Shoot Apical Meristem (SAM)

The shoot apical meristem (SAM) is a population of cells located at the tip of the shoot axis. It produces lateral organs, stem tissues and regenerates itself. In most plants little to no shoot tissue results from embryogenesis: essentially the entire shoot system of plants derives from postembryonic development in the SAM. Shoot growth consists of primary and secondary growth. Primary growth is the addition of tissue to the shoot by the apical meristem. Secondary growth is tissue derived from zones other than the meristem, such as cambial zones.

**Properties of meristems**

Meristems contain a population of cells with characteristics of stem cells; cell division serves to constantly replenish the meristem and to provide cells that will differentiate into plant organs and tissues.

Vegetative meristems are indeterminate. That is they can theoretically grow indefinitely. In plants that normally produce a relatively fixed number of nodes, if the meristem is removed and allowed to reform a plant in culture, the plant will have the normal number of nodes. Thus node number is a property of the whole plant and not intrinsic to the meristem.

Vegetative meristems are highly regulative. If portions are surgically removed, the remainder will reorganize into a functioning meristem.

The activity of vegetative meristems is repetitive, often described as iterative or meristic (from mer meaning unit as in polymer). The meristem produces modular units consisting of a lateral organ (leaf), axillary bud, node (point of leaf attachment) and subtending internode (stem segment). Each unit is called a phytomer. Thus primary shoot growth involves the repetitive addition of stem segments and associated leaves to the end of the shoot.

**Meristem structure**

SAMs are usually dome shaped and have a layered structure described as a tunica and corpus. The tunica consists usually of 2 cell layers (often only 1 in monocots) where cell division occurs primarily in the anticlinal plane (perpendicular to the surface). The corpus is the mass of cells in the central part of the meristem and cell divisions occur in all planes. An alternative nomenclature is to number the layers from the outside in. Thus plants with a 2 cell layered tunica would have an L1 layer on the outside, L3 layer in the center and an L2 layer in between, with both the L1 and L2 being part of the tunica and the L3 comprising the corpus. Monocots such as maize that have only a single layer tunica would have an outer L1 layer and an inner L2 layer with nothing in between.

SAMs also show cytological zonations independent of the layered structure. There is a central zone that stains lightly with histological stains. The central zone includes both tunica and corpus cells. The peripheral zone is a ring of densely staining cells that surround the central zone and also includes both tunica and corpus. Below the central zone is an area called the rib meristem. Several approaches have shown a correlation in the mitotic activity with the zonation. Mitotic activity is most prevalent in the peripheral region as shown by incorporation of tritiated thymidine which reflects DNA replication and therefore mitotic activity.
The central zone is the region of the **apical initials** (ie, the stem cell population). These divide infrequently and occasionally contribute cells to the rapidly dividing peripheral zone. Because most genetic mistakes are generated during mitosis, this is probably a mechanism to minimize the occurrence of genetic mistakes in the permanent cell population of the meristem.

**Initiation and maintenance of meristems**

Initiation of the shoot meristem in the embryo requires the action of several genes that were identified in genetic screens for mutants that lack a SAM. One such gene is **SHOOT MERISTEMLESS** (**STM**). Mutations in *stm* result in embryos without a SAM. Occasionally, in weak *stm* alleles, adventitious shoots will form but the shoots progressively lose their meristem indicating that **STM** is required for both the initiation and maintenance of SAMs. **STM** encodes a homeodomain transcription factor of the **KNOX** class. **KNOX** genes are expressed in the SAM and are important for promoting SAM identity. Ectopic expression of **KNOX** genes in leaves induces ectopic SAM formation on the leaf.

Maintenance of shoot apical meristems requires regulating the balance between cell proliferation and differentiation. This is especially important in long lived plants such as trees where the SAM may have to function hundreds of years. Several genes have been identified that regulate this balance. One gene called **WUSHEL** (**WUS**) has a similar mutant phenotype to **STM** and also encodes a homeodomain transcription factor. However the function of **WUS** is to promote cell division (stem cells). Thus, in loss of function **wus** mutants the SAM cell population becomes gradually depleted. **WUS** is normally expressed in the corpus region of the central zone. Overexpression of **WUS** creates an enlarged SAM.

Three genes called **CLAVATA** (**CLV**) 1, 2 and 3 have the opposite effect to **WUS** in that loss of function mutations cause an overproliferation of cells in the SAM (the SAM gets too big). Therefore the function of these genes is to restrict cell proliferation or to promote cell differentiation. **CLV1** is a receptor kinase and **CLV2** is another receptor-like protein. These 2 are proposed to form a heterodimeric receptor complex. **CLV3** is a small secreted protein that is hypothesized to function as the activating signal ligand for the **CLV1/2** receptor complex. **CLV1** is expressed mostly in the corpus region of the SAM and **CLV3** is expressed predominantly in the tunica region of the SAM, highlighting the interaction among layers of the SAM.

The **CLV** pathway acts to inhibit the expression of **WUS** which acts to promote cell proliferation. The **WUSHEL** gene acts downstream of **CLV** because **clv / wus** double mutants are phenotypically the same as **wus** single mutants. In **clv** single mutants, **WUS** is overexpressed resulting in overproliferation. However, if **wus** is mutant, then it doesn’t matter whether **CLV** is around to restrict it or not. Overexpression of **CLV3** results in loss of **WUS** expression and a SAM depletion phenotype, just like a **wus** loss of function mutant.

Thus, signaling from the tunica to the corpus regulates SAM proliferation. There is also an signal back from the corpus to the tunica. Evidence for this is that in **wus** mutants, there is no **CLV3** expression, while ectopic **WUS** expression can induce ectopic **CLV3** expression. Thus there is a feedback regulatory loop where **WUS** expression promotes the expression of **CLV3** which activates a signal transduction pathway that
inhibits *WUS* expression. This loop maintains the relative balance between cell proliferation and differentiation (the stem cell population) in the SAM.

**Organ initiation**

Leaf initiation involves an increased rate of cell division on the flank of the meristem. This involves both tunica and corpus cells. Cell divisions in the L1 remain anticlinal whereas inner layers divide both anticlinally and periclinally. The proliferation of cells leads to a bulge on the flank of the meristem called a leaf buttress. Localized apical growth in the leaf buttress generates a leaf primordium. Apical leaf growth continues until the primordium is approximately 1mm long (the length is variable among species) at which point the leaf pattern of cell division begins.

The time between the initiation of two leaves is called a **plastochron**. Primordia are referred to by counting from the youngest to oldest. Thus the 4th youngest primordium would be referred to as a plastochron 4 leaf (designated P4) because it is 4 plastochrons old.

**Patterns of organ initiation (phyllotaxy)**

Different plants have characteristic arrangements of leaves such as alternate, opposite (decussate), spiral or whorled. The arrangement of leaves on the plant is called the **phyllotaxy** and is determined by the pattern of leaf initiation on the meristem. In plants with opposite or alternate arrangement, leaves occur in files on the stem called orthostichies. Plants with spiral leaf arrangement have an arrangement that can be described with 2 opposite sets of helical rows called contact parastichies. Phyllotaxy is described by counting the spirals in each direction. For example, potato has 2 clockwise parastichies and 3 counterclockwise. This phyllotaxy is described as 2+3. Phyllotaxy usually consists of consecutive numbers of the Fibonacci series: 1,1,2,3,5,8 where each successive number is derived by adding the 2 preceeding numbers.

A divergence angle between leaf primordia of 137° is nearly constant among plants with spiral leaf arrangement. The generation of the different phyllotaxies is a property of differences in the relative rates of growth and leaf initiation in the meristem and meristem size. This will affect which primordia are in contact with one another.

**Control of organ initiation patterns**

At least three major factors contribute to the control of organ initiation. These include the establishment of morphogen fields, alterations in the biophysical properties of the SAM surface, and regulation of gene expression patterns.

**Morphogen fields:**

Organ primordia have been hypothesized to produce an inhibitory field that prevents organ initiation within a certain proximity. This is based on classical surgical manipulations in which the position of organ initiation was assessed following removal of a primordium. Subsequent organs that would normally have contacted the removed primordium initiated at positions closer than normal to the missing primordium. A corollary is that the apical dome should also produce an inhibitory field.

The phytohormone, auxin, appears important for leaf initiation. Auxin transport inhibitors, such as NPA, inhibit primordia initiation on a SAM. Localized auxin application on NPA treated SAMs induce leaf initiation at the site of application. This
and other results suggest that a localized high auxin concentration promotes organ initiation and determines the size of the field occupied by the primordium.

**Biophysical surface properties:**

According to biophysical explanations of phyllotaxy, patterns of stress and reinforcement in the surface layer of the apex determine the pattern of organ initiation. Polarized light microscopy shows a correlation between phyllotaxy and cellulose microfibril orientation in the apex surface. The inner tissues are hypothesized to provide the driving pressure, much like the air in a balloon. The protein *expansin* is able to induce cell wall loosening. Expansin coated beads placed on meristems induce the formation of ectopic primordia. I.e. the pattern of phyllotaxy was altered. The new phyllotaxy was propagated in subsequent primordia. Expansin genes are expressed at sites of organ initiation in the SAM, supporting the possible role of expansin in normal organ initiation. This evidence supports the role of biophysical properties of the surface in organ initiation.

**Genetic control:**

There are several genes known to be important for meristem function. The *KNOX* genes, such as *KNOTTED1* of maize and *STM* of Arabidopsis, encode homeodomain proteins that promote apical meristem cell identity. Both are expressed throughout the meristem except in organ primordia, indicating that the acquisition of primordium cell identity involves the suppression of SAM gene expression. Loss of expression precedes visible morphological appearance of the primordium.