Apical meristems: the plant's fountain of youth

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Summary

During postembryonic development, all organs of a plant are ultimately derived from a few pluripotent stem cells found in specialized structures called apical meristems. Here we discuss our current knowledge about the regulation of plant stem cells and their environments with main emphasis on the shoot apical meristem of *Arabidopsis thaliana*. Recent studies suggest that stem cells are localized in specialized niches where signals from surrounding cells maintain their undifferentiated state. In the shoot meristem, initiation of stem cells during embryogenesis, regulation of stem-cell homeostasis and termination of stem-cell maintenance during flower development appear to primarily involve regulation of the stem-cell niche. ***BioEssays*** **25:961–970, 2003.**

Introduction

One of the fundamental differences between plants and animals is their mode of development. While the outcome of animal embryogenesis is a mini-edition of the adult animal, with all organs being at least initiated, plant embryogenesis results in a simple structure consisting of the root apical meristem (RAM), the embryonic root, the hypocotyl, one or two cotyledons (embryonic leaves), and the shoot apical meristem (SAM) (Fig. 1). All other organs of the mature plant are formed postembryonically. These distinctive developmental strategies of plants and animals concur with different tasks of their stem cells. Whereas the major task of an animal stem cell is to replenish highly specialized body cells with a limited life span such as blood and skin cells, plant stem cells provide the material for the formation of entire new organs such as leaves, flowers and roots.

Stem cells are relatively undifferentiated cells defined by their abilities for self-renewal and for generating differentiated cells. In animals, the emerging picture is that stem-cell populations are maintained in an undifferentiated state by signals from surrounding cells in a microenvironment termed stem-cell niche.[1–3] The stem-cell niche comprises the stem cells, their signaling neighbor cells, hereafter referred to as ''inductive niche cells'', and the effective range covered by the signal.[2]

What is the location and function of stem cells in higher plants? Stem cells in the SAM provide the cells required for continuous formation of the shoot axis and lateral organs, such as leaves, flowers and side branches. Similarly, all cells in the root are ultimately derived from stem cells in the RAM. Floral meristems are specialized axillary shoot meristems where the stem cells give rise to a limited number of floral organs. While stem cells in apical meristems increase the height and the number of organs of the plant, stem cells in lateral meristems provide the cells that result in an increase in the girth of the shoot axis and ultimately enable the Plant kingdom to include the largest land organisms.[4,5] These lateral meristems have the shape of cylindrical sheets, which encircle the plant axis and give rise to specialized tissues: the vascular cambium, which is sandwiched between the xylem and phloem gives rise to the wood and the bast, and the phellogen or cork cambium, which generates a protective layer on the outside of the shoot axis.

Most of our current knowledge about the mechanisms regulating stem-cell activity in plants has been obtained from studies of the apical meristems and we will focus on these in this review. We will mainly draw on results from the herbaceous thale cress *Arabidopsis thaliana*, which is a favorite organism of plant geneticists, but will include work from other species where appropriate.

General properties of the SAM

Based on clonal studies in several species, all cells during postembryonic shoot development are ultimately derived from no more than three long-term stem cells in each of the three histogenic cell layers (L1–L3) of the shoot meristem.[6–8] For geometrical reasons, the stem cells must reside at the summit of the central zone (CZ) of the dome-shaped shoot meristem where cells divide relatively infrequently (for a detailed

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DOI 10.1002/bies.10341
Published online in Wiley InterScience (www.interscience.wiley.com).

Abbreviations: CZ, central zone; GA, gibberellin; LRR, leucine-rich repeats; OC, organizing center; PZ, peripheral zone; QC, quiescent center; RAM, root apical meristem; SAM, shoot apical meristem.

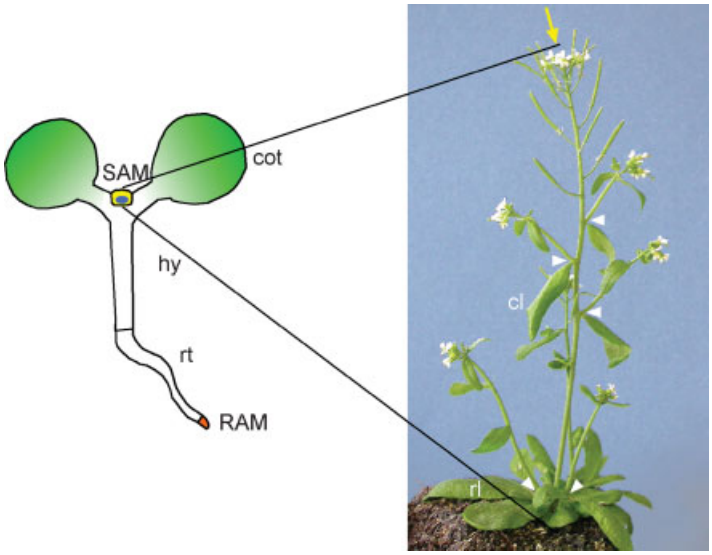

Figure 1. *Arabidopsis* shoot development. The seedling (left) comprises two cotyledons (cot), the SAM (yellow with blue *WUS* expression domain), the hypocotyl (hy), the root (rt) and the RAM (red). In the mature plant (right), the main inflorescence meristem (yellow arrow) has produced cauline leaves (cl) and flowers, which in part have developed into siliques. In the axils (arrowheads) of rosette leaves (rl) and cauline leaves side inflorescences develop. All above-ground organs of the mature plant are ultimately derived from the seedling SAM.

description of the SAM organization see legend to Fig. 2).[6] The expression domain of the *CLAVATA3* (*CLV3*) gene coincides with this position and thus is used as an operational marker for stem cells in the SAM.[9] (Note, however, that *CLV3* function is dispensable for stem-cell activity, see below.) Clonal analyses also showed that stem cells are not permanent but can differentiate if they are displaced from the summit indicating that stem-cell identity is not an inherent property of a given cell lineage but rather is conferred upon a cell by positional cues.[6,10] The stem cells are surrounded by their differentiating daughters that divide more frequently before they are incorporated into organ primordia at the flanks of the meristem. Destruction of central portions of the SAM leads to regeneration of a functional meristem from the flank of the previous one, demonstrating the ability of these cells to revert to the stem-cell state and the shoot meristem's large potential for self-regeneration.[5]

Even though the constituting cells progress through a continuum of developmental states, the workings of the shoot meristem can be formally divided into three steps: (1) local maintenance of stem cells, (2) amplification of stem-cell daughters and (3) initiation of organ primordia. Recent molecular and genetic studies in *Arabidopsis* have begun to elucidate the underlying molecular mechanisms.

The first step: maintaining stem cells

How are the stem cells in the shoot meristem maintained? In *wuschel* (*wus*) mutants, no self-maintaining stem cells are established, rather the cells at the apex differentiate.[11] The *WUS* gene encodes a homeodomain transcription factor and is expressed in a small region in the center of the SAM, termed organizing center (OC).[12] Ectopic *WUS* expression in organ primordia inhibits organ formation and induces expression of the stem-cell marker gene *CLV3*.[13] These findings lead to the model that *WUS*-expressing cells non-cell autonomously specify the cells at the summit as stem cells and thus function as an inductive niche cells.

Stem-cell maintenance and the onset of differentiation occur in close proximity within the SAM and therefore need to be precisely balanced to maintain the size of the stem-cell pool throughout the plant's life. Mutations in the *CLV* genes (*CLV1, CLV2, CLV3*) disrupt this balance and result in an enlarged CZ where a surplus of stem cells accumulates.[9,14–17] Genetic and biochemical analyses showed that the three *CLV* genes act in a common signaling pathway.[15,17] *CLV1* encodes an LRR-receptor kinase, *CLV2* a similar protein lacking the intracellular kinase domain and *CLV3* encodes a small peptide.[9,18,19] CLV3 has been suggested to function as a ligand that is secreted from the stem cells and binds to the CLV1–CLV2 receptor complex thereby activating downstream signaling events.[20–24]

What is the target of *CLV* signaling and how does this affect the size of the stem-cell pool? The *WUS* expression domain is enlarged in *clv* mutants and transgenic *WUS* expression in a similarly enlarged domain in wild-type background phenocopies the *clv* mutant defect.[13] In addition, ectopic expression of *CLV3* suppresses *WUS* transcription from its own promoter but not from a heterologous promoter.[23,25] These findings suggest that *CLV* signaling restricts the size of the OC by repressing *WUS* transcription in neighboring cells. With *WUS* inducing *CLV3* expression (see above), the *WUS–CLV3* interaction establishes a negative feedback loop between

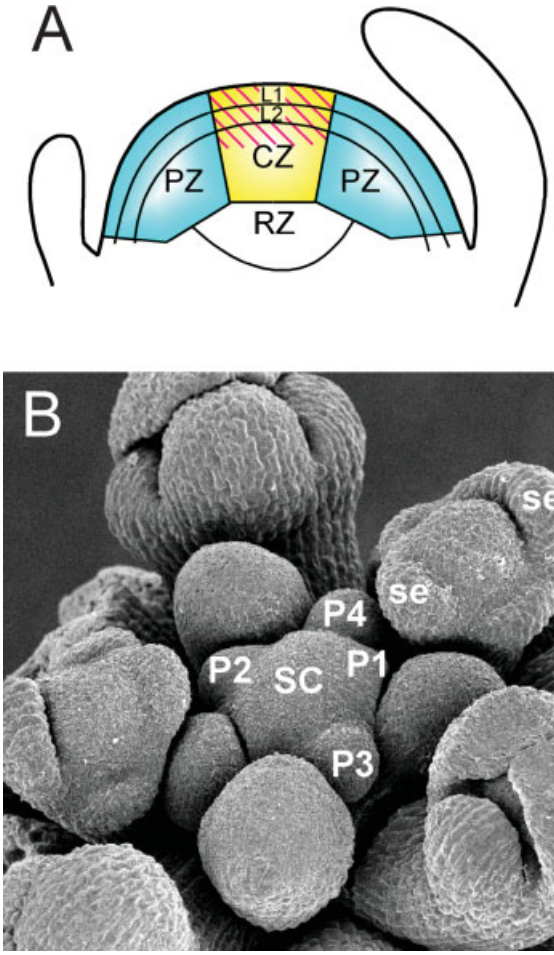

Figure 2. The *Arabidopsis* shoot meristem. **A:** Based on cytohistological studies the SAM can be subdivided into layers and zones.[5,94] The central zone (CZ), which resides at the summit of the SAM contains relatively large and slightly more vacuolated cells, which divide relatively infrequently. Surrounding the CZ is the peripheral zone (PZ) and underneath the rib zone (RZ). The PZ consists of small cells that divide frequently. The cells in the RZ and contribute primarily to the central tissues of the shoot axis. Higher plant shoot meristems have a tunica-corpus structure. In most angiosperms, the tunica consists of two layers (L1, L2) where the cells generally divide in anticlinal orientation (perpendicular to the surface) and thus form two sheets of clonally distinct tissue.[95] The L1 gives rise to the epidermis and the L2 gives rise to the subepidermal layer. Cells in the underlying L3 (corpus) divide periclinally and anticlinally and generate the internal tissues of lateral organs and shoot axis. The presumed stem-cell position is indicated by the hatched area. **B:** Scanning electron micrograph of an *Arabidopsis* inflorescence meristem. The stem cells (SC) reside in the center of the meristem. At the periphery of the meristem, formation of organs (here: flower buds) takes place. By convention, primordia are named P1, P2, P3 etc. with P1 being the youngest visible primordium and P2 the second youngest etc. In older floral buds, formation of the first set of floral organs, the sepals (se), is visible.

the stem cells and the OC with the potential to dynamically adjust the size of the stem-cell population (Fig. 3A).[13] If, for example, the stem-cell number has become too large, *WUS* expression is downregulated by the increased *CLV3* signal, resulting in a reduction of the number of stem cells and a concomitant reduction of *CLV3* expression. Findings from petunia and maize suggest that the mechanisms regulating stem-cell homeostasis are conserved in higher plants.[26,27]

Recent studies suggest that the CLV3 peptide moves away from the stem cells.[22,23] This poses the problem of how CLV3 is prevented from repressing *WUS* transcription in the OC? Ectopic expression studies indicate that binding to CLV1 can limit the range of movement of CLV3, suggesting that the CLV1 receptor on cells surrounding the OC may effectively protect the OC from CLV3 reaching it and hence from repression of *WUS* transcription.[23] A further level of CLV signaling control may take place inside the cells. For example, genetic studies suggest that protein phosphatases *KAPP* and *POLTERGEIST* dampen *CLV* downstream signaling.[28–30]

Thus, the balance between stem-cell maintenance and differentiation is struck by a fine-tuned feedback regulation between the stem cells and their inductive niche cells.

The second step: amplification of stem cells daughters

The first sign of lateral organ primordia is detected at the flanks of the SAM at some distance from the stem-cell pool, suggesting that organ formation is repressed in the intervening region of the meristem dome. How is this repression brought about? A major factor that protects the cells in the meristem dome from premature differentiation is SHOOT MERISTEMLESS (STM). *STM* encodes a homeodomain transcription factor of the KNOX (KNOTTED-like HOMEOBOX) protein family and is expressed throughout the shoot meristem but not in incipient organ primordia.[31] *KNOX* gene overexpression in various plant species results in altered leaf morphology due to delayed cell differentiation and, in severe cases, to the formation of ectopic shoot meristems, suggesting that *KNOX* genes play an important role in promoting meristematic cell

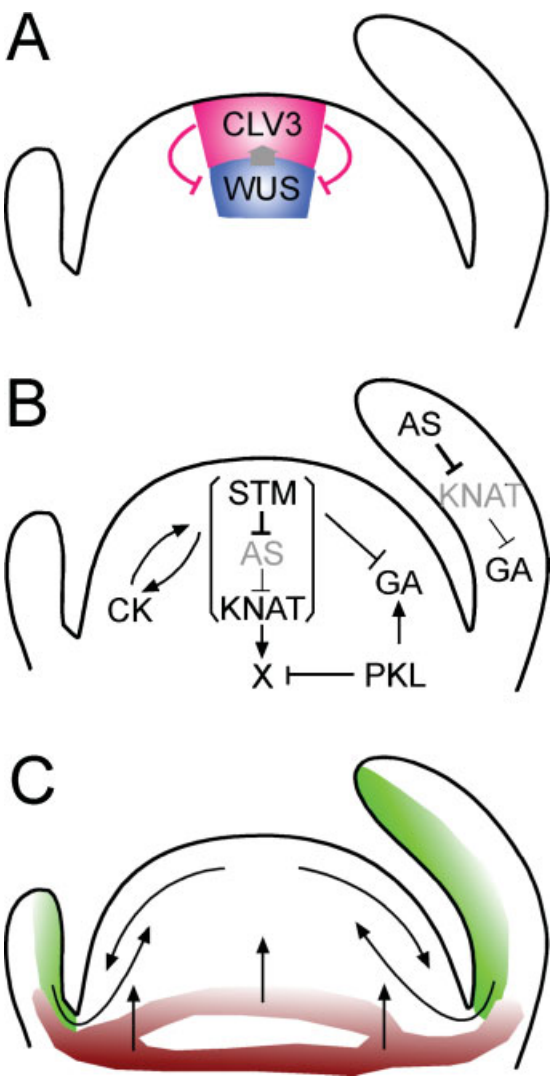

Figure 3. Regulatory pathways in the shoot meristem. **A:** Local regulation of stem-cell maintenance by the *WUS–CLV* feedback loop. The *WUS*-expressing OC (blue) confers stem-cell identity upon the overlying cells (pink), which in turn restrict the *WUS* expression domain via the *CLV* signaling pathway. **B:** Factors regulating meristem maintenance and organ initiation. In the center of the SAM, *STM* represses *AS* genes, thereby allow- ing expression of *KNAT* genes. *STM* and *KNAT* repress GA biosynthesis in the SAM. In young leaf primordia, *STM* expression is downregulated, *AS* gene products are present and repress *KNAT* gene expression while GA biosynthesis is upregulated. Positive interactions between Cytokinin (CK) and *KNOX* genes promote SAM activity. It is suggested that *PKL* promotes the transition from a meristematic to a differentiated cell state by repressing *KNOX* target genes and through GA biosynthesis. **C:** The shoot apex affects adaxial-abaxial polarity of lateral organs. Vice versa, cells in the adaxial part of the young leaves (green) and in deeper layers of the stem and the young leaves (symbolized by a brown ring of *HAM* expression) promote shoot meristem maintenance via yet unidentified signals. For details see text.

identity.[32] *stm* mutant seedlings display fused organs that appear to consume the cells of the SAM.[33,34] Genetic studies indicate that *STM* restricts organ initiation to defined sites at the meristem flanks by repressing two genes that promote organ formation, *ASYMMETRIC LEAVES1* (*AS1*) and *AS2* (see below), which in turn repress the expression of *KNOX* genes.[35–38] Thus, *STM* prevents meristem differentiation by indirectly allowing *KNOX* gene expression (Fig. 3B). In contrast to *STM,* mutation of a single downstream *KNOX* gene, *KNAT1* (*KNOTTED*-like from *ARABIDOPSIS THALIANA1*), does not result in meristem termination, suggesting redundancy at the level of these downstream components.[36,39,40]

Comparison of *STM* and *WUS* functions indicates that both genes have largely independent and complementary roles in SAM regulation despite the fact that the respective mutants display similar defects.[41,42] While *WUS* specifically functions in the local control of stem-cell identity in the SAM center, *STM* appears to be required throughout the meristem dome to restrict organ initiation to the flanks of the SAM.[41,42] A plausible effect of *STM* function is to allow the stem-cell daughters to amplify to sufficient numbers before organ formation takes place.[42] This would minimize the requirement for stem-cell divisions and the concomitant accumulation of mutations by DNA replication in the stem-cell pool.

The third step: organ initiation

Organ formation takes place in the peripheral zone (PZ) of the shoot meristem where a group of 15–30 cells derived from all three meristem layers becomes assigned to an incipient organ primordium.[7,8] One of the first signs of organ initiation is the downregulation of *STM* expression in the organ founder cells, while it continues in the rest of the SAM.[31] This presumably allows the onset of *AS1* and *AS2* gene expression in organ primordia, which in turn repress meristematic cell fates by downregulating the *KNOX* genes *KNAT1, KNAT2* and *KNAT6* (Fig. 3B).[35–38] *AS1* encodes a MYB domain protein and *AS2* encodes a novel protein characterized by cysteine repeats and a leucine zipper.[35,43] Thus, the decision between meristem and organ cell fates depends on the balance between two antagonistic sets of repressors, the *STM* gene and the *AS* genes. How *STM* expression is initially downregulated at the sites of organ initiation remains open. However, elegant studies have implicated the growth factor auxin in organ site selection (see below).

Epigenetic regulation of cell states

As described above, the cells at the shoot apex progress through a succession of differentiation states. How are the corresponding changes in their gene expression programs brought about? Several examples indicate that regulation of chromatin structure plays an important role.

Mutations in the *FASCIATA* (*FAS*) genes result in an enlarged SAM with a disrupted layer structure that tends to fasciate and the mutants form shorter roots.[49] The *FAS1* and *FAS2* genes encode two subunits of the CAF-1 (chromatin assembly factor-1) complex which in animals has been implicated in nucleosome assembly during DNA replication and repair. In *fas* shoot meristems, the *WUS* expression domain is variably expanded, suggesting that chromatin structure is involved in regulating the expression state of the *WUS* gene.[49] In the RAM, *FAS1* and *FAS2* are similarly required to maintain the cellular organization and the expression state of the *SCARECROW* gene.

Mutations in the *PICKLE/GYMNOS* (*PKL*) gene were identified independently in different developmental contexts: carpels of *pkl* mutants display delayed maturation, which leads to ectopic ovule formation in a *crabs claw* mutant background[44] and *pkl* mutants fail to exit embryonic identity during germination.[45] These data suggest that *pkl* mutants are delayed in the progression from relatively undifferentiated to differentiated cell fates. The *PKL* gene encodes a member of the CHD3 chromatin remodeling factor family.[44,46] The related dMi2 protein is involved in the initiation and maintenance of homeotic (*HOX*) gene repression during *Drosophila* development.[47] It is therefore plausible that *PKL* could facilitate the switch from meristematic to differentiated gene expression programs by altering the chromatin structure of the cell.[44] The effects of *PKL* on cell differentiation may in part be mediated through the hormone gibberellin (GA, see below), since a key enzyme of GA biosynthesis is repressed in the *pkl* mutant and the *pkl* seedling phenotype is enhanced by GA inhibitors.[45,48] Finally, mutations in the *SPLAYED* gene, which encodes a homolog of SWI/SNF chromatin remodeling ATPases, affect various developmental aspects, including shoot meristem maintenance, meristem identity and floral homeotic gene expression.[96]

In conclusion, these examples indicate that changes in chromatin structure play a crucial role for cell fate transitions in the shoot meristem.

Generation of the SAM during embryogenesis

How does the shoot meristem arise during embryo development? Molecular and genetic studies indicate that the establishment of a functional shoot meristem involves two largely independent steps, the development of the stem-cell niche and the central–peripheral partitioning of the embryo apex.

The earliest indication of SAM formation during embryogenesis is the onset of *WUS* expression in the four subepidermal cells of the apical domain of the 16-cell embryo (Fig. 4).[12] Subsequently, this expression domain is confined to the presumptive OC position in the shoot meristem primordium through asymmetric cell divisions. At later stages, WUS function is required for *CLV3* expression.[50] This suggests that the first event in embryonic shoot meristem formation is the generation of a cell lineage that will give rise to the inductive niche cells, which in turn induce the overlying cells as stem cells.

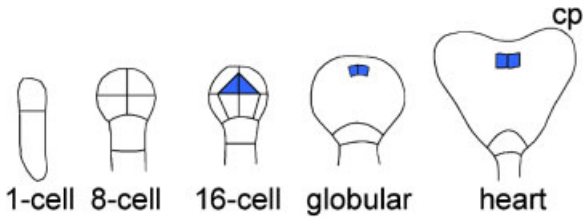

Figure 4. The early stages of *Arabidopsis* embryogenesis are characterized by a stereotypic cell division pattern. *WUS* expression (gray) starts in the 16-cell-stage embryo in the inner cells of the apical half. During further embryogenesis, *WUS* expression persists in the SAM primordium. At heart stage, the cotyledon primordia (cp) appear.

The partitioning of the embryo apex starts during the globular stage when it is divided into the peripheral cotyledonary primordia and the central shoot meristem primordium. This process requires the successive activation of a set of genes involved in repressing the outgrowth of the meristem region. Expression of the *CUP-SHAPED COTYLEDON* genes (*CUC1* and *CUC2*), both encoding putative NAC-domain transcription factors, is switched on in a few apical cells of the globular embryo, and then soon spreads in a stripe separating the two incipient cotyledon primordia.[51–53] These dynamics suggest that the *CUC* expression patterns may reflect the elaboration of bilateral symmetry, rather than following a pre-existing pattern. The *cuc1 cuc2* double mutant lacks an embryonic SAM and has almost completely fused cotyledons.[53] The spatial information for the expression of the *CUC* genes appears to be provided by the distribution of the growth regulator auxin, since it is affected in mutants disrupting directional auxin transport.[54] CUC1 and CUC2 in turn activate *STM* expression, leading to the repression of outgrowth in the region between the cotyledon primordia.[51,52] Conversely, organ-promoting genes such as *AS1* become expressed in the founder cells of the cotyledons.[35]

How is the shoot meristem integrated into the overall structure of the embryo? Studies of the *ZWILLE/PINHEAD* (*ZLL*) gene may provide some insights. During embryonic development, a certain percentage of *zll* mutants fail to establish a functional SAM, and, in addition, postembryonically axillary meristems are not always formed.[55–57] At the molecular level, the expression of SAM regulators in *zll* embryos is spatially deregulated and eventually terminates entirely resulting in differentiation of the presumed shoot meristem cells.[57] *ZLL* encodes a putative RNA-binding PAZ (PIWI ARGONAUTE ZWILLE)-domain protein which displays

homology to rabbit translation initiation factor eIF2C.[55,57–59] Related proteins from several species have recently been implicated in translational repression and RNA interference,[60–64] as has the *ZLL*-homolog from *Arabidopsis ARGONAUTE1* (*AGO1*).[64–66] However, no such role could be ascribed to *ZLL*.[65] Nevertheless, *ZLL* and *AGO1* have partially redundant functions in embryonic shoot meristem initiation since, in contrast to both single mutants, the double mutant fails to progress to bilateral symmetry and does not accumulate STM protein.[55] *ZLL* mRNA expression commences in all cells of the 4-cell-stage embryo and is later confined to the provasculature and the adaxial side of the cotyledon primordia. Interestingly, ectopic expression of *ZLL* can convert cotyledon primordia into an indeterminate axis harboring a shoot meristem at its tip.[67] Together these results suggest that *ZLL* provides positional information for the initiation of a shoot meristem at the tip of the embryo axis.

By the late globular stage the stem-cell niche is established and the embryo apex partitioned. During the following stages of embryogenesis, this information is translated into morphological structures: the cotyledonary primordia grow out and the shoot meristem structure becomes evident.

The role of plant growth regulators in the SAM

Growth regulators play a crucial role during plant development. Do they also act on the cells in the shoot meristem? The relevance of the plant growth regulator cytokinin in the promotion of SAM activity has been realized since classical tissue culture experiments showed that a high cytokinin-to-auxin ratio induces shoot formation in callus tissue.[68] Only recently it was shown that depletion of endogenous cytokinin results in smaller meristems, a prolonged plastochron and dwarfed shoots.[69] Overproduction of cytokinin stimulated the expression of the meristem genes *STM* and *KNAT1*.[70] Conversely, ectopic expression of *KNOX* genes in tobacco leaves lead to elevated cytokinin levels.[71,72] These data suggest that KNOX function and cytokinin signaling reinforce each other to promote SAM activity (Fig. 3B).

KNOX proteins act in part by negatively regulating the biosynthesis of an antagonist of meristem fate, the growth factor GA (Fig. 3B).[48,73,74] Classical studies suggested that GA promotes differentiation by inducing longitudinal cell expansion.[75] Transgenic *NTH15*, a *KNOX* gene from tobacco, directly represses the expression of a key GA biosynthetic enzyme, the GA20-oxidase *Ntc12*.[73] In accordance with these data, the expression domains of *NTH15* and *Ntc12* are mutually exclusive, with *NTH15* expressed in the SAM and *Ntc12* in the developing leaves, respectively.[72–74] Furthermore, experiments in tobacco and *Arabidopsis* showed that the effect of *KNOX* gene misexpression in leaves can be suppressed by applying exogenous gibberellin or by increasing GA signaling.[48,74] Thus, antagonistic effects of *KNOX* gene activity and GA signaling appear to be central in balancing meristem versus determinate cell fates.

Surgical experiments demonstrated that the initiation of new leaf primordia is inhibited by already existing organ primordia in their vicinity, suggesting that signals from more mature cells influence the sites of organ initiation.[97] Recent findings suggest that the growth factor auxin (indole-3-acetic acid) is involved in this process.[76–78] If polar auxin transport is severely disturbed, no lateral organ primordia grow out; instead marker genes of organ primordia and organ boundaries are co-expressed in a ring around the shoot apex suggesting that these cells have hybrid identity.[76,78] This block to organ outgrowth can be relieved by local application of exogenous auxin.[76] Interestingly, only the cells at a certain distance from the tip appear to be competent to respond to auxin by organ outgrowth, whereas the cells in the summit are not. This suggests that local auxin maxima produced by polar transport regulate the circumferential position of an organ, but appear unable to override the repression of organ formation at the shoot meristem summit.

Interactions between the shoot meristem and its descendants

Leaves, like all other lateral organs, display a dorsoventral asymmetry. While the adaxial (top) side of a leaf is optimized for light capture and photosynthesis, the abaxial (bottom) side is optimized for gas exchange. Classical surgical experiments have shown that signals from the meristem are required for the establishment of adaxial–abaxial polarity in lateral organ anlagen.[79–81] Vice versa, at least in some species, the subtending leaf is required for proper axillary meristem formation.[82] Recent studies of genes implicated in polarity control of leaves indicate that adaxial cell fates promote SAM maintenance and axillary meristem formation whereas loss of adaxial cell fates or gain of abaxial ones leads to arrest of the SAM (Fig. 3C).[83] This is consistent with the observation that axillary meristems form at the adaxial side of a leaf base.

In addition to signaling from adaxial leaf cells, signals from internal cells of the shoot axis and the lateral organs are required for SAM maintenance. In the petunia *HAIRY MERISTEM* (*ham*) mutant, the meristem differentiates postembryonically as shoot axis-like tissue.[26] The expression of *WUS* and *STM* orthologs in *ham* mutants is initiated but not maintained. The *HAM* gene encodes a GRAS-family transcription factor and is expressed ''outside'' the meristem in internal tissue of lateral organ primordia and in the provasculature of the shoot axis, suggesting that signaling from the *HAM*-expressing cells prevents meristem cells from adopting determined fates (Fig. 3C).

In conclusion, although the nature of the signals remains to be elucidated, it becomes increasingly clear that the activity of the SAM and the development of its differentiating descendents are intimately coordinated.

Termination of stem-cell activity in floral meristems

Flowers are produced from floral meristems, specialized axillary shoot meristems that give rise to a limited number of modified leaves, which protect the bud before opening (sepals), attract pollinators (petals) and serve as reproductive organs (stamens and carpels). The supply of cells necessary to initiate these organs is provided by a stem-cell population similar to that in the SAM and governed by the same regulatory circuitry. However, in contrast to the indeterminate shoot meristem, the floral meristem terminates at the end of flower development and the stem cells differentiate. This poses the problem of how to overcome the self-regulatory *WUS–CLV3* feedback loop. The MADS-box transcription factor AGAMOUS (AG) is a central regulator of this process.[84,85] Flowers mutant for the *AGAMOUS* (*AG*) gene are indeterminate and repeatedly initiate new whorls of organs.[84] In wild type, AG ensures floral meristem termination by repressing *WUS* transcription.[86,87] Intriguingly, WUS in turn participates in the activation of *AG* transcription in the center of the floral meristem, setting up a suicidal feedback loop: *WUS* expression in early flowers contributes to increasing levels of AG, which in turn represses *WUS*.[86,87] However, in contrast to the *WUS–CLV3* loop that establishes a stable boundary between two spatially separated cell populations, the *WUS–AG* interaction acts temporally in the same cell population to transform its fate from indeterminate to determinate. Thus, analogous to stem-cell formation during embryogenesis, stem-cell termination in flower development appears to be mediated primarily via the regulation of the inductive niche cells.

Stem cells at the root apical meristem of *Arabidopsis thaliana*

Are the function and the organization of the root meristem comparable to that of the shoot meristem? The Arabidopsis root displays a stereotypic arrangement of concentric tissue layers consisting of (from outside to inside) epidermis, cortex, endodermis, and pericycle, encompassing the central vascular tissue (Fig. 5).[88,89] Each of the cell files and the distal root cap are produced by stem cells that reside at the far end of each file surrounding four mitotically largely inactive cells, the quiescent center (QC). Every stem cell divides strictly asymmetrically into a daughter cell that remains in contact with the QC and retains stem-cell identity and a daughter cell that is untethered from the QC and undergoes differentiation. Upon ablation of single QC cells, the adjacent stem cells differentiate.[90] This indicates that the QC acts as an inductive niche for stem cells by producing a short-range signal that inhibits differentiation in its immediate neighbors. The nature of this signal remains elusive. Recently, it has been shown that expression of the GRAS-family transcription factor *SCARECROW* (*SCR*) in the QC is required for proper specification of the stem-cell niche.[91] However, how it does so is unresolved up to now and this analysis may be further complicated by the fact that *SCR* is not only expressed in the QC but also in the stem cells that generate cortex and endodermis and in the endodermis cells.

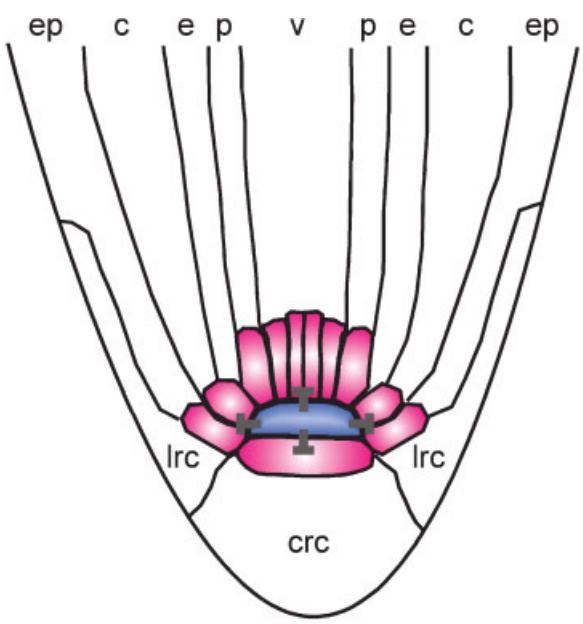

Figure 5. Schematic view of a median longitudinal section through the root tip. The root consists of concentric rings of tissue layers. From the center to the periphery these are: vascular tissue (v), pericycle (p), endodermis (e), cortex (c), epidermis (ep) and lateral root cap (lrc). The central root cap (crc) is located at the very tip of the root and protects the RAM. The QC (blue) inhibits differentiation of the neighboring stem cells (pink). The endodermal and the cortical cell layers as well as the epidermal and the lateral root cap cell layers are each derived from common stem cells.

How are the stem-cell daughters that are not in contact with the QC instructed to differentiate? Cell lineage analysis in seedling roots indicates that despite the stereotypic cell division pattern in root development, cell fate is determined by positional information.[92] The signals that confer this information upon the stem-cell daughters originate from more mature cells within the respective cell file, as demonstrated by elegant ablation experiments.[93]

Thus, the functional organization of the root and the shoot apical meristem is similar in that stem cells are located in a niche where signaling from neighbor cells prevents their differentiation. So far it is unclear, however, whether SAM and RAM regulation is governed by related sets of genes.

Conclusions and perspectives

Plant apical meristems are complex stem-cell systems that are closely linked to their differentiating progeny in developing

organs. The stem cells are maintained in an undifferentiated state in specialized niches. The differentiation of the stem-cell progeny outside the niche is affected by signals from more mature tissues. So far, genetic approaches have identified some of the key players of meristematic signaling; however the majority of signals that relay the positional information await elucidation.

While we have some clues on meristem functioning and specification of plant stem cells, we know virtually nothing about the intracellular factors that confer stem-cell identity. So far, no gene has been identified that is specifically expressed in stem cells and is essential for stem-cell function. Thus one can speculate that ''stemness'' could reflect the mere absence of differentiating factors rather than the presence of specific stem-cell determinants. Alternatively, stemness might be achieved by the concerted action of many genes. This hypothesis can be tested by the isolation of stem cells and the subsequent analysis of their gene expression pattern or by performing refined genetic screens.

Acknowledgments

We would like to thank Cris Kuhlemeier, Michael Lenhard and members of the Laux laboratory for critically reading the manuscript. We apologize to all colleagues whose work could not be cited due to space constraints.

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