Review

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Organogenesis in plants: initiation and elaboration of leaves

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Plant organs initiate from meristems and grow into diverse forms. After initiation, organs enter a morphological phase where they develop their shape, followed by differentiation into mature tissue. Investigations into these processes have revealed numerous factors necessary for proper development, including transcription factors such as the *KNOTTED-LIKE HOMEOBOX* (*KNOX*) genes, the hormone auxin, and miRNAs. Importantly, these factors have been shown to play a role in organogenesis in various diverse model species, revealing both deep conservation of regulatory strategies and evolutionary novelties that led to new plant forms. We review here recent work in understanding the regulation of organogenesis and in particular leaf formation, highlighting how regulatory modules are often redeployed in different organ types and stages of development to achieve diverse forms through the balance of growth and differentiation.

Plant organogenesis

Plant development proceeds as an iterative process of organ initiation from meristems. Above-ground tissues originate from the shoot apical meristem (SAM), which initiates lateral organs in regular phyllotactic patterns (see Glossary). In all cases, a lateral organ is formed along with an axillary meristem, and often one or the other is suppressed [1]. The lateral organs produced from the SAM can be simple leaves, dissected leaves that maintain organogenic capacity along their margins, or branches that reiterate the main shoot system. In the reproductive phase of development, inflorescence meristems produce floral meristems, which produce floral organs. Continual organ initiation allows plant architecture to be flexible and respond to changing environmental conditions, resulting in plants of the same species often differing greatly in organ number or organ size.

Organogenesis involves a subset of meristematic cells transitioning to determinate growth, requiring broad changes in cell physiology, transcriptional regulatory networks, and hormones. Cells that transition to organ initiation are within the peripheral zone (also called the morphogenetic zone) while cells that replenish the meristematic cells are in the central zone (Figure 1). The site of incipient organ formation is called the Plastochron 0 (P_0), used to index leaves at a point in development. As organs develop, various asymmetries are established that determine tissue identity across proximal/distal, abaxial/adaxial, and medial/lateral axes. The establishment of a boundary between the initiating organ and the rest of the meristem is essential to organogenesis and meristem maintenance.

Mutant studies have been essential in teasing apart the various spatial and temporal interactions that regulate organogenesis. Mutants can be identified at various stages of organ development; some fail to form a P_0, others have aberrations in the organ boundary, and many show affected shape and morphology. Furthermore, comparative studies often reveal interesting phenotypes and functions not present in common model species. Together, these approaches continue to yield insight into the core mechanisms of plant organogenesis.

Initiation

The first step in organogenesis is establishing where organ primordia will form. The Hofmeister principle states that new organs form in the location maximally distant from previous primordia [2]. The plant hormone auxin is thought to be a central regulator of organ patterning given its concentration gradient patterns across the SAM [3]. This patterning is achieved through polar auxin transport by PIN efflux proteins, which are named after their mutant phenotype of pin-like inflorescences devoid of lateral organs [4,5]. Although the inflorescence phenotype is the most conspicuous, subtle phyllotactic defects exist in the vegetative shoot of *pin* mutants [6]. Feedback between auxin and PIN patterning leads to the formation of local auxin concentration maxima as PIN polarization in the epidermis directs auxin flow toward regions of higher

Glossary

Abaxial: the abaxial surface of a leaf faces away from the meristem.
Adaxial: the adaxial surface of a leaf faces the meristem.
***bzr1-d*:** a dominant mutation in the *Arabidopsis BRASSINAZOLE RESISTANT1* gene.
Cauline: leaves formed along the bolted inflorescence stem in *Arabidopsis*.
Fasciated: an enlarged meristem that appears as if many meristems are bundled together.
***MAB4*:** the *MACCHI-BOU 4* family of genes encode NON-PHOTOTROPIC HYPOCOTYL 3 (NPH3)-like proteins.
***MYB*:** plant *MYB* genes contain a conserved DNA-binding domain from the myeloblastosis family of transcription factors.
Phyllotaxy: the spatial pattern of lateral organ initiation from shoot meristems.
Plastochron: refers to the amount of time between organ initiation events and is used to describe organs at different stages.
***Shootmeristemless* (*STM*):** a *KNOX* gene in *Arabidopsis*.

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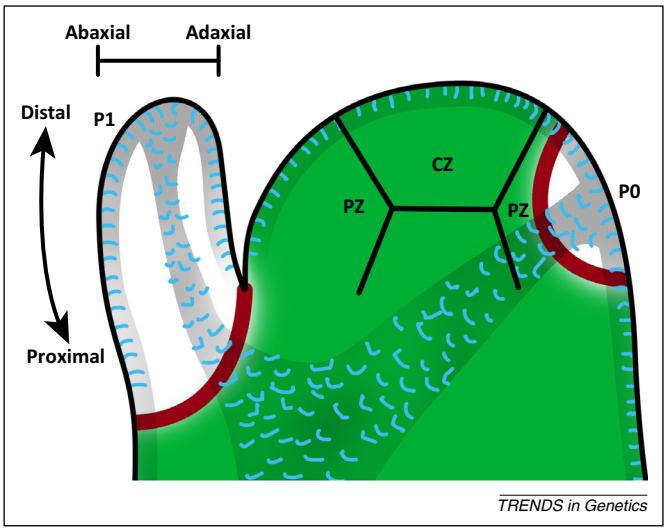

Figure 1. The shoot apical meristem. **(A)** A cartoon of a shoot meristem and initiating leaf. The shoot meristem has a central zone (CZ) where cell divisions are slower and a peripheral zone (PZ) where organogenesis occurs. *KNOTTED-LIKE HOMEOBOX* (*KNOX*) genes are expressed throughout the meristem (green) except at the position of the incipient leaf [Plastochron 0 (P_0)]. Boundary genes are expressed at the boundary of the meristem and leaves (P_0 and P_1). As the leaf initiates, the meristem provides proximal/distal and abaxial/adaxial polarity. PIN polar auxin transporters, designated with blue lines, move auxin in the epidermis and into the incipient vasculature. Regions of higher auxin levels are in dark green.

auxin concentration [7]. At the position of auxin maxima in the P_0, a switch occurs in which auxin moves internally, thus creating an incipient vascular strand that will connect with older strands and form an auxin sink. These feedback systems lead to the dynamic pattern formation necessary to create auxin maxima, which ultimately lead to the observed phyllotactic patterns (Box 1) [8].

In addition to auxin, cytokinin (CK) plays a role in organ initiation. The maize protein ABPHYL1 is part of a family of two component response regulators that negatively regulate CK signaling [9]. *abph1* mutants have enlarged SAMs and initiate two leaves at the same time instead of a single leaf. In *Arabidopsis*, mutants for the CK inhibitor *ARABIDOPSIS* HISTIDINE PHOSPHOTRANSFERASE PROTEIN 6 (AHP6) have phyllotactic defects that affect the order in which organs initiate [10]. Thus, it appears that CK signaling is required for robust initiation patterning in addition to its role in maintaining meristematic activity.

Auxin is not the sole promoter of organ initiation, as downregulation of class 1 *KNOX* (*KNOX1*) genes is also critical for this process [11]. KNOX1 homeodomain transcription factors are expressed throughout the meristem except the P_0. In multiple species with dissected leaves, reactivation of *KNOX1* gene expression during leaf elaboration is an important component of leaf dissection [12], but even in these species *KNOX1* is not expressed in the P_0. Similarly, *KNOX* gain-of-function mutants in maize misexpress *KNOX1* genes in lateral organs; however, they still show downregulation in the P_0 [13]. *KNOX1* gene expression in the P_0 has been documented only in cases where lateral organs fail to form, such as in severe *semifore* mutants [14], or in the presence of polar auxin transport inhibitors [15]. Thus, just as auxin signaling needs to occur at the site of leaf initiation, it is equally important that *KNOX1* genes are downregulated. *KNOX* expression is regulated by auxin [16] and KNOTTED1 binds and modulates many genes in auxin signaling [17]; thus, there are likely to be regulatory interactions between these transcription factors and the auxin signaling pathway.

Box 1. Auxin

Models have attempted to reconcile how one protein, PIN1, can move auxin flow up concentration gradients toward the P_0 as well as flow along paths of auxin flux away from maxima in the vasculature [84,85] (see Figure 1 in main text). The recently described *MAB4* gene family should aid in understanding the switch in auxin flow during organogenesis. MAB4 was found to play a critical role in PIN patterning by directing polarization internally at the site of organ initiation in the direction of auxin flux [86]. MAB4, along with the related proteins MEL1 and MEL2, is involved in the regulation of PIN polarity [87]. *mab4 mel1 mel2* triple mutants have a pin-like inflorescence and altered PIN1 localization. PIN1 patterning is normal in the epidermis of the triple-mutant meristem and leads to an auxin convergence, but internal expression from the auxin maximum to sink tissue is absent. Without a sink, auxin accumulates at the meristem surface. MAB4 expression depends on auxin signaling and is upregulated at the site of organ initiation in wild type plants. Together, these observations demonstrate a mechanism that reconciles different modes of PIN1 polarization and auxin distribution: high auxin levels turn on MAB4 and trigger a switch from up-the-gradient to with-the-flux PIN polarization at the site of initiation [86].

The importance of auxin concentration is also exemplified by its mechanism of action (reviewed in [88]). At low concentrations of auxin, a repressor (AUX/IAA) blocks auxin-induced transcription. At high concentrations, auxin becomes a molecular glue between its receptor, a component in the SCF^{TIR1} complex [89,90], and AUX/IAA, leading to destruction of the repressor [91] and simultaneously allowing ARF-mediated transcription. The knockout mutant of *ARF5*/*MONOPTEROS* (*MP*) in *Arabidopsis* has defects in embryo development and floral organ initiation, making bare or sparse inflorescences much like the *pin1* mutant [92]. A more general role for auxin signaling in organ initiation is found when *mp* is combined with *pin* mutants or mutants in the serine/threonine kinase PINOID, required for PIN1 polarization. These mutant combinations result in plants that make no lateral organs, instead making abnormal, large SAMs [93]. Inhibiting auxin transport alone with the use of chemicals such as 1-N-naphthylphthalamic acid is not enough to make bare shoots, but application to *mp* mutants dramatically reduces organ initiation. Together, these results show that organ initiation requires the formation of an auxin maximum and transcriptional responses mediated by ARFs.

Balancing lateral organ initiation with meristem homeostasis

Lateral organs are initiated in defined intervals that allow a balance between meristem renewal and organ initiation. Mutants that fail to maintain the meristem, such as *knox* or *wuschel*, terminate prematurely, often producing no lateral organs or a limited number [18–21]. Another class of mutants has too many indeterminate cells and makes additional organs. In *Arabidopsis*, *CLAVATA3* (*CLV3*), which encodes a secreted peptide, is expressed at the apex of the central zone, directly above the zone of *CLV1* expression, which encodes a leucine-rich receptor kinase. Through CLV1 and other receptors, CLV3 negatively regulates *WUS*, which is required for stem cell renewal [22,23]. In *clv* mutants, an increase in *WUS* expression leads to an enlarged, often fasciated meristem and additional organs.

Components of this classic feedback loop have been identified through mutant phenotypes in maize and rice,