

Stem cell regulation in the shoot meristem

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Summary

A small group of pluripotent stem cells in the shoot meristem is the ultimate source for all aerial parts in higher plants: the shoot axis, side branches, leaves and flowers. The stem cells are maintained in an undifferentiated state by signals from an underlying cell group, the organizing center. Genetic and molecular analyses have shown that a feedback signaling loop between stem cells and the

organizing center balances stem cell renewal versus differentiation, which allows the plant to maintain the organization of the shoot meristem despite a changing cellular context. Emerging common principles indicate that plant and animal stem cells are functionally equivalent.

Key words: Stem cells, Shoot meristem, *Arabidopsis*

Introduction

Stem cells are small populations of relatively undifferentiated dividing cells whose daughters can either remain stem cells or differentiate. In most, but not all cases, they are pluripotent and give rise to several differentiated cell types. The balance between self renewal and differentiation can be coordinated either at the single cell or population level (Fig. 1) (Spradling et al., 2001). In the single cell mechanism, each stem cell division is strictly asymmetric, producing one new stem cell and one differentiating cell. This can occur by asymmetric distribution of intracellular components or by placing of the daughter cells into different microenvironments. By contrast, in a population-based mechanism, the outcome of an individual stem cell division cannot be predicted, but the total number of stem cells is regulated. Regardless of the division mode, however, the emerging picture is that stem cell identity is regulated by signals from surrounding cells (Mayer et al., 1998; Spradling et al., 2001). This is in line with Newman's proposal that the founder cells in the shoot apex are the 'temporary occupants of a permanent office' (Newman, 1965), which anticipated Schofield's 'niche concept' derived from studies of hematopoietic development (Schofield, 1978; Spradling et al., 2001).

The caveat of the above stem cell definition is, however, that it is based on something the stem cell daughters do but tells us little about the nature of a stem cell itself. Can we define a stem cell in molecular terms? In many cases, molecular markers have been identified that allow enrichment for stem cells (Blau et al., 2001; Kornblum and Geschwind, 2001; Lagasse et al., 2000). However, it is often unclear whether such molecules are necessary components of stem cell identity.

In plants, all postembryonically formed cells and organs are ultimately derived from small stem cell populations in the apical shoot and root meristems, which allow generation of new organs over a life span that can be more than a thousand years. In addition, plants possess stem cells that have more tissue-specific functions, such as to provide cells for gird

growth. The apical meristems are especially suitable for studies of stem cell biology. For example, mutant sectors can be generated and the progeny of a single stem cell can be followed. This has allowed determination of the numbers, potency and proliferative properties of shoot meristem stem cells (Furner and Pumfrey, 1992; Irish and Sussex, 1992; Ruth et al., 1985; Stewart and Dermen, 1970). Here, we discuss our current knowledge of stem cell regulation in the shoot meristem. For information on general shoot meristem development and function, the reader is referred to several excellent reviews (Barton, 1998; Bowman and Eshed, 2000; Clark, 1997; Meyerowitz, 1997).

Stem cells in the shoot apical meristem

The shoot meristem contains a central zone (CZ) that harbors pluripotent stem cells and surrounding regions in which cells start to differentiate and organ primordia are initiated (Fig. 2). In most angiosperms, it is also divided into three separate 'germ' layers, L1-L3 (Satina et al., 1940). Cells in the outer two layers, L1 and L2, divide predominantly anticlinally (vertical to the surface) to form two sheets of tissue, the epidermis and subepidermal tissue, respectively. By contrast, the underlying L3 cells divide in all directions and give rise to the internal tissues.

Elegant clonal studies demonstrated that within a given layer all cells are ultimately derived from 2-3 long-term stem cells (Furner and Pumfrey, 1992; Irish and Sussex, 1992; Schnittger et al., 1996; Stewart and Dermen, 1970). In addition, their immediate daughter cells can still act as transiently active short-term stem cells, giving rise to a more restricted part of the plant (Stewart and Dermen, 1970). In privet, it has been estimated that the short-term stem cells are replaced on average every 14 days, whereas the long-term stem cells can be active throughout the plants life (Stewart and Dermen, 1970). What could distinguish long-term from short-term stem cells? It is possible that the only difference is their position: the short-term

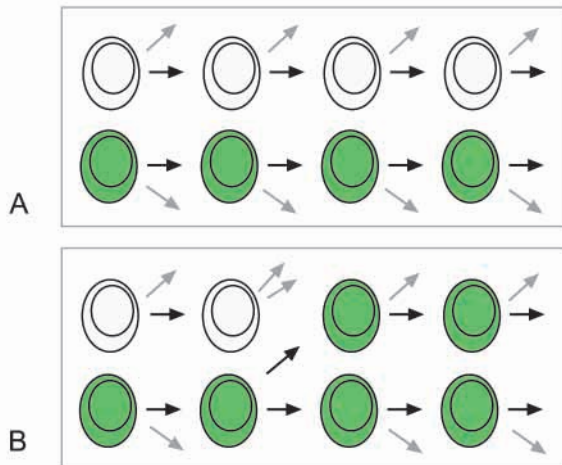


Fig. 1. Mechanisms of stem cell divisions. If individual stem cells in a niche are differently marked (color coded green or white) the mechanism of division can be assessed (for clarity, only stem cells but not differentiating cells are depicted). (A) If regulated at the single cell level, each stem cell division is strictly asymmetric to give rise to an new stem cell and a differentiating cell (shaded arrows, cells not shown). The distribution of genetic sectors does not change. (B) If regulated in a population mode, symmetric and asymmetric divisions occur. Owing to symmetric divisions genetic sectors can enlarge at the expense of others. See text for details.

cells at the rim of the stem cell pool are next to be ‘pushed out’ by divisions of central cells, whereas the central ones have a better chance of staying for a longer time (Ball, 1960). This division of labor might allow plants to reduce the number of cell divisions of their long-term stem cells and thus maintain them as a relatively error-free genetic blueprint.

The CZ is identified by its relatively weak cytoplasmic staining and low rate of cell division. Although the stem cells cannot be recognized histologically, on the basis of geometrical considerations one can predict that they are located in the three outermost cell layers of the CZ. Thus, if one assumes that the CZ in *Arabidopsis* it is about 5-6 cells high (Fig. 2) (Vaughan, 1955), the stem cells occupy only about the upper half of it. Are the stem cells different from surrounding cells in the shoot apex? The presumed stem cell region is characterized by the

expression of the *CLAVATA3 (CLV3)* gene, which is thus used as an operational stem cell marker for the shoot meristem (Fletcher et al., 1999). This indicates that the stem cells of the shoot meristem are distinct from the other cells. Note, however, that mutants lacking *CLV3* activity possess stem cells, indicating that *CLV3* is not essential for stem cell identity (Clark et al., 1995).

Cells that exit the stem cell pool initiate differentiation and form lateral organs (leaves, side shoots and flowers) in the ring-shaped peripheral zone surrounding the CZ, and central stem tissue from the rib zone underneath the CZ. The earliest molecular changes known to occur are the downregulation of *CLV3* expression (Fletcher et al., 1999) and the subsequent activation of several genes, such as *ZWILLE (ZLL)/PINHEAD* (Lynn et al., 1999; Moussian et al., 1998). Eventually expression of the *SHOOTMERISTEMLESS (STM)* gene is downregulated in organ anlagen, and organ development takes place (Long et al., 1996) (see below).

In summary, the shoot meristem displays a succession of distinct cell states, from the central stem cells to the cells in organ primordia at the periphery, whose proper specification is essential for meristem function.

Specification of stem cell identity in the shoot meristem

What keeps the stem cells in an undifferentiated state? Clonal studies have shown that in plants the fate of a dividing cell is determined by its position and not by its origin. Does this also hold true for stem cells?

During shoot meristem initiation, the first organs appear to be formed independently of the stem cells but subsequent organs are not (Fig. 3) (Sussex and Rosenthal, 1973). One can therefore predict that mutants defective in stem cell specification may form seedlings that have a normal pair of first, non-stem-cell-derived ‘leaves’ (the cotyledons) but no functional stem cells. This is the case in *Arabidopsis wuschel (wus)* mutants, where the putative stem cells are partially differentiated (Laux et al., 1996). *WUS* encodes a homeodomain protein and is expressed in a group of ~10 cells in the CZ of the meristem (Fig. 4) (Mayer et al., 1998), which are located underneath the three outermost cell layers that contain the stem cells in the vegetative shoot meristem and one

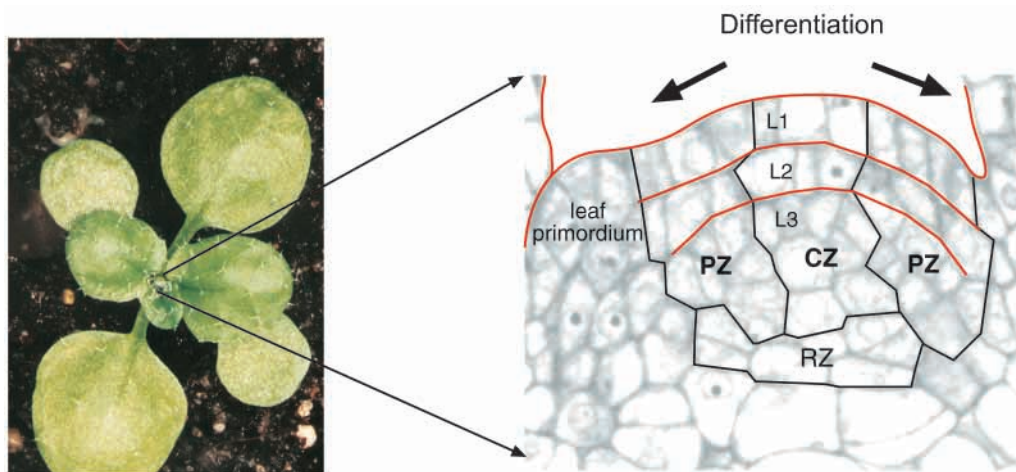


Fig. 2. Organization of the *Arabidopsis* shoot meristem. (Left) An *Arabidopsis* seedling is shown from a top view. (Right) A longitudinal section through the shoot apex is shown (figure adapted from Laux and Schoof, 1997). The approximate outlines of the central zone (CZ), peripheral zone (PZ) and rib zone (RZ) are given. For details, see text.

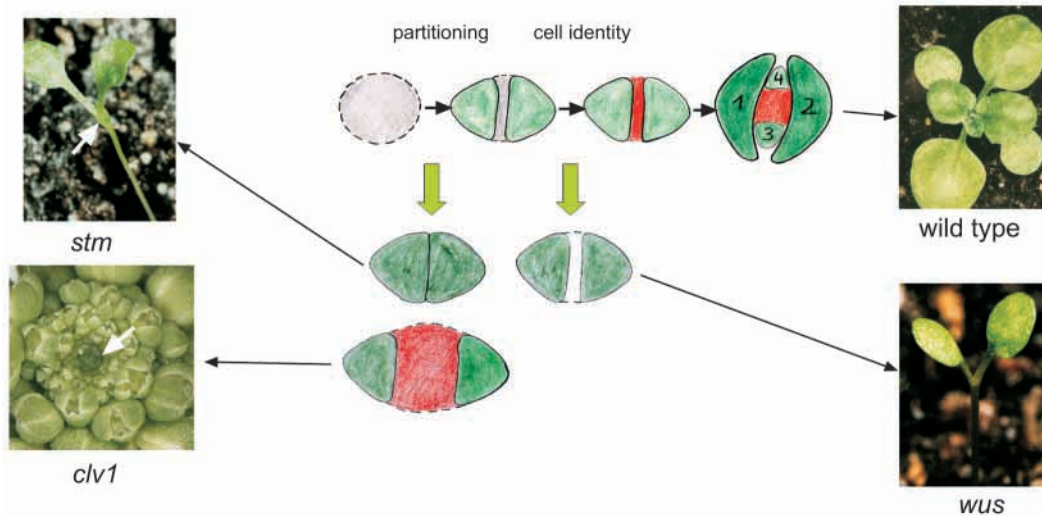


Fig. 3. Isolation of shoot meristem stem cell mutants in *Arabidopsis*. In this model of shoot meristem initiation, the first leaf primordia (green) arise before the central cells appear to be specified (red), whereas subsequent leaves are derived from the stem cells. Mutations affecting partitioning of the shoot meristem primordium could result in an altered size of the stem cell region, represented by *stm* (with fused organ primordia, arrow) and *clv* (with an increased meristem, arrow) mutants. Mutations affecting stem cell specification would result in altered cells in the center but normal first leaves, represented by a *wus* seedling.

cell layer higher in embryonic and floral meristems. Together with the finding that *WUS* is sufficient to induce expression of *CLV3* (Schoof et al., 2000), these observations suggest that the stem cells of the shoot meristem are specified by signaling from the *WUS*-expressing cells, termed the organizing center (OC; Fig. 4). Studies of the function of the putative *WUS* orthologs in *Petunia* and *Antirrhinum* support this model (M. Kieffer, H. Cook, Y. Stern et al., unpublished; Stuurman et al., 2002). Thus, the shoot meristem provides one of the few examples where the presence of a stem cell niche can be shown

genetically by the requirement for non-cell-autonomous activities of neighboring cells to specify stem cell identity.

Feedback from the stem cells establishes a self-regulatory system

How are stem cell self renewal and differentiation coordinated in plants? In ferns and other lower plants, the shoot apex displays a single large apical stem cell, the apical mother cell (Golub and Wetmore, 1948). Each division of this cell is strictly asymmetric, giving one differentiating cell while retaining the mother cell at the apex (Fig. 1A). But, the stem cells in multicellular shoot meristems of higher plants work differently: clonal studies indicate that stem cell divisions are not strictly asymmetric but regulated at the population level (Fig. 1B) (Ruth et al., 1985). This requires that the boundaries of the stem cell pool are constantly assessed and stably maintained in a changing cellular context. How is this achieved?

The results of surgical destruction of the shoot meristem apical cells suggested that the stem cells inhibit stem cell fate in their daughters by lateral suppression and thus themselves are involved in controlling the boundaries of the stem cell pool (Loiseau, 1959; Pilkington, 1929). Genetic analysis in *Arabidopsis* has verified this and revealed that the size of the stem cell population is regulated through size regulation of the OC. Mutations in the *CLV* genes result in largely increased shoot and floral meristem sizes owing to accumulation of *CLV3*-expressing stem cells (Fig. 3) (Clark, 1997; Fletcher et al., 1999; Laufs et al., 1998). The *CLV3* gene encodes a small peptide that is secreted into the extracellular space, where it presumably acts as a ligand for the *CLV1* receptor-like kinase, which is expressed throughout most of the shoot meristem (Clark et al., 1997; Fletcher et al., 1999; Rojo et al., 2002; Trotochaud et al., 1999).

Besides *CLV1* and *CLV3*, additional proteins and genetic

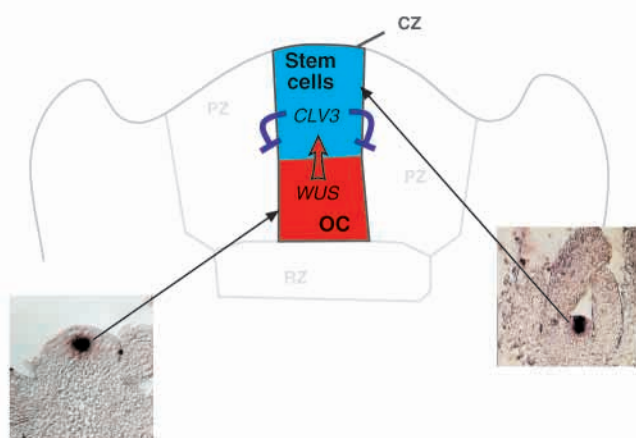


Fig. 4. Model for the regulation of stem cells in the *Arabidopsis* shoot meristem. The CZ comprises both the stem cells (blue) and the OC (red), expressing *CLV3* and *WUS*, respectively (insets: in situ hybridizations). *WUS* activity in the OC results in specification of stem cell identity in the three outermost cell layers, which in turn signal back by *CLV3*, restricting the *WUS* expression domain. Note that the expression domains in this figure are estimated based on comparison of separate experiments and not on simultaneous detection of the genes.

loci that participate in CLV-dependent signaling have been identified. CLV1 protein is stabilized by CLV2, a receptor-like molecule lacking the intracellular kinase domain that might form heterodimers with CLV1 (Jeong et al., 1999). Furthermore, SHEPHERD, an HSP90-related protein that is thought to act as an ER-localized chaperone (Ishiguro et al., 2002), is required for CLV signaling, which suggests that it is either necessary for secretion of functional CLV3 protein or for assembly of a functional CLV1-CLV2 receptor complex. Inside the cell, CLV1/CLV3 signaling is negatively regulated by KAPP, a protein phosphatase (Trotochaud et al., 1999), and antagonized by POLTERGEIST, which appears to act downstream of the *CLV* loci (Yu et al., 2000).

What are the targets of CLV signaling? *wus* mutations are epistatic to *clv*, mutations in any of the three *CLV* genes result in an expanded *WUS* expression domain, and ectopic expression of *CLV3* is sufficient to repress *WUS* (Brand et al., 2000; Laux et al., 1996; Schoof et al., 2000). These results indicate that CLV3 signaling restricts the size of the stem cell pool by restricting the size of the *WUS* expression domain. Thus, the balance between stem cells and differentiating cells appears to be dynamically regulated by a negative feedback loop between the OC and stem cells that involves the *WUS* and *CLV3* genes: signaling from the OC confers stem cell identity to the cells in the three outermost layers, which in turn signal back to limit the size of the OC (Fig. 4) (Schoof et al., 2000). If, for example, the number of stem cells is too small, a reduced amount of CLV3 signal will lead to an expanded *WUS* expression domain, which in turn will result in the induction of more stem cells. This model for size regulation of the stem cell population by the OC is in line with previous findings from periclinal chimaeras in tomato, which showed that the floral meristem size is determined by the genotype of the L3 cells (Szymkowiak and Sussex, 1992)

Targeting the signals

Within the shoot meristem, signaling between stem cells and the OC must be targeted to the right cells and kept away from others. For example, how is stem cell identity restricted to the cells above the OC but not those surrounding it? Since all cells in the shoot apex are able to respond to ectopic *WUS* expression (Schoof et al., 2000), the *WUS*-mediated signal must be directed specifically to the apical neighbors. Interestingly, L1 cells within the CZ are effectively coupled by cytoplasmic connections, plasmodesmata, that facilitate intercellular movement of molecules, whereas they are relatively insulated from surrounding peripheral cells (Rinne and van der Schoot, 1998). If the same were true for all cells of the CZ, such a cytoplasmically insulated CZ could explain the restriction of the *WUS*-dependent signal to target cells within the CZ.

Turning meristem cells into organs

Once cells have left the stem cell pool, changes in their expression pattern indicate the onset of differentiation. However, the cells are kept in a relatively undifferentiated state, and organ formation appears to be inhibited by the activity of the *STM* gene. *STM* acts independently of *WUS*, but both genes together are required for maintaining the shoot meristem

(Gallois et al., 2002; Lenhard et al., 2002). *STM* encodes the *Arabidopsis* ortholog of the maize homeodomain protein KNOTTED1 (KN1) (Long et al., 1996; Vollbrecht et al., 1991). Both genes are expressed throughout the shoot apex to keep cells in an undifferentiated state but are downregulated in the incipient lateral organ primordia (Jackson et al., 1994; Long et al., 1996). Loss of *STM* function allows organ formation to consume the entire apex (Fig. 3) (Barton and Poethig, 1993; Endrizzi et al., 1996), suggesting that *STM* negatively regulates organ formation. This regulation involves repression of the organ-promoting *ASYMMETRIC LEAVES1* and *ASYMMETRIC LEAVES2* genes (Byrne et al., 2000; Byrne et al., 2002). Thus, in contrast to *WUS*, whose function appears to be restricted to maintaining the stem cells, *STM* appears to be required in cells throughout the meristem dome to prevent premature organ formation. This regulation could allow meristem cells to amplify to sufficient numbers before organs are initiated (Lenhard et al., 2002).

In addition to *STM* and *WUS* activities, maintaining the shoot meristem cells in an undifferentiated state requires signaling from the organ primordia back to the shoot apex (Waites et al., 1998). In general, adaxial (upper) leaf identity appears to be required for meristem maintenance, whereas abaxial leaf identity is incompatible with it (Bowman and Eshed, 2000; McConnell and Barton, 1998).

Making stem cells

Embryogenesis

Wardlaw named the shoot meristem stem cells 'embryonic initials', taking into account the fact that both embryo cells and stem cells give rise to many cell types and complete organs (Wardlaw, 1957). However, shoot meristem stem cells are not apparent until half way through embryogenesis and their expression profile shows they are different from early embryo cells. The earliest sign of shoot meristem formation is the onset of *WUS* expression in four subepidermal apical cells of the 16-cell embryo (Fig. 5) (Mayer et al., 1998), which subsequently undergo a series of asymmetric cell divisions with respect to continuing *WUS* expression. In mid-stage embryos, the shoot meristem becomes discernable and *CLV3* expression is initiated (Fig. 5) (Brand et al., 2002). Loss of *WUS* function results in a failure to express *CLV3* and to form a shoot meristem (Brand et al., 2002; Laux et al., 1996). Thus, embryonic formation of shoot meristem stem cells can be divided into two steps: first, a cell lineage that will give rise to the OC is established and presumably preserved by *WUS* function; and second, stem cell identity is induced in mid-stage embryos. Mutations in other genes important for embryonic shoot meristem development, such as *STM*, seem not to be required for stem cell initiation but instead to maintain them (Brand et al., 2002; Long and Barton, 1998).

Analysis of the *zwille* (*zll*, also named *pinhead*) mutant has given some indication of how stem cell initiation is coordinated with the rest of the embryo. In *zll* embryos, the expression pattern of shoot meristem genes is randomized but still restricted to the shoot apex (Lynn et al., 1999; Moussian et al., 1998). At late stages, shoot meristem gene expression is shut off, and the cells at the stem cell position differentiate (Endrizzi et al., 1996; McConnell and Barton, 1995; Moussian et al., 1998). During early embryo development, *ZLL* is expressed in

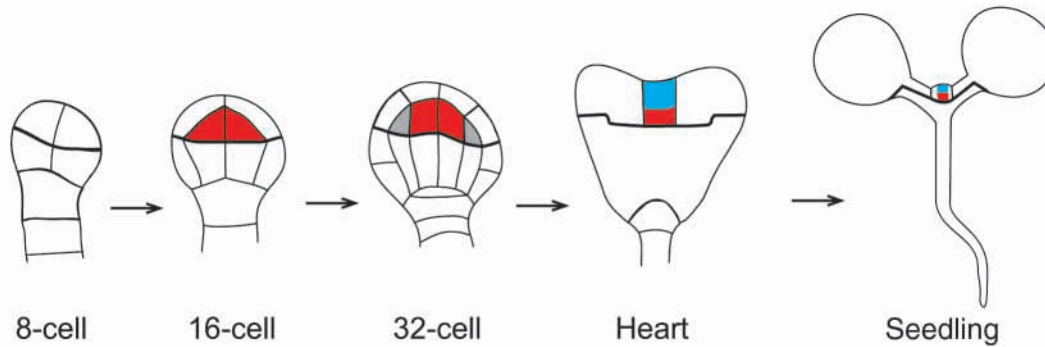


Fig. 5. Initiation of shoot meristem stem cells in the *Arabidopsis* embryo. An OC precursor cell lineage expressing *WUS* (red) is established in the four subepidermal cells of the 16-cell embryo. As judged from *CLV3* expression (blue), stem cells are induced in heart stage embryos. The thick line indicates the clonal boundary between apical and basal derivatives of the 8-cell embryo (figure adapted from Schrick and Laux, 2000).

the precursor cells of the vasculature, directly underneath the shoot meristem primordium, and later in the adaxial (upper) side of cotyledonary primordia (Lynn et al., 1999; Moussian et al., 1998). Expression of *ZLL* in the abaxial (lower) instead of the abaxial side of the cotyledonary primordia resulted in their transformation into indeterminate axes with ectopic shoot meristems (Newman et al., 2002). Together these observations indicate that stem cell formation requires *ZLL*-dependent signals from neighboring embryonic cells. Similarly, postembryonic *ZLL* activity in the vascular primordia and/or the adaxial part of leaf primordia is important for the formation of axillary meristems (Lynn et al., 1999).

ZLL encodes one of the name-giving members of a protein family with a PAZ (PIWI ARGONAUTE ZWILLE) domain, which is highly conserved throughout the animal and plant kingdom (Cerutti et al., 2000). Recent evidence shows that some PAZ proteins are members of complexes that bind micro RNAs – small RNA molecules of ~20 nucleotides (Hammond et al., 2001; Mourelatos et al., 2002) – which in turn allow binding to homologous sequences of specific mRNA species. Since one member of the PAZ family is the putative translation initiation factor eIF2C from rabbit (Zou et al., 1998), and double mutants of *zll* and its relative *argonaute* produce *STM* mRNA but no *STM* protein, an attractive hypothesis is that *ZLL* functions in translational regulation of specific mRNA species required for shoot meristem development (Lynn et al., 1999).

Formation of stem cells from differentiated cells

Plants can also form stem cells *de novo* from differentiated cells. For example, the expression pattern of meristem genes suggests that floral meristems that arise at the periphery of the inflorescence meristem are not contiguous with the inflorescence meristem but are initiated *de novo* (Mayer et al., 1998; Otsuga et al., 2001). While the cells that give rise to floral meristems are still relatively undifferentiated, plants clearly can also form meristems from fully differentiated cells. For example, differentiated leaf epidermal cells can give rise to leaf-borne embryos with apical meristems (Taylor, 1967). In the root, lateral root meristems are initiated from differentiated pericycle cells (Malamy and Benfey, 1997). The capacity of differentiated plant cells to dedifferentiate and to give rise to stem cells, either through the induction of

meristems or through somatic embryo formation, provides an important tool for regenerative biology. It should be noted, however, that in many cases the cells from which the stem cells ultimately originate and their differentiation state are not precisely known.

Termination of stem cells

Flowers are homologous to shoots and it is thus not surprising that the regulatory circuitry that governs stem cell maintenance in the shoot meristem also functions in floral meristems. However, one important difference between shoot and flower development is that the *Arabidopsis* shoot meristem is indeterminate whereas the floral meristem is determinate and gives rise to only a limited number of organs. Thus, at the end of floral meristem activity, the stem cells differentiate and contribute to the central organ, the gynoecium. The MADS box transcription factor *AGAMOUS* (*AG*) is required for both specifying floral organ identity and termination of the floral meristem (Yanofsky et al., 1990). The latter involves repression of *WUS* expression in the OC (Laux et al., 1996; Lenhard et al., 2001; Lohmann et al., 2001). *WUS* in turn can activate transcription of the *AG* gene, setting up a suicidal feedback mechanism by which the OC and eventually the stem cells differentiate. Obviously, the correct temporal and spatial control of this mechanism is important. First, stem cells have to be active for long enough to ensure that sufficient cells are generated for all floral organs. A possible mechanism is suggested by the finding that floral determinacy appears to require high levels of *AG* activity, which might be reached only gradually during flower development (Sieburth et al., 1995). Second, stem cell termination must be restricted to the floral meristem and not occur in the shoot meristem. Genetic interactions indicate that *WUS* requires the presence of the floral regulator *LEAFY* in order to activate *AG* expression (Lenhard et al., 2001). However, *in vitro* both proteins appear to be able to bind the *AG* promoter independently (Lohmann et al., 2001).

Common principles in stem cell regulation

How similar are shoot meristem stem cells to other stem cell systems? The root meristem is thought to have evolved independently of the shoot meristem, but the organization of

both meristems is very similar: In the root meristem, differentiation of stem cells is prevented by signals from a small group of cells, the quiescent center, similar to the function of the OC in the shoot meristem (van den Berg et al., 1997). Whether the regulatory molecules and mechanisms involved are similar to those in the shoot meristems has to await further analysis.

Traditionally plant stem cells have been considered to be fundamentally different from animal stem cells. However, recent advances suggest that plant and animal stem cells are functionally equivalent and regulated by common principles. A major perceived difference is that plant stem cells are regulated by positional information, whereas animal stem cells have been viewed as cell lineages of a fixed fate. However, it now has become clear that at least many animal stem cells are similarly specified by signals from their neighborhood (Spradling et al., 2001). For example, the germ line stem cells in the oviduct of *C. elegans* are specified by signals from a single cell the distal tip cell, that suppress differentiation (meiosis) and maintain mitotic divisions (Berry et al., 1997; Henderson et al., 1994).

A second apparent difference is that plant stem cells are considered to have a much broader developmental program (they give rise to complete organs) than their animal counterparts, which regenerate cells restricted to one tissue type. However, recent findings have shown that in both cases the developmental capacity of stem cells is not an inherent property of the stem cell but instead dictated by the environment the daughter cells are exposed to, and can be dramatically expanded if this environment is altered (Blau et al., 2001; Morrison, 2001; Steeves and Sussex, 1989).

Are there also molecular similarities? This question is still difficult to answer since we know too little of the molecules that are important for stem cell generation and maintenance. Interestingly, one of the first *ZLL*-related genes identified, *PIWI*, functions in the regulation of stem cell maintenance in the *Drosophila* germlarium (Cox et al., 1999), which suggests that there are at least some similarities. However, there are no obvious candidates for the *WUS* or *CLV3* genes in animals, which could reflect the different cellular constitutions of plant and animal stem cell niches, i.e. a dividing cell population versus a stable cavity.

Conclusion and perspectives

In the past few years, we have gained insight into how stem cells of the shoot meristem are specified and how self-renewal and differentiation are balanced. Most findings demonstrate that a major function in stem cell specification is to suppress differentiation pathways. However, the expression of *CLV3* exclusively in the stem cells indicates that the activation of stem-cell-specific gene expression could also play a role. We have obtained an overview about the players and the mechanisms but many details are still to be discovered and genetic screens in selected genetic backgrounds may enable us to identify components missed so far.

Many of the regulatory principles appear to be common to a wide range of stem cell systems in plants and animals. But still we do not know what a stem cell is in molecular terms. What are the molecular factors that impart pluripotency on a cell? Many hypotheses have implicated chromatin structure in

cell potency but, although this is an attractive possibility, robust experimental data are not yet available. Do stem cells express specific genes that confer 'stemness' or is it sufficient to protect them against differentiation signals? Is there a universal stem cell factor? Now that we are able to mark and to purify stem cells, their molecular profiles can be obtained that may allow answers to these questions (Phillips et al., 2000; Terskikh et al., 2001).

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References

- Ball, E. (1960). Cell divisions in living shoot apices. *Phytomorphology* **10**, 377-396.
- Barton, M. K. (1998). Cell type specification and self renewal in the vegetative shoot apical meristem. *Curr. Opin. Plant Biol.* **1**, 37-42.
- Barton, M. K. and Poethig, R. S. (1993). Formation of the shoot apical meristem in *Arabidopsis thaliana*: An analysis of development in the wild type and in the *shoot meristemless* mutant. *Development* **119**, 823-831.
- Berry, L. W., Westlund, B. and Schedl, T. (1997). Germ-line tumor formation caused by activation of *glp-1*, a *Caenorhabditis elegans* member of the *Notch* family of receptors. *Development* **124**, 925-936.
- Blau, H. M., Brazelton, T. R. and Weimann, J. M. (2001). The evolving concept of a stem cell: entity or function? *Cell* **105**, 829-841.
- Bowman, J. L. and Eshed, Y. (2000). Formation and maintenance of the shoot apical meristem. *Trends Plant Sci.* **5**, 110-115.
- Brand, U., Fletcher, J. C., Hobe, M., Meyerowitz, E. M. and Simon, R. (2000). Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by *CLV3* activity. *Science* **289**, 617-619.
- Brand, U., Grunewald, M., Hobe, M. and Simon, R. (2002). Regulation of *CLV3* expression by two homeobox genes in *Arabidopsis*. *Plant Physiol.* **129**, 565-575.
- Byrne, M. E., Barley, R., Curtis, M., Arroyo, J. M., Dunham, M., Hudson, A. and Martienssen, R. A. (2000). Asymmetric leaves1 mediates leaf patterning and stem cell function in *Arabidopsis*. *Nature* **408**, 967-971.
- Byrne, M. E., Simorowski, J. and Martienssen, R. A. (2002). ASYMMETRIC LEAVES1 reveals knox gene redundancy in *Arabidopsis*. *Development* **129**, 1957-1965.
- Cerutti, L., Mian, N. and Bateman, A. (2000). Domains in gene silencing and cell differentiation proteins: the novel PAZ domain and redefinition of the Piwi domain. *Trends Biochem Sci.* **25**, 481-482.
- Clark, S. E. (1997). Organ formation at the vegetative shoot meristem. *Plant Cell* **9**, 1067-1076.
- Clark, S. E., Running, M. P. and Meyerowitz, E. M. (1995). *CLAVATA3* is a specific regulator of shoot and floral meristem development affecting the same processes as *CLAVATA1*. *Development* **121**, 2057-2067.
- Clark, S. E., Williams, R. W. and Meyerowitz, E. M. (1997). The *CLAVATA1* gene encodes a putative receptor-kinase that controls shoot and floral meristem size in *Arabidopsis*. *Cell* **89**, 575-585.
- Cox, D. N., Chao, A., Baker, J., Chang, L., Qiao, D. and Lin, H. (1999). A novel class of evolutionary conserved genes defined by *piwi* are essential for stem cell renewal. *Genes Dev.* **12**, 3715-3727.
- Endrizzi, K., Moussian, B., Haecker, A., Levin, J. and Laux, T. (1996). The *SHOOTMERISTEMLESS* gene is required for maintenance of undifferentiated cells in *Arabidopsis* shoot and floral meristems and acts at a different regulatory level than the meristem genes *WUSCHEL* and *ZWILLE*. *Plant J.* **10**, 967-979.
- Fletcher, J. C., Brand, U., Running, M. P., Simon, R. and Meyerowitz, E. M. (1999). Signaling of cell fate decisions by *CLAVATA3* in *Arabidopsis* shoot meristems. *Science* **283**, 1911-1914.
- Furner, I. J. and Pumfrey, J. E. (1992). Cell fate in the shoot apical meristem of *Arabidopsis thaliana*. *Development* **115**, 755-764.
- Gallois, J. L., Woodward, C., Reddy, G. V. and Sablowski, R. (2002). Combined *SHOOT MERISTEMLESS* and *WUSCHEL* trigger ectopic organogenesis in *Arabidopsis*. *Development* **129**, 3207-3217.
- Golub, S. and Wetmore, R. (1948). Studies of the development in the

- vegetative shoot of *Equisetum arvense* L. II. The mature shoot. *Am. J. Botany* **35**, 767-781.
- Hammond, S. M., Boettcher, S., Caudy, A. A., Kobayashi, R. and Hannon, G. J.** (2001). Argonaute2, a link between genetic and biochemical analyses of RNAi. *Science* **293**, 1146-1150.
- Henderson, S. T., Gao, D., Lambie, E. J. and Kimble, J.** (1994). lag-2 may encode a signaling ligand for the GLP-1 and LIN-12 receptors of *C. elegans*. *Development* **120**, 2913-2924.
- Irish, V. F. and Sussex, I. M.** (1992). A fate map of the *Arabidopsis* embryonic shoot apical meristem. *Development* **115**, 745-753.
- Ishiguro, S., Watanabe, Y., Ito, N., Nonaka, H., Takeda, N., Sakai, T., Kanaya, H. and Okada, K.** (2002). SHEPHERD is the *Arabidopsis* GRP94 responsible for the formation of functional CLAVATA proteins. *EMBO J.* **21**, 898-908.
- Jackson, D., Veit, B. and Hake, S.** (1994). Expression of maize KNOTTED1 related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. *Development* **120**, 405-413.
- Jeong, S., Trotochaud, A. E. and Clark, S. E.** (1999). The *Arabidopsis* CLAVATA2 gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase. *Plant Cell* **11**, 1925-1934.
- Kornblum, H. I. and Geschwind, D. H.** (2001). Molecular markers in CNS stem cell research: hitting a moving target. *Nat. Rev. Neurosci.* **2**, 843-846.
- Lagasse, E., Connors, H., Al-Dhalimy, M., Reitsma, M., Dohse, M., Osborne, L., Wang, X., Finegold, M., Weissman, I. L. and Grompe, M.** (2000). Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat. Med.* **6**, 1229-1234.
- Laufs, P., Grandjean, O., Jonak, C., Kieu, K. and Traas, J.** (1998). Cellular parameters of the shoot apical meristem in *Arabidopsis*. *Plant Cell* **10**, 1375-1390.
- Laux, T., Mayer, K. F. X., Berger, J. and Jürgens, G.** (1996). The WUSCHEL gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* **122**, 87-96.
- Laux, T. and Schoof, H.** (1997). Maintaining the shoot meristem – the role of CLAVATA1. *Trends Plant Sci.* **2**, 325-327.
- Lenhard, M., Bohnert, A., Jürgens, G. and Laux, T.** (2001). Termination of stem cell maintenance in *Arabidopsis* floral meristems by interactions between WUSCHEL and AGAMOUS. *Cell* **105**, 805-814.
- Lenhard, M., Jürgens, G. and Laux, T.** (2002). The WUSCHEL and SHOOTMERISTEMLESS genes fulfil complementary roles in *Arabidopsis* shoot meristem regulation. *Development* **129**, 3195-3206.
- Lohmann, J., Huang, R., Hobe, M., Busch, M., Parcy, F., Simon, R. and Weigel, D.** (2001). A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. *Cell* **105**, 793-803.
- Loiseau, J. E.** (1959). Observation et expérimentation sur la phyllotaxie et le fonctionnement du sommet végétatif chez quelques Balsaminacées. *Ann. Sci. Nat. Bot. Ser.* **11**, 201-214.
- Long, J. A. and Barton, M. K.** (1998). The development of apical embryonic pattern in *Arabidopsis*. *Development* **125**, 3027-3035.
- Long, J. A., Moan, E. I., Medford, J. I. and Barton, M. K.** (1996). A member of the KNOTTED class of homeodomain proteins encoded by the STM gene of *Arabidopsis*. *Nature* **379**, 66-69.
- Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P. and Barton, M. K.** (1999). The PINHEAD/ZWILLE gene acts pleiotropically in *Arabidopsis* development and has overlapping functions with the ARGONAUTE1 gene. *Development* **126**, 469-481.
- Malamy, J. E. and Benfey, P. N.** (1997). Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development* **124**, 33-44.
- Mayer, K. F. X., Schoof, H., Haecker, A., Lenhard, M., Jürgens, G. and Laux, T.** (1998). Role of WUSCHEL in regulating stem cell fate in the *Arabidopsis* shoot meristem. *Cell* **95**, 805-815.
- McConnell, J. R. and Barton, M. K.** (1995). Effects of mutations in the PINHEAD gene of *Arabidopsis* on the formation of shoot apical meristems. *Dev. Genet.* **16**, 358-366.
- McConnell, J. R. and Barton, M. K.** (1998). Leaf polarity and meristem formation in *Arabidopsis*. *Development* **125**, 2935-2942.
- Meyerowitz, E. M.** (1997). Genetic control of cell division patterns in developing plants. *Cell* **88**, 299-308.
- Morrison, S. J.** (2001). Stem cell potential: can anything make anything? *Curr. Biol.* **11**, R7-R9.
- Mourelatos, Z., Dostie, J., Paushkin, S., Sharma, A., Charroux, B., Abel, L., Rappsilber, J., Mann, M. and Dreyfuss, G.** (2002). miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. *Genes Dev.* **16**, 720-728.
- Moussian, B., Schoof, H., Haecker, A., Jürgens, G. and Laux, T.** (1998). Role of the ZWILLE gene in the regulation of central shoot meristem cell fate during *Arabidopsis* embryogenesis. *EMBO J.* **17**, 1799-1809.
- Newman, I. V.** (1965). Patterns in the meristems of vascular plants. III. Pursuing the patterns where no cell is a permanent cell. *J. Linn. Soc. Bot.* **59**, 185-214.
- Newman, K. L., Fernandez, A. G. and Barton, M. K.** (2002). Regulation of Axis Determinacy by the *Arabidopsis* PINHEAD Gene. *Plant Cell* **14**, 3029-3042.
- Otsuga, D., DeGuzman, B., Prigge, M. J., Drews, G. N. and Clark, S. E.** (2001). REVOLUTA regulates meristem initiation at lateral positions. *Plant J.* **25**, 223-236.
- Phillips, R. L., Ernst, R. E., Brunk, B., Ivanova, N., Mahan, M. A., Deanehan, J. K., Moore, K. A., Overton, G. C. and Lemischka, I. R.** (2000). The genetic program of hematopoietic stem cells. *Science* **288**, 1635-1640.
- Pilkington, M.** (1929). The regeneration of the stem apex. *New Phytologist* **28**, 37-53.
- Rinne, P. L. and van der Schoot, C.** (1998). Symplasmic fields in the tunica of the shoot apical meristem coordinate morphogenetic events. *Development* **125**, 1477-1485.
- Rojo, E., Sharma, V. K., Kovaleva, V., Raikhel, N. V. and Fletcher, J. C.** (2002). CLV3 is localized to the extracellular space, where it activates the *Arabidopsis* CLAVATA stem cell signaling pathway. *Plant Cell* **14**, 969-977.
- Ruth, J., Klekowski, E. J. and Stein, O. L.** (1985). Impermanent initials of the shoot apex and diplontic selection in a juniper chimera. *Am. J. Bot.* **72**, 1127-1135.
- Satina, S., Blakeslee, A. F. and Avery, A.** (1940). Demonstration of three germ layers in the shoot apex of *Datura* by means of induced polyploidy in periclinal chimeras. *Am. J. Botany* **27**, 895-905.
- Schnittger, A., Grini, P. E., Folkers, U. and Hülskamp, M.** (1996). Epidermal fate map of the *Arabidopsis* shoot meristem. *Dev. Biol.* **175**, 248-255.
- Schofield, R.** (1978). The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* **4**, 7-25.
- Schoof, H., Lenhard, M., Haecker, A., Mayer, K. F. X., Jürgens, G. and Laux, T.** (2000). The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell* **100**, 635-644.
- Schrick, K. and Laux, T.** (2000). Zygotic embryogenesis: The formation of an embryo from a fertilized egg. In *Current Trends in the Embryology of Angiosperms* (ed. S. S. Bhojwani and W. Y. Soh), pp. 249-277. Dordrecht: Kluwer Academic Publisher.
- Sieburth, L. E., Running, M. P. and Meyerowitz, E. M.** (1995). Genetic Separation of Third and Fourth Whorl Functions of AGAMOUS. *The Plant Cell* **7**, 1249-1258.
- Spradling, A., Drummond-Barbosa, D. and Kai, T.** (2001). Stem cells find their niche. *Nature* **414**, 98-104.
- Steeves, T. A. and Sussex, I. M.** (1989). *Patterns in Plant Development*. Cambridge, UK: Cambridge University Press.
- Stewart, R. N. and Dermen, H.** (1970). Determination of number and mitotic activity of shoot apical initial cells by analysis of mericlinal chimeras. *Am. J. Bot.* **57**, 816-826.
- Stuurman, J., Jaggi, F. and Kuhlemeier, C.** (2002). Shoot meristem maintenance is controlled by a GRAS-gene mediated signal from differentiating cells. *Genes Dev.* **16**, 2213-2218.
- Sussex, I. M. and Rosenthal, D.** (1973). Differential ³H-thymidine labelling of nuclei in the shoot apical meristem of *Nicotiana*. *Bot. Gaz.* **134**, 295-301.
- Szymkowiak, E. J. and Sussex, I. M.** (1992). The internal meristem layer (L3) determines floral meristem size and carpel number in tomato periclinal chimeras. *Plant Cell* **4**, 1089-1100.
- Taylor, R. L.** (1967). The foliar embryos of *Malxis paludosa*. *Can. J. Bot.* **45**, 1553-1556.
- Terskikh, A. V., Easterday, M. C., Li, L., Hood, L., Kornblum, H. I., Geschwind, D. H. and Weissman, I. L.** (2001). From hematopoiesis to neurogenesis: evidence of overlapping genetic programs. *Proc. Natl. Acad. Sci. USA* **98**, 7934-7939.
- Trotochaud, A. E., Hao, T., Wu, G., Yang, Z. and Clark, S. E.** (1999). The CLAVATA1 receptor-like kinase requires CLAVATA3 for its assembly into a signaling complex that includes KAPP and a Rho-related protein. *Plant Cell* **11**, 393-406.
- van den Berg, C., Willemsen, V., Hendriks, G., Weisbeek, P. and Scheres, B.** (1997). Short-range control of cell differentiation in the *Arabidopsis* root meristem. *Nature* **390**, 287-289.

- Vaughan, J. G.** (1955). The morphology and growth of the vegetative and reproductive apices of *Arabidopsis thaliana* (L.) Heynh., *Capsella bursa pastoris* (L.) Medic. and *Anagallis arvensis* L. *J. Linn. Soc. Bot.* **55**, 279-301.
- Vollbrecht, E., Veit, B., Sinha, N. and Hake, S.** (1991). The developmental gene *Knotted-1* is a member of a maize homeobox gene family. *Nature* **350**, 241-243.
- Waites, R., Selvadurai, H. R., Oliver, I. R. and Hudson, A.** (1998). The *PHANTASTICA* gene encodes a MYB transcription factor involved in growth and dorsoventrality of lateral organs in *Antirrhinum*. *Cell* **93**, 779-789.
- Wardlaw, C. W.** (1957). On the organization and reactivity of the shoot apex in vascular plants. *Am. J. Bot.* **44**, 176-185.
- Yanofsky, M. F., Ma, H., Bowman, J. L., Drews, G. N., Feldmann, K. A. and Meyerowitz, E. M.** (1990). The protein encoded by the *Arabidopsis* homeotic gene *AGAMOUS* resembles transcription factors. *Nature* **346**, 35-39.
- Yu, L. P., Simon, E. J., Trotochaud, A. E. and Clark, S. E.** (2000). *POLTERGEIST* functions to regulate meristem development downstream of *CLAVATA* loci. *Development* **127**, 1661-1670.
- Zou, C., Zhang, Z., Wu, S. and Osterman, J. C.** (1998). Molecular cloning and characterization of a rabbit eIF2C protein. *Gene* **211**, 187-194.