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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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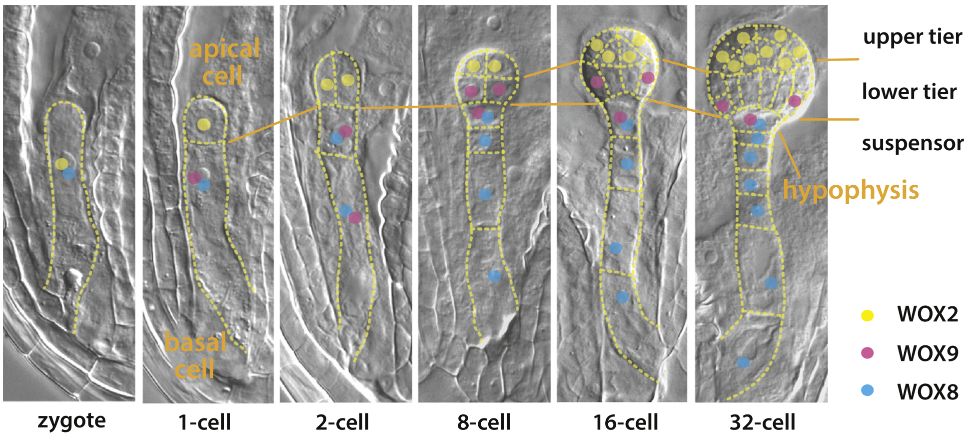

Fig. 1. Developmental progression of early Arabidopsis embryos. Images of whole-mount cleared seed from the zygote to the 32-cell stage; the expression of three WOX genes is indicated by colored dots.

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;