Minireview

**Leaving the meristem behind: regulation of KNOX genes**

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**Abstract**

The mechanism by which the plant reserves some cells as pluripotent stem cells while partitioning others into differentiated leaf tissue is fundamental to plant development. New work in *Arabidopsis* elucidates the genetic circuitry that distinguishes meristem from leaf.

Although leaf shape and arrangement may differ greatly between species, all plants share a similar body plan, consisting of repeating units of stem and leaf. These units of stem and leaf have their origin in the apical meristems located at the growing tips of shoots.

The shoot apical meristem contains a set of self-renewing stem cells at its center. When the stem cells divide, daughter cells are pushed out into the peripheral zone of the meristem where clusters of cells are specified as leaf founder cells. The mechanism by which the plant reserves some cells as pluripotent stem cells while partitioning others into differentiated leaf tissue is currently being explored in three model organisms - snapdragon, maize and *Arabidopsis*. Here, the recent advances from work in *Arabidopsis* are described and compared to work done in other species. The results suggest a conserved mechanism for gene regulation in leaf development.

When leaf founder cells are set aside, genes responsible for stem-cell specification and/or function must be inactivated. One set of genes down-regulated in the leaf are the class 1 KNOX genes [1]. Class 1 KNOX genes are a family of homeobox-containing genes found in all plant species in which they have been sought.

Two observations initially suggested the importance of class 1 KNOX gene regulation for meristem and leaf development. First, KNOX gene products are found in the meristem and are down-regulated in leaves [1,2]. Second, ectopic expression of KNOX genes in the developing leaf is associated with a syndrome of characteristics that includes leaf lobing, increased leaf dissection, ectopic meristem formation and pattern changes along the proximal-distal axis of the leaf [1,3-6].

For some members of the class 1 KNOX family, a role in meristem development has been confirmed, whereas for others it remains hypothetical. In *Arabidopsis*, the *KNAT1*, *KNAT2* and *SHOOTMERISTEMLESS (STM)* genes make up the class 1 KNOX genes. Lack of STM function in *Arabidopsis* results in failure to form a meristem [7]. For the *KNAT1* and *KNAT2* genes, *in vivo* functions have not yet been ascertained, as mutants for these genes have not yet been found. The tight down-regulation of *KNAT1* and *KNAT2* transcripts in the leaf founder cells and the effects of *KNAT1* or *KNAT2* ectopic expression do indicate, however, the importance of keeping these genes turned off in the developing leaf. It follows that the gene products responsible for keeping the KNOX genes off in the developing leaf are essential for normal plant development.

In a quest for such negative regulators of KNOX expression, Ori *et al.* [8] and Byrne *et al.* [9] examined mutants that have characteristics of the KNOX ectopic expression syndrome. The asymmetric1 (as1) and asymmetric2 (as2) mutations were found during the early days of *Arabidopsis* research but the associated phenotypes have not been well understood until now. Similar to plants that ectopically express KNOX genes, asymmetric mutants may have lobed leaves, develop ectopic meristems from leaves and show changes in pattern along the proximodistal axis of the leaf.
It is especially satisfying to find that KNAT1 and KNAT2 are up-regulated in the leaves of asymmetric mutants. Interestingly, down-regulation of KNAT1 and KNAT2 in leaf founder cells is normal in asymmetric mutants, indicating that AS1 and AS2 maintain KNOX genes in an off state in the leaf but do not mediate their initial down-regulation.

Not all KNOX genes are affected in the same way in asymmetric mutants. Loss of AS1 or AS2 function does not cause derepression of STM in the leaf [8,9]. This is the first hint that different class 1 KNOX genes act at distinct points in leaf development.

The ASYMMETRIC1 gene encodes a myb-like transcription factor [9] and, as expected since as1 mutants are predominantly defective in leaf development, is expressed in developing leaves where KNOX genes are turned off but not in meristems where KNOX genes are thought to be active. So what keeps AS1 from being expressed in the meristem? STM does. In the absence of STM function, AS1 transcript is found in the meristem [9]. In fact, the data from Byrne et al. [9] suggest that the inactivation of AS1 may be one of the principal roles of STM. In the absence of both STM and AS1, the shootmeristemless phenotype is suppressed and the plants are competent to develop vegetative meristems.

This leads to the model for gene action shown in Figure 1. In the wild-type meristem STM is on and it keeps AS1 off. This allows the KNAT genes and other targets required for meristem function to be on. In leaf founder cells, all class 1 KNOX genes are down-regulated by some unknown mechanism. In slightly older leaf primordia (P2 stage and beyond) the presence of AS1 maintains KNAT gene repression while another, as yet unknown, factor maintains STM repression. In as1 mutants, there is no effect on the meristem since AS1 is not active there normally. In the leaf, lack of as1 function causes expression of the KNAT1 and KNAT2 genes, which in turn causes the observed alterations in leaf development. In stm mutants, STM is off, which causes AS1 to be active. AS1 expression in the meristem blocks expression of genes required for meristem function (perhaps KNAT1 or others as yet to be identified), causing meristem termination. Finally, in stm; as1 double mutants, STM activity is missing from the presumptive meristem. This is partially ameliorated by the absence of AS1 from the meristem, allowing expression of other genes required for meristem function.

This model begs the question: what turns STM off in the leaf founder cells? A recent set of experiments points to localized fluxes in auxin concentration [10]. Auxin transport was blocked in shoot apical meristems either genetically or through the use of inhibitors. These meristems failed to initiate lateral organs and expressed STM-like genes throughout the meristem dome. When auxin was applied to developing shoot apical meristems, new leaf primordia developed near the site of auxin application. Connecting this result with the emerging framework of genetic regulation will be one of the next challenges in this area of research.

There are several parallels between this newly published work and earlier work done on as1 homologs in snapdragon (PHANTASTICA, PHAN) and in maize (ROUGH SHEATH2, RS2). Both the maize rs2 mutant and the snapdragon phan mutant ectopically express KNOX genes [11-13]. Also, rs2 and phan transcripts are found in the leaf founder cells. These similarities suggest that a conserved pathway governs this aspect of leaf development in the rather distantly related monocots and dicots. Given the conservation of KNOX genes in primitive plants such as ferns, it will be interesting to find out when this regulatory subprogram arose in plant evolution.

The alteration in pattern that exists in all as1, rs2 and phan mutants is less well understood. Maize rs2 mutant leaves exhibit distal to proximal transformations, snapdragon phan mutants exhibit adaxial-to-abaxial and distal-to-proximal transformations, and Arabidopsis as1 mutants exhibit lateral-to-medial and distal-to-proximal transformations. It is not clear how KNOX derepression causes these defects. One interpretation is that KNOX genes specify proximal
fates and their misexpression causes a disruption in the proximal-distal axis. If this is true, KNOX genes should be expressed in proximal regions of the developing leaf primordium. Are they? They may be. The exact boundaries that delineate the leaf founder cells from the rest of the meristem are not known. If the incipient leaf includes not only the cells that show strong down-regulation of KNOX genes but also adjacent KNOX-expressing cells, the latter cells would be in a position predicted to give rise to proximal regions of the leaf. Once loss-of-function mutations of KNAT1 and KNAT2 are available, it will be important to determine whether the corresponding mutants show defects in the development of proximal leaf domains.

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