Shoot apical meristem maintenance: the art of a dynamic balance

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The aerial structure of higher plants derives from cells at the tip of the stem, in the shoot apical meristem (SAM). Throughout the life of a plant, the SAM produces stem tissues and lateral organs, and also regenerates itself. For correct growth, the plant must maintain a constant flow of cells through the meristem, where the input of dividing pluripotent stem cells offsets the output of differentiating cells. This flow depends on extracellular signaling within the SAM, governed by a spatial regulatory feedback loop that maintains a reservoir of stem cells, and on factors that prevent meristem cells from differentiating prematurely. The terminating floral meristem incorporates the spatial regulation scheme into a temporal regulation pathway involving flower patterning factors.

A specialty of plants is that they can modulate their body plan after embryogenesis, an ability that helps them to deal with the environmental changes that affect their growth. This flexibility in development and organogenesis is provided by pools of dividing, pluripotent cells that reside in structures called meristems. Two meristematic cell populations arise during embryogenesis [1] and grow in polar directions throughout plant life: the shoot apical meristem (SAM) generates the aerial part of the plant, whereas the root apical meristem (RAM) generates the underground part. The SAM continuously produces cells that will become incorporated into stem tissue, lateral organs (leaves and flowers) and axillary meristems. The above-ground part of the plant can be divided into iterative modules, called phytomers, each containing a leaf (or leaves) attached at a node, an axillary meristem at the base of the leaf(s) and a subtending internode (Fig. 1a). After a phase of vegetative growth during which stem tissues and leaves are formed, the SAM and each axillary meristem turns into an inflorescence meristem that bears flowers [2] (Fig. 1b).

The flowers are generated by specialized types of meristems, the floral meristems (FMs), which are also products of the SAM (Fig. 1c). Floral meristems have the same organization in terms of cellular domains as the SAMs [3] (see below) but they differ in two important ways. First, FMs do not generate vegetative structures but only flowers, which are composed of sepals, petals, stamens and carpels in four rings (whorls) from the outside to the inside of the flower (Fig. 1d). Second, FMs are terminating structures: they have a transient activity that stops after enough cells have been produced for their recruitment into the most internal organs, the carpels [2]. Termination of the FM requires the activity of specific factors that are not present in the SAM.

Architecture of the SAM: layers and zones

Cytohistological analyses of several angiosperm species reveal two main architectural aspects of the SAM [2]. The first and most evident feature is its organization into cell layers (Fig. 1c). The surface region or tunica consists of one to five clonal layers – two in most dicots (L1 and L2) and one in monocots – in which cells divide in a single plane, whereas the underlying corpus (L3) is an arrangement of cells that divide in all planes. The different layers generally maintain distinct cell lineages, each generating specific derivatives. In *Arabidopsis*, the L1 contributes to the epidermis of shoots, leaves and flowers, the L2 produces the ground tissues and germ cells, and the L3 contributes to the vascular tissues of the stem and the most internal tissues of leaves and flowers. However, the development of each clonal layer is flexible and can adapt to cell proliferation changes occurring in other layers, as shown by studies of genetic mosaics [4].

Juxtaposed over the SAM cell layers are three zones that have distinct functions (Fig. 1c). The peripheral zone (PZ) and the rib zone (RZ) contain cells that will become incorporated into lateral organs and the stem core, respectively. The central zone (CZ), which is characterized by a lower mitotic activity [5], constitutes the self-renewing stem cell reservoir that is the source of cells for the PZ and RZ. During primordia initiation, sets of cells in the PZ and RZ become specified as the founder cells of young plant tissues. These cells are replaced by dividing pluripotent cells provided by the CZ, which correspondingly replenish themselves through new cell divisions. In this way, the SAM sustains itself as a stable structure, in spite of the constant flow of cells passing through it.

To sustain the modular growth of the shoot and simultaneously maintain SAM homeostasis, shifts in gene expression must occur within cells as their positions within the meristem change. This implies that SAM cells determine their appropriate gene expression states based on signal exchanges with other cells in the meristem rather than through a strict lineage-based predetermination of cell fate [4]. How groups of cells in the SAM signal one another to learn their fates and coordinate proper meristem

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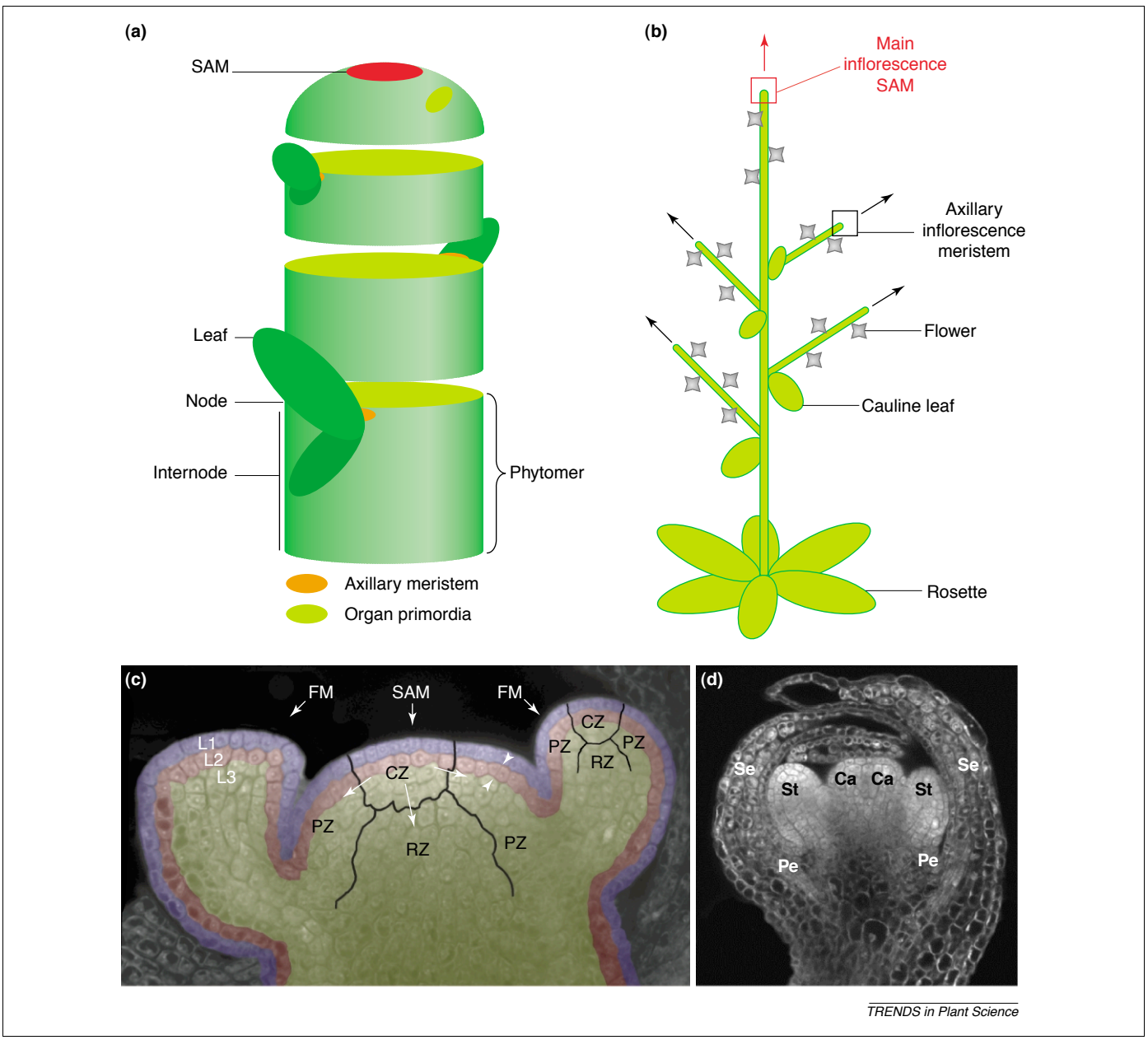

Fig. 1. The shoot apical meristem (SAM) is a cell supply system for the aerial part of the plant. (a) The modular growth of the shoot is illustrated in this three-dimensional model of a growing stem with leaves. The plant body generates from the iterative initiation of units called 'phytomers' composed of a leaf (or leaves) attached at a node, a subtending internode and a bud – an axillary meristem – at the base of the leaf. Depending on their position on the shoot, phytomers differ in leaf size and shape, internode length, and axillary meristem potential. Axillary meristems differ from the SAM in that they are formed postembryonically but both types of meristems can produce an indefinite number of structures such as branches, leaves and flowers. (b) A mature *Arabidopsis* plant. The postembryonic growth of the aerial parts of the plant starts with the rosette stage, during which the SAM initiates a variable number of leaves in a spiral phyllotaxy with unexpanded internodes. At the end of this stage, in response to endogenous and environmental cues, the stem elongates and the SAM produces a variable number of cauline leaves that each develop an axillary meristem at its base. The SAM is now an inflorescence, and initiates floral meristems (FMs) that will produce the flowers. (c) Confocal laser-scanning micrograph through an *Arabidopsis* inflorescence SAM and its adjacent floral meristems (FMs). Continuous organogenesis is made possible by the activity of meristems, which are sources of pluripotent dividing cells. Superimposed on the micrograph are the meristem layers and zones. The colored domains depict the different cell layers: in *Arabidopsis*, the tunica corresponds to two layers of cells, the epidermal (L1) and subepidermal layers (L2), whereas the corpus corresponds to the internal layers (L3). The two arrowheads point at an anticlinal cell division occurring in L2. The black outlines represent the approximate boundaries between the different meristematic zones: the peripheral zone (PZ) contributes cells to the formation of lateral organs, the rib zone (RZ) contributes cells to stem growth and the central zone (CZ) acts as a 'factory' of cells for the PZ and RZ, but also for its own replenishment with new cells. Although the cells of the PZ and the CZ are histologically distinguishable, there is no sharp boundary between each zone. The PZ and the CZ contain both tunica and corpus cells, whereas the RZ is 'buried' beneath them in the deeper layers of the corpus. (d) Confocal laser-scanning micrograph of an *Arabidopsis* FM. After the production of cells that are incorporated into the four whorls of organs – sepals (Se), petals (Pe), stamens (St) and carpels (Ca) – the FM terminates.

function is the subject of intense study. Here, we mainly focus on recent data from *Arabidopsis*, which have revealed that SAM maintenance involves an extracellular signaling network, the CLAVATA (CLV) pathway, that communicates cell fate information between different SAM domains.

CLAVATA signal transduction pathway maintains stem cell identity

CLAVATA ligand–receptor complex

The discovery of plants producing broader and distorted meristems has caught naturalists' interest for centuries. Early reports describe these plants as fasciated (from the

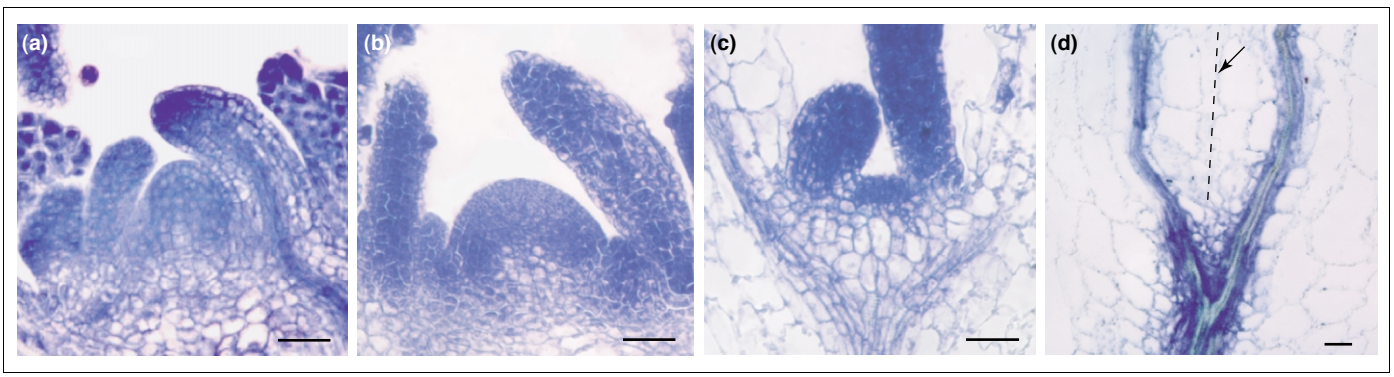

Fig. 2. Phenotypes of *Arabidopsis* shoot apical meristem (SAM) mutants. Longitudinal sections through the vegetative SAMs of 7-day-old (a) wild type, (b) *clavata1-4* (*clv1-4*), (c) *wuschel-1* (*wus-1*) and (d) *shootmeristemless-11* (*stm-11*) plants. (b) The SAM of *clv1-4* plants is both broader and taller than the wild-type SAM (a). (c) In *wus-1* mutants, the shoot apex is flat, yet has a tunica–corpus organization. The SAM shown in (c) was probably about to terminate after the initiation of the two leaf primordia. Eventually, this *wus-1* seedling would have repetitively initiated axillary meristems, all terminating prematurely in a similar flat structure. (d) Plants carrying the *stm-11* mutation lack a SAM and the two cotyledons are fused at their base. The arrow indicates the boundary between the two cotyledons. Scale bars ? 50 μm.

Latin *fascia*, meaning 'bundle'), because their meristems grow as a band or a ring instead of as a point [6]. In *Arabidopsis*, recessive loss-of-function mutations at three *clv* loci cause the formation of fasciated meristems [7–9]. Whereas *clv2* alleles display additional phenotypes, *clv1* and *clv3* mutations specifically affect the development of above-ground meristems of all types such that they broaden progressively, starting during embryogenesis. Consequently, the mutant phenotypes become more dramatic with time: the enlarged vegetative SAM generates a fasciated stem, the inflorescence meristem produces many extra floral meristems and the flower meristems produce extra floral organs. *clv* mutants' FMs often form many extra carpels, giving a club-like shape to the gynoecium [7–9]. The *clavata* mutants obtain their name from this last characteristic, because *clavatus* means 'shaped like a club' in Latin. Cell number is greatly increased in the SAMs and FMs of the *clv* mutants [7–10] (Fig. 2), which is more likely to be caused by a defect in the transition of cells out of the CZ than by higher cell division rates in the CZ [5]. Genetic studies have demonstrated that *CLV1*, *CLV2* and *CLV3* act in the same pathway to limit the expansion of the undifferentiated stem cell population in SAMs and FMs [8,9]. These studies also revealed that the *CLV1* and *CLV3* gene products have a quantitative interdependence [8], which argues for CLV1 and CLV3 physically interacting in the same complex.

The cloning of the *CLV* genes identified them as components of an extracellular signaling pathway. *CLV1* and *CLV2* encode a receptor-like kinase (RLK) and a receptor-like protein (RLP), respectively, and are both members of large gene families [11,12]. CLV1 contains an extracellular region consisting of 21 leucine rich repeats (LRRs), a transmembrane region and an intracellular serine/threonine kinase domain. CLV2 contains 20 LRRs and a transmembrane domain but, unlike CLV1, has a short cytoplasmic tail lacking any signaling domain. CLV3 encodes a secreted polypeptide of 96 amino acids [13] and is mainly expressed in the L1 and L2 of the central zone, whereas *CLV1* mRNA is mostly found in the L3 of the central zone [11,13] (Fig. 3a). RNA blot experiments show that *CLV2* transcripts can be detected in many different tissues, including SAMs and FMs, consistent with *clv2* mutants displaying a pleiotropic phenotype [12]. Altogether, analyses of the *CLV* sequences, expression domains and mutant phenotypes have led to the proposal that the CLV factors act in a cell–cell signaling pathway that extends between layers in the central zone of the SAM. Moreover, mutations in a *CLV2*-like gene from *Zea mays* called *FASCIATED EAR2* (*FEA2*) cause fasciated inflorescence meristem phenotypes similar to those of *clv2* mutants [14], suggesting that the CLV pathway might be conserved throughout the angiosperms.

The CLV cell signaling model is now supported by genetic and biochemical studies jointly demonstrating that CLV2 and CLV3 are required for the assembly of CLV1 into an active 450 kDa signaling complex [12,15]. This complex cannot be isolated from *clv2* mutant protein extracts [12], meaning that CLV2 probably associates as a heterodimer with CLV1. Analyses of chimeric RLKs show that the strong *clv1* mutations, which are caused by mis-sense mutations within the CLV1 extracellular domain, are dominant negative [16]. The dominant negative activity of these alleles can be accounted for if the mutant CLV1 proteins interact physically via their extracellular domains with other receptors, and thus block their function. These findings suggest that other RLKs overlap functionally with CLV1 to fine tune meristem development [16]. A simultaneous study of dominant negative alleles of the ERECTA RLK arrived at a similar conclusion about the redundancy of RLK signaling pathways involved in organ shape regulation [17].

The mode of CLV3 action has also been investigated. Genetic and immunological studies demonstrated the requirement for CLV3 in the extracellular space for its function *in vivo* to activate the CLV complex [18]. Furthermore, CLV1 and CLV2 are required to obtain the gain-of-function phenotypes observed in *CLV3*-overexpressing plants, indicating that CLV3 signaling depends upon CLV1 and CLV2 [19]. These results provide evidence that the CLV3 polypeptide is secreted from the overlying L1 and L2 cell layers, and moves into the underlying L3, where it is likely to act as the ligand for a CLV1/CLV2 signaling complex (Fig. 3). Nevertheless, at this time, additional evidence supporting the whole scheme is still missing, such as proof of a physical interaction between CLV1 and CLV2 or between CLV1/2 and CLV3.

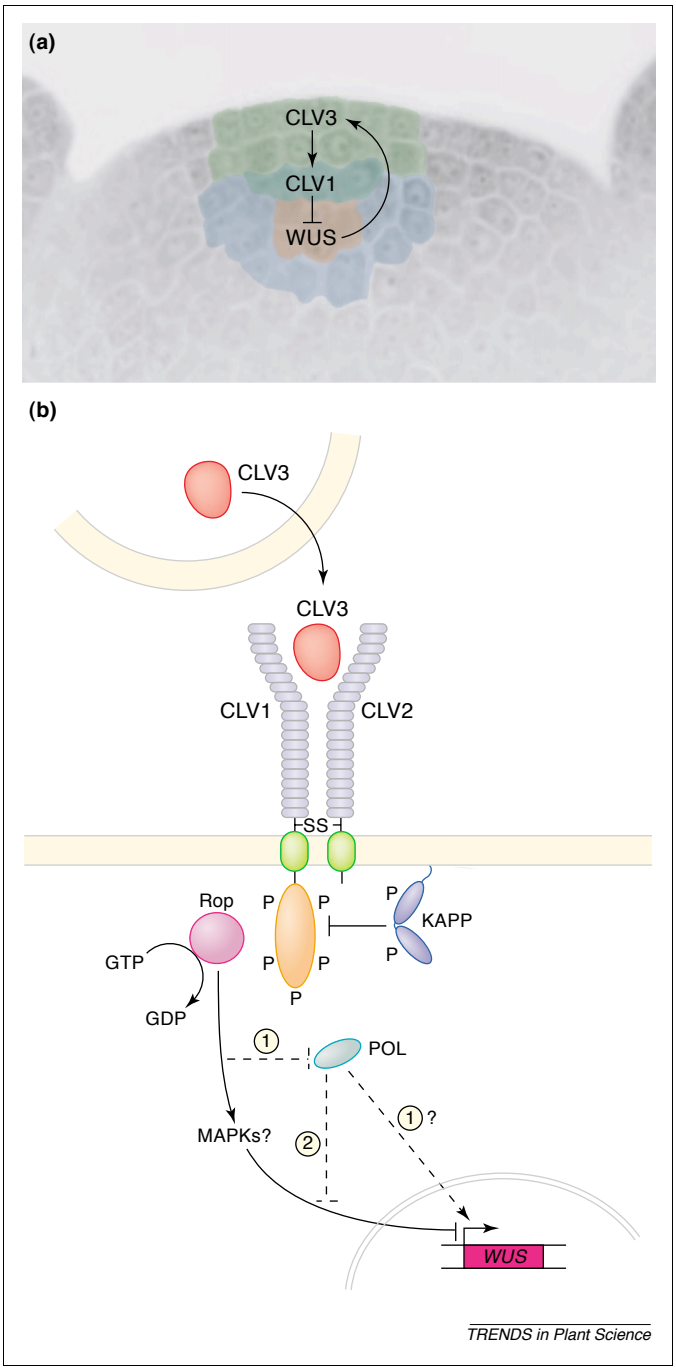

Fig. 3. Coordination of cell proliferation and cell fate decisions across the shoot apical meristem (SAM). (a) Stem cell regulation in the SAM via the CLAVATA–WUSCHEL feedback loop. *CLAVATA3* (*CLV3*) expression is restricted mainly to the L1 and L2 of the central zone [13], whereas *CLV1* mRNA can only be detected in the L3 of the central zone and rib zone [11]. *WUSCHEL* (*WUS*) is expressed in few central cells of the rib zone [21]. The CLV–WUS feedback loop consists of a WUS-mediated signal from the organizing center (OC) that specifies stem cell identity in the outermost layers, which signal back via the CLV pathway to limit the size of the *WUS*-expressing OC. (b) CLV transduction pathway at the cellular level. The CLV1 and CLV2 leucine-rich repeat (LRR) receptor proteins might homo- or heterodimerize through the formation of disulfide bridges between the conserved cysteines pairs (SS) flanking the LRR domain. The binding of CLV3 to CLV1 and/or CLV2 could promote the assembly of the 450 kDa complex, which also contains a protein phosphatase (KAPP) and a Rho-like GTPase (Rop). KAPP is a negative regulator of CLV signaling, capable of dephosphorylating CLV1. Rop transduces the signal from the CLV complex to downstream targets. By analogy with animal systems [56], the binding of Rop to CLV1 might be mediated by a linker protein, the nature of which remains to be determined. The signaling cascade from the plasma-membrane-bound CLV complex leads to the downregulation of *WUS* transcription in the nucleus. This intracellular signaling cascade might involve mitogen-activated protein kinases (MAPKs), based on the example of other LRR receptor-like kinase signaling pathways [57]. The protein phosphatase 2C POLTERGEIST (POL) acts either (1) as a positive regulator of *WUS* transcription that is antagonized by CLV signaling or (2) as a direct inhibitor of CLV signal transduction, analogous to KAPP but acting at a different point in the pathway.

CLV–WUS feedback loop maintains the central reservoir of stem cells

For the SAM to remain in a balanced state, the CLV pathway, which restricts stem cell accumulation, has to be counterbalanced by a factor or factors that promote stem cell production. This function resides with the WUSCHEL (WUS) homeodomain transcription factor. Nonsense mutations in the *WUS* gene result in the mis-specification of stem cells and the premature termination of the SAM and FMs after the formation of a few organs [20,21] (Fig. 2). Eventually, *wus* mutants undergo iterative processes of meristem initiation and premature arrest, leading to the production of disorganized groups of leaves and shoots. Thus, these mutants were given the name *wuschel*, meaning 'tousled hair' in German. It has been proposed that the *WUS*-expressing domain, which is restricted to the L3 of the CZ, acts as an organizing center (OC) that specifies the overlying neighbor cells as stem cells [21]. Because *wus* mutations are epistatic to *clv* mutations, *WUS* is likely to act downstream of the CLV pathway [20,22]. *WUS* produces a non-cell-autonomous signal to activate cell division, because mosaic expression of *WUS* leads to the development of outgrowths that do not express *WUS* themselves but appear in the vicinity of *WUS*-expressing cells [23].

Several major works have shed considerable light on how the antagonistic processes driven by the CLV complex and WUS are coordinated within the SAM. In *clv* SAMs, the reduction in CLV signaling leads to the expansion of the *WUS* expression domain [22]. Mis-expression of *WUS* in an enlarged domain is sufficient to cause a *clv*-like phenotype, confirming that the *clv* meristem phenotype is caused by the deregulation of *WUS* expression. Conversely, ectopic expression of *CLV3* is sufficient to repress *WUS* expression throughout the meristem and thus leads to a *wus*-like phenotype [19]. In wild-type plants, CLV3 protein spreads laterally from the stem cells, signaling to both the stem cells themselves and their lateral neighbors to repress transcription from the *WUS* promoter [24]. The intercellular movement of CLV3 into the meristem center is limited by the CLV1 receptor kinase, which is proposed to sequester the CLV3 protein and to prevent its signal from causing *WUS* transcriptional repression in the OC [24]. Altogether, these results demonstrate that the CLV pathway negatively regulates stem cell accumulation by limiting *WUS* expression. Moreover, *WUS* expression is sufficient to induce the expression of *CLV3* and to promote meristem cell identity [22].

Thus, a picture emerges of a feedback loop between the stem cells and the OC that is mediated by CLV3 and WUS. WUS-mediated signaling from the OC specifies stem cell identity in the outermost cell layers, which signal back via the CLV pathway to limit the size of the *WUS*-expressing OC (Fig. 3a). The net result is meristem homeostasis in which the output of cells into new organs is balanced by the input of new stem cell derivatives (Box 1). New questions

Box 1. Factors that maintain the organization of the shoot apical meristem

Independent of the CLAVATA (CLV) pathway are other factors that regulate the cellular organization of the shoot apical meristem (SAM). Two such factors are the FASCIATED1 (FAS1) and FAS2 proteins, which were identified from the *fas* mutants, in which the cellular and functional organization of both the SAM and the root apical meristem (RAM) are disturbed [10,58]. The *fas1* and *fas2* SAM phenotypes are linked to the distortion of *WUSCHEL* (*WUS*) expression pattern in a broader and more random manner than in *clv* mutants, regardless of the cell layers and zones. The FAS proteins are components of *Arabidopsis* chromatin assembly factor 1 and thus are likely to ensure the appropriate state of gene expression, including *WUS* expression, by facilitating reformation of the correct chromatin structure after replication [58]. A third factor is an APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) type transcription factor that was recently isolated [59] from the activation tagged mutant *dornröschen* (*drn*). Ectopic expression of *DRN* in *drn-D* mutants causes a dramatic increase in SAM size, followed by premature meristem arrest. The terminated *drn-D* meristems display perturbations in cell layering and expanded and reorganized *CLV3* and *WUS* expression domains [59]. *DRN* is expressed in the meristem central zone at all stages of development and also in lateral organ primordia. Double mutant analysis shows that ectopic DRN can function independently of SHOOTMERISTEMLESS (STM), WUS and CLV activities. A *drn* loss of function mutant is aphenotypic, giving DORNRÖSCHEN its name ('sleeping beauty'). A second, closely related gene is also present in the genome. Thus, DRN is likely to be a redundant component of a new SAM regulatory pathway, independent of the CLV–WUS signaling system. In this scheme, DRN would act to repress stem cell fate, counteracting WUS function.

arise from these results. What is the intracellular pathway leading to the inactivation of *WUS*? How does WUS send a signal from the OC to the overlying stem cells? Does WUS itself migrate between the cell layers or is the signal relayed by other factors? If a WUS-induced target gene encodes the signal, how is it perceived?

The group of CLAVATA interactors has recently enlarged

Two immediate downstream components of the CLV signaling complex are a kinase-associated protein phosphatase (KAPP) [25] and a Rho-like GTPase (Rop) [26,27]. These proteins interact directly with CLV1 in the 450 kDa active signaling complex [15] (Fig. 3b). KAPP functions *in vivo* as a negative regulator of the CLV pathway through direct dephosphorylation of CLV1, preventing downstream components from receiving the CLV1 signal [28,29]. The Rop GTPase is proposed to transduce the signal from the receptor complex toward the nucleus [15]. However, the question of how the signal reaches the nucleus from the plasma-membrane-bound signaling complex is as yet unresolved.

Within the past year, several new factors involved in CLV signaling have been identified. The SHEPHERD (SHD) protein might interact directly with the CLAVATA complex, assisting in its assembly [30]. The *shd* mutation causes the same meristem phenotypes as weak and intermediate *clv* alleles, although it also has more pleiotropic effects. Mutations in *CLV* and *WUS* are epistatic to *shd*, suggesting that SHD functions in the CLV pathway. Moreover, the *CLV3* and *WUS* expression domains are greatly enlarged in *shd* mutants and the effect of *CLV3* overexpression is abolished. Thus, the function of the CLV pathway in suppressing *WUS* expression depends on SHD activity. *SHD* encodes an ortholog of the mammalian GRP94 chaperone protein and is expressed throughout the SAM and FMs. By analogy with GRP94, SHD is likely to be involved in achieving the proper conformation or association of the CLV proteins. *SHD* is also expressed in the root and the RAM of *shd* mutants is disorganized, suggesting that SHD might promote the assembly of CLV1-like RLKs that are expressed in roots.

POLTERGEIST (POL) is a newly identified intracellular component of the CLV signaling pathway. Mutations in *POl* are suppressors of *clv* mutant phenotypes and genetic evidence places POL as a downstream regulator of CLV signal transduction [31]. In a *pol* background, *wus* mutations are semidominant and are no longer epistatic to *clv1* mutations [32], suggesting that POL might function downstream of CLV through two pathways, one WUS dependent and the other WUS independent. Because *pol* reveals its effect only when associated with other mutations, it has been named 'the noisy ghost'. *POL* encodes a nuclear-localized protein phosphatase 2C (PP2C) that is expressed broadly throughout the plant and is predicted to act in multiple signaling pathways [32]. It is not clear yet whether POL functions in the SAM as a direct inhibitor of CLV signal transduction or whether it is a positive regulator of *WUS* that is repressed by the CLV pathway (Fig. 3b). The identification of POL as a new player in CLV signaling emphasizes the idea that meristem balance is achieved by complex mechanisms that often involve negative interactions between factors.

Combined effects of homeobox genes prevent stem cell differentiation

The prevention of stem cell differentiation involves several KNOX (KNOTTED1-like homeobox) genes. KNOTTED1 (KN1), the first homeodomain transcription factor identified in plants [33], was isolated from a maize (*Zea mays*) gain-of-function mutant that produced outgrowths ('knots') of undifferentiated tissue on the leaves [34]. SHOOTMERISTEMLESS (STM) is the *Arabidopsis* ortholog of KN1 [35] and was isolated from loss-of-function mutants that fail to establish and maintain the SAM such that no lateral organs are produced once the cotyledons have formed [36] (Fig. 2). Thus, STM is required to maintain the indeterminate cell fate and to prevent cell differentiation in the meristem, where it acts independently of CLV3 and WUS [23,37,38]. *STM*, which is expressed throughout the SAM, is not absolutely required to activate *CLV3* transcription [39]. However, its activity is necessary in addition to that of WUS to maintain an appropriately high level of *CLV3* expression at the meristem apex.

Three other *Arabidopsis* KNOX genes (*KNAT1, KNAT2* and *KNAT6*) are, like *STM*, expressed in the shoot apex and function redundantly with it [40–42]. The role of these KNOX genes is to restrict the expression of the *ASYMMETRIC LEAVES1* (*AS1*) and *AS2* genes to organ primordia, and thus to prevent inappropriate leaf development at the shoot apex. In turn, AS1 and AS2 negatively regulate KNOX gene expression, excluding them from

Box 2. HAM from *Petunia* reveals a function for differentiating primordial cells in maintaining the uncommitted state of meristematic cells

The HAIRY MERISTEM (HAM) factor from *Petunia hybrida* constitutes a relay between the differentiating cells in new organ primordia and the uncommitted cells at the shoot apex. Shoot apical meristems (SAMs) of *ham* mutants terminate prematurely in differentiated stem tissues that fail to respond to *Petunia hybrida SHOOTMERISTEMLESS* (*PhSTM*) and *WUSCHEL* (*PhWUS*) activities [60]. *HAM* encodes a putative transcription factor of the GRAS family [61], and is expressed in the L3 cells of organ primordia and the provasculature of the stem. Because it is expressed in differentiating cells but affects the SAM, HAM functions in a non-cell-autonomous manner, in parallel with PhWUS. These data reveal that differentiating cells, as they leave the meristem field, actively maintain a pool of uncommitted cells in the SAM through HAM activity. Such a mechanism, revealing a new level in the maintenance of the meristem cells in an undifferentiated state, is likely to be shared by other plant species.

organ primordia [41–43]. *AS1* encodes a Myb domain transcription factor [40], and *AS2* encodes a putative transcription factor containing a leucine zipper domain [44]. Reciprocal negative interactions between KNOX transcription factors expressed in meristems and Myb transcription factors expressed in organ primordia have also been reported in *Zea mays* [45,46] and *Antirrhinum majus* [47], where they are likewise required to distinguish between stem cells and organ founder cells.

Ectopic expression of *STM* is sufficient to induce the cell division machinery as well as *KNAT* gene expression [23,38]. However, ectopic *STM* expression does not activate *CLV3* expression [38], showing that STM is not capable of inducing stem cell identity on its own. Both STM and WUS functions are in fact required and sufficient to activate *CLV1* and *CLV3* expression in non-meristematic cells [23,38,39]. Altogether, these observations suggest that STM and WUS serve distinct yet complementary functions in the SAM. STM prevents meristem cells from differentiating prematurely, whereas WUS specifies a subset of cells at the apex of the meristem as stem cells. In conclusion, the joint functions of these two factors are essential for a continuous formation of organ primordia and stem tissues from cells provided by the self-maintaining meristem (Box 2).

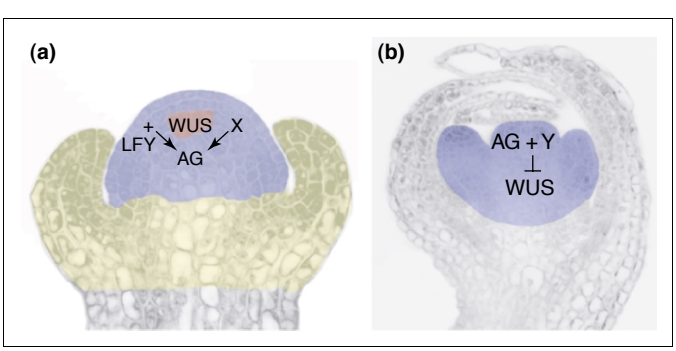

Fig. 4. Floral meristem (FM) termination via a WUSCHEL–AGAMOUS temporal feedback loop. (a) In the early stages of flower development, initiation of *AGAMOUS* (*AG*) expression in the center of the FM is mediated by WUSCHEL (WUS) and LEAFY (LFY). Because the *AG* expression domain (blue) is larger than the *WUS* expression domain (orange) and because *LFY* is expressed throughout the meristem (yellow and blue domains), another factor (X) or factors is likely to be involved in the spatial activation of *AG*. (b) At the time of carpel initiation, AG and at least one other factor (Y) represses *WUS* expression to terminate stem cell activity. The last meristematic cells are then consumed in the formation of the gynoecium. Repression of *WUS* by AG alone is not sufficient to lead to FM termination, as observed in plants overexpressing *AG* [54].

Particular case of the terminating FM

The FM constitutes a particular case in which stem cell activity must be turned off for morphogenesis to be completed. The FM sequentially produces sepal, petal and stamen primordia from stem cell daughters on its flanks. The remaining meristematic cells in the center of the FM then differentiate and form the carpel organs. The AGAMOUS (AG) MADS domain transcription factor, one function of which is to specify flower organ identity, is also required for FM termination [48]. Early in flower development, WUS activates *AG* transcription, thus leading to its own transcriptional demise: AG later switches off OC activity by repressing *WUS* expression, resulting in the differentiation of the last stem cells [20,49,50]. Interestingly, AG seems to repress *WUS* independently of the CLV pathway [50].

The discovery of this 'suicidal' temporal feedback mechanism raises two questions. First, why does this phenomenon occur specifically in the FM and not in the SAM? At least part of the answer is that the activation of *AG* by WUS requires the presence of a third element [49], the LEAFY (LFY) transcription factor, which specifies FM identity [51] and is not expressed in the SAM [52]. WUS and LFY bind separately to adjacent enhancer sequences in the *AG* second intron [50]. Second, what makes the arrest of meristem activity such a time-specific event, so that enough cells have been produced to ensure the correct formation of each whorl? This question seems to find its answer in the increasing level of *AG* expression observed during flower development [53]. There might be a threshold amount of AG necessary for efficient repression of *WUS*, so only at the time of carpel initiation has enough AG accumulated in the FM to repress *WUS* expression. Another possibility is the participation of an additional factor, the activity of which begins at the time of carpel differentiation. The study of plants overexpressing *AG* favors this last hypothesis [54]. In addition, because the *AG* expression domain is larger than the *WUS* domain and because *LFY* is expressed throughout the FM, there might be other factors involved in the spatial activation of *AG*, unless WUS protein itself moves from cell to cell. Thus, FM development involves two negative feedback loops, one the spatial CLV–WUS feedback loop shared with the SAM and the other a WUS–AG temporal feedback loop specific to the FM (Fig. 4).

Recently, the histone acetyltransferase AtGCN5 has been shown to be required for proper FM activity and to act by regulating the WUS–AG pathway [55]. Plants carrying a T-DNA insertion into the bromodomain of *AtGCN5* display homeotic transformations of flower organs, and the conversion of the inflorescence meristem into a terminal flower. These phenotypes correlate with upregulation of *WUS* and *AG* transcription in FMs, expansion of the *WUS* and *AG* expression domains within FMs, and ectopic induction of *AG* in the inflorescence apex. These data

Box 3. Are common maintenance mechanisms shared by the shoot apical meristem and the root apical meristem?

Shoot apical meristems (SAMs) do not share their set of regulatory factors with root apical meristems (RAMs), yet they adjust their cell populations according to the same basic mechanisms, such as intercellular signaling. In both SAMs and RAMs, these mechanisms involve interactions between two groups of cell populations, the pluripotent undifferentiated cells –in the organizing center of the SAM and in the quiescent center (QC) in the RAM – and the differentiating cells that will be incorporated into the plant body [62]. A recent elegant work shows that in the *Arabidopsis* root, the stem cell population depends on the activity of the adjacent QC, which positions the stem cell niche properly in the RAM. This activity depends on the function of the SCARECROW putative transcription factor [63]. The SAM and the RAM might thus be derivative forms of a common meristem organization that depending on the developmental context would produce shoot or root organs. Some SAM and RAM factors belong to the same genes families, which is consistent with such a hypothesis. For example, the *Arabidopsis* SHORTROOT factor, which is expressed in roots and controls cell fate by intercellular movement [64], is a homolog of HAIRY MERISTEM [60] (Box 2). Moreover, the FASCIATED (Box 1) and SHEPHERD (see text) protein examples suggest that, even if the SAM and the RAM have converged on independent but parallel mechanisms for regulating their cell populations, they seem to share common upstream regulators.

reveal an important role for chromatin modification in regulating the level and domain of expression of key components of the temporal feedback loop.

Prospects

The continuous production of new lateral organs by the SAM of growing plants depends upon the presence of mechanisms that permit a constant supply of new cells for organogenesis while preventing those cells from differentiating prematurely. Several recent studies have provided exciting new insights into the molecular mechanisms of *Arabidopsis* SAM maintenance. These analyses have revealed that SAM homeostasis, rather than relying upon genetically predetermined cell fate specification, requires the active exchange of signals between cells to maintain meristem organization and function as the shoot tip grows.

Even though key genes have been identified that are involved in several elegant spatial and temporal meristem feedback loops, our understanding of meristem maintenance mechanisms has not reached its apogee yet (Box 3). A major unanswered question is how, or indeed if, the *CLV1*, *CLV2* and *CLV3* gene products physically interact in a protein signaling complex. The most parsimonious hypothesis is that CLV3 acts as a ligand that directly binds to the extracellular LRR domains of CLV1, CLV2 or a heterodimer of both receptor proteins. However, other models cannot be ruled out, such as CLV3 facilitating ligand binding to CLV1 and/or CLV2, or being involved in producing the ligand, perhaps by proteolytic processing of a ligand precursor protein. Further biochemical analysis will be required to resolve this crucial issue.

In addition, many other areas of plant stem cell signaling research will continue to yield exciting results. Identification of the complete set of CLV–WUS and WUS–AG signal transduction components is ongoing, and will require creative experimental designs to find factors that have not been uncovered in traditional forward genetic screens. The nature of the signals driven by the CLV–WUS factors is still not completely elucidated, and neither are the modes of regulation to which these factors are subjected. The recent characterization of several new factors in the CLV pathway opens the door to the isolation of many more that will fit into the current picture.

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