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. 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. 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.

As a result of histological analyses the SAM has been subdivided into 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 (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 (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. For example, leaves in most eudicot species are composed of derivatives from the epidermal layer (L1), the subepidermal layer (L2) and corpus (L3). One of the earliest markers of leaf initiation from the peripheral zone is the periclinal cell division 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).
Embryonic origin of the shoot apical meristem

The origin of the SAM during embryogenesis has been the subject of controversial debate. 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), the apical histological zonation (Fig. 1) is visible before cotyledon initiation in some species. 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 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. 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 region of the SAM as it forms (Fig. 2), suggesting that cell–cell interactions probably dictate the boundaries of its expression region of the SAM as it forms (Fig. 2), suggesting that cell–cell interactions probably dictate the boundaries of its expression region of the SAM as it forms (Fig. 2), suggesting that cell–cell interactions probably dictate the boundaries of its expression region of the SAM as it forms (Fig. 2).

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.

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 fate. 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.
initiation is affected in the mgoun mutants, and the location of accumulation of excess cells is not presently clear34. Mutants of both classes also appear to have enlarged rib zones15,16.

Based on morphology, histology and gene expression patterns, mutations in CLAVATA1 (CLV1) or CLV3 lead to an accumulation of cells in the central zone11,18,19. 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 mutants20. In wild-type SAMs, cell division rates are slower in the central zone than in the adjacent peripheral zone, which will give rise to the SAM17,18,20–22. Such a phenotype could 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 than those of the wild type28. 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 day29 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 gene30. 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 zone25. 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-like protein, and is restricted to the epidermal layer (L1) and subepidermal layer (L2) of the central zone 20,21 . Its expression is also present in 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 22,23 . 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 and 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 11,18,20,21 .

However, several key questions remain. First, although CLV1/CLV3 activity is partially restricted to the L2 of the central zone, encodes a leucine-rich repeat (LRR) receptor-like protein, and is restricted to the epidermal layer (L1) and subepidermal layer (L2) of the central zone 20,21 , it is unclear how the expression of WUS in the L3 of the central zone (L3) promotes stem-cell, and fate cell division in the overlying cells? Second, what is the significance of the dynamic WUS expression pattern within the meristem 11 ? The pattern correlates well 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 24 . Fourth, and perhaps more interestingly, how is the relative activity of the CLV1/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.

Regulation of meristem function by the lateral organ primordia

The effects of signals 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 anlagen of live tomato apices promoted organ primordium formation at the site of application 32 . 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 by 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 Arabidopsis mutant 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 phyllotaxis-1d (phy-1d) are radial with ubiquitous adaxial cell types 34 . In phy-1d mutants, the apical meristem is enlarged and auxiliary 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 34,35 . Each family member is expressed in the abaxial domains of one or more...
above-ground lateral organs: Ectopic expression of either of two members of the YABBY gene family, FILAMENTOUS FLOWER (FLS) or YABBY1, 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 partial conversion of adaxial tissues into abaxial ones. The nature of the proposed signals, their transduction (e.g., via plasmodesmata or secreted ligands), and the precise points of origin and perception (e.g., central or peripheral zones) are presently an enigma. Approximate boundaries of the central, peripheral, and rib zones are shown in blue. AN, leaf anlagen.

Fig. 4. Model for interactions between lateral organ primordia and the shoot 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 primordium developed into radially symmetric, apparently abaxialized, organs36 (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 primordia because older primordia can develop autonomously into phenotypically normal leaves37. 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 abaxialized phb-1d mutant, in which auxiliary meristems are formed around the basal circumference of the radial leaves and the apical meristem is itself enlarged38. Thus, meristems produce lateral organs that in turn stimulate meristem formation or regeneration39. The failure to maintain a functional meristem when lateral organs are abaxialized is also consistent with this proposed signaling40,41. The nature of the proposed signals, their transduction (e.g., via plasmodesmata or secreted ligands) and the precise points of origin and perception (e.g., central or peripheral zones) 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 (FLS) or YABBY1, 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 arrest42. Because YABBY gene family members appear to promote abaxial cell fate43, 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 organs44. 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 SHEATH (RS2)45. Leaves of rs2 mutants appear similar to gain-of-function alleles of the SAM-specific KNOTTED class I genes46–48. Indeed, several genes of that group were shown to be misregulated in either phan or rs2 mutants49–51, leading to the concept that PHAN and RS2 might have different functions in Antirrhinum and Zea leaves, respectively45. Specifically it was suggested that RS2 could be involved in establishing the proximal-distal axis rather than the abaxial-adaxial axis in developing leaves50. 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 development51. 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 41,42), 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 U.S. Dept of Agriculture (NRRCGP), 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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