Cell–cell signaling in the shoot meristem

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The shoot meristem is a proliferating, changing cell population yet displays a stable organization. Recent studies have addressed how signaling processes coordinate the behaviour of shoot meristem cells.

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Abbreviations

AG	*AGAMOUS*
ANT	*AINTEGUMENTA*
CLV	*CLAVATA*
CZ	central zone
LFY	*LEAFY*
OC	organizing center
NPA	N-(1-naphthyl)phtalamic acid
PID	*PINOID*
pin1	*pin-formed1*
pol	*poltergeist*
PZ	peripheral zone
RZ	rib zone
SAM	shoot apical meristem
STM	*SHOOTMERISTEMLESS*
WUS	*WUSCHEL*

Introduction

The shoot apical meristem (SAM) comprises three domains that fulfill distinct functions: the central zone (CZ) harbors the stem cells, which continually produce new cells, whereas initiation of lateral organs and central stem tissue occurs in the surrounding peripheral zone (PZ) and underlying rib zone (RZ), respectively (Figure 1; [1,2]).

The outer cells of the SAM always divide perpendicularly to the surface, giving rise to clonally distinct cell layers. In most angiosperms, the outermost L1 layer will form epidermis, and an underlying L2 layer will produce the subepidermal tissues of the stem and lateral organs. Underneath, the L3 cells divide in all planes and form the pith of the stem and interior tissues of organs. Despite this apparently pervasive clonal separation in wild-type development, there is ample evidence from chimeric plants to show that cells develop according to their actual position and not their origin. For example, an L1 cell displaced into the subepidermal layer will develop into a subepidermal cell type [3]. In addition, the growth and differentiation of all three layers must be coordinated to maintain meristem shape and produce normal organs. Together, these data imply that meristem cells exchange signals to coordinate their behavior.

In this review, we discuss papers from the past year that provide new insights into the cell–cell signaling mechanisms that direct the two meristem functions: stem cell homeostasis and organ initiation.

Regulation of stem cells

Clonal analyses have demonstrated that all of the postembryonic structures of the plant shoot are derived from about three stem cells in every layer of the meristem [3]. We use the term stem cells in the operational sense that they give rise to both daughters that renew the stem cell pool and that differentiate into a variety of cell types. The stem cells cannot be distinguished histologically but, for geometric reasons, are thought to be located at the very tip of the CZ. This position roughly coincides with the expression domain of the *CLAVATA* (*CLV*)*3* gene, which has been used as a stem-cell marker (Figure 1a,b; [4]).

The stem cells can be replaced by neighboring cells [5], suggesting that they are not permanent but, rather, are specified as stem cells as a result of their position in a niche where appropriate signals are provided from the surrounding cells. This situation is similar to stem cell regulation in the root meristem or in animals [6,7]. It should be noted that daughters of the apical stem cells can act as transient stem cells, which give rise to a more restricted part of the shoot, until they are displaced by a division of a more apical cell and undergo differentiation [3].

Setting up a niche for stem cells

Mutations in the homeobox gene *WUSCHEL* (*WUS*) result in a mis-specification of stem cells [8], whereas overexpression of *WUS* can repress organ formation and induce expression of the stem-cell marker *CLV3*, demonstrating that WUS is necessary and sufficient to induce stem cell identity [9••]. *WUS* is not expressed, however, within the stem cells but in an underlying group of cells, termed the organizing center (OC) (Figure 1a,b; [10]). This has led to the model in which WUS activity in the OC promotes a signal that specifies the overlying neighbors as stem cells.

A question arises from this model: why are only the overlying neighbors induced to be stem cells? One possibility is that only these cells are competent to respond to the WUS-dependent signal. Either additional factors could be required to enable cells to answer to WUS or differentiation signals could suppress the response to WUS outside the stem-cell region. The idea of a restriction on cell competence is supported by observations that ubiquitously expressed *WUS* induces *CLV3* expression only in some cell types (M Lenhard, T Laux, unpublished data).

An alternative model is that the WUS-dependent signal may be communicated via plasmodesmata in one direction only. Injection studies and microscopic analysis have revealed that cell–cell signaling may be restricted to regions of the

Figure 1
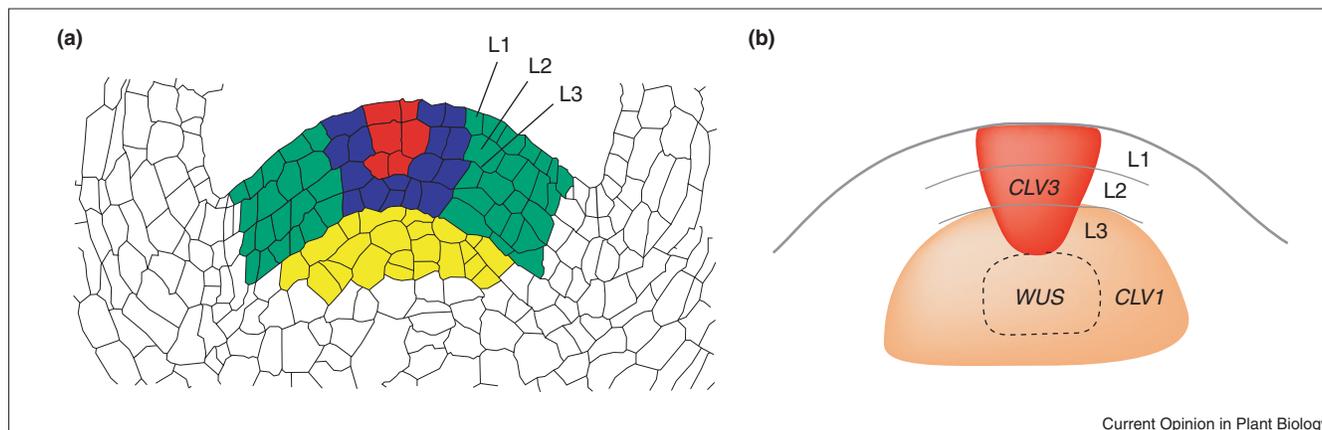
Organization of the shoot apical meristem. **(a)** Schematic view of SAM domains. The CZ (blue) contains slowly dividing cells that stain weakly with cytoplasmic dyes. The apical stem cells (red) form part of the CZ. Cells in the PZ (green), where initiation of organ primordia takes place, divide more rapidly and stain more strongly. In the RZ (yellow), differentiation of central pith tissue is initiated. Redrawn after [37]. **(b)** Outline of the central part of (a), showing the approximate mRNA expression domains of *CLV1*, *CLV3* and *WUS.*

shoot meristem by regulating the plasmodesmal coupling of meristem cells [11,12]. At least for the epidermal cells, facilitated transport was observed in the CZ domain [11,12]. Thus, it is conceivable that the movement of the WUS-dependent signal is directed by preferential coupling between stem cells and the OC.

The reply from the stem cells

The function of WUS is counteracted by the CLV signaling pathway [8,9••]. Mutations in any one of the three *CLAVATA* genes (*CLV1*, *CLV2* and *CLV3*) lead to a progressive enlargement of the stem-cell population, and genetic data suggest that all three genes act in the same pathway [13]. Several results suggest that one of the main targets of the CLV pathway is *WUS*. The effects of *wus* mutations are epistatic to *clv1*, *clv2* and *clv3* phenotypes; the *WUS* expression domain is enlarged in *clv* mutants, indicating that the CLV pathway suppresses *WUS* at the transcript level; and enlarging the *WUS* expression domain in wild-type plants can phenocopy the *clv* mutant defect [9••]. Genetic analysis of the *poltergeist* (*pol*) mutant suggests that POL acts downstream of CLV signaling and may have functions that overlap with those of *WUS* [14••]. In double mutants, *pol* mutations suppress the *clv* phenotype whereas *pol* alone has no effect, and *pol* and *wus* show dominant interactions.

CLV1 encodes a putative receptor kinase with an extracellular leucine-rich repeat receptor domain and an intracellular serine/threonine kinase region. It is expressed mainly in the corpus of the SAM, but possibly also in the L2 (Figure 1b; [15]). *CLV2* encodes a protein with an extracellular domain similar to that of CLV1 but that lacks the kinase domain and probably forms a membrane-associated complex with CLV1 [16]. *CLV3*, which as mentioned above is expressed in the apical stem cells, encodes a small polypeptide with a putative secretory signal peptide [4] that appears to be present *in planta* as a small soluble complex [17••].

Does CLV3 act as a signal in the shoot meristem? There is convincing biochemical evidence that CLV3 represents a ligand for the CLV1 receptor kinase: the formation of the apparently active CLV1 complex of 450 kiloDaltons is dependent on the presence of CLV3 [18]. CLV3 co-immunoprecipitates with CLV1 and this binding requires an active intracellular kinase domain, suggesting that kinase activity stabilizes the receptor–ligand interaction [17••].

In addition, CLV3 specifically binds to intact yeast cells expressing *CLV1* and *CLV2*, suggesting that CLV3 may function as an extracellular signal that binds to CLV1–CLV2 at the cell surface. It should be noted, however, that other scenarios are also possible: as *WUS* is repressed by the CLV pathway in the L3 stem cells, in which *CLV1* and *CLV3* expression overlap (Figure 1b; [4,9••,15]), the possibility that the CLV1–CLV3 interaction is intracellular cannot be excluded.

The above results suggest the following model for the regulation of stem-cell homeostasis (see also 'Update'). Stem cells are specified by, as yet unknown, WUS-dependent signals from the underlying OC and in turn signal back via the CLV3 molecule, delimiting the OC by repression of *WUS*. This negative-feedback loop enables the stem cells to autoregulate their number indirectly through size control over the OC (Figure 2a).

Localized CLV3 signal

Transgenic plants that express *CLV3* throughout the SAM mimic the *wus* mutant phenotype [19••], indicating that *CLV3* activity is sufficient to repress *WUS*. This implies that the availability of CLV3 function is the rate-limiting

factor in the repression of *WUS*; however, assuming that CLV3 can travel within the meristem, why is *WUS* repressed only in the cells above and possibly lateral to the OC, but not in the OC itself? There must be a mechanism that prevents CLV3 from being active in OC cells. One conceivable model is that all CLV3 protein produced in the apical stem cells is bound — for example in the third cell layer — preventing it from entering the OC cells underneath (Figure 2b). Consistent with this idea, 75% of the total amount of CLV3 protein in cauliflower meristem extracts was found to be associated with CLV1 [17••]. Doubling the number of *CLV3* gene copies was not sufficient to overcome this barrier and repress *WUS* in the OC [19••], suggesting that, in accordance with the model, the OC is safely protected from CLV3 signaling by a large excess of binding sites.

Anchoring the organizing center

Although the shoot meristem consists of a population of dividing cells, expression domains remain stable at the same position relative to the organization of the shoot apex. In the case of the OC, periclinal divisions of the overlying L3 stem cells result in a flow of cells through the OC: cells that enter the OC from above activate *WUS* expression, whereas cells that leave the OC towards the RZ switch it off.

This raises the question of how the organizing center is maintained at a given distance from the shoot summit, underneath the third cell layer. One possibility would be that the stem cells not only send a repressive signal but also a positive signal that induces *WUS* expression in the OC. Although the strength of the repressive signal in this model would drop sharply because of CLV3 sequestration at the third cell layer, the activating signal would reach the OC.

An alternative, though not mutually exclusive, scenario is that the position of the OC depends on signals from underlying or lateral cells. This view is consistent with findings that meristem maintenance requires the presence of young leaf primordia and is influenced by genes expressed in the leaves and the vasculature [20–22].

Organ initiation

Cells that leave the CZ lose stem cell identity and initiate differentiation and organ formation. How are the cells instructed to do so? Consistent with the niche concept, loss of stem cell identity could simply result from the cell leaving the range of WUS signaling; however, absence of WUS signaling is not sufficient to initiate organ formation, as the central cells in *wus* mutant apices do not do so. Given the continuity of tissues, it is conceivable that differentiated cells instruct the new cells. Such a mechanism is indicated by experiments in the root; when undifferentiated root cells were disconnected from older tissue by cell ablation, they lost their ability to differentiate according to their position [23].

Several recent studies have addressed the role of signaling in organ initiation in the SAM. Reinhardt and coworkers [24••] exposed excised tomato apices to the auxin transport

Figure 2

Current Opinion in Plant Biology
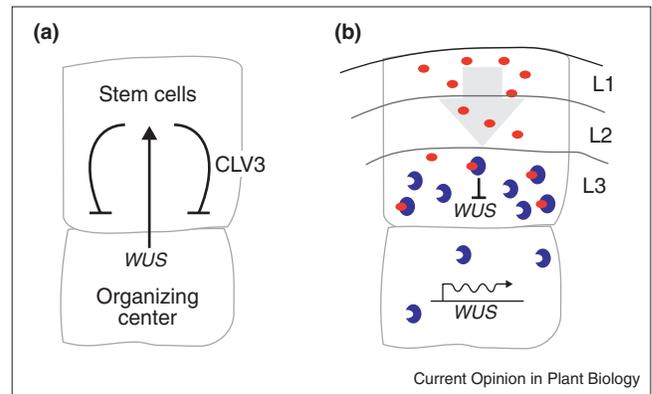
Signaling between stem cells and the organizing center (OC). **(a)** *WUS* expression in the OC promotes a yet unidentified signal that specifies the overlying cells as stem cells. The stem cells signal back via CLV3 and restrict the size of the OC. **(b)** Model for the protection of the OC from CLV3 signaling. Binding of CLV3 (red circles) to the CLV1 complex (blue crescents) results in repression of *WUS* in L3. An excess of CLV1 receptor complex prevents CLV3 from entering the underlying OC cells, allowing *WUS* to be expressed there.

inhibitor NPA (N-[1-naphthyl]phtalamic acid). Although the histological organization of the SAM remained unaffected, organ formation was blocked, indicating a requirement for auxin transport. This phenotype is reminiscent of the *pin-formed1* (*pin1*) mutant, in which polar auxin transport is reduced by about 90% as a result of a defect in the putative auxin efflux carrier PIN1 [25,26].

As the site of auxin production in the apex is unknown, the phenotype of NPA-treated or *pin1* shoots could be caused by an increased or decreased concentration of auxin. To address this question, auxin was locally applied at the periphery of naked NPA-treated and *pin1* mutant apices, and in both cases induced organ formation at the corresponding sites. The number of cells that were incorporated into an organ primordium increased with the amount of auxin applied; however, only the PZ was competent to respond to auxin, whereas the CZ of the meristem was not. Together, these experiments indicate that local maxima of auxin are sufficient to induce organ initiation in the PZ and that polar auxin transport is required to establish such maxima.

This model is supported by an analysis of marker gene expression in the *pin1* mutant [27••]. In wild-type plants, *SHOOTMERISTEMLESS* (*STM*) is expressed throughout the PZ but is absent from sites of incipient organ primordia [28]. In contrast, *AINTEGUMENTA* (*ANT*) and *LEAFY* (*LFY*) are expressed in incipient and outgrowing organ primordia in a pattern that is complementary to that of *STM* expression [29,30]. In the *pin1* mutant, *STM* expression was completely absent in the PZ, whereas *ANT* and *LFY* were expressed throughout the PZ. PZ cells showed a mixed identity, however: the same PZ cells that expressed *LFY* and *ANT* also expressed *CUP-SHAPED COTYLEDON2*,

which in wild-type plants marks the boundary of organs [31]. This indicates that PIN1 is required to separate these expression domains — that is, to delimit organ anlagen. Target genes of LFY were not expressed in *pin1* mutants, suggesting that the cells in the periphery had initiated organs but were blocked soon thereafter. Whether this block is a consequence of the mixed cell identity or whether PIN1 is directly required for progression of organ development remains to be determined.

Mutations in *PINOID* (*PID*), which encodes a serine/threonine kinase, also result in naked shoot apices [32••]; however, overexpression analysis suggests that *PID* antagonizes auxin signaling. As *PID* is expressed in young organ anlagen on the flanks of the SAM, these results suggest that dampening of auxin signaling is also required for organ formation.

Together, these findings indicate that polar auxin transport is required for the organization of the peripheral zone into primordia and non-primordial cells and, furthermore, that auxin is necessary for organ outgrowth. What is the source of auxin in the shoot apex and by which method is it transported? If NPA-treated shoots are allowed to recover in the absence of NPA and auxin, they first form leaves at random positions but, later, phyllotaxis became normal, confirming that wild-type phyllotaxis requires signals from existing organs. It is questionable, however, whether this signal is auxin as organ formation is repressed in the vicinity of existing organs. This signal could, therefore, antagonize auxin activity.

Conclusions

Genetic and molecular analyses have revealed a complex network of signaling between cells within the SAM and between the SAM and other parts of the plant.

What are the roads along which the signals travel? Smaller secreted molecules, such as CLV3, could travel through the extracellular space and bind to receptors at the cell surface. Larger molecules, however, may have to use plasmodesmata in order to move from cell to cell [33]. For example, the transcription factor LFY was shown to be present in cells in which the *LFY* gene was not expressed, indicating that LFY protein itself can travel [34••]. Recently, a transient increase in plasmodesmal coupling between all meristem cells has been observed immediately after floral induction [35••]. This increase was due to establishment of secondary plasmodesmata and occurs independently of cell division. What is the significance of a varying density of cell–cell connections? A conceivable model, taking into account the growing number of reports of non-cell-autonomous functions of floral regulators, holds that during floral induction the roads between meristem cells are opened briefly to allow a rapid and co-ordinated response of all cells to the floral stimulus [36••]. In this view, the regulation of cell–cell coupling can provide a means with which to spatially and temporally modulate cell–cell communication.

Update

In contrast to the *Arabidopsis* shoot meristem, floral meristems are determinate and produce only a limited number of organs. Recent work suggests that floral meristem determinacy is regulated by a negative feedback mechanism, in which *WUS* activates expression of the *AGAMOUS* (*AG*) gene, which in turn represses *WUS* and thus terminates stem-cell maintenance [38••,39••].

In vitro WUS protein binds to consensus homeodomain target sites within the regulatory region of the *AG* gene. These sites are necessary for expression of an *AG* reporter gene construct *in planta* [38••]. However, as the *AG* gene is still active in *wus* mutants [8], other yet unknown proteins could also bind to the target sites or other *cis*-regulatory elements could play a role. The activation of *AG* expression by *WUS*, and thus stem-cell termination, is restricted to determinate floral meristems, apparently because of the requirement for additional flower-specific factors. As endogenous *WUS* does not appear to activate *AG* in *lfy* mutants, one of these factors could be the floral meristem identity gene *LFY* [39••]. However, overexpressed WUS can still activate the *AG* promoter in the absence of LFY [38••], suggesting that this requirement is not absolute.

Acknowledgements

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