Maintenance of Shoot and Floral Meristem Cell Proliferation and Fate

Vijay K. Sharma and Jennifer C. Fletcher*
United States Department of Agriculture-Plant Gene Expression Center, Plant and Microbial Biology Department, University of California at Berkeley, 800 Buchanan Street, Albany, California 94710

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.

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 the coordination between accumulation and 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.

1 This work was supported by the U.S. Department of Agriculture and the National Science Foundation.

* Corresponding author; e-mail fletcher@nature.berkeley.edu; fax 510–559–5678.

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 fami-

![Figure 1](image-url)
Maintenance of Shoot and Floral Meristem Cell Proliferation and Fate

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.

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 FLOWERING 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 homotrimer 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-
expression 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 specificit 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.

ACKNOWLEDGMENTS

We thank Eden Abram for providing the illustrations in Figure 1, and we apologize to those whose work was not cited because of length restrictions.

Received October 31, 2001; accepted January 28, 2002.

LITERATURE CITED


Aida M, Ishida T, Tasaka M (1999) Shoot apical meristem and cotyledon formation during Arabidopsis embryogen-

esis: interaction among the CUP-SHAPED COTYLEDON and SHOOTMERISTEMLESS genes. Development 126: 1563–1570


Maintenance of Shoot and Floral Meristem Cell Proliferation and Fate


