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Axillary meristem initiation — a way to branch out

Ying Wang[1] and Yuling Jiao[1,2]

Plants differ from most animals in their retained ability to initiate new cycles of growth and development, which relies on the establishment and activity of branch meristems. In seed plants, branching is achieved by axillary meristems, which are established in the axil of each leaf base and develop into lateral branches. Research into axillary meristem initiation has identified transcription factors and phytohormones as key regulators. Based on these findings, a mechanistic framework for understanding axillary meristem initiation has emerged. Taking recent research into account, we discuss mechanisms underlying stem cell fate regulation that enable axillary meristem formation.

Addresses
[1] College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
[2] State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, and National Center for Plant Gene Research, Beijing 100101, China

Corresponding author: Jiao, Yuling (yljiao@genetics.ac.cn)

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Introduction

An iconic feature of plant development is the branching growth habit, an innovation considered crucial for the conquest of land by plants [1,2]. Plants maintain meristems with undifferentiated stem cells, which are responsible for the life-long organogenesis of growing plants. Branching occurs through the periodic initiation of new meristems. In seed plants, secondary growth axes arise from axillary meristems (AMs, also termed lateral meristems) in or near the leaf axils [3,4,5•]. The leaf axil is an organ boundary region with many unique characteristics, such as slow cell division, that are important for AM initiation [5•]. Branching is a major determinant of plant architecture and significantly affects crop yield by influencing tiller and spike numbers as well as spike branching complexity [6–8]. Axillary bud development comprises two stages: initiation in the leaf axil and subsequent outgrowth or dormancy [3]. Recent breakthroughs, including the identification of strigolactones and the elucidation of its biosynthesis and signaling, have provided a relatively clear picture of the molecular regulation of axillary bud outgrowth [3,9,10]. On the other hand, our understanding of AM initiation at the molecular level remains incomplete.

Genetic studies in tomato (*Solanum lycopersicum*), *Arabidopsis thaliana*, rice (*Oryza sativa*), and maize (*Zea mays*) have shown that AM initiation is regulated by several transcription factor-encoding genes, such as *CUP-SHAPED COTYLEDON* (*CUC*), *LATERAL SUPPRESSOR* (*LAS*), *REGULATOR OF AXILLARY MERISTEMS* (*RAX*), and *REVOLUTA* (*REV*) in *Arabidopsis* [5•,11–20]. Many of these genes have conserved functions in the regulation of AM initiation in dicots and monocots [7]. Nevertheless, the genetic regulation of AM initiation in monocots is clearly distinct from that in dicots, and several recent reviews examine this divergence more exhaustively [7,8,21,22].

Notably, some AM initiation regulatory genes are also involved in leaf dissection, the process by which a leaf blade is subdivided or lobed [23••,24,25]. These AM initiation regulators have been found to modulate compound leaf formation (such as in tomato) or simple leaf serration (such as in *Arabidopsis*). Strikingly, tomato and *Cardamine pratensis* occasionally form ectopic meristems at the base of leaflets, while ectopic leaf base meristem formation requires the same set of genes as AMs [26•]. These observations suggest that cells in the leaf axil and in the base of leaflets (for compound leaves) or in the sinus of leaf margin serration (for simple leaves) share similar cell identity, gene expression, and developmental potential [23••].

In this review, we focus on recent findings on cell fate determination during AM initiation and highlight transcriptional and phytohormonal regulation. We discuss the importance of cell lineage in AM initiation and the advantages of AM initiation for studying stem cell fate determination. In this review, we limit ourselves to AM-specific regulation and further refer readers to recent excellent reviews on the primary shoot apical meristem (SAM) [27–30].

A stem cell lineage acting in AM initiation

During AM initiation, a morphologically detectable bump forms in the leaf axil and develops into a bud to enable branching. AMs share the same developmental potential as the SAM, and AM initiation requires the establishment

of new meristems harboring stem cells. This phenomenon raises questions about the precise regulation of cell fate. The definition of new stem cells in the leaf axil is fundamental to this process, but the underlying mechanism remains elusive. There are two long-standing models for AM initiation. The 'detached meristem' model proposes that a few pluripotent stem cells detach from the primary SAM and associate with the leaf axil as the leaf differentiates from the SAM [31]. Histological analysis shows that leaf axil cells likely remain undifferentiated, providing support for the detached meristem theory. *In situ* hybridization analysis of fixed samples also identified expression of the meristem marker *SHOOT MERISTEMLESS* (*STM*) in the leaf axil at all stages [14,32,33]. In addition, the detached meristem theory is further supported by the expression of boundary genes [34]. On the other hand, analysis of the *A. thaliana phavoluta-1d* (*phv-1d*) mutant supports the alternative '*de novo* induction' model [35], in which an AM initiates from differentiated leaf cells. Specifically, the model proposes that positional signals enable *de novo* formation of the AM.

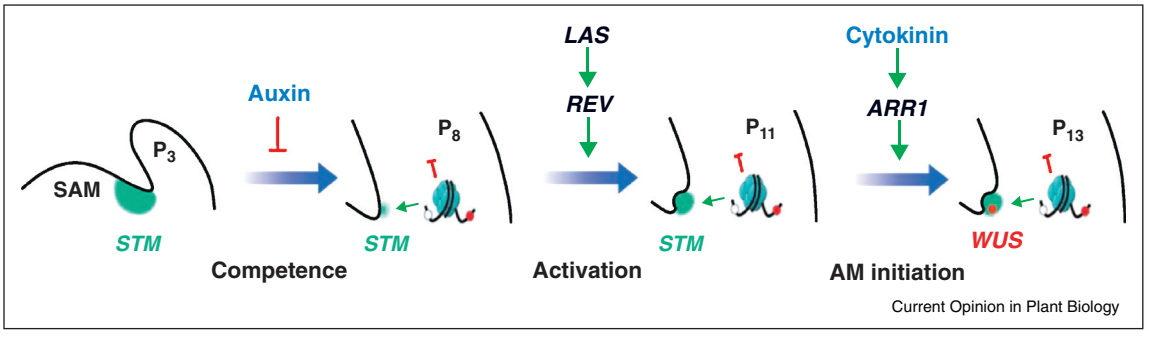
Recent studies using live cell imaging of leaf axil cells in *Arabidopsis* and tomato support that AMs derive from a stem cell population [36••,37•]. In one study, live cell imaging showed that the leaf axil maintained a cell population continuously expressing the meristem marker *STM*, with the progenies of these cells forming the axillary buds (Figure 1) [36••]. Furthermore, laser ablation of the *STM*-expressing cells, but not neighboring cells, abolished axillary bud formation. Thus, AM initiation requires the *STM*-expressing cell population. The *STM*-expressing cells fulfill the criteria of stem cell fate insofar as they maintain an undifferentiated state, can differentiate into specialized cells, and can divide to produce more stem cells. Notably, ectopic *STM* expression was insufficient to activate axillary bud formation from leaf axil cells that had lost *STM* expression [36••]. This clearly suggests that some cells undergo irreversible fate changes. An independent study investigating the origin of AMs combined live cell imaging and modeling and showed that AM progenitor cells are set aside early in the SAM, with only seven to nine cell divisions prior to AM initiation [37•]. The initial cell division rate of AM progenitor cells was found to be much lower than that of non-boundary cells [37•], but rapid cell division was observed immediately prior to AM formation [36••]. It should be noted that auxin reporter DR5 was used in the second study to mark the stem cell lineage in plants after floral transition (see below for further discussion).

Two-step regulation of the stem cell population

The stem cell population exhibits two phases of cell division, each associated with different levels of *STM* expression. This phase transition supports a 'threshold model' in which the maintenance of low *STM* expression levels is required but not sufficient for AM initiation, with a subsequent increase in *STM* expression resulting in AM initiation induction (Figure 1) [36••]. The early low *STM* expression level is likely required for stem cell competence. Cells that have lost *STM* expression are no longer competent in terms of meristem formation, based on the finding that they are insensitive to ectopic *STM* activities at a later stage. Cells with weak *STM* expression also have low cell division rates.

Before AM initiation, *STM* is up-regulated in the center of the leaf axil, which depends on previously maintained *STM* expression. Elevated *STM* expression is associated with active cell division and the formation of a morphologically detectable bump. Subsequently, *WUSCHEL* (*WUS*) expression is activated *de novo* in the center to establish a new SAM organization center (Figure 1)

Figure 1



Conceptual model showing the two-step regulation of AM initiation. Early leaf primordium axils maintain low levels of *STM* expression to enable leaf axil cell competence to form AMs. High levels of auxin suppress the maintained *STM* expression and cell competence. In more mature leaf primordia, the expression of *REV*, which is regulated by *LAS*, up-regulates *STM* expression to promote AM initiation. Subsequent cytokinin signaling then activates *WUS* expression *de novo* through B-ARRs to enable stem cell specification and axillary bud formation. Chromatin organization is involved in restricting gene expression in the leaf axil. In each part of the diagram, the red inhibition symbols indicate transcriptional repression in mature leaf cells, and the green arrows indicate transcriptional activation in leaf axil cells.
Part of the figure is adapted from Shi *et al*. [36••].

[38•,39]. The expression of the stem cell marker *CLAVATA3* (*CLV3*) becomes detectable about one plastochron after *WUS* expression [38•,39], which is consistent with the direct activation of *CLV3* by WUS [40]. Establishment of the *WUS-CLV3* module indicates that the AM is characterized by the same structure and developmental potential as the SAM. The AM forms its own leaf primordia that cover the AM to become a bud. Taken together, these new findings support a threshold model of *STM* expression that further supports and elaborates the detached meristem model of AM initiation.

Auxin regulation of AM initiation

Auxin plays pivotal roles in plant development, and recent studies have shown that an auxin minimum is required for AM initiation during vegetative growth [41••,42••]. Using the DII and DR5 auxin signaling reporters [43,44], AMs were found to initiate from leaf axil cells with low auxin through stereotypic stages. Consistently, ectopic overproduction of auxin in the leaf axil efficiently inhibited AM initiation [41••,42••], while ectopic inhibition of auxin signaling resulted in supernumerary axillary buds [42••]. Furthermore, these studies showed that auxin efflux is required for the auxin minimum and AM initiation in the leaf axil [41••,42••].

The leaf axil auxin minimum is required for AM initiation at least partially through the regulation of *STM* expression [36••]. Ectopic overproduction of auxin in the leaf axil diminishes the otherwise maintained expression of *STM* and stem cell differentiation (Figure 1). Thus, AMs can no longer form without the leaf axil auxin minimum.

The gene regulatory network underlying AM initiation

Most genes that regulate AM initiation encode transcription factors. Not surprisingly, gene regulatory networks (GRNs) play central roles in the regulation of AM initiation via cell type-specific gene expression and interactions between transcription factors and regulatory promoter regions. Genetic analysis has suggested a regulatory hierarchy for some of these transcription factors and provides a foundation for further understanding the GRNs underlying AM initiation [12,25].

A recent analysis produced a cellular-resolution genome-wide gene expression map for low-abundance *Arabidopsis* organ boundary cells. Additionally, a genome-wide protein-DNA interaction map was constructed and integrated with the boundary cell-specific gene expression map [45]. The resulting prototype GRN uncovers transcriptional signatures, predicts cellular functions, and identifies promoter hub regions, which are bound by multiple transcription factors upstream of *LAS* and *CUC2*. This study further resolved the regulatory hierarchy among transcription factors and identified new regulators of AM initiation. The GRN for floral meristem formation and patterning has also been extensively studied [46] and may be informative about AM initiation.

From the organ boundary GRN, the HD-ZIP III transcription factor REV was identified as a direct regulator of *STM* expression [45]. Before AM initiation, locally expressed REV up-regulates *STM* expression in the center of the leaf axil [36••]. The leaf axil-enriched *REV* expression in turn requires *LAS* activity [14]. This up-regulation of *STM* expression depends on the previously maintained *STM* expression and also requires a permissive epigenetic environment [36••]. In organ boundary-enriched samples, the *STM* locus has high levels of H3K4me2 and/or H3K4me3, chromatin modifications associated with transcriptional activation. In mature leaves, however, the *STM* locus is instead enriched for H3K27me3, a modification associated with transcriptional repression. Notably, this finding explains the ectopic abaxial AM formation in *phv-1d* mutants, a key support to the '*de novo* induction' model [35]. PHV is homologous to REV and can bind to the *STM* promoter region in yeast [45]. Continuous ectopic *PHV* expression maintains and further activates ectopic *STM* expression on the abaxial leaf side, resulting in ectopic AM formation [36••].

RAX1 is another key regulator of AM initiation [13,19], and recent studies have shed light on its regulation. EXCESSIVE BRANCHES1 (EXB1) is a WRKY transcription factor, and its overexpression leads to a bushy phenotype [47•]. *EXB1* is expressed in the leaf axil and directly activates *RAX1* expression. After floral transition, the master floral meristem identity regulator *LEAFY* (*LFY*) directly activates *RAX1* expression [48•]. Fairly little is known about the downstream targets of *RAX1*, but the *Arabidopsis* gene *REGULATOR OF AXILLARY MERISTEM FORMATION* (*ROX*), the ortholog of rice *LAX PANICLE1* and maize *barren stalk1*, is at least one of them [49]. In *rax* mutant plants, *ROX* expression is no longer detectable [49].

Cytokinin regulation of AM initiation

In addition to the low auxin environment associated with AM initiation, a subsequent cytokinin signaling pulse has been observed prior to AM initiation [41••]. Genetic analysis suggests that cytokinin perception and signaling are both required for AM initiation. Furthermore, cytokinin overproduction in the leaf axil partially rescues the AM initiation deficiency in *rax* mutants [41••].

Expressed in the organizing center, the homeodomain transcription factor *WUS* defines the shoot stem cell niche in the SAM and in AMs. In addition to its role in embryonic SAM formation, *WUS* in *Arabidopsis* and its ortholog in rice are also required for AM formation [38•,39,50,51]. During AM initiation, cytokinin signaling activates *de novo WUS* expression in the leaf axil [38•]. Type-B ARABIDOPSIS RESPONSE REGULATORs

(ARRs), a group of transcriptional activators in the cytokinin signaling pathway [52], directly bind to the *WUS* promoter to activate its expression (Figure 1). Similar to *STM* expression regulation, a permissive epigenetic environment is required to restrict *WUS* expression to highly defined meristematic tissues with existing *STM* expression. Notably, the same regulatory circuits are also utilized in adventitious shoot meristem formation [53,54].

Perspectives

Although it is a fundamental aspect of plant development, especially body planning, AM formation is difficult to study, due in part to its tight connection with apical dominance. Nevertheless, genetic analysis and systems biology approaches have provided a mechanistic framework for understanding AM initiation (Figure 1). A notable feature is the requirement of a stem cell population for AM initiation, making it a good system for the study of cell fate specification. In this context, the 'detached meristem' model better describes the AM initiation process. One open question concerns the nature of the positional cues that separate the leaf axil cells from differentiated cells and also maintain stem cell competency. Another important question is how the regulation of AM initiation changes after floral transition, especially for AMs in cauline leaf axils. A different auxin regulatory mechanism is likely employed after the floral transition. For example, in maize, an auxin maximum is required for inflorescence branching during inflorescence development [55–57]. In *Arabidopsis*, floral meristem formation similarly requires an auxin maximum [58]. It has also been reported that AM initiation during the vegetative stage and the reproductive stage requires different sets of genes [5•,59]. It may therefore be necessary to consider other signaling pathways as relevant players in cauline leaf AM and even floral meristem fate determination.

Conflict of interest

The authors declare no conflict of interest.

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