Maintenance of Shoot and Floral Meristem Cell Proliferation and Fate[1]

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To cope with environmental changes, animals respond by altering their behavior, but sessile plants respond by altering their growth and development pattern. One of the major differences between plant and animal development is that plants have the capacity to develop new organs postembryonically. This potential to develop new organs is attributed to sets of cells, called meristems, which are found at the growing tips of the plants. Two meristematic cell populations are generated during embryogenesis. The shoot apical meristem (SAM) generates all of the aerial parts of the plant, whereas the root apical meristem generates the underground parts. The SAM produces lateral organs from the cells on its flanks while simultaneously maintaining a central pool of pluripotent stem cells for future organogenesis. Thus, maintenance of a functional SAM requires coordination between loss of cells from the meristem by differentiation and their replenishment by stem cell division (Steeves and Sussex, 1989).

Different types of lateral organs are generated by the SAM during successive phases of development. The SAM produces leaves and axillary meristems during the vegetative phase and floral meristems during the reproductive phase. Floral meristems produce flowers that usually consist of four whorls of organs. After producing these whorls, the activity of the floral meristem ceases, unlike the SAM, which continuously proliferates and produces organ primordia from its flanks. Several unanswered questions about meristem function are generating considerable interest: How do stem cells originate? How is the coordination between accumulation and loss of stem cells maintained? What are the signaling mechanisms involved in the communication between stem cells and with cells in the meristem flanks? How are shoot and floral meristems distinguished? Here, we provide an update of recent developments that address some of these key questions of SAM and floral meristem maintenance and development in Arabidopsis.

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ORGANIZATION OF THE SAM

The SAM forms during embryonic development, and the specific way in which it is organized enables plants to produce organs throughout their lives. The SAM of flowering plants consists of three radially distinct domains depending on the relative position of the cells (Fig. 1A). A central zone of cells at the very tip of the SAM corresponds to the small population of pluripotent stem cells (Steeves and Sussex, 1989). These cells divide slowly to maintain themselves as a reservoir of stem cells. Cells in the central zone are surrounded by a region of more frequently dividing cells, called the peripheral zone. Cells from the peripheral zone are incorporated into lateral organs on the flanks of the meristem, entering pathways leading to determined cell fate, and their numbers are replenished by cells from the central zone. Beneath the central zone in the deeper layers of the meristem, columns of large, vacuolated cells in the rib zone provide cells for the developing pith, which makes up the inner tissues of the meristem.

SAM cells can also be arranged into distinct layers called tunica and corpus on the basis of their cell division patterns (Kaplan and Cooke, 1997). In plants such as maize (*Zea mays*), the tunica is a single-cell monolayer of epidermal cells. In Arabidopsis, a model plant for genetic and molecular studies, the tunica consists of an overlying epidermal L1 layer and a subepidermal L2 layer that provides the mesophyll cells for leaves and the germ tissue found in pollen grains and ovules (Fig. 1B). Both of these layers are one cell thick, and each remains clonally distinct from the others because the cells within them divide only in an anticlinal orientation, perpendicular to the plane of the meristem. The corpus, or L3, is a multilayer group of cells that lie beneath the tunica and divide in all planes, allowing the plant to grow upward and outward. The L3 contributes cells for the vasculature and pith. Although highly regular patterns of cell division are detected in the SAMs, mosaic analysis reveals that the fate of a meristem cell cannot be determined from its lineage (Poethig et al., 1986; Furner and Pumfrey, 1992; Irish and Sussex, 1992). Instead, the fates of SAM cells are determined by their positions in the meristem rather than by instructions from their ancestors. Constant communication among SAM cells both within and between

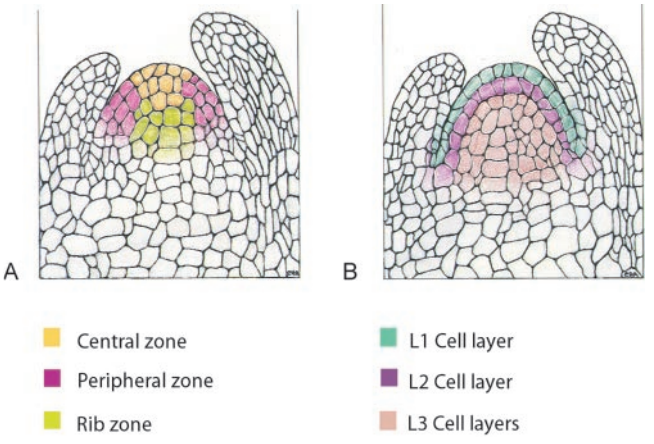

Figure 1. A schematic depiction of the organization of the SAM. A, Radial domains. Lateral organs are produced from cells recruited from peripheral zone, whereas cells from the rib zone contribute to the bulk of the stem. The central zone acts as a reservoir of stem cells that replenishes the cells of the peripheral and rib zones, which are lost during the formation of stem and lateral organs. At the same time, the central zone also maintains the pool of cells for itself. B, The clonally distinct layers of cells. The epidermal (L1) and subepidermal (L2) layers maintain their distinctness by anticlinal cell division. The L1 and L2 layers are collectively referred to as the tunica. Cells interior to the L2 constitute the corpus (L3) in which cell divisions take place in various planes, resulting in growth in all directions.

layers is, therefore, critical for the cells to assess their location in the meristem and to determine their behavior appropriately. As described below, some of the pathways that communicate cell fate information between neighboring SAM cells are now beginning to be understood.

ORIGIN OF THE EMBRYONIC SAM

The Arabidopsis embryo starts as a single cell after the fertilization of the egg cell by the sperm cell. The embryo begins dividing in organized fashion, producing apical cells that pass through a series of recognizable morphological stages (Goldberg et al., 1994) before reaching dormancy (Fig. 2). During this progression, the apical region of the embryo becomes divided into domains that are demarcated by different gene expression patterns and assume distinct developmental fates. Recent advances using SAM-specific transcripts as histological markers to analyze the development of the apical portion of the embryo reveals that the characteristic transcriptional domains of the SAM develop gradually during embryogenesis. Here, we discuss recent information generated about key meristem regulatory genes whose expression at very early stages of embryo development has been investigated.

One key meristem regulatory gene is the homeobox gene *WUSCHEL* (*WUS*), whose expression in the mature SAM is restricted to a small domain beneath the stem cells. *WUS* is first expressed in the apical subepidermal cells at the 16-cell stage of embryogenesis (Fig. 2A) before an organized shoot meristem is evident (Mayer et al., 1998). *WUS* transcripts gradually become limited to deeper regions of the SAM as it forms, suggesting that cell-cell interactions probably dictate the boundaries of the *WUS* expression domain. *WUS*-expressing cells in the interior of the shoot meristem signal to their overlying neighbors to specify them as pluripotent stem cells (Brand et al., 2000; Schoof et al., 2000). Loss-of-function *wus* mutations cause premature termination of both shoot and floral meristems after the formation of a few organs. Thus, the meristems are initiated but are not correctly maintained, indicating that other factors are also involved in keeping the meristems active.

The *SHOOTMERISTEMLESS* (*STM*) gene is also critical for proper embryonic SAM formation. In *stm* mutant embryos, the three precursor layers of the SAM fail to perform the cell divisions that generate the tunica/corpus arrangement (Barton and Poethig, 1993), and a functional SAM is never organized. *STM* encodes a member of the *knox* family of homeodomain proteins that are likely to act as transcriptional regulators in promoting SAM development. *STM* expression is first detected in a few cells in the globular stage embryo; these cells are slightly displaced from the center of the embryo (Long and Barton, 1998). The domain of *STM* expression subsequently enlarges until expression is detected on both sides of the globular/transition stage embryo. By the early heart stage, *STM* expression is detected as a continuous band between the presumptive cotyledons, where it remains throughout the rest of embryonic and postembryonic development (Fig. 2, B–D). Genetic and molecular analyses indicate that *STM* is required in the central region of the initiating SAM to inhibit differentiation and initiate a SAM-specific program of development (Long and Barton, 1998). On the meristem periphery, *STM* appears to inhibit organ outgrowth and subsequent differentiation by negatively regulating the expression of organ-specific Myb genes (Byrne et al., 2000).

The *CUP-SHAPED COTYLEDON1* (*CUC1*) and *CUC2* genes are also important for establishing a functional embryonic meristem, because *cuc1 cuc2* double mutants form fused cotyledons but fail to develop a SAM (Aida et al., 1997). The SAM of *cuc1* and *cuc2* single mutants develops normally, and *CUC1* is expressed in *cuc2* mutants and vice versa. These results suggest that the activity of either *CUC1* or *CUC2* is sufficient for embryonic SAM formation and that the two genes are regulated independently. *CUC1* and *CUC2* are highly homologous to each other and to the petunia (*Petunia hybrida*) *NAM* gene, which is also required for floral organ development, cotyledon separation, and embryonic SAM formation (Souer et al., 1996). The N-terminal halves of *CUC1*, *CUC2*, and *NAM* contain highly conserved sequences called the NAC domain. *NAC* domain-encoding genes (*NAC* genes) constitute a large plant gene fam-

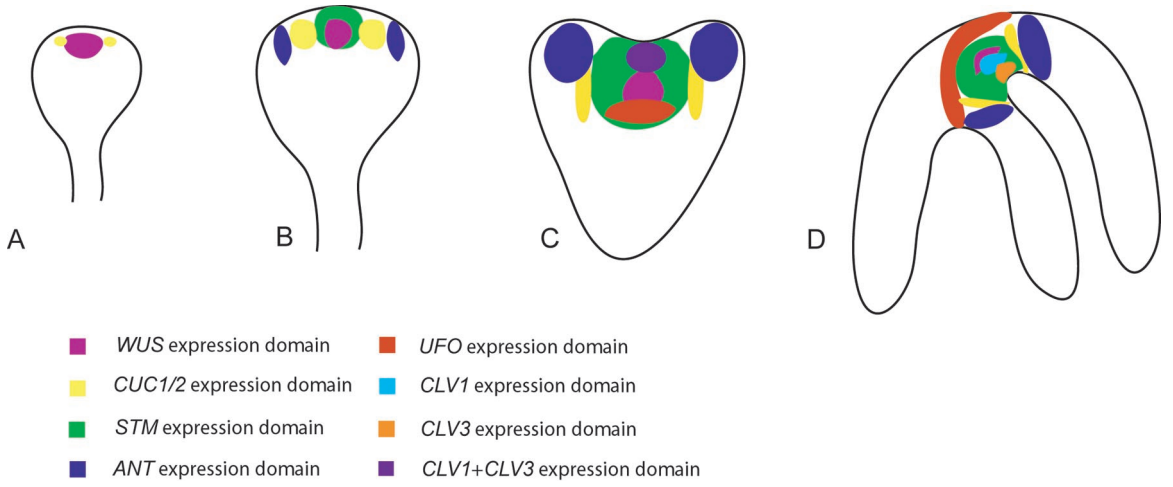

Figure 2. Schematic representation of the dynamic embryonic expression patterns of genes involved in initiation and maintenance of the SAM. The domains of gene expression shown here are representative, but for exact expression profiles refer to the references cited in the text. A, Early globular stage embryo. The *WUS* gene is expressed in a few apical cells, and *CUC1* and *CUC2* (*CUC1/2*) gene expression is initiated at two places flanking the *WUS*-expressing cells. B, Late globular-transition stage embryo. *STM* expression initiates in the *WUS* expressing cells, and by the transition to heart stage, the domain of *WUS* expression reduces to few cells. *ANT* is expressed in the peripheral regions flanking the *STM* domain. C, Heart stage embryo. *UFO* is expressed in *STM*-expressing cells mainly in the L3. The expression domain of *CUC1/2* moves further toward the flanks and is almost at the base of the presumptive cotyledons, overlapping with the *ANT* expression domain. In the SAM primordium, *CLV3* is induced in the superficial cells, whereas the *CLV1* and *WUS* expression domains overlap in the interior. D, Torpedo stage, at which the histological structure of the SAM is evident. By this stage, *UFO* is expressed as a ring beneath the cells expressing *STM*, forming the lower boundary of the SAM. *ANT* and *CUC1/2* expression domains mark the flanks of the SAM. *CLV3* is expressed in L1 and L2 layers and *CLV1* in L2 and L3 cells of the SAM, with *WUS* mRNA restricted to a few cells in the L3.

ily and are not found in other organisms, suggesting that they play unique roles in plant development (Aida et al., 1997; Takada et al., 2001).

CUC1 and *CUC2* are functionally redundant genes with nearly identical embryonic expression patterns that appear to maintain a population of undifferentiated cells during SAM formation. *CUC1* mRNA is detected in a few cells at the globular stage that are predicted to form the embryonic SAM, suggesting that *CUC1* functions within these cells and regulates SAM formation. *CUC2* mRNA expression is observed in the late globular stage embryo, in the same domain as *CUC1* (Aida et al., 1999). By the early transition stage, the *CUC1/2* expression pattern is restricted to the boundary region between the presumptive cotyledons and the SAM, where it remains through later stages (Fig. 2, C and D). *CUC1*, *CUC2*, and *STM* are expressed in overlapping regions in globular stage embryos, but *STM* transcripts are not detected in *cuc1 cuc2* double mutant embryos, whereas *CUC1* and *CUC2* are expressed in *stm* mutant embryos (Takada et al., 2001). Thus, *CUC1* and *CUC2* function upstream of *STM* and are required for its expression. Overexpression of *CUC1* induces ectopic *STM* expression, even in the absence of *CUC2*. The fact that *STM* is ectopically expressed in 35S::*CUC1* cotyledons strongly suggests that *CUC1* is sufficient to activate *STM* and acts as a positive regulator of *STM* transcription. However, it remains to be determined whether *CUC1* directly or indirectly regulates *STM* expression.

STM function, in turn, is required by the early heart stage to induce the transcription of *UNUSUAL FLORAL ORGANS* (*UFO*), which is expressed in meristems throughout development. *UFO* expression, although limited to a subset of *STM*-expressing cells, is dynamic during embryogenesis (Long and Barton, 1998). At the heart stage, *UFO* transcripts are found in a small group of cells at the presumptive shoot apex (Fig. 2C). At this early stage, *UFO* expression is limited to the presumptive L2 and L3 layers and does not extend into the L1 layer of the embryo. During the torpedo stage, *UFO* expression resolves into a cup-shaped domain at the base of the embryonic meristem (Fig. 2D). In the mature embryo, *UFO* expression persists in a cup-shape around the SAM, and, after germination, *UFO* transcripts are found at low levels in the center of the SAM and at higher levels in a ring around the periphery. This expression pattern suggests that *UFO* may be involved in demarcating the boundary between meristem cells and organ founder cells. *UFO* encodes an F-box protein that interacts with ASK1, another F-box protein that has been demonstrated to be part of a complex involved in protein degradation via the ubiquitin pathway (Zhao et al., 2001). It can be speculated that UFO may be involved in protein degradation and helps degrade stem cell factors and/or cell cycle regulators

that are not required by cells in the peripheral zone, once they enter specific cell fate pathways.

The *AINTEGUMENTA* (*ANT*) gene is involved in ovule development and in the initiation and growth of floral organs. Expression of *ANT* is first detected in the 32-cell stage globular embryo where it is found in a few cells in the apical portion of the embryo (Long and Barton, 1998). By the late globular to transition stage, *ANT* expression forms a ring in the periphery of the apical region around the presumptive SAM (Fig. 2B). The ring-shaped pattern of *ANT* expression at this stage is consistent with expression of this gene in presumptive organs because the cotyledons encircle the apical portion of the embryo. However, the *ANT* expression pattern is not completely complementary to that of *STM* because the domains overlap in the peripheral region. As cotyledon outgrowth continues through the later stages, the region of *ANT* expression (Fig. 2D) eventually becomes limited to a plane that separates the cotyledons into nearly equal upper and lower halves (Elliot et al., 1996). Observations of *ANT* expression in *stm* mutants revealed that *ANT* is not negatively regulated by *STM* activity in the center of embryo. In addition, because *STM* and *ANT* transcripts coexist in the peripheral region, the presence of *STM* mRNA alone is not sufficient to negatively regulate *ANT* expression. Therefore, these two genes appear to be independently regulated.

Taking together all the information we have at present, a sketchy picture can be drawn of the origin of the Arabidopsis SAM (Fig. 2). *WUS* expression, which is detected at the very early 16-cell stage, marks the onset of the presumptive SAM. *WUS* induction is followed by the expression of *CUC1* and *CUC2* at the apex of the globular stage embryo, and *CUC1* activates *STM* expression in the same cells. During the transition stage, the expression of *CUC1/2* becomes restricted to peripheral region of the apical embryo to demarcate the boundary between the SAM and the *ANT*-expressing cells of the initiating cotyledons. Once the presumptive SAM is organized, *STM* induces *UFO* expression, which eventually marks the boundary of the SAM. In this way the molecular features of the SAM are elaborated gradually during embryogenesis, rather than all at once, and further studies will provide additional insights into how these genes interact with one another and with other factors during SAM initiation.

MAINTENANCE OF THE SAM STATE

Once the SAM has been initiated, a meristem signal transduction pathway mediated by the *CLAVATA* loci maintains the proper number of stem cells to enable ongoing organogenesis. Recessive loss of function mutations at the Arabidopsis *CLAVATA* (*CLV1*, *CLV2*, and *CLV3*) loci cause the accumulation of excess stem cells in the center of the SAM. Stem cell accumulation in *clv* mutants takes place progressively and results in the fasciation (overgrowth) of the SAM at the time of the transition to flowering. This trend of excess stem cell accumulation continues in the floral meristem, suggesting that both shoot and floral meristems use the same mechanism to restrict stem cell activity. Genetic analyses have shown that *CLV1*, *CLV2*, and *CLV3* act together in a single-stem cell-restricting pathway in shoot and floral meristems, although *CLV2* also functions more broadly to regulate other aspects of development. Recent studies have shed light on how the *CLV* genes act to maintain the pool of pluripotent stem cells in shoot and floral meristems and have identified other players involved in this process.

The three *CLV* genes encode key components of a meristem signaling pathway. The *CLV1* gene encodes an extracellular Leu-rich repeat (LRR) receptor Ser/Thr kinase (Clark et al., 1997). The *CLV2* gene encodes an LRR receptor-like protein with a short cytoplasmic tail (Jeong et al., 1999), and *CLV3* encodes a small polypeptide with an amino-terminal putative signal sequence (Fletcher et al., 1999). CLV3 has been shown biochemically to act as the ligand for a receptor complex containing CLV1 and CLV2 that is presumed to be membrane-bound (Trotochaud et al., 2000). In cauliflower meristem extracts, 75% of CLV3 protein is bound to the CLV1/CLV2 receptor complex. The other 25% is not receptor-associated and can be detected as a putative multimer of approximately 25 kD. It is not known whether this putative complex consists of a CLV3 homomultimer or whether other proteins are present. In addition to the CLV proteins, the active receptor complex also contains one or more members of the Rop subfamily of plant Rho/Rac small GTPase-related proteins and a kinase-associated protein phosphatase. On the basis of the roles of Ras GTPases in animals, it has been proposed that Rop GTPases may respond to CLV1 kinase activation by activating a mitogen-activated protein kinase-like signal transduction cascade (Trotochaud et al., 1999), although there currently is no direct evidence to support this hypothesis.

The mRNA expression patterns of the *CLV* genes provided insights into how the CLV signal transduction pathway controls the accumulation of shoot and floral meristem cells. The *CLV1* and *CLV3* mRNAs are initially expressed at the heart stage of embryogenesis in a small group of presumptive SAM cells between the cotyledons (Fig. 2). The *CLV1* and *CLV3* transcripts subsequently are restricted to neighboring subdomains of shoot and floral meristem cells. *CLV3* mRNA is found only in the stem cells at the meristem apex (Fletcher et al., 1999), whereas *CLV1* mRNA is detected in a group of central, interior L3 cells beneath but slightly overlapping the *CLV3* expression domain (Clark et al., 1997). Thus, the stem cells at the apex of shoot and floral meristems communicate cell fate information via CLV3 signaling to the underlying cells that contain the CLV1 receptor

complex. *CLV2* transcripts are detected in meristems and also in other tissues, consistent with the broader realm of function for this gene (Jeong et al., 1999).

Another key element of the CLV signaling pathway is the *WUS* gene product. *WUS* encodes a novel subtype of the homeodomain transcription factor family (Laux et al., 1996). Both the SAM and floral meristems of *wus* mutant plants terminate prematurely after the formation of few organs, indicating that *WUS* is necessary to promote stem cell activity throughout development. During embryogenesis, *WUS* is expressed in the apical portion of the embryo before the initial appearance of *CLV1* and *CLV3*. The *WUS* transcripts become restricted gradually to a small group of cells that lie beneath the *CLV3* expression domain and overlap the *CLV1* domain in the central L3 cells of the shoot and floral meristems. The *WUS* expression domain is maintained by *FAS1* and *FAS2*, which encode components of chromatin assembly factor-1 (Kaya et al., 2000). FAS1 and FAS2 are likely to facilitate chromatin assembly after DNA replication, thereby promoting stable transcription of the *WUS* gene.

WUS is a direct target of the negative, stem cell-restricting *CLV* pathway. *wus clv* double mutants have the same phenotypes as *wus* single mutants (Schoof et al., 2000). This epistatic interaction reveals that *WUS* is a component of the CLV signaling pathway and that its activity is essential for CLV function. *WUS* is, therefore, thought to function at the transcriptional level to promote stem cell fate and that this activity is down-regulated by signaling through the CLV complex. In *clv3* mutant meristems, the *WUS* expression domain expands upward into the subepidermal layer and also laterally toward the meristem flanks. In *CLV3* overexpressing plants, which form arrested meristems and phenocopy the *wus* loss-of-function mutant phenotype, *WUS* mRNA is not detected (Brand et al., 2000). Thus, signaling through the CLV pathway in shoot and floral meristems restricts the size of the cell population that expresses *WUS*, limiting the ability of *WUS* to promote stem cell activity.

A recent study has shown that *WUS* activity is sufficient and necessary to specify stem cell fate. When *WUS* is mis-expressed on the meristem periphery under the control of the *ANT* promoter, the resulting transgenic seedlings fail to produce lateral organs (Schoof et al., 2000). Instead, the shoot apex consists entirely of undifferentiated meristematic cells, indicating that *WUS* is sufficient to confer stem cell fate. *CLV3* mRNA, which is restricted to the central region of the SAM in wild-type plants, is detected on the periphery of the meristematic cell mass in *pANT::WUS* seedlings. Thus, *WUS* activity is also sufficient to induce *CLV3* transcription, indicating that *WUS* is a critical component of a stem cell-promoting pathway that preserves the *CLV3*-expressing stem cell reservoir at the SAM apex.

The *POLTERGEIST* (*POL*) gene appears to function in the stem cell-promoting pathway along with *WUS* (Yu et al., 2000). *pol* mutants were isolated as recessive suppressor mutations in a genetic screen for second-site modifiers of intermediate *clv3* and *clv1* alleles. *pol* single mutants are nearly indistinguishable from wild-type plants, but *POL* appears to promote stem cell fate because *pol clv* double mutants have fewer stem cells in their shoot and floral meristems than *clv* single mutants. *pol* mutations also enhance the *wus* shoot and floral meristem phenotypes, and dosage effects are observed between *POL* and *WUS*. These data suggest that *POL* acts redundantly with *WUS* to promote shoot and floral meristem cell fate, but the precise role of *POL* in the CLV-WUS pathway remains to be determined once the gene is cloned.

The *STM* and *CUC* genes also promote SAM function, but genetic and molecular studies show that these genes function largely independently of the *CLV* pathway. *stm clv* double mutants develop some vegetative and floral organs, revealing that the *clv* mutations partially suppress the *stm* mutant phenotype (Clark et al., 1996). Suppression of the *stm* mutant phenotype by the *clv* mutations occurs in a dominant fashion, and *stm* mutations can also partially suppress the *clv* mutant phenotypes in a dominant fashion. These genetic interactions suggest that the *STM* and *CLV* loci function to regulate some of the same processes, but act in different pathways. *STM* and the *CUC* genes are expressed in embryos before the appearance of *CLV1* and *CLV3. CLV1* expression initiates normally in the absence of *STM* (Long and Barton, 1998), so *STM* is not required to induce *CLV1* transcription. However, *CLV1* expression is reduced or absent in *stm* mutants at later stages of embryogenesis, indicating either that *STM* is directly required to maintain *CLV1* expression or that cells in the *stm* shoot apex eventually lose *CLV1* expression as they undergo differentiation. Overall, the evidence favors the conclusion that *STM* and the *CLV* genes appear to play opposite and largely independent roles in regulating meristem development.

With the data currently in hand, a rudimentary picture of the feedback regulatory loop required for the postembryonic maintenance of Arabidopsis shoot and floral meristems is coming into focus (Fig. 3A). In wild-type plants, the CLV3 signal is produced by the stem cells at the apex of shoot and floral meristems. The CLV3 ligand binds to the CLV1-CLV2 receptor complex in the underlying cells, probably by interacting with the extracellular LRR domains of these proteins. Ligand binding in the presence of the active CLV1 kinase domain induces assembly of the active receptor complex, permitting downstream signal transduction that restricts *WUS* expression to a small domain in the interior of the meristem. Activity of the positive pathway mediated by *WUS* with poorly understood input from *POL*, in turn, promotes the ex-

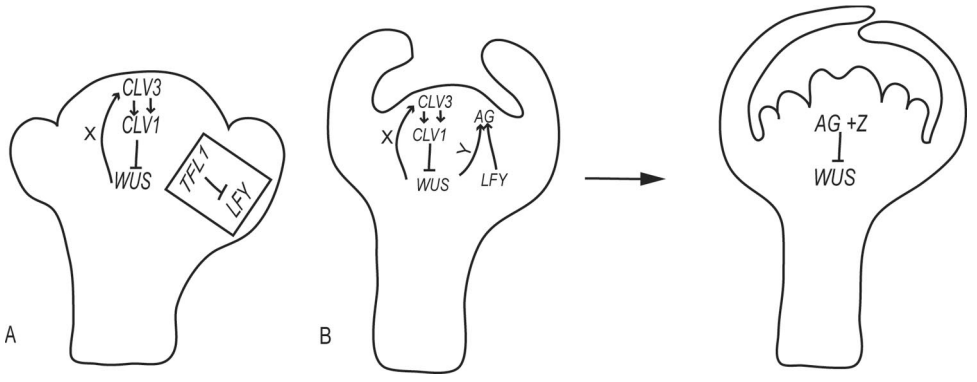

Figure 3. Model for the interactions between regulatory genes in the SAM and the floral meristem. A, During the reproductive phase, the SAM produces floral meristems from its flanks, whereas its central pool of stem cells is maintained by *CLV* pathway. Signaling by CLV3 through the CLV1 receptor complex limits the size of the *WUS* expression domain in the interior of the meristem. In turn, *WUS* activity, perhaps with the help of a diffusible signal (X), preserves the population of *CLV3*-expressing stem cells in the superficial cell layers. TFL simultaneously represses the expression of the floral meristem identity gene *LFY* in the SAM. B, *LFY* is up-regulated in early floral meristems, along with other genes that specify floral meristem identity. *LFY* is expressed throughout the floral meristem, and, along with *WUS*, it activates *AG* in the center of floral meristem where the stamens and carpels will form. *LFY* and *WUS* may require another factor (Y) to activate *AG* in the correct expression domain. C, Later in floral meristem development, when the carpel primordia are due to form, AG and another unidentified factor (Z) repress the expression of *WUS*, terminating stem cell maintenance and allowing gynoecium differentiation.

pression of *CLV3* and the persistence of the *CLV3*-expressing stem cell pool. In wild-type meristems, *WUS* mRNA is not detected in the stem cells in the L1 and L2, so their activity in these superficial cell layers may be maintained by an non-cell autonomous, extracellular signal induced by *WUS*. As an alternative, the *WUS* mRNA or protein itself may move into the superficial layers to regulate *CLV3* expression directly.

Recent evidence suggests that the CLV meristem regulatory feedback loop is conserved in other species of plants. Taguchi-Shiobara et al. (2001) have isolated a novel mutant of maize, *fasciated ear2* (*fea2*), which causes shoot and floral meristem enlargement and massive over-proliferation of the ear inflorescence meristem. The FEA2 protein is closely related to the Arabidopsis CLV2 LRR receptor-like protein and localizes to the plasma membrane. A *CLV1*-related gene, *OsLRK1*, has also recently been isolated from rice (*Oryza sativa*; Kim et al., 2000). Antisense *OsLRK1* expression in rice caused the formation of extra floral organs, a characteristic phenotype of *clv1* mutant plants, without significantly affecting the SAM. These exciting results indicate that the components of the CLV pathway are also present in monocots, and this may be the conserved pathway in angiosperms to regulate meristem size, at least in flowers. More work still needs to be done to isolate the orthologs of the *CLV* genes and other factors from various plant species to understand fully the underlying mechanism for maintaining stem cell activity during development.

Finally, the cloned components of the CLV pathway are all members of gene families in Arabidopsis. The WUS homeodomain protein is distantly related to the KNOTTED1 class of eukaryotic transcription factors (Laux et al., 1996), and CLV1 and CLV2 are members of large families of receptor proteins (Initiative, 2000). Many CLV1-like receptor kinases have been shown to function in signaling cascades, such as Arabidopsis FLS2 and HAESA (RLK5) and the brassinosteroid receptor BRI1 (Li and Chory, 1997; Gomez-Gomez and Boller, 2000; Jinn et al., 2000). With the completion of the Arabidopsis genome sequence, CLV3 has now been discovered to be a member of the CLE family of small, putative secreted proteins that has two dozen members in Arabidopsis and others from maize, rice, tomato (*Lycopersicon esculentum*), and several other plants (Cock and McCormick, 2001). Only a few of the Arabidopsis *CLE* genes are represented by expressed sequence tags, but all of them are expressed during development (V.K. Sharma and J.C. Fletcher, unpublished data). CLE proteins have not been found in animals or fungi, suggesting that these proteins may act as signaling molecules in pathways that are specific to plants. Because Arabidopsis plants seem to use a very large number of CLV1- and CLV2-like proteins for transducing signals, it seems quite plausible that the CLE proteins may act as ligands that interact with different combinations of LRR receptor kinases.

REGULATION OF FLORAL MERISTEM CELL FATE

Floral meristems are formed from the lateral margins of the SAM and produce the most beautiful parts of the plant, the flowers, which are usually comprised of four types of organs. At the structural and organizational level, both the SAM and the floral meristem are similar, because both contain a stem cell

reservoir at the apex that contributes cells to organogenesis on the flanks. As a result, both types of meristems share a number of regulatory genes and mechanisms for development and maintenance. Yet, despite these similarities, shoot and floral meristems also differ in several ways. One difference between them is the type and arrangement of the lateral organs that they produce. The SAM generally forms leaves and their associated meristems in a spiral arrangement, whereas floral meristems generate sepals, petals, stamens, and carpels in concentric rings called whorls. Another critical difference is that the SAM is indeterminate and grows indefinitely, whereas the floral meristem is determinate and terminates once all the floral organs are made. Thus, the stem cell reservoir in floral meristems is transient, and floral meristems must overcome the mechanisms that ensure stem cell maintenance at the correct stage of development to allow carpel formation in the center of the flower. Several recent papers have provided exciting insights into the mechanisms that regulate stem cell fate in floral meristems and into how the determinacy of the floral meristem is regulated with respect to indeterminacy of the SAM.

Floral meristems develop from the flanks of the SAM similar to leaf primordia, but with different fates. Floral meristem identity is specified combinatorially by the *APETALA1* (*AP1*), *CAULIFLOWER* (*CAL*), and *LEAFY* (*LFY*) genes. AP1 and CAL encode members of a regulatory protein family (Mandel et al., 1992; Weigel et al., 1992; Bowman et al., 1993). Another group of genes, including *TERMINAL FLOWER1* (*TFL1*), prevents the shoot from becoming a flower by retarding progression through all growth phases (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). The opposing functions of *TFL1* and the floral meristem identity genes are reflected in their complementary expression patterns and phenotypic effects.

One function of *LFY* is to activate the expression of the homeotic gene *AGAMOUS* (*AG*; Busch et al., 2000). *AG* is expressed in the floral meristem cells that will produce the stamens and carpels in the center of the flower and encodes a flower-specific MADS domain transcription factor (Yanofsky et al., 1990). Mutations in *AG* cause the generation of indeterminate flowers in which petals form in the third whorl instead of stamens, and a new flower is formed in place of the carpels. *ag* mutant flowers, therefore, resemble shoots because they remain indeterminate and continue to produce organs (Bowman et al., 1989). Conversely, transgenic plants that constitutively express *AG* form SAMs that terminate in a solitary flower (Mizukami and Ma, 1997). These data indicate that *AG* is required to specify the formation of stamens and carpels, and that it is sufficient to convert an indeterminate meristem into a determinate meristem.

LFY directly activates *AG*, but other factors are also required because LFY protein is present throughout the floral meristem, whereas *AG* is activated only in the center. Two recent papers have shown that *WUS*, which is expressed in the center of floral meristem in a subset of the cells that eventually express *AG*, encodes a factor that contributes regional specificity to *AG* induction (Lenhard et al., 2001; Lohmann et al., 2001). Like LFY, WUS binds directly to regulatory sequences in one of the *AG* introns. The WUS and LFY binding sites are adjacent to one another, but LFY and WUS appear to bind independently to the *AG* enhancer sequence. In *lfy* mutant plants, *AG* is not expressed in the majority of flowers. In other words, first-produced flowers have lower or no *AG* expression and the later produced flowers have some *AG* expression. Thus, the endogenous level of WUS is not sufficient to activate *AG* when *LFY* is absent. However, overexpression of *WUS* in *lfy* mutants causes ectopic expression of *AG*, which suggests that the requirement for *LFY* can be circumvented by a sufficiently high level of WUS. Yet neither LFY nor WUS appears to be sufficient to activate *AG*, because neither protein alone can activate an *AG* reporter construct in vitro or in yeast cells (Busch et al., 2000; Lohmann et al., 2001). In addition, one stamen usually forms in *wus* mutant flowers, indicating that LFY may stimulate a sufficient level of *AG* mRNA for limited floral organ identity specification even in the absence of WUS. These results reveal that *LFY* provides floral specificity and *WUS* provides regional specificity to *AG* induction, so that *AG* is only activated in floral meristem and not in the SAM (Fig. 3B). Note, however, that the *AG* expression domain is larger than the *WUS* expression domain, indicating either that other factors are also involved in inducing *AG* outside the *WUS* domain or that WUS itself acts as a diffusible signal.

Once *AG* is activated in the center of the floral meristem, one of its major functions is to convert the developing flower to a determinate structure. When *AG* function is absent, *WUS* expression in prolonged in the center of indeterminate *ag* flowers even after the production of many organs. Plants with reduced *AG* function have a partial floral indeterminacy phenotype and resemble plants that moderately overexpress *WUS* at the floral apex. *ag wus* double mutant flowers resemble *wus* flowers, indicating that *ag* indeterminacy is dependent upon the ectopic activity of *WUS*. From the collected data, it can be concluded that prolonged expression of *WUS* is sufficient to make floral meristems indeterminate and that *AG* regulates their determinacy by repressing *WUS* and terminating stem cell activity before carpel formation (Fig. 3B). The *CLV* signal transduction pathway also acts in the floral meristem to down-regulate *WUS*, but *AG* repression of *WUS* appears to occur at least partially independently of CLV function because the

size of the *WUS* expression domain is larger in *ag clv1* flowers than in *ag* flowers.

WUS and *AG*, which are key regulators of indeterminate and determinate growth, respectively, therefore have a complicated interaction in the floral meristem. *WUS* promotes stem cell proliferation in the floral meristem, and, together with the meristem identity gene *LFY*, it activates the floral homeotic gene, *AG*, which specifies floral organ identity and determinate growth of the floral meristem. Once expression of *AG* is established in the center of flower, it represses *WUS* to terminate the stem cell population in preparation for carpel formation. This simple feedback loop in which WUS and the floral-specific LFY protein activate *AG*, which then represses *WUS* and terminates the WUS-promoted stem cell population, provides an elegant mechanism for changing an indeterminate growth pattern into a determinate pattern for proper development to occur.

CONCLUDING REMARKS

The SAM is the stem cell maintenance center that caters to the need of the plant for additional growth and development depending on the cues it receives from the environment. To be able to provide constant supply of cells for organogenesis, the SAM must be under tight regulation to maintain the balance between the accumulation and differentiation of cells. In the recent years, there has been an explosion of information about how the SAM is initiated and maintained and about how the transition from indeterminate to determinate patterns of growth is regulated. Key genes involved in these pathways have been isolated, but we still have a long way to go to understand fully the mechanisms underlying them, how the different molecules and pathways interact, how signals are transmitted and perceived in various regions of the SAM, and the other factors that are involved in determining the fate of shoot and floral meristems. Pursuing these various fields of study should provide many more insights in the years to come.

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