more apically in 8-cell mutant embryos, with PIN7 accumulating in the lower tier and PIN1 the upper tier. Consistent with the idea of a fate map shift, han mutants initiate a root meristem at the boundary between the upper and lower tier instead of at the boundary between the lower tier and suspensor.

Clearly, pre-globular embryos generate complex and dynamic expression domains that can direct local differentiation, including cell type-specific differences in auxin production, transport and perception (Rademacher et al., 2012). But when it comes to understanding the networks underpinning gene transcription in early development, it seem like we are just scratching the surface. The functional relationships between known factors remain largely open, and it stands to reason that many important factors have not been identified yet. Progress is likely to require a more comprehensive view, and it is encouraging that a number of approaches for generating transcriptional profiles of whole-mount embryos as well as specific domains of the embryo have been reported (reviewed in Palovaara et al. (2013); recent contributions include Belmonte et al., 2013; Slane et al., 2014; Zhang et al., 2014). Such studies confirm that whole-mount embryos produce transcriptional profiles of similar complexity as other cell or tissue types; furthermore, the profiles change rapidly and profoundly as the embryos develop. Information about cell type or domain-specific expression profiles is still very sparse. Using fluorescent-based sorting of nuclei to collect suspensor and proembryo-specific samples, Slane et al. (2014) report that approximately 5% of all detected transcripts were differentially expressed; due to limitations with the sensitivity of the approach, this estimate is quite possibly conservative.

6. Basipetal auxin flux

Auxin flow is directed by efflux carriers of the PINFORMED (PIN) family. The intra-cellular localization of PIN proteins in the early embryo implies that transport is organized in two waves and relies on domain-specific expression of different PIN proteins (Fig. 3; Friml et al., 2003). After the first division, PIN7 becomes expressed in the basal cell and later the suspensor, localizing to apical domain of the plasmamembrane. DR5, a synthetic reporter of auxin-dependent transcription, is weakly expressed in the apical cell and its descendents, implying that PIN7 funnels auxin, presumably from the maternal seed coat, into the proembryo. PIN1 protein begins to accumulates in the proembryo domain before the 16-cell stage but intracellular localization is initially non-polar. Only at about the 32-cell stage does PIN1 protein become preferentially localized to the basal membranes of vascular and ground tissue precursors in the center of the proemrbyo. Strong expression of DR5 in the hypophysis indicates that apical-to-basal auxin transport across the proemrbyo has been established. Mutants disrupting auxin transport or perception generally show variable and comparatively minor defects before the globular stage (for example Hamann et al., 1999; Friml et al., 2003), such that it is not clear what the role of auxin accumulation in the apical cell and pre-globular proembryo is. However, auxin is strictly required for axis and root initiation, as block of auxin transport or auxin signaling by genetic or pharmacological means consistently results in root-less embryos lacking a vascularized hypocotyl (reviewed in Weijers and Jürgens (2005)).

Two recent studies highlight the contribution of auxin synthesis and uptake into the cell to the overall distribution of auxin in the embryo. Proteins of the AUX1/LAX family greatly increase auxin-permeability of the plasmamembrane and thus a cell's capacity for auxin relay (Bennett et al., 1996). LAX1 and LAX2 accumulate in the upper tier and the center of the proembryo, respectively (Robert et al., 2015). Loss of the AUX/LAX transporters causes abnormal divisions in the incipient root (albeit with a low frequency) and reduced expression of DR5, implying that efficient

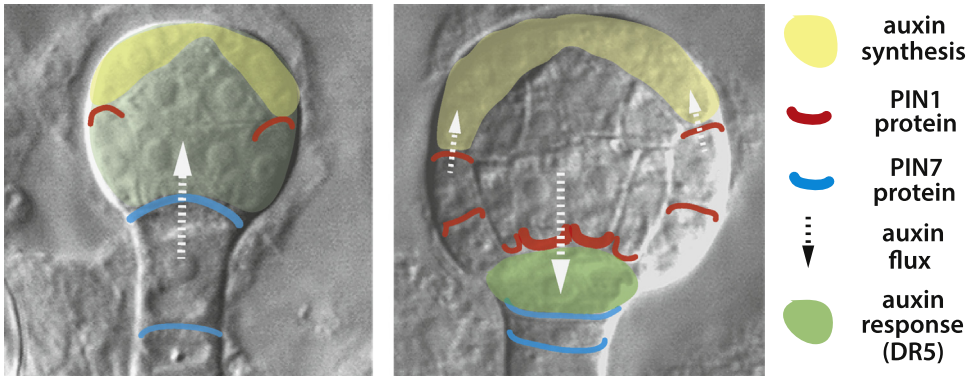

Fig. 3. Auxin production, transport, and responses in the early embryo. At the 16-cell stage (left), PIN7 protein (blue) localizes to the apical membranes of suspensor cells, directing auxin flux into the proembryo, where a reporter for auxin-dependent transcription (DR5) is expressed weakly (green). PIN1 protein (red) starts to accumulate at the apical sides of surface cells in the lower tier, and auxin production (yellow) is initiated in the outer cells of the upper tier. At the 32-cell stage, PIN1 protein is strongly expressed in the central cells of the lower tier and localizes to the basal membrane, directing basipetal auxin flux across the proembryo; PIN7 shows similar basal polarity in the suspensor. DR5 expression becomes high in the uppermost cell of the suspensor, the hypophysis.

auxin uptake by the cells in the center of the proembryo aids axialization.

What is the source of auxin in the early embryo? The bulk of indoleacetic acid, the active auxin, is synthesized from tryptophan in two steps (reviewed in Zhao (2012)); step one is catalyzed by TRYPTOPHANE AMINOTRANSFERASE OF ARABIDOPSIS 1 (TAA1) and the TAA1-related enzymes TAR1/TAR2, step two by YUCCA monoxigenases (YUC1–11). Transcription of these biosynthetic genes has been interpreted as a proxy for auxin production. A detailed survey (Robert et al., 2013) found that TAA1 expression first becomes detectable in the upper tier of 16-cell embryos and YUC1/YUC4 in a similar domain slightly later (Fig. 3). Furthermore, embryos lacking TAA1/TAR activity show defects in axis formation, such as aberrant divisions in the incipient root primordium, incomplete polarization of PIN1, and weak expression of DR5. These defects could not be complemented by broad expression of TAA1, implying that a local source of auxin at the apex of the embryo may be important for initiating basipetal auxin flux.

Wabnik et al. (2013) have incorporated these observations into a computer model that nicely recapitulates the dynamic localization of PIN proteins as determined by immuno-fluorescence. The model rests on three core assumptions: (1) localized auxin sources at the base of the suspensor and, beginning with the 16-cell stage, at the apex of the proembryo; (2) positive regulation of PIN gene transcription by auxin; and (3) a cell surface receptor for auxin that can regulate the localization of PIN efflux carriers in response to auxin concentration in the apoplast. The existence of auxin receptors at the plasmamembrane is controversial (Xu et al., 2014; Gao et al., 2015; Michalko et al., 2015), and it is not clear by which mechanism auxin distribution influences PIN localization. However, the idea that auxin transport is regulated by positive feedback is well supported, and the model suggests that auxin production may well be a trigger for polarization of PIN1 in the early embryo. The model draws on auxin-independent input for regulating cell-type specific expression of PIN transporters and initiating the production of auxin at the right place and time. Such cues may be provided by transcription factors like HAN, WOX8/WOX9, and WOX2, as outlined above. WOX8 transcription, for example, is robust to perturbations of auxin signaling (Ueda et al., 2011), as would be expected of a “pre-pattern” for basipetal auxin transport. A closer examination of how transcriptional networks contributes to setting up the auxin transport and signaling machinery and how auxin signaling in turn affects transcription will be important for teasing apart the molecular mechanisms driving axialization.

7. Perspective

The embryonic axis of plants appears to be organized through a concerted interaction of cellular polarity pathways, transcriptional networks carving out spatial domains, and an auxin transport machinery with self-organizing properties. Thus, it may not be surprising that progress in understanding this process has come intermittently and through contributions employing diverse approaches. Several key regulators have now been identified (although very likely more remain to be discovered). Importantly, the information at hand can be combined into models that, while tentative, have a realistic core and provide specific hypotheses. Technical advances with seed culture, live imaging, and image analysis (Yoshida et al., 2014; Gooh et al., 2015; Liao et al., 2015) now enable more objective and quantitative descriptions of embryonic development; similar advances with transcriptional profiling promise to dramatically increase the depth of detail. Such improvements should particularly benefit the comparison of mutant phenotypes, an important step in clarifying the functional relationships between relevant factors. It should be interesting to see how the fragments will fall in place.

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