

Plant Meristems: A Merry-Go-Round of Signals

Review

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Recent studies have provided significant new insights into the gene actions that specify and maintain stem cells in plant shoots and roots. New layers of genetic control and potential signalling pathways and effector mechanisms have emerged from these new studies and will be reviewed here. These new findings refine the current model in which stem cells in plant meristems are regulated by negative feedback loops and uncover a fundamental mechanism for stem cell maintenance that might be common to shoots and roots.

Introduction

Growth and organ formation in plants occur post-embryonically, mediated by meristems located on the tips of growth axes in shoots and roots. Meristems are unique structures made up of pluripotent stem cells, a transitory population of indeterminate cells and determinate organ primordia formed at the periphery. Secondary growth, which increases the girth of stems, is mediated by cambial cells, which continue to add vascular cells to the circumference of the central, vascular cylinder of the plant.

Shoot and root meristems are generated during embryogenesis, but do not contribute to the construction of the embryo and are not activated until the seedling germinates. Following germination, the plant passes through several developmental phases that culminate in flowering and reproduction. In the course of these phase changes, shoot meristems change their identity. This is most conspicuous in the lateral structures made on the flanks of the shoot apical meristem. In *Arabidopsis*, these structures are leaves during the initial vegetative growth, leaves and axillary meristems during the transition to flowering, and floral meristems and bracts by the inflorescence meristem during reproductive growth. In contrast, root meristems do not apparently change their identity during development.

Roots and shoots also make lateral structures differently. Lateral appendages of the shoot are initiated on the flanks of the apical meristems. The regular temporal sequence of organ initiation gives rise to the characteristic spiral phyllotaxy of leaves, and to a concentric organisation of floral organs into whorls in flowers. Production of leaves in a regular, phyllotactic sequence is a good indication of a functioning apical meristem, and distinguishes these from adventitious structures transiently capable of leaf production. Lateral roots, in contrast are formed only at a distance

from the root apex, and appear in stochastic patterns with no regular spatial relationship to each other.

In this review I shall focus on meristems and recent progress in our understanding of how they function. The emphasis will be on vegetative meristems of dicotyledonous plants, specifically on *Arabidopsis thaliana*, the best-characterised representative.

Shoot Meristems

Vegetative shoot meristems have very similar structures in different dicotyledonous plant species (Figure 1). They are organised into cell layers and concentric zones. The outermost, single-celled layer, the L1, is defined by molecular markers and regular patterns of anticlinal divisions, which preserve its continuity. The L1 layer subsequently gives rise to the epidermis. The L2 layer is internal to the L1, with predominantly anticlinal divisions. The interior of the meristem is made up of the L3 layer, with variable patterns of division. At the meristem base, the L3 layer transitions into the stem.

The meristem centre comprises very slowly dividing cells in all three layers. Low rates of cell division reduce the likelihood of mutations affecting the large sectors of the aerial plant body produced by individual stem cells [1–3]. Stem cells are surrounded by slightly more rapidly dividing cells on the flanks. At sites of primordium formation at the meristem periphery, proliferation increases markedly. Cells from all layers are incorporated into nascent primordia. Accelerating proliferation towards the meristem periphery progressively displaces the shoot apical meristem centre away from its progeny.

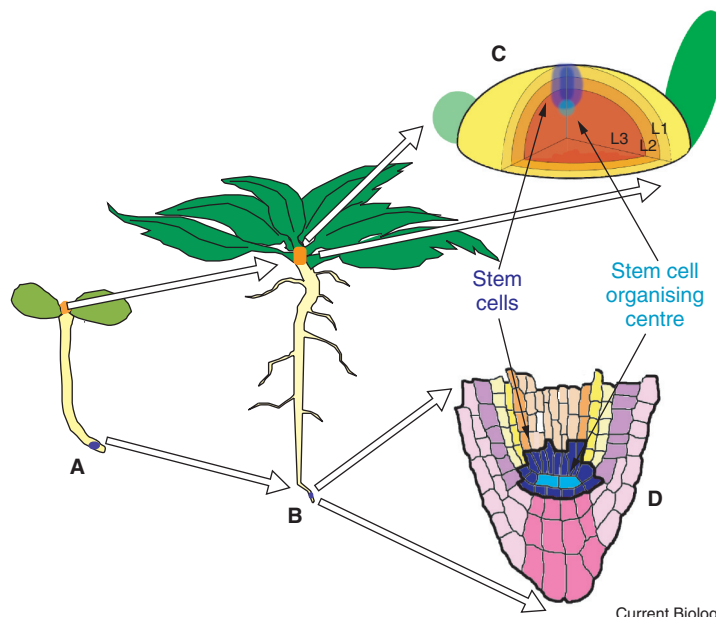
Meristems mediate plant growth and hence are dynamic structures in which cells transit through zones with distinct developmental potential. The coordination of growth with development in such a dynamic structure requires extensive short and long distance intercellular signalling. A conceptual framework for meristem function must include at least the following elements. First, meristems must have a capacity to specify an indeterminate cellular ground state. Second, a subset of these indeterminate cells must acquire stem cell identity, ultimately replenishing cells lost to organs and maintaining genetic integrity. Cells in this stem cell niche must self-regulate their activity to not disappear or overproliferate. Third, indeterminate cells must have the ability to acquire determinate fates associated with organogenesis.

How is the Shoot Meristem Maintained?

The indeterminate state of cells within the shoot apical meristem is dependent on the *SHOOT MERISTEMLESS (STM)* gene [4]. Recessive *stm* mutants are unable to maintain a shoot apical meristem and terminate development as seedlings with differentiated shoot apices and cotyledons that are fused at their base. With weaker *stm* alleles, adventitious meristems are occasionally formed which always originate from the position

Figure 1. The structure and function of meristems during plant development.

(A) Activation of apical meristems at germination initiates post-embryonic growth and organogenesis. (B) The shoot meristem generates all aerial tissues, while the root meristem extends the primary root. (C) Shoot meristems are organised into zones and layers. The identity of cells in layers — L1, L2, L3 — is specified during embryogenesis and maintained throughout development. Stem cells inhabit the central zone, while organ primordia are initiated at the flanks. (D) Root stem cells surround the quiescent centre, which maintains their indeterminate state.



of the embryonic shoot apical meristem between cotyledons. *STM* is expressed in the shoot apical meristem (Figure 2), but excluded from incipient organ primordia, and encodes a putative transcription factor with a homeodomain-type DNA binding domain [5].

The closely related *KNAT1* gene is partially redundant with *STM* [6]. Moreover, overexpression of *KNAT1* in leaves results in the formation of ectopic meristems on leaf margins and the adaxial leaf surface [7]. Taken together, these observations suggest that *STM* and *KNAT1* confer an indeterminate cellular state in the shoot apical meristem. *STM* expression is first observed in a few apical cells during the late globular stage of embryo development, and is subsequently observed in cells at the position of the future shoot apical meristem between the cotyledons [8].

Recessive *clavata* (*clv*) mutants have a phenotype opposite to that of *stm* plants [9]: they all have enlarged meristems which lead to distorted patterns of organ formation. Analysis of cell division patterns and frequencies in *clv* shoot apical meristems showed that the central zone dramatically expands [10], suggesting that this syndrome specifically affects a subset of indeterminate cells at the centre of the shoot apical meristem. The three *clavata* loci, *clv1–3*, have virtually identical mutant phenotypes, suggesting that the cognate gene products form a functional complex. The *CLV1–3* genes encode a leucine-rich receptor-like protein kinase, a leucine-rich receptor and a peptide ligand, respectively, and their products associate for activity [11–14]. The *CLV* genes are expressed in the central zone of the meristem in a partially overlapping pattern, with *CLV3* expressed in L1 and L2, and *CLV1* in abutting cells in L3 [11,13]. Their expression domain thus delineates cells with stem cell identity (Figure 2). Extracellular movement through the apoplastic space is required for the cell non-autonomous function of the *Clv3* peptide [15].

The discovery of the *wuschel* (*wus*) mutant identified a third activity required for meristem function. Recessive *wus* mutants are able to form meristems, but these are not sustained. However, *wus* mutants repeatedly reinitiate meristems from the axils of previously produced leaves. This results in an episodic, ‘stop-and-go’ pattern of development [16], which markedly contrasts with the *stm* phenotype. The *wus* phenotype implies that *WUS* is required to initiate and maintain stem cells in the central zone of the meristem. *WUS* encodes a homeodomain transcription factor from a different subclass than the *STM* product [17]. *WUS* expression is first observed at the 16 cell stage, where it identifies apical cells in the interior of the wild-type embryo [17]. *WUS* expression initiates, but cannot be maintained, in *stm* embryos [17]. In vegetative meristems, *WUS* is expressed in a few L3 cells at the centre of the meristem, below the expression domain of the *CLV* genes [17] (Figure 2).

WUS thus acts non-cell autonomously as an organising centre to position, promote and maintain stem cell fate in the overlying cell layers. The *wus* phenotype and the low division rates of stem cells imply that stem cells must only be able to lose their identity during their rare divisions. *wus*, *clv* double mutants are indistinguishable from *wus* single mutants during vegetative development, suggesting that these genes operate in a common pathway and that *WUS* might be a target of *CLV* signalling [16]. The *WUS* expression domain is enlarged in *clv* mutants, indicating that *CLV* negatively regulates *WUS* RNA abundance. Forced expression of *CLV3* under the control of the 35S *CaMV* promoter, which expands the *CLV3* expression domain throughout the meristem, leads to plants with a *wus* phenotype that do not accumulate *WUS* RNA, suggesting that *Clv* signalling represses *WUS* expression [18], and that *CLV3* expression is limiting for *Clv* signalling. *WUS* expression under the control of the *CLV1* promoter leads to expansion of

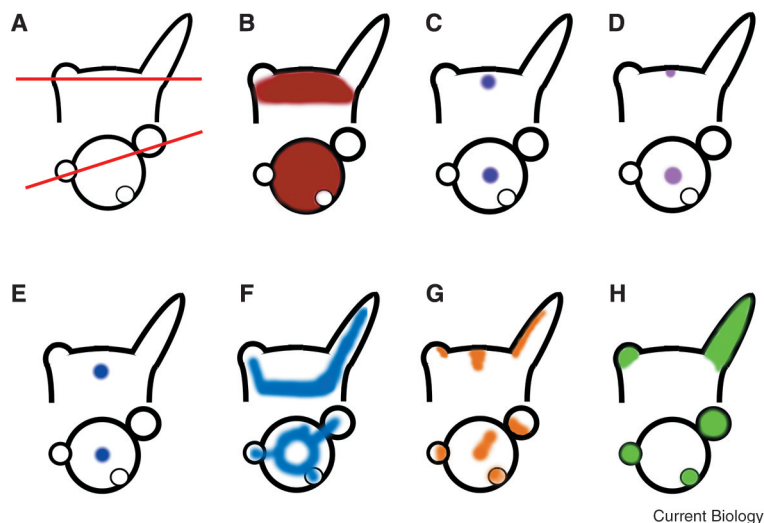


Figure 2. Structure and gene expression patterns in the shoot meristem.

Expression patterns of genes involved in meristem function. (A) Schematic showing the orientation of sections in panels B–H. (B) *STM* is expressed in the indeterminate cells of the meristem. (C) *CLV3* is expressed by stem cells in the L1 and L2. (D) *CLV1* is expressed by stem cells in L2 and L3. (E) *WUS* is expressed in an organising centre below the stem cells. (F) *HAM* expression in the pro-vasculature. (G) *PHB* expression in stem cells and the adaxial domain of incipient organs. (H) *AS1* expression exclusively in incipient organs.

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the meristem and the expression domain of *CLV3* [19], suggesting that *CLV3* is controlled by *WUS*.

Stem Cell Homeostasis through Feedback Loops

These observations led to the development of a model for meristem maintenance and stem cell specification (Figure 3). This model has two key elements: *STM* and related genes are necessary to specify the indeterminate state of cells in the shoot apical meristem, and hence allow for cells in the shoot apical meristem to acquire opposing developmental fates — stem cell or determinate cell in organ primordia. *WUS* and *CLV3* comprise a regulatory loop, such that *WUS* promotes *CLV3* expression, while the Clv complex negatively regulates *WUS*. Within this loop, *WUS* promotes stem cell identity, while Clv activity restricts stem cell identity and promotes the transition of stem cells to the indeterminate ground state, from which cells are recruited into organ primordia.

The dramatic *wus* and *clv* phenotypes best illustrate the requirement for such a negative feedback loop to tightly control stem cell numbers. If stem cell numbers increased too much, meristem expansion would disrupt normal phyllotaxy in leaf organogenesis. While this does not acutely threaten the plant's survival — some ornamental plants have fasciated meristems similar to *clavata* mutants — it is likely to strongly affect the individual's ability to compete for light in complex, tiered plant communities. Conversely, a minimum number of stem cells must be maintained to ensure continuous, as opposed to episodic, growth. Particularly for plants in temperate zones with distinct growing seasons, continuous growth is a competitive advantage when seasonal conditions conducive for growth prevail. Stem cell homeostasis is maintained astonishingly well within tight bounds, as the longevity of the shoot apical meristem of trees demonstrates.

Specifying Indeterminacy

To determine whether either *WUS* or *STM* suffices to specify stem cells and indeterminacy, they were

expressed in lateral organs [20–22]. Expression of *STM* under the control of the 35S *CaMV* promoter disrupted the shoot apical meristem and inhibited leaf growth and development [23], and expression of *WUS* in leaf primordia completely suppressed their development [19]. Activation of just *STM* in organ primordia after germination was sufficient to inhibit their differentiation, induce the expression of *KNAT1* and *KNAT2* and promote leaf lobbing, similar to the effects seen when *KNAT1* was overexpressed [7]. But ectopic meristems were not observed, and *STM* expression did not turn lateral organs into meristems, activate the stem cell marker *CLV3*, or lead to uncontrolled proliferation. So although *STM* is required to maintain *WUS* expression, *STM* is not sufficient to induce stem cell identity when expressed in leaf primordia.

Activation of *WUS* in leaf primordia inhibited leaf expansion, did not activate *KNAT1* or *KNAT2* [22] expression, and did not consistently activate *CLV3* expression [21,22]. But when *STM* and *WUS* were both activated, the *CLV3* promoter was activated throughout leaf primordia or cotyledons [21,22], and normal leaf development was inhibited. Combined *STM* and *WUS* activities are thus required to confer stem cell identity, but why were no ectopic organs formed? In these experiments, *STM* and *WUS* were expressed or post-translationally activated uniformly throughout the organ. But when *WUS* was delivered in a localised manner in organs uniformly expressing *STM*, transient ectopic organogenesis was observed [20], indicating that organogenesis depends on a spatial restriction of stem cell fate.

Ectopic meristems and organogenesis are not observed when *STM* and *WUS* are expressed throughout a leaf primordium, because this transforms the organ into a structure akin to the central zone of the shoot apical meristem, where organogenesis is not observed. This transformation is incomplete, however, because although differentiation is inhibited in the leaf primordium, it is still recognisable as a lateral outgrowth and growth is not sustained, as in *clv* mutants. *WUS* may also have additional functions because its

overexpression throughout the plant leads to the formation of ectopic, somatic embryo-like shoots even in roots, suggesting that *WUS* specifically promotes embryogenesis or shoot development [24].

Radial Patterning and Gibberellins

Obviously, *STM* and *WUS* are not the whole story, and further players that contribute to meristem function were identified recently. Stuurman *et al.* [25] described the *hairy meristem (ham)* mutant in *Petunia*, which does not maintain its shoot apical meristem. Young *ham* plants were indistinguishable from the wild type and, when development terminated after formation of some leaves, organogenesis ceased, but the expression of a marker for indeterminate cell identity in the shoot apical meristem, *petunia STM*, decayed only gradually. But *HAM* is distinct from *petunia WUS*, which has the same episodic pattern of development as *Arabidopsis WUS*; furthermore, *ham, wus* double mutants initially have a *wuschel* phenotype, but later terminate as *ham* apices do, indicating that the two genes operate in parallel pathways.

HAM is expressed in the developing pro-vasculature of the stem and of leaf primordia (Figure 2). In *ham* mutants, the expression levels of *petunia WUS* and *STM* decay rapidly after termination of organogenesis and become spatially disorganised. It is likely that *HAM* is, or generates, a cell non-autonomous retrograde signal from the differentiating determinate tissues — stems and leaves — produced by the shoot apical meristem. *HAM* encodes a GRAS family putative transcription factor and has two presumptive orthologs in *Arabidopsis*, *AtSCL6* and *AtSCL15*, for which no mutant phenotypes have been reported yet [25].

GRAS transcription factors have been implicated in pathways mediating signalling of the plant growth regulator gibberellic acid, the radial patterning of the root and maintenance of root meristem stem cells [26–29]. Although it is not clear whether GRAS genes not yet known to be directly associated with gibberellic acid-signalling mediate gibberellic acid-dependent functions, gibberellins do appear to be closely involved in regulating meristem function [30–32]. Expression of gibberellic acid biosynthetic genes is suppressed in the shoot apical meristem, but up-regulated in incipient primordia [30]. This is functionally significant, as gibberellic acid sprays suppress, while inhibition of gibberellic acid signalling or biosynthesis enhances, *KNAT* misexpression phenotypes, and constitutive gibberellic acid signalling enhances weak *stm* phenotypes [30]. This indicates that gibberellic acids are important effectors of the indeterminate–determinate dichotomy and may function by regulating the stability of proteins [26] encoded by indeterminacy-promoting genes.

How Do Meristems Continuously Produce Organs?

Plants entertain meristems to produce leaf and reproductive organs, not just to maintain themselves. The leaf organs produced on the flanks of the meristem are determinate organs, and so organogenesis involves a switch from indeterminate to determinate cell fate (Figure 4). A crucial regulator involved in this transition is the *ASYMMETRIC LEAVES1 (AS1)* gene

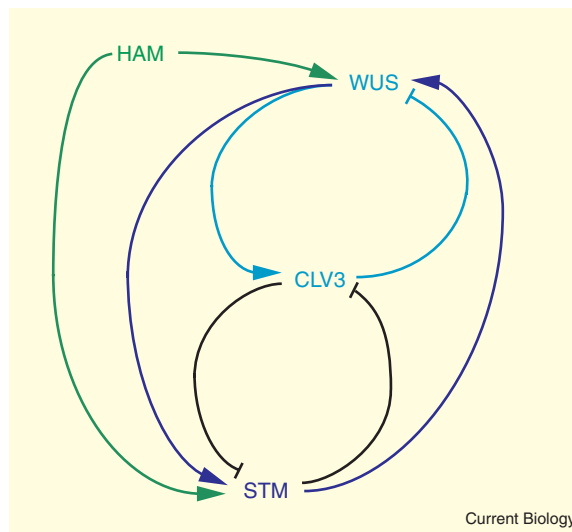


Figure 3. Model for meristem gene interactions.

WUS promotes *CLV3* expression, which in turn restricts *WUS* expression. *WUS* and *STM* are mutually required for each others' expression and both are necessary to specify stem cells. *CLV* and *STM* antagonise each other. *HAM* is also required to maintain *STM* and *WUS* expression.

[33–35]. *AS1* encodes a putative transcription factor, a member of the Myb family and closely related to the *Antirrhinum PHANTASTICA (phan)* gene product [33,36]. Mutant *as1* plants have patches of small, proliferating cells on their leaf lamina and form occasional shoot apical meristems on the leaf petioles, indicating that at least some indeterminate cells persist within the determinate lateral organ. This phenotype is reminiscent of the effects of forced ectopic expression of *KNOX* genes such as *KNAT1* [7].

KNAT1 and *KNAT2* are indeed ectopically expressed in leaves of *as1* plants [33–35], although they are initially down-regulated just as in the wild type upon establishment of the leaf primordia [34]. This suggests that *AS1* is involved in enforcing the switch from indeterminate to determinate identity by ensuring that *KNOX* gene expression remains extinguished. But *as1* leaves are quite normal compared to those in plants expressing *STM* in lateral primordia, indicating that only some aspects of indeterminacy are under control of *AS1*. In *as1, stm* double mutants, the *stm* meristem phenotype is suppressed, and in *stm* plants, *AS1* expression extends from its normal domain in determinate primordia into the shoot apical meristem, indicating that a key function of *STM* in the shoot apical meristem is to suppress the expression of *AS1* [33].

The maintenance of shoot apical meristem activity is tightly coupled to the progression of lateral organ development (Figure 4). One aspect of organ development — the acquisition of appropriate dorso-ventral polarity — appears to be under particularly close surveillance, and lack of dorsal identity generates a retrograde signal that shuts down the shoot apical meristem. Developing leaf organs in *Antirrhinum phan* mutants lack dorsal (adaxial) attributes and the shoot apical meristem arrests [36]. Forced and ectopic

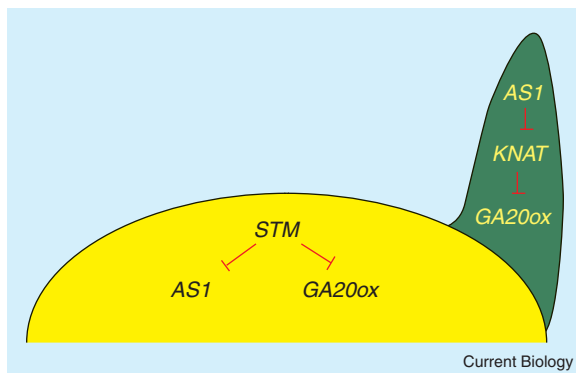


Figure 4. The transition from indeterminate to determinate cell identity.

In meristems *STM* suppresses *AS1*, while in incipient primordia *AS1* suppresses *KNOX* genes such as *KNAT1*. Gibberellin (GA) synthesis correlates with determinate cell fate and is suppressed in the meristem.

expression of the ventral (abaxial) factors *KANADI*, *YABBY3* or *FILAMENTOUS FLOWER* under control of the 35S *CaMV* promoter leads to abaxialisation of the leaf and extinguishes the *Arabidopsis* shoot apical meristem [37–39]. In contrast, semi-dominant alleles of *PHABULOSA (PHB)* and *PHAVOLUTA (PHV)* have adaxialised leaves that form axillary meristems around the base of both the upper and lower leaf surfaces [40,41], but the shoot apical meristem remains active.

Taken together, these observations, together with lineage analysis and the ontogeny of embryonic shoot apical meristem patterning [2,8], highlight the fact that the shoot apical meristem and the adaxial side of developing leaf primordia have inseparable, shared functions that promote meristem formation. The hypothesised retrograde signalling pathway has not been identified, but could involve genes such as *HAM*, *REVOLUTA (REV)*, *PHB*, *PHV* and *PINHEAD/ZWILLE* [25,42] (see below).

Morphogens and Enforcers in Meristem Patterning

One of the major unresolved questions of meristem function is the nature of the mechanisms by which signalling occurs to establish specific patterns of gene expression. Recently, two candidate mechanisms have come to the fore involving diffusible lipophilic molecules and RNA.

As yet unidentified small hydrophobic molecules have been proposed as ligands for the Rev, Phb, Phv class of putative transcription factors with homeo-domain-related DNA binding motifs and START-domains [41], with a *modus operandi* analogous to animal steroid hormone receptors. START domains have been shown to bind sterols in animal systems [43]. *PHB* is initially expressed at a low-level in the central zone and at a low and uniform expression in P1 organ primordia (Figure 2). This pattern evolves towards high-level expression on the adaxial side of incipient organs [41]. Given this expression pattern, it was proposed that a diffusible, hydrophobic ligand for Phb or Phv START domain originates from the central zone. Ligand binding would then activate the

cognate factor, which would autoactivate its own expression in addition to that of dependent genes. This would result in a positive feedback loop and lead to preferential accumulation of *PHB* on the adaxial side of the primordium [41]. The *PHB-d* mutant phenotype would then be explained by a constitutive activation of the transcription factor when the START domain is mutated, resulting in autoactivation throughout the developing organ and hence adaxialisation of the leaf. This model is further supported by the semi-dominant and temperature-sensitive nature of the mutants [40].

A second candidate mechanism for patterning emerged from the startling observation that one of the micro RNAs (miRNAs) identified in *Arabidopsis* is complementary to the sequence encoding the small region of the START domain affected in all dominant alleles [44]. miRNAs are a recently discovered, novel class of non-coding, regulatory RNAs found in animals and plants [45,46]. These ~22 nucleotide long RNAs function as guide RNAs for the DICER enzyme to degrade specific target sequences 100% complementary to the miRNAs [45]. Two of the more abundant and conserved miRNAs identified to date in plants are complementary to *AtSCL6* and to *PHV*, *PHB* and *REV* [44,47]!

This indicates that small RNA-mediated processes are involved in the control of stem cell maintenance and leaf polarity. This is supported by the phenotypes of plants with recessive mutations in the related *ARGONAUTE (AGO)* and *PINHEAD/ZWILLE (PNH)* genes [42,48–50], which are involved in RNA-mediated gene silencing processes [51]. *AGO* and *PNH* have partially redundant functions required for sustained meristem activity and *STM* accumulation [42]. Conversely, forced *PNH* expression exclusively outside its normal expression domain conditions ectopic indeterminacy and converts the cotyledon axis into a rudimentary branch with a meristem at the centre [52], indicating that *PNH* is involved in promoting meristem establishment.

Small, short interfering RNAs (siRNA) are distinct from miRNAs and are involved in epigenetic regulation mediated by DNA and histone methylation. At least one member of the ARGONAUTE family, *AGO4*, is involved in specifying epigenetic regulation relevant to floral patterning [53]. Therefore, it is likely that several small RNA-mediated processes participate in precipitating, enforcing or maintaining patterning decisions. If miRNAs are morphogens that mediate the fixation of leaf polarity, the *phb-d* phenotype might be explained as follows: because of the mismatch to the mutant *phb-d* target, the miRNA would be unable to direct the destruction of the transcript on the abaxial side of the leaf, thus leading to its adaxialisation.

It is difficult, however, to imagine how miRNAs could function as morphogens without a satisfactory answer as to how their own expression pattern is generated. So a simpler interpretation of the role for RNA degradation of *PHB* or *AtSCL6* might be that miRNA-mediated RNA turnover is required to enforce developmental decisions, reminiscent of the requirement for the ubiquitin-dependent turnover of proteins during the cell cycle.

Root Meristems

Root and shoot meristems have a fundamentally similar radial organisation, in that they both produce cells destined for epidermal, ground and vascular tissue fates (Figure 1). In the root apical meristem, however, stem cells entirely surround the cells that position the stem cell niche. The cells in the quiescent centre position the stem cell niche and are required cell-nonautonomously to prevent the differentiation of the surrounding stem cells, or initials [54]. Polar auxin transport is required to position and restrict stem cells along the proximal-distal primary organ axis, and an expanded zone of elevated auxin concentration leads to an expansion of the stem cell population formally similar to the *clv* phenotype [55].

While radial and proximo-distal patterning mechanisms in roots are beginning to be understood, the genes responsible for patterning the meristem and maintaining stem cells have remained obscure, particularly as none of the mutations affecting the shoot apical meristem appeared to affect root meristems. Recently, however, the function of *SCARECROW* (*SCR*) in stem cell maintenance and positioning of the stem cell niche has been more closely characterised [29]. Selective expression has shown that *SCR*, which is expressed in the quiescent centre, is required here to position the stem cell niche and, therefore, to maintain the root apical meristem. This raises the exciting possibility that *SCR* and related genes have a shared, more fundamental role in root and shoot meristem establishment or maintenance. In terms of maintaining the stem cell niche, the *scr* and *ham* mutant phenotypes are similar [25,29,56], and, moreover, both are expressed in proximity to the developing vascular tissue. However, no overt defect in leaf polarity — the shoot equivalent of radial patterning — has been reported for *ham* mutants.

Perspectives

Taken together, the continued analysis of meristems and meristem gene function has revealed some fundamental properties. Meristems thrive on antagonism: negative feedback loops and juxtaposition of cells with divergent developmental fates are characteristic features. Stem cell homeostasis depends on the antagonism between *CLAVATA* and *WUSCHEL* genes. Continued activity of indeterminate meristem cells depends on proper development of the determinate organ. Growth of the determinate organ depends on the juxtaposition of adaxial and abaxial leaf surfaces. No single ‘master regulator’ has been identified sufficient to specify shoot or root meristems originating from any single cell, and from the emerging paradigm for meristem function it is unlikely that such a gene exists. Regulatory systems based on negative feedback loops are inherently quite stable because they are self-correcting. The capacity to maintain a dynamic equilibrium in meristem cell populations is critical for the ability to adapt to changing environments.

Elaborate signalling and effector networks are required to convey and respond to such antagonising signals. No clear candidates for morphogens have yet emerged, but small RNA-dependent processes are

good candidate mechanisms to effect changes in expression levels or patterns in the short term or for longer periods by epigenetic mechanisms. Root and shoot meristems may share fundamental mechanisms, perhaps coupling radial patterning to maintenance of their respective stem cell niches.

Acknowledgements

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