Cell signaling within the shoot meristem

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Shoot apical meristems are self-renewing stem cell populations that generate all of the above-ground organs (i.e. stems, leaves and flowers) of higher plants. Recent studies have identified new molecular components required for proper shoot meristem activity, and they have revealed that complex, intercellular communication pathways play important roles in coordinating meristem function.

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Abbreviations

AGO	*ARGONAUTE*
BRI	*BRASSINOSTEROID INSENSITIVE*
CLV	*CLAVATA*
CUC	*CUP-SHAPED COTYLEDONS*
ER	*ERECTA*
KAPP	kinase-associated protein phosphatase
LRR	leucine-rich repeat
NTH	*Nicotiana tabacum* homeobox
PDF1	*PROTODERMAL FACTOR 1*
PNH	*PINHEAD*
RTK	receptor tyrosine kinase
SAM	shoot apical meristem
STM	*SHOOTMERISTEMLESS*
WUS	*WUSCHEL*
ZLL	*ZWILLE*

Introduction

Intercellular communication is critical for coordinating the growth and development of both animals and plants. In higher plants, unlike many animals, most of development occurs post-embryonically through the activity of shoot (and root) apical meristems. Shoot meristems are formed during embryogenesis and have the capacity to continuously renew themselves. They also produce progeny cells that can differentiate into leaves, stems and flowers [1]. A key to understanding, and ultimately, to manipulating plant form lies in unraveling the communication machinery that enables shoot apical meristem (SAM) cells to continuously coordinate the processes of stem-cell proliferation and organ primordia initiation. One clear theme emerging from papers in this field published over the past two years is that localized signaling between different cells and regions of the SAM is a critical component of SAM function. In this review we examine papers from the past two years in the field of SAM development and function, concentrating on signaling between different regions of the SAM.

Shoot meristem organization

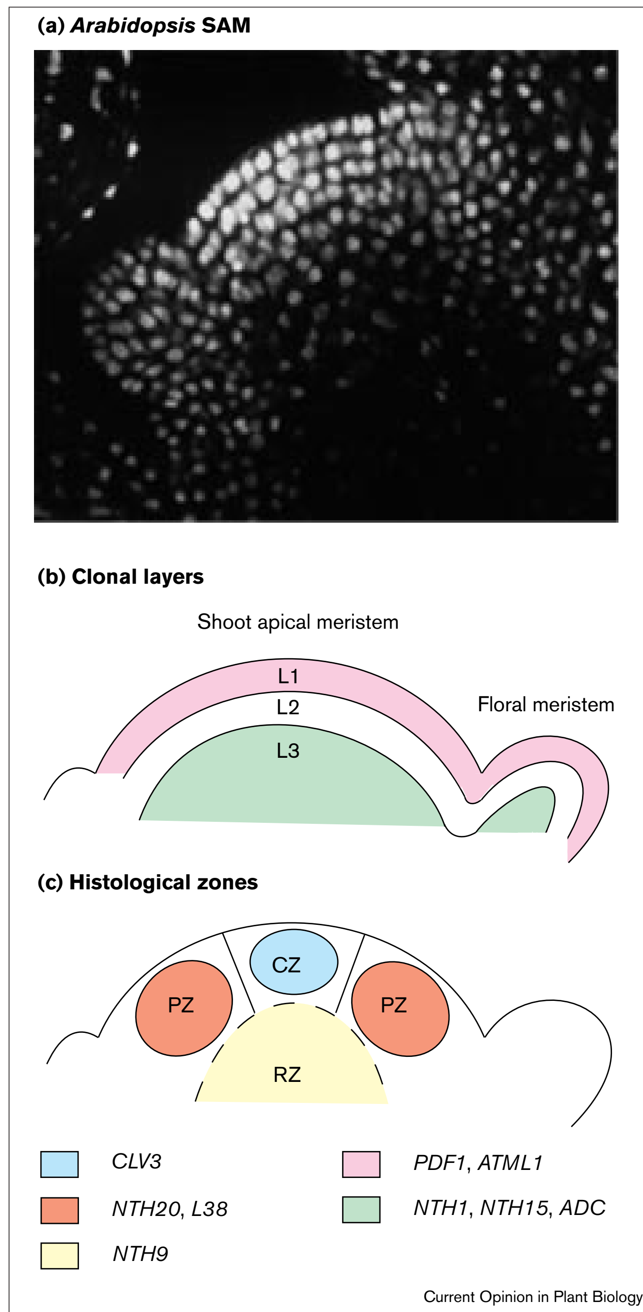
The angiosperm SAM consists of a small dome of cells with specific structural features (Figure 1a). One level of organization established in the embryo is the arrangement of SAM cells into tunica and corpus layers [2,3]. In *Arabidopsis* and most other dicots, the tunica consists of an overlying epidermal 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). L1-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 symplastically 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 *knotted1*-type homeobox genes has provided molecular evidence supporting the zonation model (Figure 1b,c). 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, *NTH1* and *NTH15* gene expression is

Figure 1



Shoot apical meristem structure. **(a)** Confocal laser scanning microscope optical section through an *Arabidopsis thaliana* wild-type (Landsberg *erecta)* shoot apical meristem (SAM) and developing floral meristem. The nuclei have been stained with propidium iodide.
(b) Diagram of the *Arabidopsis* SAM labelled to show the clonal layers (L1–L3) and the expression of various layer-specific genes.
(c) Diagram of the *Arabidopsis* SAM labelled to show the histological zonation and expression of various zone-specific genes. *ADC*, *arginine decarboxylase*; *ATM1*, *Arabidopsis thaliana* meristem layer 1; *CLV3*, *CLAVATA3*; CZ, central zone; *PDF1*, *PROTODERMAL FACTOR 1*; PZ, peripheral zone; and RZ, rib meristem zone.

localized to the corpus [12••], whereas the *Arabidopsis* homeobox gene *ATML1* is expressed exclusively in the L1 [13]. 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 after they form — in *wus* mutants, these SAM stem cells are misspecified and undergo differentiation without becoming incorporated into organ primordia [25]. Adventitious meristems are then generated, but these form only a few organs and terminate prematurely. *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 *wus* 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 that resemble those of the wild-type. The *ZLL/PNH* gene encodes a member of a novel family of proteins [30••,31••] that is found in many eukaryotes and that includes the product of the *ARGONAUTE1* (*AGO1*) gene, which is involved in leaf development and meristem cell maintenance [32•]. The rabbit translation initiation factor eIF2C is another family member [33], suggesting a role for *ZLL/PNH* and *AGO1* in translational control during development. Expression of the two genes begins early during embryogenesis, with *ZLL/PNH* expressed in the presumptive SAM and the provascular tissue and *AGO1* more broadly [30••,31••]. These two genes also encode partially redundant functions [31••]. *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 *prg-1* and *prg-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 fluctuations 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 pleiotropic 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,

Table 1

Genes regulating *Arabidopsis* shoot apical meristem development.

Gene	Product	Initial stage of expression	References
Proliferation-promoting			
CUC1	Unknown	–	[23••]
CUC2	NAC domain transcription factor	Early- to mid-globular stage	[23••]
STM	KN1-like homeodomain transcription factor	Late globular stage	[17]
WUS	Homeodomain transcription factor	16-cell stage	[26••]
ZLL/PNH	AGO1-like protein	4-cell stage	[30••]
Proliferation-restricting			
CLV1	LRR receptor kinase	Heart stage	[40]
CLV2	LRR receptor-like protein	Unknown	[61]
CLV3	Putative secreted protein	Heart stage	[46••]

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 signficant 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 tunica cells of the region corresponding to the central zone. The *CLV3* expression domain thus overlies the *CLV1* expression domain, suggesting that *CLV3* may signal in a non-cell autonomous fashion from overlying to underlying 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 the number of SAM central zone cells, the *CLV3* signal has been proposed to inform these cells either of their proper rate of cell division [37,47] or of the rate at which they should exit the central zone to enter pathways leading to differentiation [48••].

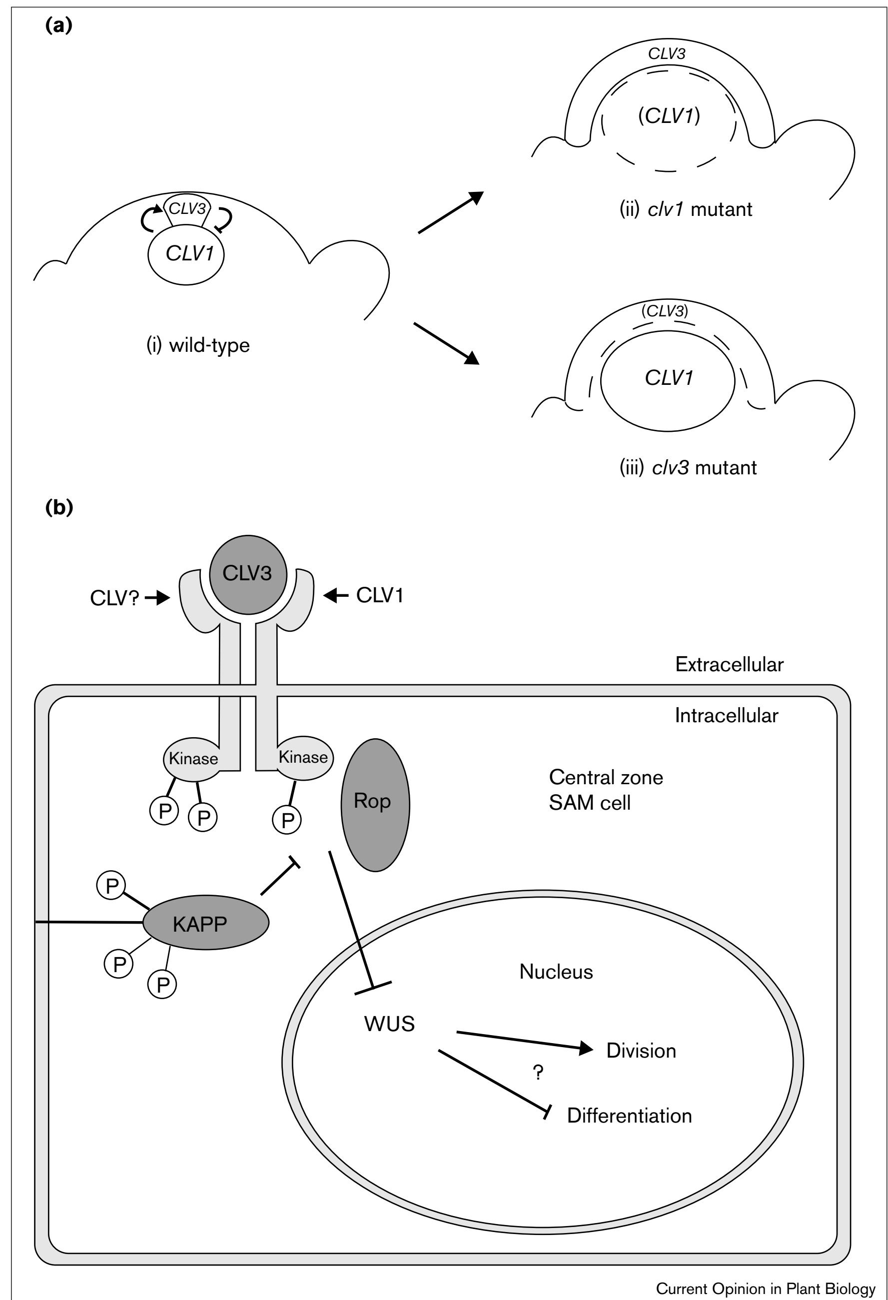
Additional studies indicate a close biochemical association between CLV1 and CLV3, and shed light on intracellular events during CLV signaling (Figure 2b). Trotochaud *et al.* [49••] found that the active form of CLV1 is present in a large heteromeric complex *in vivo*, and that the formation of this 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, many of which are expressed in stems [50••]. Rho GTPases are conserved signaling proteins that interact with downstream serine/threonine and tyrosine protein kinases to control many key cellular processes in animals and fungi, including actin cytoskeletal reorganization, cell polarity establishment and polarized cell growth [51,52]. Rho GTPases are related to Ras GTPases, examples of which have yet to be found in plants. Thus, Rho GTPases such as the Rops might functionally substitute for Ras GTPases in plant signal transduction, initiating a mitogen-activated protein-kinase-like cascade in response to CLV1 kinase activation.

Conclusions and perspectives

CLV1 is a member of a plant-specific family of receptor protein kinases [41•,53•] 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*). *er* 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 hypocotyls; 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 are known to be expressed in SAMs (RW Williams, EM Meyerowitz, unpublished data). Thus, 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.

CLV3 is the first protein to be identified as a possible ligand for a member of the plant receptor kinase family. Its synthesis in one region of the SAM and its action on a receptor in another region allows for coordinated growth between the two regions. Other plant LRR receptors may each respond to different ligands, made in and secreted from regions adjacent to those expressing the receptors. Several observations — that some plant homeobox genes also display meristem region-specific expression patterns, and that homeobox genes such as *WUS* are predicted targets of LRR-receptor signaling pathways — suggest a possible mechanism for meristem growth control. The observations suggest that meristem growth may be coordinated through localized transmembrane receptor kinase–ligand signaling pathways that operate through changes in effector gene expression mediated by unique subsets of homeodomain proteins.

Figure 2



Models of *CLV* signal transduction pathway function in the control of meristem proliferation. **(a)** Signaling between shoot apical meristem (SAM) cell layers. (i) In the wild-type *Arabidopsis* SAM, the *CLV3*-expressing region of the outer cell layers conveys an inhibitory cell proliferation signal to the *CLV1*-expressing region of the underlying cell layers. Evidence suggests that the inner region, in turn, conveys a positive signal, independent of the CLV pathway, to the overlying region to coordinate the size of the two domains [46••]. (ii,iii) In *clv1* or *clv3* loss-of-function mutants, the CLV-dependent inhibitory signal is abolished but the CLV-independent positive signal is not affected. The result is an enlargement of both the *CLV3* and *CLV1* RNA expression domains and, consequently, of the entire SAM. **(b)** Intracellular pathway in a SAM central zone cell. It is proposed that secreted CLV3 protein is bound by the CLV1 transmembrane receptor kinase, which probably acts either as a dimer or a heterodimer, *via* its extracellular LRRs. Ligand binding leads to phosphorylation (P) of the CLV1 kinase domain, and formation of an active complex that includes the Rop GTPase-related protein and the protein phosphatase KAPP, which dephosphorylates CLV1 and acts as a negative regulator of the pathway. KAPP is reported to have an amino-terminal signal anchor that results in cytoplasmic orientation of the membrane-anchored protein [43]. The active CLV1 complex negatively regulates cell proliferation by repressing WUS, which is proposed to act as a transcription factor to promote the division of the cell and/or inhibit it from entering pathways leading to differentiation.

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 subunit activation and 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* [*btl*] 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 *branchless* 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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