

# Shoots and Buds

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The shoot of higher plants carries the branches, foliage and flowers. Repetitive organ formation takes place at the tip of shoots and in floral buds by the activity of stem cell systems, the shoot and flower meristems.

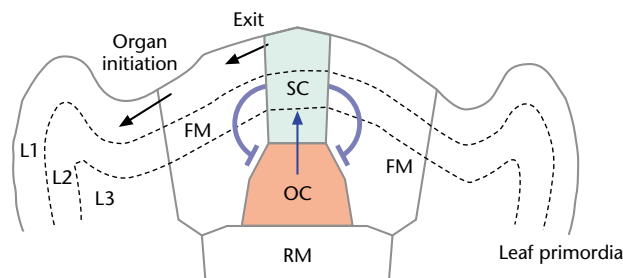
## Introduction

The shoot represents the above-ground part of higher plants. It is composed of repeated units, the phytomers, which consist of a node at which leaves and axillary meristems insert and the part of the stem between two nodes. The stem raises foliage and flowers for optimal light exposure and seed dispersal. The leaves are the main photosynthetic organs. They carry meristems in their axils that can give rise to side branches or flowers. The flowers themselves are modified shoots with specialized leaf-like organs: petals and sepals that mainly serve to protect the flower and in many cases to attract pollinators, and the male and female reproductive organs – stamens and carpels, respectively.

In the case of some trees the shoot can grow for more than a thousand years and elaborate an extensive organ system. This repetitive development is accomplished by the activity of a stem cell system, the shoot meristem. This review focuses on our current understanding of the mechanisms that govern shoot development.

## The Shoot Meristem

The ultimate source of all above-ground organs is a small population of stem cells in the central zone of the shoot apical meristem (**Figure 1**). The stem cells are pluripotent in



**Figure 1** Shoot meristem homeostasis. The stem cells are specified by yet unidentified signalling from the underlying organizing centre (OC). Cells that exit the stem cell region initiate differentiation and are recruited into organ primordia (RM, rib meristem; FM, flanking meristem; SC, stem cells; OC, organizing centre).

## Introductory article

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that they give rise to a variety of different cell types. Stem cell divisions result in two populations of daughter cells. The daughters that stay in the apical position remain pluripotent and renew the stem cell population. By contrast, daughter cells more distant from the apex ultimately undergo differentiation: in the peripheral zone of the meristem they initiate primordia for leaves, side branches and flowers, and in the rib zone they differentiate into the central tissue of the stem.

The shoot meristem organization reflects specific patterns of cell divisions: the outermost cells typically divide perpendicular to the surface, resulting in separate cell layers. The number of the cell layers varies between species: many dicotyledonous plants have two, named L1 and L2 (together termed tunica). Cells underneath these layers divide in different orientations, and this region is named L3 (or corpus). Because the outer cell layers grow as sheets, cells of L1, L2 and L3 usually do not mix but behave as clonally independent compartments and contribute differently to the plant tissues: the L1 exclusively forms the epidermis, whereas the L2 and the L3 give rise to internal tissues. All cells within L1, L2 or L3 are ultimately derived from only 1–3 apical stem cells per cell layer. The fate of each shoot meristem cell, however, is only predictable in a stochastic way: if a daughter cell from one layer is displaced into another layer, this cell will adopt the fate of its new position.

## Cell Fate Specification in the Shoot Meristem

How is a cell's decision between pluripotency and differentiation regulated. Stem cell identity is restricted to the meristem centre (**Figure 1**). However, if a stem cell becomes displaced from the centre, it enters differentiation. This indicates that stem cell identity is not a heritable trait, but that positional information defines a niche in which the cells maintain a pluripotent state. This specification

process requires expression of the homeobox gene *WUSCHEL* (*WUS*) in the underlying cells. Mutations in *WUS* result in the misspecification of stem cells and the premature termination of meristem activity. The misspecified apical cells in *wus* mutants, however, do not become integrated into organs, indicating that *WUS* functions as a positive regulator of stem cell identity rather than as a repressor of organ formation. It has been suggested that the *WUS*-expressing cells act as an organizing centre that signals to its overlying neighbours, specifying them as stem cells.

By contrast, mutations in the *SHOOTMERISTEMLESS*, *ZWILLE* or *TERMINAL FLOWER* genes result in ectopic organ formation in the meristem centre, indicating that maintenance of shoot meristem activity also involves mechanisms that repress the formation of organs or flowers in the centre.

Cells that become displaced from the meristem centre initiate differentiation (**Figure 1**). In a first step, they exit stem cell identity. This step is regulated by the *CLAVATA* receptor kinase signalling pathway: mutations in any of the three *CLV* genes result in a delayed entry of cells into differentiation and consequently in a gradual accumulation of meristem cells. In a second step, cells become recruited into organ primordia. This step requires the *MGOUN* genes, mutations in which result in defective organ formation.

The balance between the two processes of stem cell maintenance and differentiation, and thus the homeostasis of the shoot meristem, appears to be regulated by a feedback loop between stem cells and the *WUS* expressing cells underneath. It has been suggested that the latter act as an Organizing Centre that signals to its overlying neighbours, specifying them as pluripotent stem cells. The stem cells in turn express *CLV3*, the putative ligand of the *CLV1* receptor kinase, which functions as a feedback signal to repress *WUS* and thereby restricts the Organizing Centre. By this regulatory feedback loop, the size of the stem cell population can continually be checked and kept constant during development.

Within each organ primordium, a number of different cell types are needed: for example, in a leaf, L1-derived cells form epidermal structures, whereas L2 and L3 progeny give rise to mesophyll and vasculature. Which differentiation pathway a cell follows depends on specific signals. Some of those signals may be derived from older, already differentiated, tissues in order to integrate the newly formed organ into the plant architecture.

## Integration of Cell Fate Decisions by Cell–Cell Communication

How do cells communicate with each other to coordinate their development? There are two main routes by which

plant cells can communicate and both appear to function in the shoot meristem. One way is to exchange molecules via cytoplasmic connections, the plasmodesmata. It has been shown that cells of distinct regions of the shoot meristem, e.g. the cells in the central zone, are effectively coupled via plasmodesmata, allowing facilitated exchange of molecules, whereas the exchange between cells of different domains may be less efficient. Studies of the maize *Knotted 1* (*KN1*) protein suggest that the plasmodesmata are indeed used as molecular routes in the shoot meristem to exchange important regulatory molecules. The *KN1* gene appears to prevent apical cells of the shoot meristem from differentiating. Its mRNA is present in subepidermal cells, whereas *KN1* protein is also detected in the epidermis. Injection of *KN1* protein into mesophyll cells indicate that the *KN1* protein itself can pass through plasmodesmata and can also promote selective plasmodesmal transport of its own mRNA.

A second way of communication between cells is signalling through the intercellular space, the apoplast. Mobile molecules secreted by one cell can be recognized by specific receptors on another cell's surface. The *CLV* signalling pathway is a candidate for this type of communication in the shoot meristem.

## Position of the Shoot Meristem: Distal Organizer or Self-regulatory Loop

What designates the shoot tip as the place where the shoot meristem resides? The shoot meristem receives signals from the rest of the plant that affect, for example, its maintenance, the position of new primordia or flowering time. Thus it is conceivable that the position of the shoot meristem itself could also be determined by distal information constantly provided to the shoot apex. This information could be a molecule transported towards and thus accumulating at the shoot pole, promoting stem cell identity. There is no evidence for such a mechanism in shoot meristem regulation, but findings imply that a similar mechanism may be involved in regulating the root meristem. The growth factor auxin is transported basipetally and accumulates in a cell group at the root pole, which may function as a distal organizer of the root meristem.

An alternative mechanism is that once the shoot meristem is established, it is able to maintain itself without continuous information from the rest of the plant. Such an autonomous shoot meristem would simply stay at the apex through its polar mode of growth, whereby cells are given off towards the meristem base.

## Shoot Meristem Formation

The primary shoot meristem is formed during embryogenesis and it only becomes apparent when the cotyledonary primordia are evident (**Figure 2**). The precise origin of the shoot meristem and the cotyledons is controversial. One view, based on comparative morphological analysis, holds that the apical half of the globular embryo represents the shoot meristem, which as its first products gives rise to the cotyledons, similar to the way true leaf primordia are initiated later. In an alternative view, a functional shoot meristem is the outcome of a gradual patterning process that is not completed until after the cotyledons have been formed. In this view, plants would have two different ways of making leaf-like organs: cotyledons made during embryo patterning, and leaves derived from the shoot meristem.

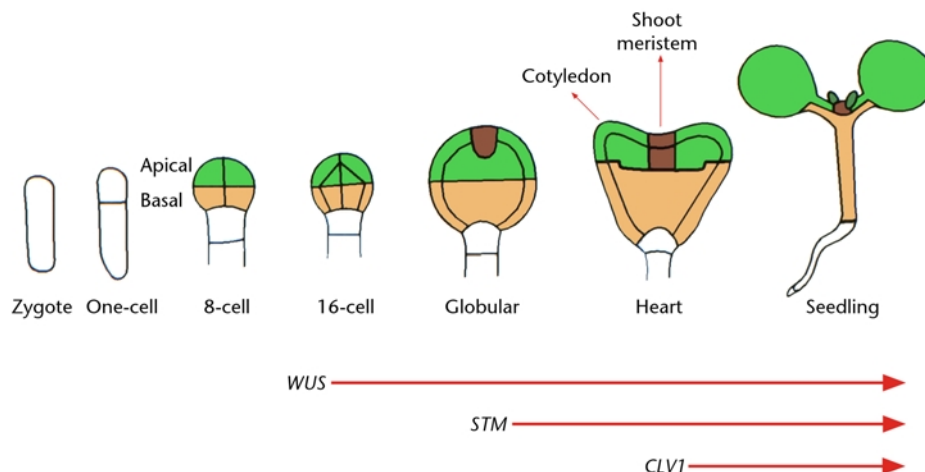
There is growing evidence in favour of the latter model: although expression of the *WUS* gene is already initiated in the apex of the 16-cell stage embryo, the *WUS* expression domain only gradually becomes confined to its prospective domain of function in the shoot meristem. Other shoot meristem genes, such as *STM*, *UFO* and *CLV1* are switched on successively and also display dynamic expression patterns.

Plants can also form shoot meristems in the axils of the leaves, which will give rise to side branches and flowers. In principle, axillary meristems could be derived either from cells of the main shoot meristem that have never entered differentiation but are locked in a meristematic state, or could arise *de novo* from already differentiated cells in the leaf axil. In any case, formation of axillary meristems appears to require information from the subtending leaf. In the *Arabidopsis phabulosa-d* (*phb-d*) mutant, the abaxial

(lower) side of the leaf has features usually found on the adaxial (upper) side and axillary meristems develop ectopically at the lower side of the leaf. This has been taken as an indication that cells on the adaxial leaf side promote shoot meristem formation. An apparent contradiction to this proposal is that floral meristems in some species, e.g. in *Arabidopsis*, are formed in the absence of a subtending leaf. However, it is possible that in those cases a leaf primordium is present that does not grow out but may be sufficient to initiate a floral bud.

## Programming the Shoot Meristem

At an appropriate time that depends on environmental stimuli and/or internal cues, the shoot meristem switches from the production of axillary shoot meristems to floral meristems. Genetic analysis in *Arabidopsis* has led to a model according to which flowering is repressed early in development until factors that promote flowering have accumulated to overcome this block. A central repressor of flowering is the *EMBRYONIC FLOWER* (*EMF*) gene. Mutation in *EMF* results in precocious flowering in the embryo. The *TERMINAL FLOWER* (*TFL*) gene and several early flowering genes prevent an early switch to flower formation before the normal vegetative programme has been completed. This inhibitory block is gradually whittled away by the accumulation of flowering-promoting factors until the floral meristem identity genes *LEAFY* (*LFY*) and *APETALA* (*API*) have reached a critical threshold and specify axillary shoot lateral primordia as floral buds. One part of their function is to activate a set of floral organ identity genes that lead to the formation of floral organs instead of leaves. The antagonism between



**Figure 2** Initiation of the shoot meristem. The shoot meristem is initiated early in embryo development as indicated by the onset of *WUS* expression. The expression domains of *WUS* and other genes undergo dynamic changes before the shoot meristem organization is in place.

vegetative development and flowering is reflected in the reciprocal negative regulation of their key genes. *TFL*, for example, inhibits flower initiation by delaying the upregulation of *LFY* and *API* and by lowering the responsiveness of the meristem to these genes. *API* and *LFY* in turn repress *TFL* expression in floral primordia.

## Perspectives

Shoot development requires the continuous production of undifferentiated cells and the integration of these cells into the growing plant. The first task is fulfilled by pluripotent stem cell populations that are being maintained by a neighbouring organizing centre. Studies of the underlying regulatory mechanisms will not only increase our understanding of shoot development but may reveal fundamental parallels between plant and animal stem cell systems. For example, the status of haematopoietic stem cells appears to depend on their cellular neighbourhood, with the regulatory mechanisms being unknown. The similarity between *ZWILLE* and *PIWI* proteins, non-cell-autonomously required for maintaining stem cells in the *Arabidopsis* embryo and the *Drosophila* germarium, respectively, is consistent with regulatory mechanisms being conserved in different stem cell systems. The second task, differentiation of specific cell types, depends on a network of positional information. In this network each cell signals and perceives signals at the same time to

integrate individual developmental choices into a meaningful context. This positional information can change with time, and – as at the flanks of the shoot meristem – may result in the formation of leaves early in development and floral buds later on.

## Further Reading

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