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Formation and maintenance of the shoot apical meristem

John L. Bowman and Yuval Eshed

Development in higher plants is characterized by the reiterative formation of lateral organs from the flanks of shoot apical meristems. Because organs are produced continuously throughout the life cycle, the shoot apical meristem must maintain a pluripotent stem cell population. These two tasks are accomplished within separate functional domains of the apical meristem. These functional domains develop gradually during embryogenesis. Subsequently, communication among cells within the shoot apical meristem and between the shoot apical meristem and the incipient lateral organs is needed to maintain the functional domains within the shoot apical meristem.

Post-embryonic development in higher plants is characterized by the reiterative formation of lateral organs from the flanks of apical meristems[1]. A shoot apical meristem (SAM) is initially formed during embryogenesis, and derivatives of this meristem give rise to the above-ground portion of the plant. The SAM contains a population of pluripotent stem cells, which serve three primary functions[1–4]:

(1) Lateral organs, such as leaves, are produced from the peripheral regions of the SAM.
(2) The basal regions of the SAM contribute to the formation of the stem.
(3) The stem cells of the SAM must replenish those regions from which cells have been recruited and maintain the pool of stem cells required for further growth.

In general, we focus on SAMs in this review, although extrapolation of concepts to other shoot meristems, such as flower meristems, will be discussed when pertinent.

As a result of histological analyses the SAM has been subdivided in two different manners. First, three distinct zones of the SAM are defined by cytoplasmic densities and cell division rates: the peripheral zone, the central zone and the rib zone[1–4] (Fig. 1). These three zones might represent a functional subdivision of the SAM although direct evidence for this is lacking. Lateral organs are produced from cells recruited from the peripheral zone whereas stem tissue is derived from cells recruited from the rib zone. The central zone acts as a reservoir of stem cells, which replenish both the peripheral and rib zones, as well as maintaining the integrity of the central zone. It should be noted that these cells do not act as permanent initials, but rather their behavior is governed in a position-dependent manner. Second, the SAM is also composed of clonally distinct layers of cells[5] (Fig. 1). The fact that the peripheral and central zones, as well as the lateral organs produced, contain cells from the three clonally distinct layers indicates that communication between cell layers is required to coordinate developmental processes[5,6]. For example, leaves in most eudicot species are composed of derivatives from the epidermal layer (L1), the subepidermal layer (L2) and corpus (L3)[6]. One of the earliest markers of leaf initiation from the peripheral zone is the periclinal cell divisions in specific regions in the L2. Cells in the L1 and L3 adjust their growth accordingly, with the entire region acting coordinately to produce a leaf primordium.

In this review, we discuss some recent advances in our understanding of three aspects of meristem functioning: the origin of the SAM during embryogenesis, the maintenance of the stem cell population in the central zone, and the relationships between lateral organ primordia and the meristems from which they are produced. Several excellent reviews cover broader views of the biology of the SAM (Refs 2–4).

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Embryonic origin of the shoot apical meristem

The origin of the SAM during embryogenesis has been the subject of controversial debate[7,8]. The primary point of contention is whether the cotyledons are formed from the SAM, or if the SAM and cotyledons arise independently. Resolution of this question has major implications, influencing ideas on the homology of leaves and cotyledons. We will not attempt to resolve this question here, but rather argue that the complex histology of the mature SAM is built up gradually during embryogenesis.

Although the tunica–corpus structure, which is characteristic of the SAM (Fig. 1), is not evident until the torpedo stage of embryogenesis in *Arabidopsis* (well after the initiation of the cotyledons)[8], the apical histological zonation (Fig. 1) is visible before cotyledon initiation in some species[7]. This has led to competing hypotheses: either the SAM is formed by the apical portion of the globular embryo, or alternatively, the SAM is not formed until the tunica–corpus structure is evident at the late-heart or early-torpedo stage of embryogenesis. Two recent studies[9,10] have addressed this issue using gene expression patterns as histological markers to analyze the development of the apical portion of the *Arabidopsis* embryo from the globular through the torpedo stages. The primary conclusions from these studies (Fig. 2) are that:

(1) The complex gene expression patterns (histology) of the SAM develop gradually during embryogenesis.
(2) Both independent and interdependent relationships exist among genes directing SAM establishment and maintenance.
(3) The apical portion of the globular embryo is divided into domains, demarcated by gene expression patterns, with distinct developmental fates.

One of the earliest genes expressed is *WUSCHEL* (*WUS*), whose mature SAM expression is limited to a small group of cells underneath the outer three layers (in the L3), but is first expressed in the apical subepidermal cells at the 16-cell stage of embryogenesis[11]. The *WUS* expression pattern gradually becomes limited to deeper regions of the SAM as it forms (Fig. 2), suggesting that cell–cell interactions probably dictate the boundaries of its expression domain. *SHOOT MERISTEMLESS* (*STM*), *CUP-SHAPED COTYLEDON2* (*CUC2*) and *AINTEGUMENTA* (*ANT*) are all first expressed in the late globular embryo[9,10]. By the early transition stage the expression patterns of these genes divide the apical portion of the embryo into three regions[9,10] (Fig. 2):

- A central region destined to give rise to the SAM (*STM* and *CUC2*).
- A peripheral region, which is further subdivided into (i) regions that will produce cotyledons (*ANT*) and (ii) regions where growth will be suppressed, which form the boundaries between the cotyledons (*STM*, *CUC2* and *ANT*).

Later during the heart stage, *CUC2* expression becomes restricted to the boundary regions between the cotyledons and the SAM (Fig. 2) – this restriction is dependent upon *STM* activity[10]. Although *CUC2* and *STM* have complementary expression patterns in the mature SAM, they are expressed in overlapping domains during embryogenesis. This implies that other factors are involved in establishing the complementary expression patterns of the two genes[10]. *CUC2*, which acts redundantly with *CUC1*, is proposed to have a role in the separation of organs from the meristem and from each other[12], in a manner analogous to that of *NO APICAL MERISTEM* in petunia[13].

By early heart stage, after cotyledon primordia have formed, *UNUSUAL FLORAL ORGANS* (*UFO*) and *CLAVATA1* (*CLV1*) are activated in the L2 and L3 of the presumptive SAM (Ref. 9). Although the function of *UFO* in the SAM is unknown, *CLV1* acts with *CLV3* and *WUS* to maintain the integrity of the central zone.

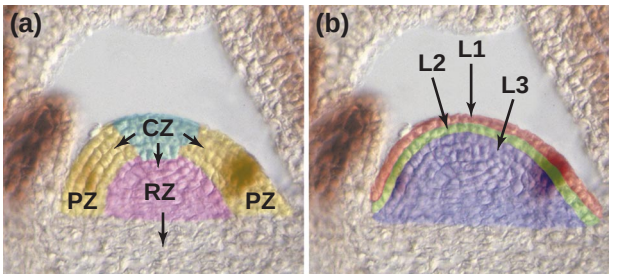

Fig. 1. Histology of the shoot apical meristem (SAM). (a) Lateral organs are produced from cells recruited from the peripheral zone (PZ), whereas the bulk of the stem is derived from cells recruited from the rib zone (RZ; the outermost layers of the stem are derived from the peripheral zone). The central zone (CZ) acts as reservoir of stem cells, which replenishes both the peripheral and rib zones as well as maintaining the integrity of the central zone itself. (b) The SAM is composed of clonally distinct layers of cells. In the SAMs of eudicot plants, there are typically three layers. However, the SAMs of many monocots, including grasses, are composed of only two layers. The epidermal layer (L1) forms one clone, its integrity being maintained by the almost exclusively anticlinal orientations of cell division within the layer. The subepidermal layer (L2) also exhibits almost exclusive anticlinal orientations of cell division, which maintain its clonal distinctness. The L1 and L2 are collectively referred to as the tunica. Cells interior to the L2 constitute the corpus (L3), in which various planes of cell division are observed. *FILAMENTOUS FLOWER* (*FIL*) expression (brown color) demarcates lateral organ anlagen in the peripheral zone and the abaxial domains of leaf primordia in *Arabidopsis*[37,38].

Both initial *UFO* expression and maintenance of *CLV1* expression requires *STM* activity, implying that *STM* acts to initiate a developmental program required to establish or maintain several components of the SAM (Ref. 9), consistent with the loss-of-function phenotype of *stm* mutants[8].

From these studies it is clear that the apical region of the globular embryo is progressively subdivided during development, and that the establishment of the functional regions of the SAM is a gradual and dynamic process that occurs during embryonic pattern formation. In general, it appears that the earliest acting genes are required for establishment or maintenance of stem cell fate or alternatively, repression of differentiation (e.g. *WUS*, *STM*). Whereas genes whose expression is initiated later might be involved in regulating the size of the central zone (e.g. *CLV1*).

Maintenance of the central zone

One striking property of SAMs is their ability to remain relatively constant in size. For example, the SAM of a several-hundred-year-old mountain ash (*Sorbus aucuparia*) does not differ significantly in size from the SAM of its cognate sapling. This is all the more remarkable considering the continual production of lateral organs from the peripheral zones and the lack of cell lineage restriction in determining cell fates[3,14–16]. These properties suggest that cells within the SAM must continually assess their positions relative to others, and subsequently decide to divide, differentiate or remain as they are. Failure to choose appropriately leads to either an accumulation of cells within the SAM, or alternatively, loss of cells from the SAM, which in turn eventually leads to a failure of SAM maintenance. Several mutants accumulating too many cells in the SAM have been identified in *Arabidopsis*, and these mutants fall primarily into two classes. The *clavata* mutants accumulate excess cells in the central zone[17,18]. By contrast, organ

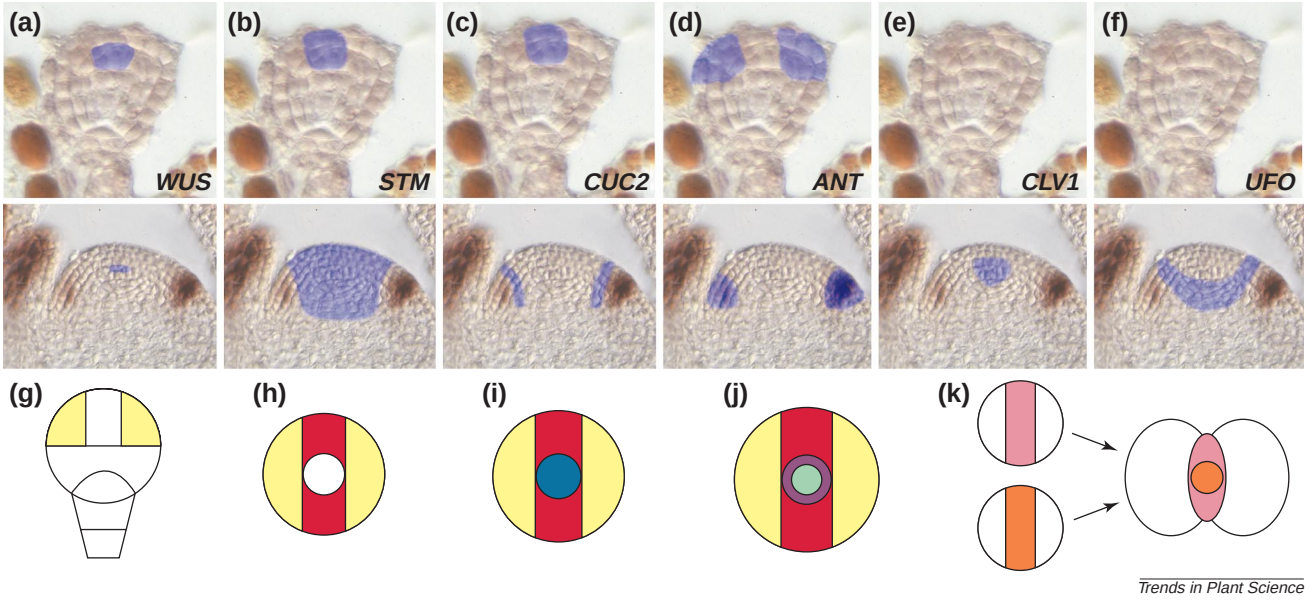

Fig. 2. Expression patterns of genes directing the establishment and maintenance of the shoot apical meristem (SAM) form gradually during embryogenesis. (a–f) Expression patterns of six genes: the upper panel depicts the expression in a frontal section[9] of a late globular-transition stage of embryogenesis and the lower panel shows expression in a vegetative SAM. *WUSCHEL* (*WUS*)[11], *SHOOT MERISTEMLESS* (*STM*)[9,47], *CUP-SHAPED COTYLEDON2* (*CUC2*)[10] and *AINTEGUMENTA* (*ANT*)[9] are all expressed by the transition stage, whereas expression of *CLAVATA1* (*CLV1*)[9,20] and *UNUSUAL FLORAL ORGANS* (*UFO*)[9,48] is not detected until the heart stage. The expression pattern shown for *CUC2* in the vegetative SAM is an extrapolation of its reported embryonic expression[10] and that observed for *NO APICAL MERISTEM* (*NAM*)[13]. *PHANTASTICA* (*PHAN*)[33] and *FILAMENTOUS FLOWER* (*FIL*)[37,38] (brown staining in each of the vegetative SAM panels) also mark the lateral organ anlagen in a manner similar to that of *ANT1* (Refs 2,49). It should be noted that the expression patterns depicted here are extrapolated from different sections and that the precise patterns might differ from those shown. In addition, only qualitative patterns are shown here, but quantitative variations might be present as well. (g–k) Complex and dynamic subdivision of the apical portion of the embryo. *STM* expression is depicted in orange; *CUC2* depicted in pink; *ANT* depicted in yellow; *STM* + *ANT* depicted in red; *STM* + *UFO* depicted in blue; *STM* + *CLV1* depicted in green; *STM* + *CLV1* + *UFO* depicted in purple. (g and h) Depiction of the globular stage embryo in which *ANT* is expressed around the periphery and *STM* is expressed at the periphery between the cotyledon anlagen[9]. By the early heart stage (i), *STM* expression is also expressed in the central region and, along with *UFO*, marks the site of the presumptive SAM (Ref. 9). During the heart stage (j), *CLV1* is expressed in the central region whereas *UFO* is restricted to the margins of the central region[9]. Although *STM* and *CUC2* have similar expression patterns at the transition stage[9,10], during the heart stage *STM* and *CUC2* resolve to complementary patterns, with *STM* expressed in the central region (which will give rise to the SAM) and *CUC2* expressed at the boundaries between the SAM and the cotyledon primordia[10] (k). (g–k) Adapted from Refs 9 and 10.

initiation is affected in the *mgoun* mutants, and the location of accumulation of excess cells is not presently clear[19]. Mutants of both classes also appear to have enlarged rib zones[17–19].

Based on morphology, histology and gene expression patterns, mutations in *CLAVATA1* (*CLV1*) or *CLV3* lead to an accumulation of cells in the central zone[17,18,20–22]. Such a phenotype could either be because of an increase in cell division rates in the central zone, or alternatively, a reduction in the rate of recruitment of cells from the central zone to the peripheral zone. A reduction in the rate of recruitment has been argued based on observations of cell division rates in the central zone of SAMs in *clv1* mutants[22]. In wild-type SAMs, cell division rates are slower in the central zone than in the adjacent peripheral zone, whereas in *clv1* mutants, cell division rates within the central zone of both inflorescence meristems and meristems of seven-day-old seedlings were measured to be lower than that of the wild-type central zones[22]. Although this would suggest that the accumulation of cells in the central zone is caused by a reduction in the rate of cells being recruited into the peripheral zone, a possible caveat is that observations on already enlarged meristems could be misleading because of developmental epistasis. That is, that the reduction in cell division rates in mature inflorescence meristems might be a consequence of earlier alterations in the functioning of the meristem. A more conclusive experiment would be to analyze the structure of the SAM late in embryogenesis before the production of the first set of leaves. In this case, it is apparent that SAMs of *clv3* embryos contain many more cells that those of the wild type[18]. Likewise, slightly later in development, after the initiation of the first pair of leaves, there are considerably more cells in *clv1* and *clv3* SAMs than in wild-type SAMs (Ref. 23). Although these phenotypes could be caused by leaf anlagen initiation during embryogenesis, the observation that *clv* mutants produce more leaves per day[23] suggests that the accumulation of cells in the central zone in these mutants is probably caused by an increased cell division rate in the central zone itself. Further studies are needed to resolve this issue.

The converse phenotype, the inability to maintain a population of stem cells in the central zone, has been described for plants with mutations in the *WUS* gene[24]. SAM's can be initiated by *WUS* mutants, but cells within these SAMs are recruited to form lateral organs without replenishment of the stem cell population in the central zone[24]. Thus mutations in *WUS* and *CLV1*/*CLV3* have essentially opposite effects on the stem cell population of the central zone, suggesting that these genes act in pathways to promote and restrict cell division rates, respectively, within the central zone.

Genetic interactions, the expression patterns and nature of the encoded gene products of *CLV1*, *CLV3* and *WUS* has led to the development of a model of their action (Fig. 3). *CLV1*, whose

mRNA is present primarily in the L3 of the central zone (its expression might also extend into the L2), encodes a leucine-rich repeat (LRR) receptor kinase[20,21]. *CLV3*, whose mRNA is primarily restricted to the L1 and L2 of the central zone, encodes a small, putatively secreted protein[21]. Because *CLV1* and *CLV3* are proposed to act in a common pathway[18], they might act as a receptor-ligand pair in a signal transduction cascade that restricts cell division rates in the central zone[21]. The limited expression domains of *CLV1* and *CLV3* imply that these genes act non-cell-autonomously to regulate central zone size, and suggests that extensive cell–cell signaling, both within and between zones in the meristem, is required for the maintenance of SAM integrity. *WUS* encodes a homeodomain transcription factor and *WUS* mRNA is localized to the L3 of the central zone[11]. It has been proposed that *WUS*-expressing cells act as an organizing center, conferring stem-cell identity to overlying neighboring cells[11] in a manner similar to that of the quiescent center in the root meristem[25,26]. Because *wus* mutations are epistatic to *clv1* mutations[24], the *CLV1*/*CLV3* signaling pathway could potentially act to negatively regulate the activity of *WUS* directly. Thus one possible model is that *WUS* promotes stem cell fate non-cell autonomously among cells of the central zone[11], and that the *CLV1*/*CLV3* signaling pathway dampens this promotion by restricting cell division within the central zone[17,18,20–22].

However, several key questions remain. First, although *CLV1*/*CLV3* activity is mitigated by KAPP (Refs 27,28), acts through a complex that includes a Rho GTPase (Ref. 29) and is likely to be modulated by *CLV2* [another LRR receptor-like protein that might heterodimerize with *CLV1* (Ref. 30)], the ultimate targets of this signal transduction cascade are unknown. Could WUS itself be a target? Second, how does expression of *WUS* in the L3 of the central zone non-cell, autonomously influence cell division in the overlying cells? Third, what is the significance of the dynamic *WUS* expression pattern within the meristem[11]? The pattern correlates with the nature of primordia initiation by the meristem:

- Expression in the upper layers (L2 or uppermost L3) when opposite or whorled primordia are formed (e.g. floral organs by flower meristems).
- Expression deeper in the L3, when primordia are initiated in a spiral manner (e.g. leaf initiation by mature vegetative meristems).

However, it is unclear if the changes in *WUS* expression are involved in the alteration of phyllotaxy. Intriguingly, *CLV1* expression also appears to shift upward when organs are initiated in a whorled manner by the flower meristems[20]. Fourth, and perhaps more interestingly, how is the relative activity of the *CL1/CLV3* system regulated? Because the extent of cell division required in the central zone is profoundly influenced by the need to replenish the loss of cells in the peripheral zone (associated with lateral organ formation), these processes are likely to be intimately linked. One attractive hypothesis is that lateral organ primordia communicate their formation to the SAM, resulting in a replenishment of the peripheral zone from cells ultimately derived from the central zone.

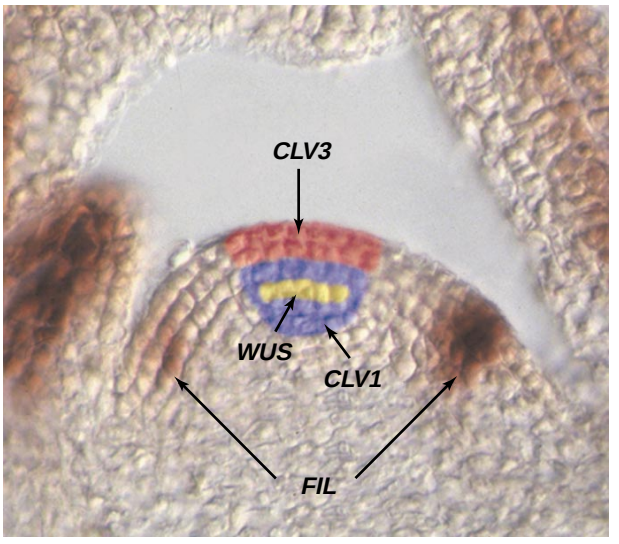

Fig. 3. Expression patterns of genes involved in maintaining the integrity of the central zone. *CLAVATA3* (*CLV3*) mRNA is restricted to the epidermal layer (L1) and subepidermal layer (L2) of the central zone[21], whereas *CLV1* mRNA is detected in the corpus (L3) of the central zone[20]. During vegetative development *WUSCHEL* (*WUS*) mRNA is restricted to a few cells within the L3, below the uppermost layer of the L3 (Ref. 11). It has been proposed that CLV3 acts as a secreted ligand for the CLV1 receptor, and that this signaling is responsible for restricting the size of the central zone[20,21]. By contrast, *WUS* is required to maintain an active central zone, possibly by non-cell autonomously conferring a stem cell identity on cells overlying its expression domain[11]. The relative overlaps in expression of these three genes in this figure are estimated based on comparisons of published data, although the simultaneous detection of these genes might alter this view. *FILAMENTOUS FLOWER* (*FIL*) expression demarcates lateral organ anlagen in the peripheral zone.

Regulation of meristem function by its lateral organ primordia

The effects of signals emanating from mature leaves on the fate of the apical meristem are already part of botany textbooks. Recently, two different approaches demonstrated that such effects also occur during primordia initiation. First, the localized exogenous application of the cell-wall-loosening protein EXPANSIN to the organ anlage of live tomato apices promoted organ primordia formation at the site of application[31]. Moreover, altering the normal positions of primordia initiation can influence the phyllotactic pattern of primordia initiation, implying primordium–SAM communication. Although the expression pattern of *EXPANSIN* mRNA is correlated with the pattern of primordia initiation[32], it is unclear whether the effects of ectopic EXPANSIN activity are mediated via biochemical or biophysical effects[33], or a combination of both.

Non-cell-autonomous relationships between the SAM and lateral organ primordia have also been uncovered in studies of the *Antirrhinum* mutation *phantastica* (*phan*)[34,35]. *PHAN*, which encodes a MYB-related protein, is expressed throughout lateral organ primordia. However, when mutant plants are grown in non-permissive conditions they develop radialized leaves and arrested SAMs (Ref. 35). The radial leaves of *phan* mutants appear to consist predominantly of abaxial cell types[34]. Thus, although *PHAN* is expressed in lateral organ primordia and appears to promote adaxial cell fate, it is required non-cell-autonomously to maintain a functional apical meristem. By contrast, leaves of the *Arabidopsis* semi-dominant mutant *phabulosa-1d* (*phb-1d*) are radial with ubiquitous adaxial cell types[36]. In *phb-1d* mutants, the apical meristem is enlarged and axillary meristems are formed around the entire circumference of the leaves. These observations led to the proposal that adaxial cell fate promotes meristem formation[36]. Conversely, abaxial cell fate might be incompatible with meristem maintenance. Consistent with this hypothesis is the failure to maintain a functional meristem in *phan* mutants[34,35]. Recently, several members of the YABBY gene family have been proposed to promote abaxial cell fate in lateral organs[37–39]. Each family member is expressed in the abaxial domains of one or more

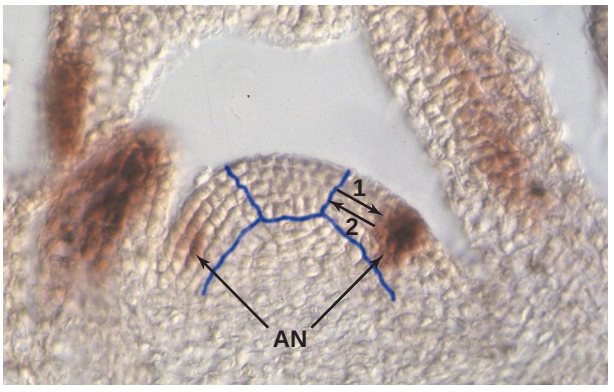

Fig. 4. Model for interactions between lateral organ primordia and the apical meristem. Experiments in which incipient leaf primordia were separated by incisions from the shoot apical meristem have suggested that the apical meristem might be the source for a signal required for the proper abaxial–adaxial development of the leaf because the isolated primordia developed into radially symmetric, apparently abaxialized, organs[50,51] (arrow 1). One interpretation is that signals emanating from the apical meristem promote adaxial cell fate, and in the absence of such signals, abaxial cell fate is the default pattern of differentiation. The establishment of the abaxial and adaxial domains occurs during the transition from leaf anlagen to leaf primordium because older primordia can develop autonomously into phenotypically normal leaves[50,51]. The suggestion that adaxial leaf fate has a positive influence on the maintenance of the meristem (arrow 2) is supported by the phenotype of the adaxialized *phb-1d* mutant, in which axillary meristems are formed around the basal circumference of the radial leaves and the apical meristem is itself enlarged[36]. Thus, meristems produce lateral organs that in turn stimulate meristem formation or regeneration[36]. The failure to maintain a functional meristem when lateral organs are abaxialized is also consistent with this proposed signaling[34,35,37]. The nature of the proposed signals, their transduction (e.g. via plasmodesmata[52–54] or secreted ligands) and the precise points of origin and perception (e.g. central or peripheral zone) are presently an enigma. Approximate boundaries of the central, peripheral and rib zones are shown in blue. AN, leaf anlagen.

above-ground lateral organs. Ectopic expression of either of two members of the YABBY gene family, *FILAMENTOUS FLOWER* (*FIL*) or *YABBY3*, throughout the plant at a low level results in partial conversion of adaxial tissues into abaxial ones. However, with higher levels of ectopic expression, plants produce only abaxialized cotyledons and display meristem arrest[37]. Because YABBY gene family members appear to promote abaxial cell fate[37–39], it suggests that abaxial cell fates and meristematic fates are incompatible.

One speculative model consistent with the above observations is that as cells are set aside to become lateral organ primordia in the peripheral zone of the SAM. Signals from the SAM itself are required for the specification of adaxial cell fate within the lateral organ anlagen (Fig. 4). Subsequently, signals emanating from the adaxial regions of emerging lateral organ primordia would stimulate the SAM to replenish the peripheral zone depleted by the recruitment of cells into the lateral organs[36]. The nature of the proposed signals and their mechanism of transduction are presently an enigma and remain a challenge for the future.

Complexities of the relationships between SAMs and lateral organs are further exposed by the analysis of the maize ortholog of *PHAN – ROUGH SHEATH2* (*RS2*)[40,41]. Leaves of *rs2* mutants appear similar to gain-of-function alleles of the SAM-specific *KNOTTED* class I genes[42–44]. Indeed, several genes of that group were shown to be misregulated in either *phan* or *rs2* mutants[40–42], leading to the concept that *PHAN* and *RS2* might have different functions in *Antirrhinum* and *Zea* leaves, respectively[40,41]. Specifically it was suggested that *RS2* could be involved in establishing the proximal–distal axis rather than the abaxial–adaxial axis in developing leaves[41]. However, the development of these two axes might be linked and one consequence of severely abaxialized lateral organs could be a concomitant loss of proximal–distal development[34,35,37]. Analysis of orthologous genes in other species might be required to clarify this issue.

Conclusions

The primary theme from the three vignettes presented is that cells within the SAM are constantly reassessing their positions and fates with respect to their neighbors to ensure proper formation and maintenance of the SAM. Thus, SAM formation and maintenance are active processes, and it is likely that extensive communication pathways exist within and between the classically defined regions of the meristem, as well as between the SAM and incipient lateral organ primordia. This view of the SAM is consistent with position-dependent rather than lineage-dependent development. Extensive communication pathways imply numerous receptors and their corresponding ligands, or perhaps morphogens as conduits for cells talking to their neighbors and beyond. Given the many candidate molecules uncovered by the *Arabidopsis* genome-sequencing project (such as Refs 45,46), a challenge for the future is to identify specific components that mediate such communication pathways, and elucidate their interactions in developing plants.

Acknowledgements

We apologize to those researchers whose work we were unable to cite because of space limitations. For those whose work is cited, we assume full responsibility for any errors in interpretation or presentation. Work in J.L.B.'s laboratory is supported by the National Science Foundation, the US Dept of Agriculture (NRICGP), the Dept of Energy (Division of Biosciences), and the Beckman Foundation. Y.E. was partially supported by a postdoctoral fellowship from BARD.

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