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Organ Formation at the Vegetative Shoot Meristem

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OVERVIEW

In higher plants, organ formation is critical for generating the vegetative portion of the plant. Above-ground organ formation occurs at a collection of stem cells termed the shoot meristem (SM), which is established during embryogenesis. How the SM functions as a site of continuous organ formation is a central question for plant developmental biology. This is not only because all above-ground organs are initiated by the SM but also because their position and identity are established there.

The SM retains the capability to form organs through two fundamental processes (Figure 1). The first function of the SM is to maintain a pool of undifferentiated cells. Without undifferentiated cells to draw upon, new organ initiation would not be possible. The second process is to direct appropriately positioned undifferentiated cells toward organ formation and eventual differentiation. Although the structure of meristems is variable across the plant kingdom, all complex multicellular plants regulate the balance between an undifferentiated and differentiated fate at their respective meristems. Thus, unraveling the mechanisms by which model plants such as Arabidopsis regulate meristem development is expected to have implications for a wide variety of plant species.

The SM has been the focus of many studies over the past several decades. These studies have investigated the diversity, morphology, histology, cell division patterns, and cell lineages of the SM. Because this work has been summarized in detail elsewhere (Steeves and Sussex, 1989; Lyndon, 1990, 1994), this review focuses on recent advances in understanding the genetic control of organ formation at the SM.

MERISTEM STRUCTURE

The SM can be divided conceptually into several regions (Figure 1C). The distinctions between the inner two regions, the central zone (CZ) and the peripheral zone (PZ), are based on classic experiments on angiosperms (Figure 1B; Steeves and Sussex, 1989; Lyndon, 1990). The CZ is located at the very center of the meristem and is characterized by a lower rate of cell division. It is surrounded by the PZ, which is characterized by a more rapid rate of cell division. Additional histological differences between the CZ and PZ have been observed in some species. During vegetative development in Arabidopsis, the SM is ~11 cells across in the epidermal layer. An analysis of cell division rates at the vegetative SM indicates that the PZ is approximately four cells across, whereas the CZ is two to three cells across (C.Y. Hung and S.E. Clark, unpublished results).

For the purposes of this review, the CZ is considered to be equivalent to the pool of undifferentiated cells of the SM, whereas the PZ is considered to be equivalent to the region in which cells are incorporated into organ primordia, although there is no direct evidence for this interpretation of the CZ/PZ distinction.

Surrounding the PZ and outside the meristem proper is a region I have termed the organ zone (OZ; Figure 1C). Organ primordia (leaf or flower) become morphologically distinct in the OZ. All three of these regions overlie the rib meristem (RM), which is postulated to give rise to the vasculature and interior stem structures (Steeves and Sussex, 1989).

In addition to these zonal distinctions, the SM in most angiosperms is also composed of clonally distinct horizontal cell layers. The layers are divided into the tunica, in which cell divisions are strictly anticlinal, and the corpus, in which cell divisions occur in a variety of orientations. In Arabidopsis, the tunica is composed of the L1 epidermal cell layer and the underlying L2 layer. The corpus, or L3, lies beneath the tunica layers. The cell layering is generally retained in organ primordia. In the leaf, for example, the epidermis is derived mainly from the L1, the mesophyll from the L2, and the vascular tissue from the L3 (Satina et al., 1940; Tinley-Basset, 1986; see also Poethig, 1997, in this issue).

These clonally isolated cell populations have been exploited experimentally in the generation of periclinal chimeras, in which the SM and the resulting organs have cell layers of differing genotypes. This technique allows researchers to assess the role that each cell layer plays in a given developmental process (Marcotrigiano and Bernatzky, 1995; see also Poethig, 1997, in this issue). The implication

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that cell signaling may direct the coordinated development of clonally distinct cell populations is discussed below.

MERISTEM FUNCTION

The SM is a very dynamic structure, constantly undergoing growth, cell division, and organ formation. Thus, although meristem structure is maintained at a near-constant level throughout the vegetative growth of a plant, the position and developmental fate of cells derived from the SM change over time. For example, cells in the CZ give rise to the cells of the CZ and PZ; cells that occupy the PZ are later found in the OZ.

A single, undifferentiated CZ cell is shown in Figure 2A (box). This cell is considered to be "undifferentiated" based on a number of criteria. First, undifferentiated cells are morphologically distinct from differentiated cells in that they are small (~5 μm across), densely cytoplasmic, and lack large vacuoles. CZ cells also exhibit remarkable developmental potential—cells derived from the CZ are capable of developing into trichomes, guard cells, petal epidermis, pollen, and all of the other cell types found above ground.

Other cells that are morphologically similar to CZ cells do not exhibit the same developmental range. For example, the cells of a leaf primordium only develop into leaf cell types under normal conditions. A corollary to this observation is that undifferentiated cells would be predicted not to express developmental regulators that would direct them toward a specific developmental fate. Although this has yet to be demonstrated for genes regulating leaf development, the expression of flower-specific regulators, such as *LEAFY* (Weigel et al., 1992) and *APETALA1* (Mandel et al., 1992), is not observed in the CZ of the inflorescence SM. Moreover, the ectopic expression of these genes in CZ cells results in the development of the SM into floral organs, with the subsequent loss of organ-forming ability. Thus, one would predict that genes directing leaf identity during vegetative development would not be expressed in the CZ.

As the SM continues to propagate itself, some of the progeny of the undifferentiated cell shown in Figure 2A become part of the PZ (Figure 2B). As these cells reach the PZ (circles), they must make a transition from their undifferentiated state in the CZ toward a more specified state in which they can become incorporated into organ primordia. This transition presumably involves the expression of specific developmental regulators and leads ultimately to complete cellular differentiation.

The trigger for the transition is presumably provided by positional information. Indeed, the location of organ formation has long been postulated to be regulated by positional information provided by the most recently formed organ primordia (Snow and Snow, 1931; Richards, 1948; Wardlaw, 1949). Perhaps the same signal provides the positional information for cell differentiation as well. However, cell fate in the PZ is not uniform. Some cells may act as nucleating centers for a primordium, whereas others may be recruited to

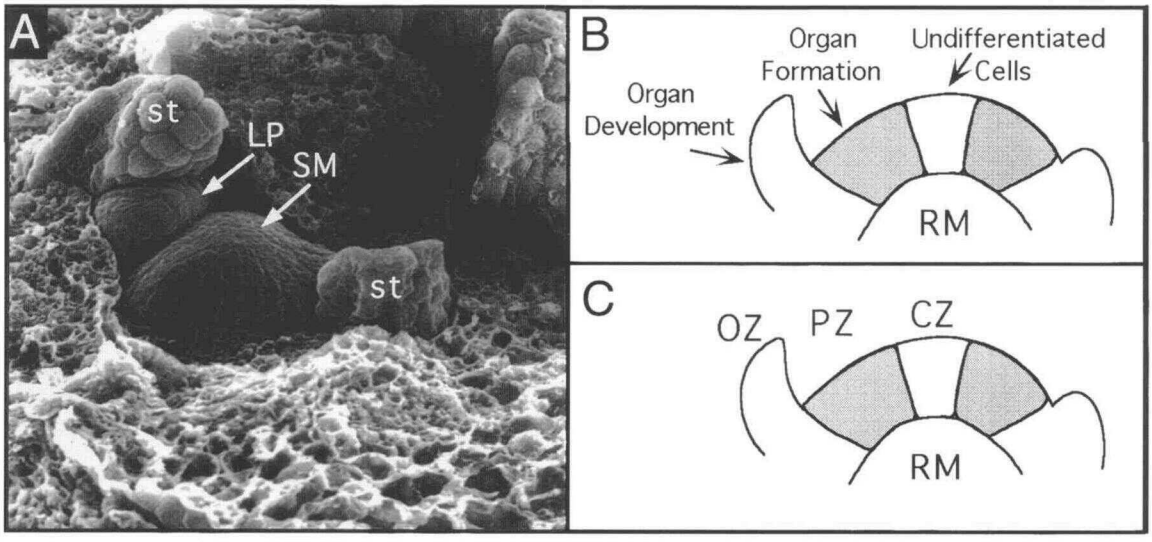

Figure 1. The Vegetative SM.

(A) Scanning electron micrograph of a 10-day-old wild-type SM. The locations of the SM, leaf primordia (LP), and stipules (st) are indicated.
(B) Functional regions of the SM.
(C) Morphological divisions in the SM. A slowly dividing CZ, a more rapidly dividing PZ, and OZ of primordial growth are all depicted lying above the RM.

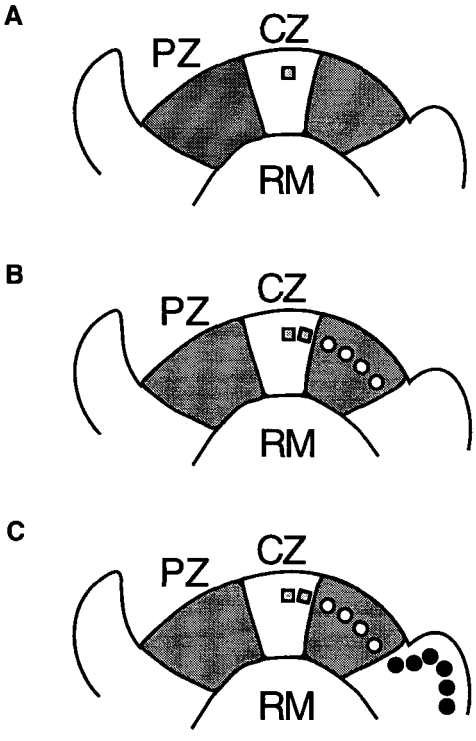

Figure 2. Dynamics of Shoot Meristem Development.

(A) A single undifferentiated cell (box) in the CZ. All of the cells in **(B)** and **(C)** are derived from this cell.

(B) Growth leads to progeny cells on the flanks (circles), where organ anlagen are organized.

(C) Cells that formerly resided in the PZ divide as the organ primordium grows (closed circles).

form the abaxial/adaxial and lateral sides of the primordium. Still others fall between the primordia and presumably go on to form the internodes between organs. So, although all of the cells are undergoing a developmental switch from an undifferentiated state, the type of developmental change may vary greatly, as would the pattern of gene expression and the rate of cell division.

Recent analysis of sectored (chimeric) Arabidopsis flowers indicates that the earliest flower anlagen may be composed of four cells in a square (Bossinger and Smyth, 1996). As organ anlagen are organized from a small number of cells in the PZ, they are presumably established as asymmetric units with abaxial/adaxial, lateral, and proximal/distal distinctions. This can be inferred from the observation that primordia growth occurs in an asymmetric fashion. Leaf primordia in the OZ, for example, rapidly form crescent-shaped primordia whose outward growth is also asymmetric. Directional growth, not cell division, appears to drive this asymmetry (Lyndon, 1990). One particularly enlightening experiment analyzed the growth of γ-irradiated wheat seedlings. Despite the lack of cell division in these seedlings, leaf primordia formed in the correct position and assumed the correct shape (Foard, 1971).

DEVELOPMENTAL GENETICS

Over the past several years, a number of mutations that disrupt specific aspects of the SM have been described, suggesting that the corresponding genes play important roles in meristem development. Two of these genes appear to affect SM initiation only, two genes are required for both SM initiation and maintenance, and three genes affect SM maintenance only (Table 1). The discussion below focuses on the processes of SM maintenance and function by describing these genes and the phenotypes of the mutants. After discussing several of the key players, the striking genetic interactions that have been observed between them are described. These experiments provide insights into the relationships between the respective gene products, although there remain several competing models for the actions of these genes. The process of SM formation during embryogenesis is addressed elsewhere in this issue (Kerstetter and Hake, 1997; Laux and Jürgens, 1997, in this issue).

SHOOT MERISTEMLESS

Mutations in two genes result in the specific failure of Arabidopsis plants to form and maintain a SM. One of these genes, *SHOOT MERISTEMLESS* (*STM*), was initially characterized from a single mutant allele, *stm-1*, that now appears to represent a null allele (Barton and Poethig, 1993). Embryogenesis in plants homozygous for *stm-1* is normal in that the cotyledons, hypocotyl, root, and root meristem develop properly, but the mature embryos lack a SM (Figures 3A and 3B). The cells that normally comprise the wild-type embryonic SM are largely absent from *stm-1* embryos, and those cells that are present at the base of the cotyledons

Table 1. Genes Regulating Meristem Development in Arabidopsis

	Required for Meristem		
Gene	Initiation	Maintenance	Encodes
ZWILLE	Yes	No	Unknown
PINHEAD	Yes	No	Unknown
STM	Yes	Yes	Transcription factor
WUS	Yes	Yes	Unknown
CLV1	No	Yes	Putative receptor kinase
CLV2	No	Yes	Unknown
CLV3	No	Yes	Unknown

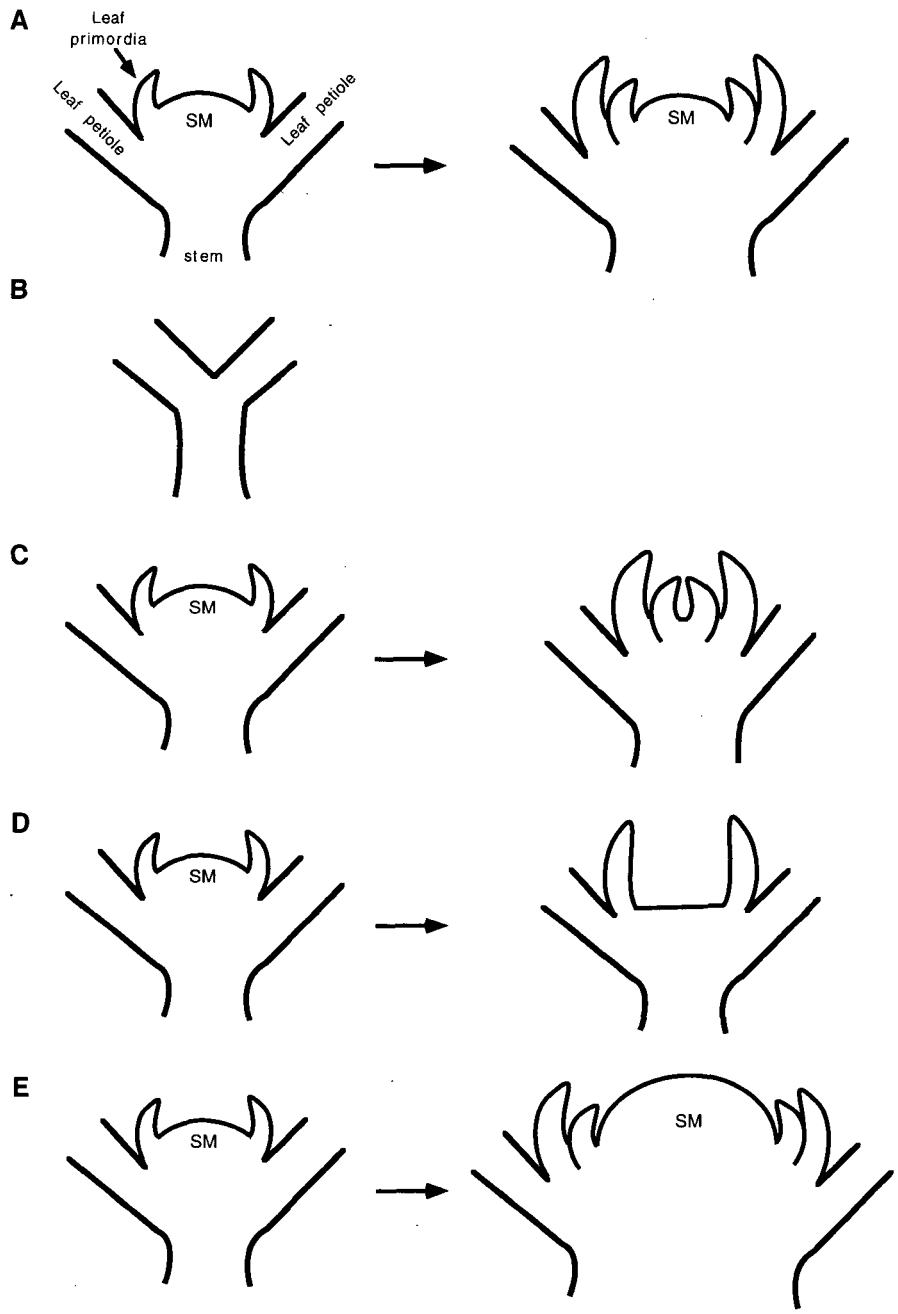

Figure 3. Comparison of SM Development in Wild-Type and Mutant Arabidopsis Plants.

The left panels represent the SM at initiation. For wild-type and *clv* plants, this would be the apical SM or any lateral SM. For *stm-2* and *wus* plants, this would be any adventitious SM. The right panels represent the SM after further growth.

(A) Wild type.

(B) Strong *stm* mutant (*stm-1*).

(C) Weak *stm* mutant (*stm-2*).

(D) *wus* mutant.

(E) *clv* (*clv1* or *clv3*) mutant.

(where the SM is normally found) lack features characteristic of meristematic cells.

In a small percentage of *stm-1* plants, growth occurs above the junction of the cotyledon vascular elements, giving rise to leaves. However, these leaves are not initiated in the organized manner characteristic of leaves formed by a meristem. Over time, these "rescued" *stm-1* plants continue to generate leaves slowly, but the leaves are generally initiated singly in the axils of existing leaves. Nevertheless, the fact that *stm-1* plants can initiate leaves in the absence of identifiable shoot meristems raises interesting possibilities. Perhaps organ formation can occur in the absence of a SM, or perhaps the initiated leaf represents the sole product of a SM that was entirely committed to the formation of a single organ. Regardless, *stm-1* plants retain a limited ability to form organs.

Further analyses have demonstrated that *STM* is not only required for initiation of the SM in the embryo but also for maintaining the ability of the SM to form organs during postembryonic growth. The isolation of the *STM* gene (Long et al., 1996) provided the first evidence for this expanded role. The predicted product of the *STM* gene is a member of the KNOTTED class of homeodomain proteins (Kerstetter et al., 1994; see below) and is therefore a putative transcription factor. *STM* expression is first observed in one or two cells of globular stage embryos, before the visible presence of a SM (Long et al., 1996). As embryo development proceeds, *STM* expression expands to include the entire embryonic SM when it becomes morphologically recognizable as such. Later, during vegetative development, *STM* is expressed continually in a central region of the SM that is larger than the CZ.

Additional evidence for a postembryonic role for *STM* has come from analyses of two weak *stm* alleles (Clark et al., 1996; Endrizzi et al., 1996). Mutants carrying these *stm* alleles also lack a fully functional embryonic SM. However, a small number of cells with meristematic features are present in the appropriate position at the apical pole of the embryo. These cells could represent an incomplete embryonic SM or the initiation of an adventitious meristem before embryonic arrest. In seedlings carrying the weak *stm* alleles, leaves are visible as early as 6 days after germination. Although growth stops after the formation of two to three leaves, new meristems are formed in the axils of the existing leaves. After the initiation of two to several leaves, the SM is apparently "used up" in the generation of organs (i.e., organ formation occurs at the summit of the meristem, across the CZ; Figure 3C). Interestingly, the organs that form at the apex of the SM of weak *stm* alleles are often fused together or are mosaic in structure, as if the organ primordia had overlapped when they were initiated.

The analyses of *stm* mutants indicate that *STM* is required to specify the SM cells of the embryo and to maintain undifferentiated cells at the center of the SM. The size of this pool of undifferentiated cells in the SM is increased through cell divisions and decreased through the commitment of cells to organ primordia. Thus, *STM* likely regulates one or both of these processes. For example, *STM* could be required to promote cell division in the CZ. If so, *stm* mutants would fail to replenish cells committed to organ formation and the CZ would quickly be consumed. Another possibility is that *STM* acts to maintain the cells of the CZ in an undifferentiated state. Under this model, differentiation would occur across both the CZ and PZ in *stm* mutants.

WUSCHEL

The phenotype of plants homozygous for mutations in the *WUSCHEL* (*WUS*) gene appears to be superficially similar to that of weak *stm* plants (Laux et al., 1996). Like *stm* mutants, *wus* mutants lack a functional embryonic SM. However, *wus* seedlings do develop two delayed leaves, although the origin of these leaves is unclear. Later, adventitious meristems, which are capable of forming two to several leaves before ceasing growth, are initiated. Repeated rounds of meristem termination followed by adventitious meristem formation lead to the development of a bushy plant very similar in appearance to plants carrying weak *stm* alleles.

However, a critical difference between *wus* and weak *stm* mutants is that whereas the CZ of weak *stm* meristems is consumed during organ formation, the centers of *wus* meristems retain a flat apex long after they cease to grow (Figure 3D). The flat region is as large as or larger than a SM but does not function as one. Moreover, the cells in this flattened region are slightly larger and more vacuolated than meristematic cells but still much smaller than differentiated cells. Perhaps as a result of the flat apex, *wus* plants do not develop the overlapping, mosaic structures that are present at the apex of plants carrying weak *stm* alleles. Nevertheless, adventitious meristems can form on the flat apex of *wus* plants.

Because the reason for meristem termination in *wus* mutants is unclear, interpreting the role *WUS* plays in meristem development is rather more complicated than it is for *STM*. The two *wus* alleles that have been identified exhibit similar phenotypes, but given the lack of a full allelic series, it is unclear if these represent null alleles. Perhaps *WUS* functions to establish the CZ/PZ distinctions within the SM. Thus, in the absence of *WUS* activity, the genes regulating CZ and PZ function would not be activated and the apical cells would neither proliferate nor differentiate. If *wus* does indeed function to establish the CZ/PZ distinctions, the ability of *wus* "promeristems" to initiate organs is quite remarkable because it suggests that organ formation can occur without the complete specification of the meristem.

CLAVATA

The phenotype of plants with mutations at the *CLAVATA1* (*CLV1*) locus is essentially the opposite of that of *stm* or *wus*

plants (Leyser and Furner, 1992; Clark et al., 1993). As early as the mature embryo stage, the *clv1* SM is significantly larger than the wild type (Running et al., 1995), and as *clv1* plants develop, their SMs continue to enlarge compared with those of wild-type plants (Figure 3E), becoming up to 1000-fold larger (by volume) at later stages of development. The enlargement is the result of the progressive accumulation of undifferentiated cells, which displace organ formation (i.e., the PZ) farther and farther from the apex.

Mutations at a distinct locus, *CLV3*, exhibit defects identical to *clv1* mutants (Clark et al., 1995). Moreover, *clv1 clv3* double mutants have phenotypes identical to strong *clv1* or *clv3* single mutants, suggesting that these genes function in the same pathway. In addition, *clv3* alleles dominantly enhance recessive and weakly semidominant *clv1* alleles. Mutations in a third locus, *CLV2*, also give rise to phenotypes similar to *clv1* and *clv3* mutants (Koornneef et al., 1983), but preliminary data indicate that *CLV2* may play a role somewhat independent from those of *CLV1* and *CLV3* (J. Kayes and S.E. Clark, unpublished data).

Because the size of the stem cell population at the SM is affected by two processes—cell division and cell differentiation—there are two possible models explaining the role of the *CLV* (i.e., *CLV1* and *CLV3*) loci in regulating meristem size. First, the *CLV* genes may restrict the rate of cell division in the CZ. This model would predict that in *clv* mutants, CZ cells would divide faster than those in the wild type, leading to their accumulation. Alternatively, *CLV* could promote the transition of cells entering the PZ from an undifferentiated state toward a differentiated state. According to this hypothesis, cells reaching the PZ in *clv* mutants would often remain undifferentiated, effectively enlarging the CZ.

The recent isolation of the *CLV1* gene indicates that it appears to code for a protein with an extracellular domain, which is composed predominantly of leucine-rich repeats, and an intracellular protein kinase domain (Clark et al., 1997). CLV1 could therefore act as a receptor kinase to relay positional information to specific cells of the SM. The *CLV1* mRNA expression pattern is quite specific. In the SM, *CLV1* is expressed in a central region (larger than the CZ), but only in the L3 layer. Thus, the accumulation of undifferentiated cells in the L1 and L2 layers of *clv1* mutants must be the result of aberrant or missing signals from the L3 layer. Incorporating the molecular data into the above models for *CLV* function, CLV1 may perceive either PZ positional information to direct differentiation or CZ positional information to limit proliferation.

Related Roles for *STM*, *WUS*, and *CLV* in Inflorescence and Flower Development

One would expect that genes regulating the fundamental process of lateral organ formation would regulate all sites of lateral organ formation. This would include the vegetative SM, the inflorescence SM, and the flower meristem (FM). The inflorescence meristem in many plants (e.g., Arabidopsis) is a continuation of the vegetative SM. In fact, there is little evidence that the inflorescence SM in Arabidopsis functions differently from the vegetative SM. Although the organs initiated by the inflorescence meristem (i.e, cauline leaves and flowers) differ from the leaves initiated by the vegetative SM, the types of organs initiated do not necessarily represent an intrinsic property of the SM itself but may instead reflect differential signaling from more mature portions of the plant. By contrast to Arabidopsis, distinct differences are observed between organ initiation at the vegetative SM and at the inflorescence SM in some species. A common difference is the pattern in which organs are initiated (i.e., the phyllotaxy; see Kerstetter and Hake, 1997; Poethig, 1997, in this issue), although this differential phyllotaxy has been postulated to result from differences in the relative sizes of leaf and flower primordia (Lyndon, 1990). The FM, on the other hand, has long been thought to be a modified SM (Goethe, 1790). Evidence from FM identity and flower organ identity genes, such as *LEAFY* and *APETALA1*, supports this idea. These genes convert a SM into a FM when ectopically expressed in the shoot and convert a FM into a SM when mutated (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995).

The defects associated with the vegetative SM for *stm*, *wus*, and *clv* mutants are also observed in any inflorescence SMs or FMs that these plants develop. Moreover, the SM termination observed in *stm* and *wus* mutants also occurs in an identical fashion at any inflorescence SMs that form. In weak *stm* mutants, the inflorescences terminate in partially fused flowers reminiscent of those forming on *terminal flower* mutant plants, in which the SM is converted to a floral fate (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). *wus* inflorescences occasionally initiate a small number of flowers before terminating in a flat apex. By contrast, the SM in *clv* mutants continues to enlarge throughout inflorescence SM development.

The flowers of weak *stm* plants develop a nearly normal number of sepals in their outer whorl but lack most of the inner-whorl stamens and carpels, presumably because of the premature termination of the FM. As in the SM, the terminal organs of weak *stm* flowers are often fused or mosaic. *wus* flowers also specifically lack the inner-whorl stamens and carpels (most flowers develop one central stamen), and these organs are not fused or mosaic. At the earliest stage of floral organ initiation (i.e., sepal initiation), the *clv* FM can be twice as large as the wild-type FM. As flower development proceeds, the *clv* FM continues to proliferate, giving rise to a large mass of undifferentiated cells at its center. Thus, *clv1* mutations result in the overproliferation of undifferentiated cells in the FM.

The fact that these genes appear to function at all sites of lateral organ formation in Arabidopsis suggests that they control fundamental processes that occur at the vegetative and inflorescence SM as well as at the FM. However, there must certainly be specific modifiers of these processes be-

cause there are clear differences between organ formation at the SM and the FM. Most obviously, whereas organs at the SM are initiated in a spiral phyllotaxy, flower organs are initiated in whorls. More fundamentally, Arabidopsis SMs are indeterminate, whereas FMs are determinate. Observations of the root meristem in these mutants support the specificity of these genes for sites of lateral organ formation. The root meristem does not initiate lateral organs (see Schiefelbein et al., 1997, in this issue), and no defects in root meristem development have been observed in *stm*, *wus*, or *clv* mutants.

Genetic Interactions

Genetic interactions among the mutations described above provide insights into possible hierarchies of gene action. The first interactions described were those between the *wus* and *clv1* mutations (Laux et al., 1996). Plants mutant for both *wus* and the strong *clv1-4* allele are indistinguishable from *wus* single mutants. This epistatic interaction suggests two possible models: *WUS* could function upstream of *CLV1* to establish the CZ/PZ distinction upon which *CLV1* acts. Alternatively, *WUS* could act downstream of *CLV1* such that the primary function of *CLV1* is to inactivate *WUS*. If the latter model is correct, and if CLV1 functions to activate a signal transduction pathway, then a key target for the CLV1 signal would be the *WUS* gene product.

Analysis of *clv* and *stm* interactions, by contrast, has revealed a competitive relationship (Clark et al., 1996). *stm* mutations provide a partial, dominant suppression of the meristem overproliferation seen in *clv* mutants. Thus, the inhibition of meristems in *stm* plants requires full CLV activity. *stm* also dominantly suppresses the semidominant phenotype of the *clv1-1* allele. Conversely, *clv* mutations dominantly suppress the phenotype of *stm-1* homozygous plants, indicating that the meristem overproliferation seen in *clv* plants requires full STM activity. Furthermore, *clv stm* double mutants show a phenotype intermediate between the two mutants: SMs often enlarge compared with those of the wild type, but they always terminate prematurely. Thus, the *clv stm* double mutant plants have dramatically lost the ability to maintain the constant meristem size seen in wild-type plants throughout development, suggesting that meristem size is maintained in part through balancing STM and CLV activities. Nevertheless, *clv stm* plants are able to generate meristems and initiate organs, indicating that other genes are capable of fulfilling these roles in the absence of both CLV and STM.

One explanation for the balanced, competitive relationship between *CLV* and *STM* is that these genes competitively regulate a common downstream target. STM could act on the promoter of this gene, and CLV-mediated signal transduction could activate a transcription factor that acts antagonistically to STM. The function of the common target would depend on the role that CLV and STM play in meristem development. If CLV and STM control proliferation of CZ cells, then this target could be a regulator of cell division. By contrast, if CLV and STM regulate the state of differentiation, then the common target could either lock cells in an undifferentiated state or promote differentiation. The balanced relationship between CLV and STM suggests that they function in an analogous, albeit competitive, manner. In other words, if CLV regulates cell proliferation, then STM should also regulate cell proliferation. Thus, a definitive identification of a role for CLV would strongly implicate the role of STM and vice versa.

Finally, *clv* mutations are unable to restore the formation of an embryonic SM in *stm* mutants. This suggests that the formation of the embryonic SM requires STM in a CLV-independent manner. Genes such as *PINHEAD* and *ZWILLE*, which, when mutated, cause SM defects that are embryo specific (Jürgens et al., 1994; McConnell and Barton, 1995), may be involved in this embryo-specific developmental pathway. This possibility is addressed in more detail in the review by Laux and Jürgens (1997, in this issue).

THE ROLE OF CELL SIGNALING IN ORGAN FORMATION

Several features of organ formation suggest a critical role for cell signaling. First, undifferentiated cells in the CZ give rise to cells with a variety of fates: undifferentiated cells, cells of organ anlagen, cells of organ primordia, and differentiated cells. Thus, cell lineage cannot be the deciding factor in determining cell fate. The tunica and corpus layers remain clonally distinct, yet they are precisely coordinated in the formation of an organ primordium, also indicating the need for cell signaling for the normal functioning of the SM (see Poethig, 1997, in this issue). In addition, organ primordia must function in a coordinated manner so as to maintain their asymmetry.

Among the most impressive of several lines of evidence that directly or indirectly support this idea are experiments using chimeric plants to demonstrate the influence of one cell layer on another (Tinley-Basset, 1986; Marcotrigiano and Bernatzky, 1995). A recent example is the use of different tomato lines to generate periclinal chimeras. In these experiments, a normal tomato cultivar was combined with the tomato *fas* mutant, which leads to enlarged SMs reminiscent of those of Arabidopsis *clv* mutants (Szymkowiak and Sussex, 1992). Evidence from a number of chimeric combinations suggests that the L3 layer is critical in determining meristem size. In other words, a plant with a *fas* L3 layer and wild-type L1 and L2 layers develops an enlarged meristem. This is of particular interest given that *CLV1* is expressed predominantly in the L3 layer of the Arabidopsis SM (Clark et al., 1997).

The identity and expression of *CLV1* provide additional indications of the role of cell signaling in meristem development. If CLV1 functions as a receptor kinase, then it

presumably detects positional information provided in the form of a ligand. Furthermore, *CLV1* is only expressed in the L3 layer of the SM, despite the fact that the *clv1* mutation affects all layers of the SM.

Evidence of a novel type of cell signaling at the SM was provided after the cloning of *KNOTTED1* (*KN1*) from maize and the analysis of its expression patterns (Vollbrecht et al., 1991; Jackson et al., 1994; Smith et al., 1996; see also Kerstetter and Hake, 1997; McLean et al., 1997, in this issue). *KN1* was isolated after the identification of dominant, gain-of-function alleles that resulted in ectopic gene expression in leaf veins and additional cell proliferation in these organs. Subsequent experiments have suggested that KN1 plays a role in maintaining shoot identity (Sinha et al., 1993). The fact that STM is closely related to KN1 and that the corresponding genes are expressed in a similar pattern raises the possibility that KN1 and STM play related roles in meristem development. Recent experiments also raise the possibility that the KN1 protein is transported between cells of the meristem, providing a possible novel mechanism for cell signaling (Lucas et al., 1995; see also Kerstetter and Hake, 1997; McLean et al., 1997, in this issue).

PERSPECTIVES

The genetic approach toward the analysis of SM structure and function in Arabidopsis has provided a solid starting point for understanding the process of organ formation and cell differentiation in plants. Work from several laboratories has identified three classes of genes that regulate meristem function: *STM*, *WUS*, and *CLV*. *STM* and *CLV* act in a balanced competitive manner to maintain the structure of the SM as it continually undergoes proliferation and organogenesis. *WUS* may establish or interpret positional informational within the SM to distinguish the CZ and PZ subdomains.

Many questions regarding the functions of these genes remain. Do *CLV* and *STM* regulate proliferation or differentiation? Does *WUS* act upstream or downstream of *CLV*? What is the nature of the flat apex in *wus* mutants? The recent cloning of two of these genes, *STM* and *CLV1*, will provide useful tools for addressing these questions. For example, if *WUS* acts upstream of *STM*/*CLV*, then *CLV1* should not be expressed in the flat apexes of *wus* mutants.

The cloning of *STM* and *CLV1* has confirmed that many other genes are likely to be involved in regulating meristem development. Based on their deduced amino acid sequences, STM is likely to be localized to the nucleus, whereas CLV1 may function at the plasma membrane. Thus, it seems certain that a number of other factors regulate *STM* expression or activity and activate *CLV1* or relay its signal. That only a limited number of genes specifically affecting meristem development have been identified to date suggests either that genetic screens have not reached saturation or that many of the other regulatory genes are redundantly encoded or are involved in a number of other processes, thereby obscuring their roles in meristem development. For example, genes with pleiotropic mutant defects, such as *REVOLUTA* (Talbert et al., 1995), *FOREVER YOUNG* (Callos et al., 1994), *FASCIATA1*, and *FASCIATA2* (Leyser and Furner, 1992), may encode proteins that function in meristem regulation.

Arabidopsis is not the only source of SM mutants. Indeed, complementary approaches are being used in a number of other plant species. Meristem mutants with *clv*-like phenotypes have been identified in tobacco (White, 1916) and tomato (Mertens and Burdick, 1954; Szymkowiak and Sussex, 1992), among many others, and the *no apical meristem* mutant of petunia lacks an embryonic shoot meristem (Souer et al., 1996). Moreover, *STM* was isolated as a homolog of the maize *KN1* gene, which is thought to play a similar role in meristem development based on overexpression phenotypes (see above).

Concurrent work on meristem development in multiple plant species has several advantages. For example, certain meristem regulatory genes may be more easily identified and/or studied in species other than Arabidopsis. Moreover, any features of meristem development that are shared by distantly related species may represent fundamental aspects of SM function. Furthermore, differences in overall morphology between species are due in large part to differences in developmental patterning at the SM. Hence, understanding how the regulation of meristem structure and function differs in morphologically distinct plant species may shed light on the evolution of morphological features.

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REFERENCES

Alvarez, J., Guli, C.L., Yu, X., and Smyth, D.R. (1992). *terminal flower*: A gene affecting inflorescence development in *Arabidopsis thaliana*. Plant J. **2,** 103–116.

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 *shoot meristemless* mutant. Development **119,** 823–831.

Bossinger, G., and Smyth, D.R. (1996). Initiation patterns of flower and floral organ development in *Arabidopsis thaliana*. Development **122,** 1093–1102.

Callos, J.D., Dirado, M., Xu, B.B., Behringer, F.J., Link, B.M., and Medford, J.I. (1994). The *FOREVER YOUNG* gene encodes an

oxidoreductase required for proper development of the *Arabidopsis* vegetative shoot apex. Plant J. **6,** 835–847.

Clark, S.E., Running, M.P., and Meyerowitz, E.M. (1993). *CLAVATA1,* a regulator of meristem and flower development in *Arabidopsis.* Development **119,** 397–418.

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., Jacobsen, S.E., Levin, J., and Meyerowitz, E.M. (1996). The *CLAVATA* and *SHOOT MERISTEMLESS* loci competitively regulate meristem activity in *Arabidopsis.* Development **122,** 1567–1575.

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.

Endrizzi, K., Moussain, B., Haecker, A., Levin, J.Z., and Laux, T. (1996). The *SHOOT MERISTEMLESS* gene is required for maintenance of undifferentiated cells in the *Arabidopsis* shoot and floral meristems and acts at a different regulatory level than the meristem genes *WUSCHEL* and *ZWILLE.* Plant J. **10,** 967–979.

Foard, D.E. (1971). The initial protrusion of a leaf primordium can form without concurrent periclinal cell divisions. Can. J. Bot. **49,** 694–702.

Goethe, J.W. (1790). Versuch die Metamorphose der pflanzen zu erklären. (Gotha, Germany: C.W. Ettinger).

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.

Jürgens, G., Torres-Ruiz, R.A., Laux, T., Mayer, U., and Berleth, T. (1994). Early events in apical–basal pattern formation in *Arabidopsis.* In Plant Molecular Biology: Molecular-Genetic Analysis of Plant Development and Metabolism, G. Coruzzi and P. Puigdomenech, eds (Berlin: Springer-Verlag), pp. 95–103.

Kerstetter, R.A., and Hake, S. (1997). Shoot meristem formation in vegetative development. Plant Cell **9,** 1001–1010.

Kerstetter, R.A., Volbrecht, E., Lowe, B., Veit, B., Yamaguchi, J., and Hake, S. (1994). Sequence analysis and expression patterns divide the maize *knotted1*-like homeobox genes into two classes. Plant Cell **6,** 1877–1887.

Koornneef, M., Van Eden, J., Hanhart, C.J., Stam, P., Braaksma, F.J., and Feenstra, W.J. (1983). Linkage map of *Arabidopsis thaliana.* J. Hered. **74,** 265–272.

Laux, T., and Jürgens, G. (1997). Embryogenesis: A new start in life. Plant Cell **9,** 989–1000.

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.

Leyser, H.M.O., and Furner, I.J. (1992). Characterization of three shoot apical meristem mutants of *Arabidopsis thaliana.* Development **116,** 397–403.

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.

Lucas, W.J., Bouche-Pillon, S., Jackson, D.P., Nguyen, L., Baker, L., Ding, B., and Hake, S. (1995). Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. Science **270,** 1980–1983.

Lyndon, R.F. (1990). Plant Development: The Cellular Basis. (London: Unwin Hyman).

Lyndon, R.F. (1994). Control of organogenesis at the shoot apex. New Phytol. **128,** 1–18.

Mandel, M.A., and Yanofsky, M.F. (1995). A gene triggering flower formation in *Arabidopsis.* Nature **377,** 522–524.

Mandel, M.A., Gustafson-Brown, C., Savidge, B., and Yanofsky, M.F. (1992). Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1.* Nature **360,** 273–277.

Marcotrigiano, M., and Bernatzky, R. (1995). Arrangement of cell layers in the shoot apical meristems of periclinal chimeras influences cell fate. Plant J. **7,** 193–202.

McConnell, J.R., and Barton, M.K. (1995). Effect of mutations in the *PINHEAD* gene of *Arabidopsis* on the formation of shoot apical meristems. Dev. Genet. **16,** 358–366.

McLean, B.G., Hempel, F.D., and Zambryski, P.C. (1997). Plant intercellular communication via plasmodesmata. Plant Cell **9,** 1043–1054.

Mertens, T.R., and Burdick, A.B. (1954). The morphology, anatomy, and genetics of stem fasciation in *Lycopersicon esculentum.* Am. J. Bot. **41,** 726–732.

Poethig, R.S. (1997). Leaf morphogenesis in flowering plants. Plant Cell **9,** 1077–1087.

Richards, F.J. (1948). The geometry of phyllotaxis and its origin. Symp. Soc. Exp. Biol. **2,** 217–245.

Running, M.P., Clark, S.E., and Meyerowitz, E.M. (1995). Confocal microscopy of the shoot apex. In Methods in Cell Biology, Vol. 49, Plant Cell Biology, D.W. Galbraith, D.P. Burque, and H.J. Bohnert, eds (San Diego, CA: Academic Press), pp. 215–227.

Satina, S., Blakeslee, A.F., and Avery, A.G. (1940). Demonstration of the three germ layers in the shoot apex of *Datura* by means of induced polyploidy in periclinal chimeras. Am. J. Bot. **27,** 895–905.

Schiefelbein, J.W., Masucci, J.D., and Wang, H. (1997). Building a root: The control of patterning and morphogenesis during root development. Plant Cell **9,** 1089–1098.

Shannon, S., and Meeks-Wagner, D.R. (1991). A mutation in the Arabidopsis *TFL1* gene affects inflorescence meristem development. Plant Cell **3,** 877–892.

Sinha, N.R., Williams, R.E., and Hake, S. (1993). Overexpression of the maize homeobox gene, *KNOTTED-1,* causes a switch from determinate to indeterminate cell fates. Genes Dev. **7,** 787–795.

Smith, L.G., Hake, S., and Sylvester, A.W. (1996). The *tangled-1* mutation alters cell division orientations throughout maize leaf development without altering leaf shape. Development **122,** 481–489.

Snow, M., and Snow, R. (1931). Experiments on phyllotaxis. I. The effect of isolating a primordium. Philos. Trans. R. Soc. Lond. Biol. Sci. **221,** 1–43.

Souer, E., Vanhouwelingen, A., Kloos, D., Mol, J., and Koes, R. (1996). The *NO APICAL MERISTEM* gene of petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. Cell **85,** 159–170.

Steeves, T.A., and Sussex, I.M. (1989). Patterns in Plant Development. (New York: Cambridge University Press).

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.

Talbert, P.B., Alder, H.T., Parks, D.W., and Comai, L. (1995). The *REVOLUTA* gene is necessary for apical meristem development and for limiting cell divisions in the leaves and stems of *Arabidopsis thaliana.* Development **121,** 2723–2735.

Tinley-Basset, R.A.E. (1986). Plant Chimeras. (London: E. Arnold).

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.

Wardlaw, C.W. (1949). Experiments on organogenesis in ferns. Growth (suppl.) **13,** 93–131.

Weigel, D., and Nilsson, O. (1995). A developmental switch sufficient for flower initiation in diverse plants. Nature **377,** 495–500.

Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F., and Meyerowitz, E.M. (1992). *LEAFY* controls floral meristem identity in Arabidopsis. Cell **69,** 843–859.

White, O.E. (1916). Studies of teratological phenomena in their relation to evolution and the problems of heredity. II. The nature, causes, distribution, and inheritance of fasciation with special reference to its occurrence in *Nicotiana.* Z. Indukt. Abstammungs. Vererbungsl. **16,** 49–185.