Plant stem cells: **The only constant thing is change**

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Recent studies in *Arabidopsis* have uncovered a negative feedback loop that couples the antagonistic functions of the *WUSCHEL* and *CLAVATA* loci to control stem cell fate in the shoot apical meristem. Abundance of the CLAVATA3 protein limits signaling through this pathway.

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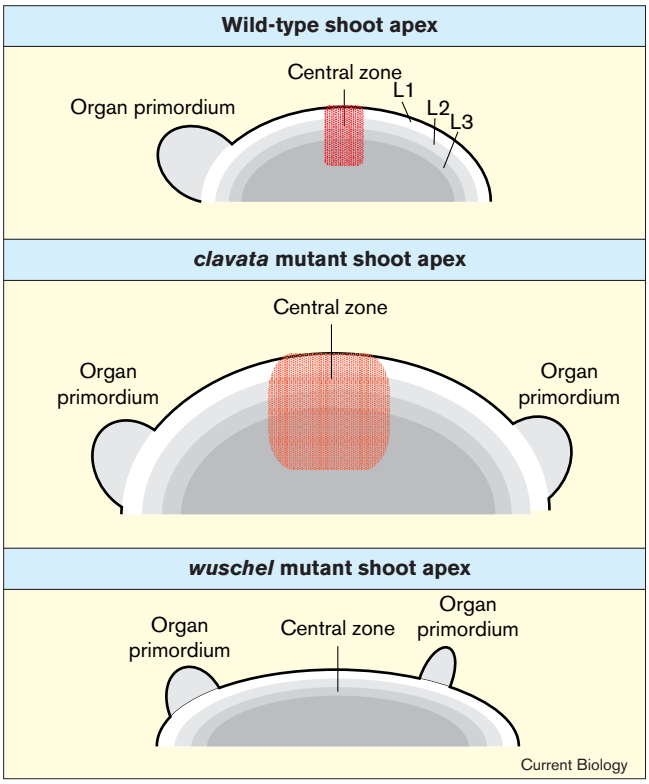
Plants grow throughout their life cycle by organ formation and enlargement. In the aerial parts of the plant, organ formation is mediated by apical meristems. Stem cells required to sustain apical growth are located at the meristem center, and their progeny are progressively displaced towards the periphery of the meristem, where they are recruited into organ primordia (Figure 1). The spatial patterns of proliferation within the apex are precisely controlled over a few cell diameters in small meristems such as that of *Arabidopsis*. Genetic analysis in *Arabidopsis* has revealed loci — *CLAVATA 1–3* — that promote cell incorporation into organ primordia, and loci — *WUSCHEL*, *ZWILLE* and *SHOOT MERISTEM-LESS* — that promote stem cell identity. Several recent papers [1–3] highlight the close interactions between some of these loci and suggest a mechanism how cellular homeostasis is maintained.

The shoot apex is organized into layers and radial domains (Figure 1). The L1 is the outer cell layer, followed internally by the L2 and L3 layers and the pith tissue, which connects the meristem with the plant stem. The L1 layer gives rise to the epidermis in lateral organs, while the L2 and L3 layers provide the precursors for the mesophyll and vasculature. As all three layers participate in organ formation, coordination of proliferation is essential to populate organ primordia with sufficient cells, as well as to maintain meristem integrity. The meristem center has low mitotic activity in all layers and is made up of stem cells. This central zone is surrounded by slightly more rapidly dividing cells at the meristem flanks. Organ primordia, in which proliferation is very rapid, arise at the meristem periphery.

Clavata mutants (*clv1–3*) all have very similar phenotypes: enlarged meristem size and an increased number of organ primordia with an apparent loss of phyllotaxy, and supernumerary carpels [4]. Histological analysis of cell division activity in meristems of the *clv3* mutant revealed

Figure 1

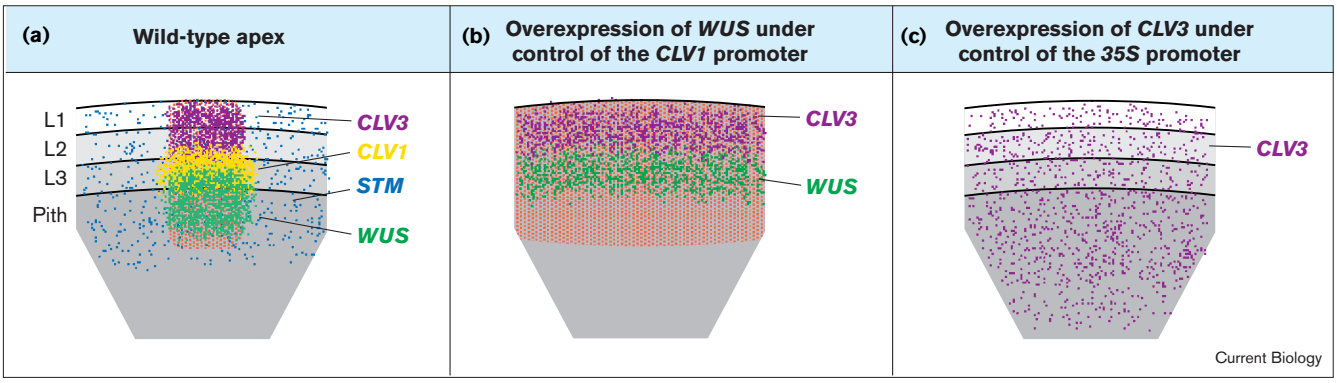


A schematic comparison of the shoot apices formed by wild-type (top), *clavata* mutant (middle) and *wuschel* mutant (bottom) *Arabidopsis* plants. The central zone (red) expands in *clv* meristems and excess organ primordia are generated along the periphery. In *wuschel* apices, stem cells are consumed in organ primordia, but sporadic initiation of adventitious meristems results in transient episodes of organ formation from random apical positions.

an expanded zone of low mitotic activity at the meristem center [5]. The *clv* mutant phenotype suggests that the wild-type gene function is to promote cell recruitment into primordia. In contrast, *wuschel* mutants are unable to sustain meristems as a result of a loss of stem cells in the central zone, although sporadically cells arise at the apex which are able to transiently re-start leaf organogenesis. These leaf primordia originate from random positions on the meristem and without appropriate phyllotaxy [6]. The antagonistic effects of *CLAVATA* and *WUSCHEL* functions raised the possibility that they might interact to control the size of meristem domains.

WUSCHEL (*WUS*) encodes a homeodomain protein and is expressed in a small group of cells below L3 in the central zone [7]. The similar phenotype of the *clv 1–3* mutants

Figure 2



(a) Accumulation of mRNAs for *CLV1*, *CLV3*, *STM* and *WUS* in wild-type meristems. The *WUS*-dependent signal must act across several cell diameters. **(b)** The *CLV3* expression domain and the central zone expand when *WUS* is expressed under control of the *CLV1* promoter. **(c)** Loss of *WUS* expression when *CLV3* is expressed under control of the 35S promoter expression of *WUS* is lost and the central zone disappears.

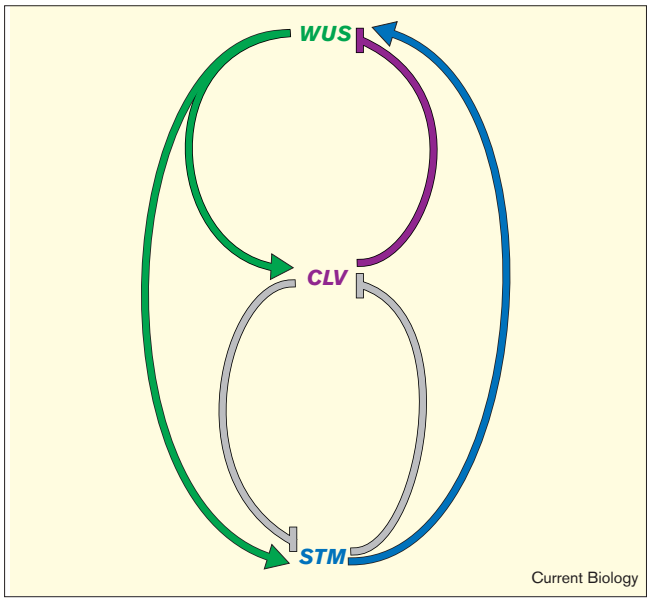
suggested that the wild-type *CLV* genes function in the same genetic pathway. *CLV1* encodes a receptor-like protein kinase and is expressed in the central zone above the *WUS* expression domain [8]. *CLV2* encodes a receptor-like protein required for the stability of the *CLV1* receptor-like kinase [9]. *CLV3* encodes a small polypeptide, and is expressed in the top cell layers of the central zone [10]. CLV3 polypeptide is the ligand for the CLV signaling complex [3]. The wild-type CLV proteins form a large signaling complex that also includes a kinase-associated protein phosphatase (KAPP) and a Rho-like GTPase [11]. It is striking that each of the *CLV* genes is expressed in distinct layers of the central zone, implying that all layers participate in the coordination of stem cell homeostasis.

Two groups [1,2] now report functional interactions between *WUS* and the *CLV* genes. The *wus clv* double mutants turn out to have a phenotype indistinguishable from that of *wus* single mutants, which suggests that *WUS* is required for the *clv* phenotype of meristem overgrowth [1]. Moreover, the expression domain of *WUS* expands subtly in *clv* mutants, suggesting that the *CLV* signaling complex might act to restrict *WUS* expression [1]. This was tested by expressing *CLV3* ectopically under control of the strong 35S promoter [2]. Transgenic plants expressing high levels of *CLV3* ceased to initiate organs after formation of the first leaves, and tissue sections suggested that cells in the position of the meristem had been recruited to organ primordia (Figure 2). Moreover, these plants did not accumulate *WUS* RNA [2]. This phenotype required all other components of the CLV signaling complex to be functional. Expression of *CLV3* under control of the *UFO* promoter in cells just under L3 also caused meristem termination, indicating that *CLV3* can activate CLV signaling from domains other than the L1 layer [2]. Ectopic expression of *WUS* under control of the *CLV1* promoter led to enhanced accumulation of *WUS* in the cells above its normal expression domain and, more importantly, uncoupled its transcriptional regulation from *CLV* signaling (Figure 2). Such plants had enlarged shoot meristems and overgrowth very similar to the *clv* phenotype [1]. The expression domain of *CLV3* in these meristems was much enlarged.

What is the role of the CLV3 polypeptide ligand in this signaling loop? Simon and collaborators [2] examined *Arabidopsis* plants with lower levels of ectopic, 35S-promoter-driven *CLV3* expression. These plants generated lateral organs in a 'stop and go' pattern, very similar to that observed in *wus* mutant plants, suggesting that the abundance of the CLV3 ligand might be the rate-determining factor in this regulatory circuit. This notion is further supported by biochemical analysis of the CLV receptor complex [3].

These observations suggest the following model (Figures 2 and 3). Stem cell identity depends on the activity of at least two genes: *WUS* and *SHOOT MERISTEMLESS (STM)*. *WUS* and *STM* start to be expressed independently of each other in distinct patterns during embryogenesis, but expression of either one cannot be maintained in the absence of the other [7]. These genes are expressed in different meristem domains, suggesting that, while *WUS* is directly involved in conferring stem cell identity, *STM* functions as a 'competence factor' to permit spatial regulation of the central zone. But as this gene pair promotes stem-cell identity, this would lead to runaway stem-cell multiplication without a negative feedback loop. The recent reports [1–3] show that the CLV signaling complex provides this negative regulation of *WUS*. The observation that meristem phenotypes in 35S::*CLV3* plants were very sensitive to CLV3 polypeptide dosage suggests that CLV3 abundance is the critical factor that determines the stem-cell pool size. This negative feedback loop acts as an

Figure 3



A negative feedback loop proposed to couple the stem cell-promoting activities – *WUS* and *STM* – and stem-cell-restricting activities – *CLV genes* – discussed in the text. The interaction between *CLV* and *STM* genes is shown in gray because it is not clear whether these are direct or mediated by *WUS*.

auto-regulating system to maintain appropriate quantities of stem cells.

What are the signaling mechanisms involved in the communication between stem cells and with cells in the meristem flanks? It is striking that the mRNAs for the various components of the CLV–WUS–STM signaling network accumulate in different, largely non-overlapping domains of the meristem (Figure 2). This strongly suggests that participation of all cell layers of the central zone is required to coordinate stem-cell homeostasis. For this regulatory circuit to operate, WUS, the mRNA for which accumulates in a stem-cell organizing domain below the L3 layer, must generate a signal that moves over several cell diameters to the L1 and L2 layers. Similarly, the signal generated by the CLV complex must also act in a non-cell-autonomous fashion. It is an exciting possibility that WUS mRNA or protein itself moves to regulate *CLV3* expression. Another homeodomain protein, product of the maize *KNOTTED* gene, has been shown to move between cells [12]. Such movement could be restricted by symplastic domains that organize meristems at the cellular level [13,14]. Does this signal network have further components? A downstream effector of CLV signalling, the *POLTERGEIST* gene, has recently been described that might be partially redundant with *WUSCHEL* [15]. It is likely that further interactions with members of the *ZWILLE* class of genes, which are also required to specify stem-cell identity [16,17], will be discovered.

The recent advances show that growth activities at the shoot apex are controlled at several levels. Stem cell homeostasis is controlled in the central zone of the meristem. Such control is necessary, because organ primordium formation on the meristem flanks occurs in discrete events and therefore cell flow across the meristem flanks is not uniform over time. There is likely also to be feedback control of meristem activity from developing primordia [18]. A further level of control of cell production lies directly in the incipient primordia, and is governed by the *AINTEGUMENTA* gene [19,20]. The further characterization of these control circuits is eagerly awaited.

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