Cell signaling within the shoot meristem
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Shoot meristem organization

The angiosperm SAM consists of a small dome of cells with specific structural features (Figure 1a). One level of organization established in the embryo is the arrangement of SAM cells into tunica and corpus layers [2,3]. In Arabidopsis and most other dicots, the tunica consists of an overlying epidermal L1 layer and a sub-epidermal L2 layer, each a single cell thick and remaining clonally distinct by continuous anticlinal cell division [4]. The corpus, or L3 layer, lies below the tunica and consists of cells that divide in all planes (Figure 1b). L3-layer derivatives give rise to the epidermis of shoots, leaves and flowers, whereas the L2 layer provides the mesodermal tissue and germ cells, and the L3 layer provides the vascular tissues and pith.

All three cell layers participate in stem growth and organ formation [5,6], thus, cell proliferation and fate specification during development must be coordinated amongst them. One way in which this may be achieved is through symplastic trafficking — recent experimental fluorescent tracer labeling of shoot apices has shown that the tunica layers of both Arabidopsis and birch exhibit symplastic connectivity, with primary and secondary plasmodesmata linking adjoining cells and providing intercellular connections for the possible regulated movement of signaling molecules [7••,8••]. The corpus appears to form a separate symplastic domain from the tunica, but it has been proposed to maintain connection to the tunica through secondary plasmodesmata in order to integrate the three clonal cell groups [9•].

Morphological and histological studies indicate that the SAM can also be divided into distinct domains or zones — a central zone, a peripheral zone and a rib zone — that cut across clonal boundaries [1,10,11] (Figure 1c). The central zone lies at the very apex of the meristem and contains relatively inactive, slowly dividing cells. The peripheral zone surrounds the central zone and consists of more rapidly dividing cells that become incorporated into organ primordia on the meristem flanks. The rib zone lies internal to the other two zones and produces the cells that form the bulk of the stem. Cells in the central zone of the tunica appear to be sympatrically isolated from those in the peripheral zone, and they may define gradient fields in which small diffusible morphogens can provide information to cells within local regions of the SAM [8••].

Analysis of the expression patterns of several related knot-like-type homeobox genes has provided molecular evidence supporting the zonation model (Figure 1c). Nishimura et al. [12••] report that the tobacco NTH20 gene is expressed in the peripheral zone of the vegetative SAM whereas NTH9 mRNA is preferentially localized to the rib zone. In addition, NTH7 and NTH13 gene expression is
The expression patterns of several effector genes have also been localized to particular meristematic regions: in tomato, the ribosomal protein L38 transcripts to the peripheral zone and the arginine decarboxylase (ADC) mRNA to the corpus [14], and in Arabidopsis, the putative cell wall component PDF1 mRNA transcripts to the L1 [15]. Such genes are candidates for downstream targets of region-specific homeobox gene regulation. These results suggest that the spatially restricted expression patterns of transcription factors such as those encoded by homeobox genes may, via the localized activation of effector molecules, specify discrete functional domains both within and between cell layers in the SAM.

Coordination of shoot meristem patterning

A recent series of genetic and molecular studies have shed light on the complex process of SAM formation. The establishment of the Arabidopsis SAM requires the activity of the SHOOTMERISTEMLESS (STM) gene. Seedlings that are homozygous for severe recessive loss-of-function stm mutations develop normal roots, hypocotyls and cotyledons, but lack an embryonic SAM [16]. STM encodes a member of the KNOTTED1 class of homeodomain proteins [17] that are expressed in the SAM [18–20] and can act as key regulators of SAM development [16,21]. STM is first expressed in one or two cells of the late globular stage embryo and has a dynamic expression pattern during embryogenesis [17,22]. STM appears to function very early during embryogenesis, as it is required for expression of the UNUSUAL FLORAL ORGANS (UFO) gene at the early heart stage [22].

A recent study by Aida et al. [23] demonstrates that the redundant function of the CUP-SHAPED COTYLEDONS1 (CUC1) and CUC2 genes is required for STM expression. cuc1 cuc2 double mutants frequently lack a SAM and exhibit fusion of the cotyledons [24], and the absence of STM expression in cuc1 cuc2 embryos may account for the meristemless phenotype. The CUC2 gene encodes a member of the NAC family of proteins [24], the biochemical functions of which are unknown but which are thought to be transcription factors. CUC2 mRNA is detected slightly earlier during embryogenesis than STM mRNA, but by the late globular stage, the two expression patterns overlap across the top half of the embryo [23]. At this stage, CUC2 mRNA is restricted to the underlying sub-epidermal cells whereas STM mRNA is also present in the overlying protoderm cells. This observation suggests either that STM activation in the protoderm does not require CUC2 or that CUC2 can function in a cell non-autonomous manner to activate STM in these overlying cells. Ultimately, the STM and CUC2 expression patterns in the mature embryo become complementary, indicating that CUC2 is not autonomously required to maintain STM expression in the center of the embryo.

Within the past year, the cloning of two additional genes that are required for SAM activity has been reported. The
WUSCHEL (WUS) gene is necessary for the maintenance of SAM stem cells [26]. After their formation in auxin mutants, these SAM stem cells are misspecified and undergo differentiation without becoming incorporated into organ primordia [25]. WUS encodes a novel subtype of the homeodomain protein family that is localized to the nucleus and is predicted to function as a transcription factor. WUS is first expressed in the 16-cell embryo, prior to the expression of STM, and becomes gradually confined to the sub-epidermal cells in the center of the embryonic and post-embryonic SAM. These WUS-expressing cells are proposed to signal to the overlying cells to maintain their specification as stem cells [26]. WUS and STM are activated independently of one another, but STM expression is lost in mutants mutant seedlings and vice versa.

In the developing SAM the ZWILLE/PINHEAD (ZLL/PNH) gene is also required for maintaining stem cells in an undifferentiated state [27–29]. ZLL/PNH mutants form defective SAMs that terminate shortly after germination, although some plants later generate adventitious meristems. The gene encodes a member of a novel family of proteins [27–29] that is found in many eukaryotes and that includes the product of the ARGONAUTE (AGO) gene, which is involved in leaf development and meristem cell maintenance [30]. The translational initiation factor eIF2C is another family member [33], suggesting a role for ZLL/PNH and AGO in translational control during development. Even though the two genes are expressed early during embryogenesis, with ZLL/PNH expressed in the presumptive SAM and the provascular tissue and AGO1 more broadly [30–32]. These two genes also encode partially redundant functions [31–33]. ZLL/PNH function is necessary to maintain high levels of STM expression late during embryonic SAM development [30], suggesting that transient ZLL/PNH expression in the embryonic SAM may prevent STM downregulation and consequent SAM differentiation. Alternatively, ZLL/PNH may play a role in signaling from the somatic provascular tissue to the overlying meristem cell population to maintain STM expression and SAM activity.

Recent work by Cox et al. [34••] on a Drosophila homolog of ZLL/PNH and AGO1, called piwi, provides evidence that this class of genes may have an ancestral function in stem-cell maintenance. The piwi gene is required for the self-renewal of germ-line stem cells, because in piwi mutant flies, all of the cells in the germ line differentiate instead of dividing. piwi is specifically expressed in the terminal filament cells adjacent to the germ-line cells, suggesting the existence of a piwi-mediated signaling pathway from the somatic tissue to germ cells. Decreasing the expression of C. elegans piwi homologs pgl-1 and pgl-2 also causes germ-line cell depletion [34••]. Signaling from differentiated cells to stem cells may therefore represent an ancient, fundamental mechanism for stem-cell maintenance among eukaryotes.

An increasing body of genetic and molecular evidence indicates that SAM patterning involves dynamic and context-dependent changes in gene expression patterns and that the generation and maintenance of an undifferentiated stem-cell population requires signaling between different regions of the embryonic SAM and potentially also between the stem cells and the surrounding somatic tissue.

**Coordination of shoot meristem proliferation**

In addition to meristem-promoting activities, such as those provided by STM and WUS, separate functions exist that restrict cell proliferation in the SAM (Table 1). Loss-of-function mutations at the Arabidopsis CLAVATA1–3 (CLV1–3) loci cause an increase in the size of both shoot and floral meristems, leading to stem fasciation and the generation of flowers with extra floral organs [35–37,38••]. Genetic analyses indicate that CLV1 and CLV3 act in the same pathway to regulate meristem-cell proliferation, as strong clv1 and clv3 alleles show mutual epistasis in double mutant analyses, and doubly heterozygous clv1/+, clv3/+ plants display a weak clv mutant phenotype [37]. clv1 and clv3 alleles also dominantly suppress stm mutant phenotypes and vice versa, indicating that CLV1/CLV3 and STM act antagonistically to regulate SAM-cell proliferation [39]. clv2 mutants display shoot and floral meristem phenotypes similar to those of clv1 and clv3 mutants, and clv1/+/clv3/– are epistatic to clv2 with regard to these traits [38••]. However, clv2 mutants display other more pleotropic phenotypes (e.g., elongated pedicels, valveless gynoecia and reduced anther locules) as well, suggesting that, while CLV2 may act in the same meristem growth control pathway as CLV1 and CLV3, it also functions more broadly during development.

The CLV1 gene encodes a leucine-rich repeat (LRR) transmembrane receptor serine/threonine kinase [40]. LRR receptor kinases constitute a large class of plant proteins, which...
many of which are involved in cell signaling [41••]. LRRs are a common motif of both plant and animal protein-binding domains [42], which suggests that the CLV1 receptor binds an extracellular protein or peptide ligand. CLV1 mRNA is expressed in the central region of shoot and floral meristems, in a region roughly coincident with the rib meristem; it is not found in the L1 cell layer and, at least in the SAM, is also absent from the L2 layer [40]. Several reports have shown that CLV1 is capable of autophosphorylation and that the CLV1 kinase domain interacts with a type-2C kinase-associated protein phosphatase (KAPP), which is expressed in an area of the meristem that is larger than, but includes, the cells expressing CLV1 [43,44,45••].

Recent molecular and biochemical analyses of members of the CLV pathway have provided new insights into this meristem regulation process. The CLV3 gene has now been cloned and found to encode a small, predicted extracellular protein with no significant homology to other known plant or animal proteins [46••]. Although a direct interaction between CLV3 and CLV1 has not yet been reported, the genetic and molecular evidence strongly indicates that CLV3 acts as, or in the production of, the ligand for the CLV1 receptor kinase. CLV3 mRNA is detected throughout development at the apex of the shoot and floral meristems, predominantly in the L1 and L2 unica cells of the region corresponding to the central zone. The CLV3 expression domain thus overlaps the CLV1 expression domain, signaling a non-cell autonomous fashion from overlapping regions of the Arabidopsis SAM (Figure 2a). Such a model is supported by a mosaic analysis of periclinal chimeras [46••], which showed that wild-type CLV3 function in the L1 layer alone is sufficient to confer a wild-type phenotype on the entire meristem. Given that clv1 and clv3 mutations cause an increase in the number of SAM central zone cells, it seems likely that each cell, or set of cells, in the SAM may be characterized by the particular combination of receptor kinases that it expresses.

Additional studies indicate a close biochemical association between CLV1 and CLV3, and shed light on intracellular events during CLV signaling (Figure 2b). Trottchaud et al. [49••] found that the active form of CLV1 is present in a large heteromeric complex of size, and that the formation of this complex requires both functional CLV1 and CLV3 proteins. The complex includes both KAPP and a member of a plant Rho GTPase-related protein subfamily termed Rop. Arabidopsis appears to contain at least 10 Rop family members, of which at least 1 is expressed in stems and in the meristem region adjacent to those expressing the receptors. Several observations suggest that plant homeobox genes also display meristem region-specific expression patterns, and that homeobox genes such as WUS are targets of LRR-receptor signaling pathways. This suggests that each cell, or set of cells, in the SAM may be characterized by the particular combination of receptor kinases that it expresses.

Conclusions and perspectives

CLV1 is a member of a plant-specific family of receptor protein kinases [41••,43•] that span the plasma membrane and allow cells to recognize and respond to their extracellular environment. Individual cloning efforts and the Arabidopsis Genome Project have so far identified over 50 LRR transmembrane receptor serine/threonine kinases in the Arabidopsis genome. On the basis of the fraction of the Arabidopsis genome sequenced and of the frequency with which members of this family are encountered in random genomic sequencing, it can be estimated that the Arabidopsis genome contains in the order of 100 family members. In addition to CLV1, only two other Arabidopsis LRR receptor kinase genes have defined mutant phenotypes: ERECTA (ER) and BRASSINOSTEROID INSENSITIVE1 (BRI1). mutant plants have compact inflorescences, as well as shortened siliques and leaf petioles. ER is expressed in SAMs and flowers, and it probably acts to increase internode and fruit length by increasing cell number [54,55••]. Plants with mutations in the BRI1 gene display light-grown characteristics (e.g., short, thick hypocotyl; open and expanded cotyledons; and anthocyanin accumulation) when grown in the dark and are markedly dwarfed when grown in the light. BRI1 is expressed ubiquitously and appears to encode a receptor for brassinolide, a plant steroid hormone [56]. It is not known if BRI1 binds brassinolide directly or via a peptide or protein intermediate, as would seem likely from the presence of extracellular LRRs (see also review by Schumacher and Chory, pp 79-84). In addition to CLV1, ER and BRI1, several other members of this family may be expressed in SAMs (RW Williams, EM Meyenowitz, unpublished data). Thus, it seems likely that each cell, or set of cells, in the SAM may be characterized by unique subsets of homeodomain proteins.
Transmembrane receptor kinases are also common components of animal signal transduction pathways, although most developmentally important receptor protein kinases in animals are tyrosine kinases. Dozens of receptor tyrosine kinases (RTKs) exist in mammals, many of which control cell growth and differentiation [57]. The RTKs are activated by secreted, soluble polypeptide ligands called cytokines, which include platelet-derived growth factor, epidermal growth factor and fibroblast growth factor. Cytokine signaling generally involves ligand-mediated receptor dimerization, which results in transphosphorylation of the receptor kinase subunits, followed by substrate phosphorylation. Protein tyrosine phosphatases have also been identified that interact specifically with RTKs, modulating the activity of downstream mitogen-activated protein kinase signaling cascades [57]. The presence of a Rop, a Rho GTPase-like protein, in an active CLV1-KAPP complex suggests that CLV1 may transduce intracellular signals into the nucleus through this GTPase in an analogous manner. Regional activation of RTK signaling in animals is mediated in many cases by ubiquitously distributed receptors that either respond to locally produced ligands or undergo
localized potentiation [58]. Several RTK signaling pathways appear to involve a localized receptor-ligand pair, however; one example is the activation of a fibroblast growth factor receptor, encoded by the *Drosophila breathless* [60] gene [59], by the Branchless ligand [60] during tracheal cell migration and branching. Transient local production of Branchless in small patches of epidermal cells activates the Breathless receptor in underlying tracheal cells, and the tight correlation observed between *breathless* expression and tracheal branching implies that Branchless instructs these tracheal cells to form branches [60]. Thus, plants and animals may send similar sorts of localized signals through independently derived but parallel mechanisms. Further exploration of receptor kinase signaling pathways in both plants and animals will be required in order to derive mechanistic models of their function during growth and development and to better understand the evolution of cell-cell communication systems in the two kingdoms.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


**Development of apical embryonic pattern**

6. Long JA, Barton MK: The development of apical embryonic pattern. In: Development 1998, 125:3027-3035.  A clear and detailed analysis of the embryonic expression patterns of the *STM UNDULATUM FLORAL ORGANS (UPD*, ANTECEDENTIA (ANT) and *CUP1* genes by in situ hybridization. This study reveals the dynamic nature of their expression patterns during shoot meristem primordium development and addresses the role of *STM* in their establishment.
Role of the meristem cells.

and floral meristems, where the cells expressing it are proposed to act as an organizing center that controls stem-cell fate in the overlying shoot apical meristem cells.


A genetic and molecular analysis of the ZL1 gene, which encodes a novel protein with homology in other eukaryotes. ZL1 is transiently expressed in the embryonic apex. The authors present evidence that ZL1 acts there to relay positional information in order to maintain shoot apical meristem cells in an undifferentiated state.


The paper reports the cloning and expression pattern of the ZL1/PNI gene. ZL1/PNI is demonstrated by genetic analysis to act pleiotropically during Arabidopsis development. Importantly, it is also shown to interact with AGOT, which encodes a related protein controlling leaf development.


A phenotypic and functional analysis of the Arabidopsis AGOT gene, which is required for leaf development and the specification of general plant architecture. The authors report that AGOT1 is expressed widely in plant tissues and belongs to a novel class of genes conserved among multicellular organisms.


Clark SE, Running MP, Mayer Ew, CLAVATA1 is a specific regulator of shoot and floral meristem development affecting the same processes as CLAVATA2. Development 1999, 126:2057-2067.


A detailed genetic analysis of the cld2 phenotype that reveals the CLV2 gene to be involved in the regulation of apical meristem function. Strong ectopic tissues resemble weak cld1 and cld3 alleles, but cld2 alleles also have other pleiotropic effects. Ectopic analysis indicates that cld2 mutations interact in a complex manner with cld1, cld3 and other meristem regulatory mutations.


A clear and concise overview of the different classes of plant receptor kinases and their proposed roles in development.


A biochemical analysis demonstrating that the CLV1 and CLV2 proteins have protein kinase activity and that the phosphorylated form interacts in vitro with the kinase-associated protein phosphatase, KAPP. Reducing KAPP mRNA levels rescues the cld1 mutant phenotype, indicating that the CLV1–KAPP association has relevance in vivo and adding to the evidence that KAPP may function as a negative regulator of CLV1 signaling in meristem cells.


The authors report the cloning of the CLY12 gene, which is required for controlling shoot meristem cell proliferation. CLY12 encodes a small, predicted extracellular protein that can function in a non-cell autonomous fashion in meristems. Evidence is presented suggesting that CLY12 acts as the ligand for the CLV1 receptor kinase.

A comprehensive review of all the classes of serine/threonine kinases identified to date and adding to the evidence that KAPP may function as a negative regulator of CLV1 signaling in meristem cells.

Stone JM, Tootscheid AE, Walker JC, Clark SE: Control of meristem cell proliferation through CLV3–CLV1 signaling is proposed.


A detailed study of actively growing apices of Arabidopsis inflorescences by confocal laser scanning microscopy and image analysis is presented. The authors quantify cell size and mitotic activity in several ecotypes and identify zones within the meristem that have different cell proliferation rates. The apices of two cld1 mutants were compared to those of the wild type. Novel conclusions about the functions of the genes are drawn.

A detailed biochemical analysis investigating the mechanism of CLV1 and CLV2 signal transduction in vivo. The authors demonstrate that CLV1 is present in two distinct multimeric protein complexes, and that assembly of the larger complex, which contains a protein phosphatase and the Rho GTPase-related protein, requires functional CLV1 and CLV3 proteins.


This paper reports the identification of an Arabidopsis Rho gene family member that is expressed specifically in anthers and has a conserved function in regulating polarized cell growth in flower organs. Other closely related Rho genes are also shown by reverse transcription-PCR to be expressed in mature pollen, suggesting that several Rho proteins may play roles in polar localization events in pollen.


Nagata K-i, Hall A: GTPase signaling: a central processor unit in plant cells is discussed.

A possible functional role for the leucine-rich repeat kinase receptor CLAVATA3/polarized response 1 (PRL) in shoot and floral meristem determination. The authors demonstrate that CLAVATA1 is present in two distinct multimeric protein complexes, and that assembly of the larger complex, which contains a protein phosphatase and the Rho GTPase-related protein, requires functional CLV1 and CLV3 proteins.


