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Axis formation in *Arabidopsis* – transcription factors tell their side of the story

Sangho Jeong¹, Matthew Volny², and Wolfgang Lukowitz¹

¹Department of Plant Biology, University of Georgia, 120 Carlton Street, Athens, GA 30602-7271, United States

²Department of Genetics, University of Georgia, Athens, GA 30602, United States

Abstract

Apical-to-basal auxin flux is a defining feature of land plants and determines their main body axis. How is the axis first set up in the embryo? Recent studies reveal that the establishment of embryonic polarity with the asymmetric first division as well as the separation of shoot and root fates within the proembryo depend on transcriptional regulation in the zygote and early embryo. Although the functional connections need to be better defined, this transcriptional network likely provides the positional information required for initiating the machinery capable of processing the systemic signal auxin in a context-dependent manner.

Introduction

In *Arabidopsis* embryogenesis, new gene expression domains arise with nearly every round of cell division suggesting a rapid specification and refinement of cell fates: division of the zygote is asymmetric and can be related directly to the apical–basal axis of the embryo; separation of shoot and root fates in the proembryo arguably begins as soon as the upper and lower tiers are created at the 8-cell stage; and apical-to-basal transport of the plant hormone auxin over the proembryo becomes apparent by the early globular stage, preceding root initiation by inductive signaling to the uppermost cell of the suspensor or hypophysis. While our understanding of these processes is still limited, many novel players have been revealed over the past few years. Most turn out to be transcription factors – does this imply that embryonic patterning is driven by a dynamic transcriptional network? Here, we review recent work on the formation of the main body axis in light of this hypothesis (for more comprehensive discussions see references [1,2]).

Creating polarity: transcriptional programs and the asymmetric first division

The newly formed zygote undergoes a series of profound cellular re-organizations before dividing asymmetrically [3]: a central vacuole inherited from the egg cell becomes fragmented, followed by rapid elongation to about three times the length of the egg cell and,

finally, a large vacuole is re-assembled at the base while the nucleus migrates to the apex. The first division then generates a small, densely cytoplasmic apical daughter and a large, highly vacuolated basal daughter.

Perhaps unexpectedly, several studies now reveal that zygote development is regulated by transcription factors acting after fertilization. Loss of *WRKY2* [4••] interferes with polar redistribution of organelles, resulting in zygotes with a central nucleus and vacuoles occupying both halves of the cell. The first division tends to be equal, producing daughters of similar size; however, these cells still adopt different fates, and by the globular stage *wrky2* embryos either look normal or show aberrations confined to the upper-most portion of the suspensor. Consistent with this phenotype, *WRKY2* is expressed in the female gametophyte, the zygote, and all cells of the suspensor (Figure 1).

WRKY2 was identified in a molecular screen for transcriptional activators of *WOX8*, one of several *WUSCHEL HOMEODOMAIN* genes that form an inter-dependent network of regional regulators [5,6] (Figure 1). According to their domains of action in the early embryo, *WOX* genes have been grouped into the ‘basal’ factors *WOX8* and *WOX9/STIMPY (STIP)* [7] and the ‘apical’ factors *WOX2*, along with *WOX1*, *WOX3/PRESSED FLOWER* [8], *WOX5* [9]. Loss of *WOX2* causes transient and relatively weak patterning defects in the proembryo, but concomitant loss of other apical *WOX* factors in a *wox2* background result in shoot-less embryos. Mutations in both basal *WOX* genes have no visible effect on elongation or division of the zygote, but subsequent patterning of the proembryo is severely disrupted: in many cases the apical cells of *wox8;wox9* embryos proliferate into finger-like cell mass without apparent organization. This phenotype is partly caused by a non-autonomous effect on *WOX2* expression, which is lost in the double mutants [5].

If *WRKY2* controls *WOX8* expression, why are the phenotypes of *wrky2* and *wox8;wox9* so different? Part of the answer seems to be that *WOX8* transcription depends on several cis-regulatory elements, only one of which is activated by *WRKY2* [4••]. Accordingly, expression of *WOX8* is reduced but not abolished in *wrky2* embryos, and the effect of *WRKY2* on the first division is probably not mediated by *WOX* factors but rather by as yet unspecified genes.

In addition to *WRKY2*, zygote development also depends on the *YODA (YDA)* MAP kinase signaling pathway. Loss of the MAPKK kinase gene *YODA (YDA)* or the two MAP kinase genes *MPK3/MPK6* blocks zygote elongation, such that the first division results in an abnormally small basal cell that typically fails to form a recognizable suspensor [10,11]. Hyperactive forms of *YDA* have the opposite effect, causing abnormally long suspensors and often completely inhibiting growth of the proembryo [10]. Recent work now shows that *YDA* signaling in the embryo is dependent on the RWP-RK-type transcription factor *GROUNDED (GRD)/RKD4* [12,13•]: *grd/rkd4* mutations have nearly identical effects as *yda* mutations; furthermore, they are epistatic over hyperactive forms of *YDA*, suggesting that *GRD/RKD4* acts downstream of the *YDA* MAP kinase cascade. Similar to *YDA*, *GRD/RKD4* has a striking synergistic interaction with basal *WOX* genes [5,12]. Triple mutant embryos arrest either as short zygotes or after the first division, with daughter cells of

virtually identical size. These observations imply that transcriptional programs initiated after fertilization are essential for the creation of embryonic polarity in the first division.

Since *GRD/RKD4* activity is not controlled by MAP-kinase phosphorylation [12], it has been proposed that *GRD/RKD4* acts in conjunction with co-factors that are direct targets of the YDA MAP kinase cascade. Intriguingly, *WRKY2* contains several consensus MAP kinase phosphorylation sites at a similar position as *WRKY33*, a known target of *MPK3/MPK6* in pathogen responses [14]. Furthermore, *wrky2* mutations and weak mutations in the *YDA* pathway are both associated with partial loss of suspensor identity. Thus, *WRKY2* perhaps provides a mechanistic link between *YDA* signaling and *WOX* factors (Figure 1), but this possibility remains to be examined.

What kind of information may a signal transduction pathway contribute to the regulation of the asymmetric first division? The receptor-like cytoplasmic kinase *SHORT SUSPENSOR (SSP)* reveals a direct link between fertilization and *YDA* signaling: *SSP* essentially acts as a trigger for the pathway that is provided to the zygote with the sperm and presumably results in transient activation [15••]. Accordingly, the main function of *YDA* may be to repress the activity of apical factors before division of the zygote, perhaps in cooperation with basal *WOX* factors. The finding that forced *WOX2* expression in a *wox8;wox9* background suppresses zygote elongation [5], similar to loss of *YDA* signaling, seems consistent with this speculation.

Sorting shoot from root fates: role of *PLT* and *HD-ZIP III* factors

The apical cell subsequently adopts an isodiametric mode of growth and, through three rounds of divisions, produces a spherical octant or 8-cell proembryo. The two tiers of the proembryo express different *WOX* factors (Figure 1) and give rise to fundamentally different organs: the upper tier produces the shoot, while the lower tier, by inductive signaling, recruits the hypophysis to generate the hypocotyl and root. *PLETHORA1 (PLT1)*, an AP-2 transcription factor that acts redundantly with closely related family members as a master regulator for root development, is already expressed in the upper suspensor and lower tier of the 8-cell embryo [16,17••] (Figure 2). How are root and shoot fates separated? Important cues have been gained by an elegant study of the co-repressor *TOPELESS (TPL)*. Dominant negative mutations in *TPL* often transform the seedling shoot into a root [18]. This homeotic effect is associated with an expansion of *PLT* expression into the upper tier of the *tpl-1* proembryos, and *tpl plt1 plt2* triple mutants restore shoot formation [19••]. Promoter binding studies indicate that *TPL* directly represses *PLT* transcription, although it remains open which co-factors recruit *TPL* to the *PLT* promoter in the upper tier.

Suppression of the *tpl-1* double roots is also observed with dominant mutations in *PHABULOSA (PHB)*, *PHAVOLUTA (PHV)* or other *HD-ZIP III* transcription factors [19••]. These mutations provide resistance to miRNA-mediated degradation and lead to generally higher as well as ectopic accumulation of transcripts, which then presumably counters the effect of ectopic *PLT* in *tpl-1* embryos. *PHB* mRNA is found throughout the proembryo at the 8–16-cell stage [20] but becomes confined to the upper tier by the globular stage. An independent study shows that this is likely due to clearing from the lower tier by

miRNA165/166. Production of miRNA165/166 is dependent on the *SERRATE* (*SE*) Zn-finger protein, and loss of *SE* function results in embryos that, among other defects, never form roots [21•]. This root-less phenotype is associated with ectopic expression of *HD-ZIP III* genes in the lower tier and can be rescued by removing *PHB* and *PHV* function in the *se* background. In a complementary approach, forced expression of miRNA resistant *HD-ZIP-III* genes in the root domain under the control of a *PLT* promoter was found to transform the incipient root into a shoot [19••]. Together, these observations cast *HD-ZIP III* and *PLT* factors as master regulators for shoot and root fates, respectively. The details of their antagonism need to be investigated—but since *PLT* and *HD-ZIP III* expression domains overlap for some time in the early embryo (and never become mutually exclusive in *se* and *tpl-1* mutants), it is likely indirect.

Synthesizing positional information: local responses to a systemic signal

Auxin coordinates continuous, reiterative growth as well as rapid responses to external stimuli throughout the plant life cycle. Although the auxin signaling machinery has self-organizing properties, independently established transcriptional domains appear to provide essential positional information for initiating auxin transport and responses in the embryo (see [22] for an excellent review).

The asymmetric division of the zygote establishes different repertoires for auxin perception and transport in the developing suspensor and proembryo (Figure 2). The first auxin transporter to show clear polar distribution is PIN-FORMED INFLORESCENCE7 (*PIN7*), localizing to the apical plasma membrane of suspensor cells and presumably promoting auxin accumulation in the apical cell and proembryo [23]. Localization of *PIN1* in the proembryo remains diffuse before the mid-globular stage, when basal accumulation in the cells of the lower tier marks the establishment of apical-to-basal auxin transport (*PIN7* localization also becomes basal) [23,24]. Auxin now presumably accumulates in the hypophysis and drives robust expression of *DR5*, a synthetic marker for auxin-dependent transcription (the auxin response factors mediating this response remain elusive).

Expression of both auxin transporters is coordinately regulated by the GATA transcription factor *HABANA TARANU* (*HAN*) [17••]. Loss of *HAN*, which is expressed in the zygote and throughout the early proembryo, causes an apical shift of gene expression from the 8-cell stage on, with *PIN7* expanding into the lower tier and *PIN1* becoming restricted to the upper tier. This shift is associated with a loss of clear polar localization of *PIN1* (but not *PIN7*) and ectopic expression of *DR5* throughout the lower tier, revealing a drastic change of auxin flux. Strikingly, the expression of transcription factors required for maintenance of the root quiescent center, such as *SHORT ROOT*, *SCARECROW*, and *WOX5*, is similarly shifted upward at the globular stage, consistent with the observation that mutant embryos initiate a root in the center of the proembryo rather than at the boundary between suspensor and proembryo. Is this fate map shift entirely due to altered auxin flux? And why does the new boundary between *PIN1* and *PIN7* expression in *han* form between the upper and lower tier? These important questions remain open. However, *PLT1* expression is unchanged in the mutants, suggesting that the pathways responsible for promoting shoot and root fates might enable differential responses in the two tiers.

Auxin serves different functions in the shoot and root; accordingly, auxin transport and responses vary significantly between the two tiers of the proembryo. *MONO-PTEROS (MP)/ARF5* acts as the main auxin response factor throughout the proembryo, with some contribution from NON-PHOTOTROPIC HYPOCOTYL4/ARF7 [25,26]; but auxin-dependent MP activity is regulated by different combinations of IAA repressors: *BODENLOS/IAA12* and *IAA13* in the lower tier [27,28], and additionally *IAA18* in the upper tier [29]. *MP* directly activates *PIN1* transcription, implying a positive feedback loop [30]. Independently, *PIN1* expression is also promoted by apical *WOX* factors [5] and the LOB-domain gene *JAGGED LATERAL ORGANS* [31].

In the upper tier, auxin acts in conjunction with NAC factors of the *CUP-SHAPED COTYLODONS* family [32,33] and the AP-2 factors of the *DORNROSCHEN* family [34] to bring about the transition to bilateral symmetry (reviewed in reference [35]). Following the establishment of apical-to-basal auxin transport, *PIN1* becomes apically localized in the upper tier to promote auxin flux toward the incipient cotyledon primordia at the late globular stage (Figure 2). Apical localization of *PIN1* is regulated by phosphorylation [36,37] and promoted by the AGC kinase *PINOID*, which becomes specifically expressed in the flanks of the upper tier of globular embryos [38,39] (several other AGC kinases show region-specific expression in the embryo but their function is not well understood [40]). Both basal and apical targeting of *PIN1* depends on dynamic cycling to and from the plasma membrane and requires the guanine nucleotide exchange factor *GNOM (GN)*. Providing *GN* function only to the epidermis of *gn* embryos restores the formation of cotyledons, implying that auxin is transported at the surface [41]. Conversely, providing *GNOM* function only in the center of the proembryo restores hypocotyl and root formation, confirming previous findings that the embryonic axis depends on downward flux through the lower tier. It seems difficult to see how this complex pattern of *PIN* localization, and thus auxin flux, can be generated without pre-existing positional information.

In the lower tier, auxin is involved in recruiting the hypophysis to the incipient root. Root formation starts with an asymmetric division of the hypophysis, producing the lens-shaped progenitor of the root meristem (Figure 2). This division is dependent on *MP* activity specifically in the center of the lower tier, revealing a non-cell autonomous effect [30]. One of the functions of *MP* is to mediate auxin accumulation in the hypophysis, which seems required for root formation; however, auxin is not sufficient to trigger hypophysis division, such that other signals must exist. A genome-wide survey of direct targets of *MP (TMO* genes) has now implicated the small bHLH factor *TMO7* [42••]. *TMO7* RNA is transcribed and translated in the proembryo, but the protein is mobile and also accumulates in the hypophysis. Forced expression of *TMO7* in the suspensor can suppress the effect of weak *mp* alleles, supporting the view that *TMO7* protein acts as an inductive signal for root initiation. Thus, signaling in the embryo can be both complex and simple – multiple signals are sent to the hypophysis but the communication pathways may be as direct as a moving transcription factor.

Conclusions

Over the past few years, a number of factors have been identified that appear to provide overlapping layers of transcriptional regulation in the early embryo: *WRKY2*, the homeodomain factors *WOX8/WOX9*, and the RWPRK factor *GRD* regulate the asymmetric first division; AP-2 factors of the *PLT* family and *HD-ZIP III* factors mediate the separation of root and shoot fates; the GATA factor *HAN* coordinately affects the expression of auxin transporters. Almost certainly there is more to be discovered, but it also seems like a good time to pause and dream a little: can we connect these factors to reveal the outlines of a dynamic network at the core of the patterning process? A systematic approach towards transcriptional profiling in the early embryo appears within reach [43–47] and a tentative framework for integrating our still rather disjoint understanding of embryonic development is badly needed. And while biased, such a transcription-centric view may serve a unifying purpose and enable a more focused attack on still elusive aspects of embryonic development, such as the regulation of cellular polarity and polar transport [48] or cell-to-cell communication pathways [49].

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Figure 1.

Three classes of transcription factors affect the asymmetric division of the zygote and the establishment of embryonic polarity. While the RWP-RK factor *GRD/RKD4* is expressed broadly in the early embryo, *WRKY2* and the homeodomain factors *WOX2*, *WOX8*, and *WOX9* are expressed in specific domains that can be overlaid on the fate map. A tentative and speculative model of genetic interaction between these factors is outlined below the drawings, with indirect effects represented as dotted arrows and inferred or hypothetical effects in grey. *YDA*-dependent signaling promotes zygote elongation and basal or suspensor fate while repressing apical or proembryo fate; this activity requires *GRD/RKD4*, which here is speculated to act in conjunction with an unknown co-factor presumably phosphorylated by the *YDA* MAP-kinase cascade. *WRKY2*, which is required for re-polarization of the zygote, has several potential MAP-kinase phosphorylation sites and may be one of the factors regulated by *YDA* and acting with *GRD/RKD4*; in addition, *WRKY2* is one of the activators of *WOX8* in the zygote and suspensor (although other activators must exist; grey arrow). Together with *WOX9*, *WOX8* non-cell autonomously promotes expression of *WOX2* in the apical cell (dotted arrow). Obviously this model is incomplete, suggesting that more factors remain to be discovered.

Figure 2.

Left: AP2 factors of the PLT family and HD-ZIP III class transcription factors function as master regulators for root and shoot fates, respectively. In the early embryo, *PLT1* expression is confined to the lower tier and upper suspensor by the TPL repressors acting in conjunction with an unknown cofactor. Conversely, transcripts of *PHB* and other *HD-ZIP III* genes are cleared from the lower tier by action of SE and miRNA165/166. **Right:** Positional information is integrated by auxin signaling. After the asymmetric division of the zygote, accumulation of PIN7 protein (orange) in the apical membranes of the basal cell and, subsequently, the cells of the suspensor direct auxin flux upward (small green arrow); auxin accumulation presumably promotes apical fate, perhaps by promoting the expression of MP, the main auxin response factor of the proembryo. At the early globular stage, PIN1 protein (red) becomes localized to the base of the lower tier, marking the establishment of apical-to-basal auxin transport (large green arrow); *DR5*, a synthetic reporter of auxin-dependent transcription becomes strongly expressed in the hypophysis and upper suspensor. TMO7 protein moves from its site of production in the proembryo to the hypophysis and, together with auxin and possibly other signals, induces the formation of the lens-shaped progenitor cell of the root meristem quiescent center. At the late globular stage, the PID kinase becomes expressed at the flanks of the upper tier and mediates apical localization of PIN1, resulting in auxin flux toward the site of cotyledon formation (small green arrows).