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REVIEW

Plant stem cells and their regulations in shoot apical meristems

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Abstract Stem cells in plants, established during embryogenesis, are located in the centers of the shoot apical meristem (SAM) and the root apical meristem (RAM). Stem cells in SAM have a capacity to renew themselves and to produce new organs and tissues indefinitely. Although fully differentiated organs such as leaves do not contain stem cells, cells in such organs do have the capacity to re-establish new stem cells, especially under the induction of phytohormones *in vitro*. Cytokinin and auxin are critical in creating position signals in the SAM to maintain the stem cell organizing center and to position the new organ primordia, respectively. This review addresses the distinct features of plant stem cells and focuses on how stem cell renewal and differentiation are regulated in SAMs.

Keywords plant, stem cell, shoot apical meristem, root apical meristem

1 Introduction

The concept of "stem cell" is initially rooted from the observation of plants, to describe the fact that cells in shoot apical meristem (SAM) are able to divide and to differentiate, thus continuously producing new stems and leaves. The structural and functional relationship of plant SAM has been studied since the1950s, when Philipson et al. observed for the first time that some of enlarged and vacuolated cells in the center of SAM divide very slowly, while the cells in the periphery zone are active in division to give rise to new organ primordia (Philipson, 1954; Sussex, 1955). He termed the central zone as "meristem regulation center" to define cells that are now known as stem cells (SCs) in the upper domain and stem cell organization center (SCOC) in the lower domain. Further, Sussex and his colleagues used microdissection in tomato to remove cells in the whole central zone and found that a new functional meristem could be re-established after a few days (Steeves and Sussex, 1989). This result was reproducibly confirmed by more precise experiments carried out using molecular markers later (Reinhardt et al., 2003). However, when the surface layer is removed, the meristem is not able to be recovered, suggesting a signal cue is being delivered through the epidermis (Steeves and Sussex, 1989).

Molecular genetic studies of SC regulation in plants have been carried out in the last two decades using the model plant Arabidopsis. Leyser and Furner (1992) firstly identified a group of mutants named *clavata*, which exhibit increased sizes of SAM and numbers of floral organs including petal, steman and carpel. These mutants are mapped to three recessive loci, *CLV1*, *CLV2* and *CLV3*, which, respectively, encodes a leucine-rich repeat receptor kinase, a leucine-rich receptor-like protein and a small extracellular protein (Clark et al., 1997; Fletcher et al., 1999; Jeong et al., 1999). Mutants with no SAM were subsequently identified in both Petunia and Arabidopsis, named *wuschel* (*wus*)(Mayer et al., 1998). *WUS* encodes a homeodomain family of transcription factors. At the mean time, roots are also used extensively for studying SC regulation due to its simple structure and translucency, which are easy for observation. Using genetic dissections, some critical transcriptional factors, such as SCARECROW (SCR), PLETHORA (PLT) and WUSCHEL-RELATED HOMEOBOX 5 (WOX5), have been identified to be functional in regulation of SC maintenance and/or differentiation in RAM (Sabatini et al., 2003; Aida et al., 2004; Scheres, 2007). Furthermore, advances have been made in hormone signaling in recent years, showing the critical importance of auxin, cytokinin, brassinosteroid

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(BR), gibberellic acid (GA) and ethylene in SC regulation (Hass et al., 2004; Müller and Sheen, 2008; Swarup et al., 2008).

2 The characteristics of plant SCs

Similar to those in animals, SCs in plants are located in close contact with SCOC, to function together with surrounding differentiated cells to establish the SC niche (Fig. 1). Unavoidably, SCOCs in both SAM and RAM provide the signal for SC maintenance, while the signal for differentiation is either from SCs themselves or surrounding differentiated cells (Singh and Bhalla, 2006). The balance of these two signals determines the homeostasis of SC, thereby maintaining the relatively fixed sizes of these two types of meristems. In Arabidopsis SAM, SCs are located in the upper layers of L1 to L3 of central domain, while SCOC is located in L3, partially overlapping with the SC domain (Fig. 1). Active cell divisions and differentiations take place in the periphery zones of SAM. In roots, the well-described centrally localized quiescent center (QC), 4 cells in Arabidopsis, is actually the SCOC, while a single layer of surrounding cells that are in direct contacts with SCOC are root SCs (Fig. 1). Thus, meristems in plants are not equal to SCs, as misunderstood by many people. Compared to their counterparts in animals, SCs in plants exhibit distinct characteristics in the following four aspects.

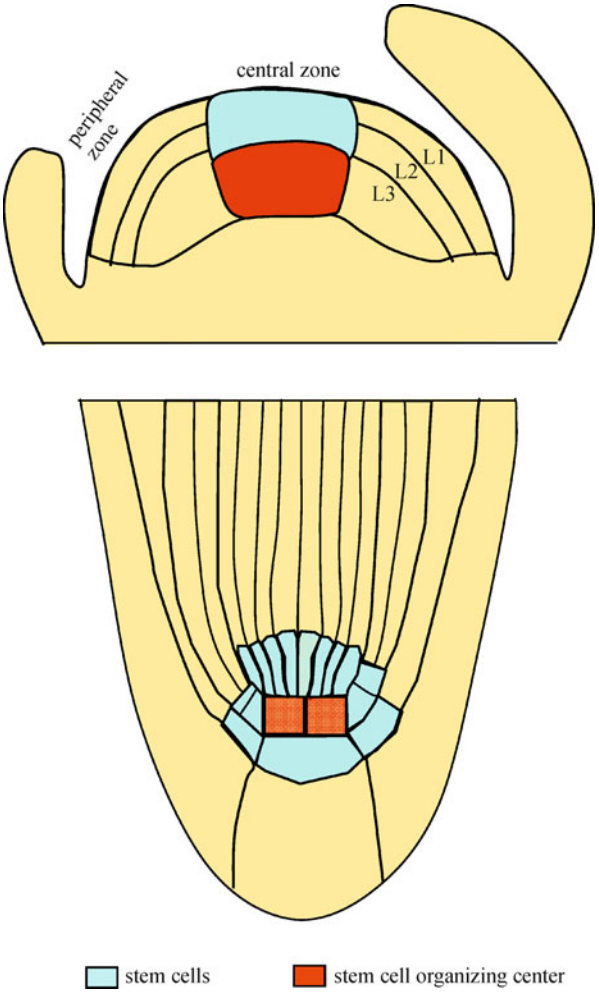

Fig. 1 Schematic illustration of plant stem cell organizing centers. Structures of shoot apical meristem (SAM, upper) and root apical meristem (RAM, below) are shown. Stem cells (in cyan) in SAM are cells located in the central zone, occupying the L1, L2 and upper L3 layers; while stem cells in RAM are cells with direct contacts with stem cell organizing center. Stem cell organizing center (in red) in SAM is located in the L3 layer of the central zone; the organizing center in RAM is quiescent center, which usually contains 4 cells in Arabidopsis. Stem cells and the cells in organizing center are usually vacuolated and slowly dividable.

2.1 Continuous involvement in construction of body plan

In animals, an almost completed body plan has been built up through SC regulation in embryogenesis, and SCs in adult organs are mainly used to compensate for the cell loss in growth and development. In plants, however, embryogenesis is only to establish two functional SC niches in SAM and RAM, and the post-embryonic development is, in a modular manner, to allow new organs to be continuously added during the whole lifespan. As such, SCs in plants are continuously active and are essential for the construction of the plant body.

2.2 The totipotency

Except in early embryonic stages, most SCs in adult animals gradually lose the full capacity of differentiation but retain the ability to form one or a few specific cell types. In plants, although terminal differentiations also occur frequently, allowing certain cell types such as xylem elements in vascular bundles and starchy endosperms in seeds to permanently lose their capacities in divisions and differentiations, most differentiated cells retain the ability to de-differentiate and then re-establish the SC niche to form a complete plant body (Gordon et al., 2007; Su et al., 2009). This can occur not only *in vitro* with the help of hormones and synthetic growth regulators, but also *in vivo* autonomously. For example, a whole plant or plantlet can be formed from a segment of roots or at the edge of leaves. No doubt, most differentiated cells in plants retain an indefinite totipotency to produce every cell types and a whole plant body.

2.3 Position signal-defined SC state

Instead of the cell autonomous regulation manner in animal SCs, SCs in plants are regulated strongly by position signals, allowing some cells in particular domains such as meristems to function continuously as SCs to

produce new tissues and organs indefinitely (van den Berg et al., 1995, 1997)(Fig. 1). In recent years, more and more evidence suggest that a position cue in RAM is mainly achieved by auxin flow and local auxin maximum, which regulate both the SC maintenance and lateral root initiation (Scheres, 2007). Nevertheless, cytokinin may act as the most important position cue in SAM for SC maintenance (Hwang and Sheen, 2001), and auxin and auxin transport are responsible for the initiation of new organs (Liu et al., 1993; Reinhardt et al., 2000; Heisler et al., 2005). These position signals are visible when new SCs are quickly generated from neighboring cells after SC organizing center and the SCs are physically removed (Reinhardt et al., 2003).

2.4 Non-fixed SC linage

Instead of a continuous cell linage of animal SCs, SCs in plants are not always following linage. Although SCs in a growing plant body are continuously being laid down in cambia and axillary buds, the SC fate is completely lost during flower formation. New SCs are re-established in zygotic embryos in a new generation. This could be seen by a complete switching off of the *WUSCHEL* (*WUS*) expression through interactions between WUS and AGAMOUS (AG) and switching on again in 16-cell staged embryos (Mayer et al., 1998; Lenhard et al., 2001). New SCs can be re-established during *in vitro* regeneration from various explants (Su et al., 2009).

3 SC regulation in shoot apical meristems

3.1 The CLV3-CLV1/CLV2/CRN signaling complex

In every individual species, the size of SAM is very much fixed. For example, Arabidopsis SAM is about 200 μm in diameter, in which SCs and SCOC are located in the central zone, while the new leaf primordia are initiated continuously in periphery zone. The size of SAM is determined by the numbers of SCs in the SAM and leaf primordia being developed. The CLV3 peptide ligand-receptor complex and WUS transcription factor-based feedback regulation loop is a well-characterized signaling network in controlling the size of SAM (Brand et al., 2000; Schoof et al., 2000; Fiers et al., 2007).

The CLV3-CLV1/CLV2/CRN ligand-receptor interaction complex was initially identified from the molecular characterization of *clavata* (*clv*) mutants, leading to discovery of a delicate signaling machinery that restricts the SC number and promotes SC differentiation in SAM. All *clv* mutants (*clv1*, *clv2* and *clv3*) have enlarged SAMs (Leyser and Furner, 1992) (Fig. 2). *CLV1* encodes a membrane-bound receptor kinase with 21 extracellular leucine-rich repeats (LRR), a transmembrane domain and an intracellular kinase domain (Clark et al., 1997). *CLV1* is expressed in the L3 layer of the central zone of the SAM. *CLV2* encodes receptor-like protein, with 18 LRR and a transmembrane domain, which is expressed constitutively and functions in both SAM and RAM to perceive the signal from peptide ligands (Jeong et al., 1999) (Fig. 3). It has been proposed that CLV1 and CLV2 form a complex through disulfide bonds (Jeong et al., 1999; Trotochaud et al., 1999); however, data obtained recently shows that it is not the case (Zhu et al., 2010). Through suppression screenings using *RCH1* promoter driven *CLE19* overexpression line, another genetic locus named *SOL2/CRN* has been identified. The *sol2* and *crn* mutants have compromised *CLE19* and *CLV3* overexpression phenotypes, occasional multi-carpel phenotype and slightly enlarged SAM (Casamitjana-Martínez et al., 2003; Müller et al., 2008). *SOL2/CRN* encodes a receptor-like kinase with only a transmembrane domain and intracellular kinase domain (Müller et al., 2008). The both *in vitro* and *in vivo* interaction of SOL2/CRN with CLV2, but not CLV1, suggests that a new model of peptide perception involves two parallel complexes, one CLV1 homodimer and another CLV2/CRN heterodimer (Zhu et al., 2010) (Fig. 4). Of course, this model does not exclude the possibility that they two may form a more complicated complex.

The SCs in SAM are accurately marked by *CLV3* expression (Fletcher et al., 1999). As a small 96-AA extracellular protein, CLV3 was proposed to act as a peptide ligand to interact with CLV1 and CLV2 receptors

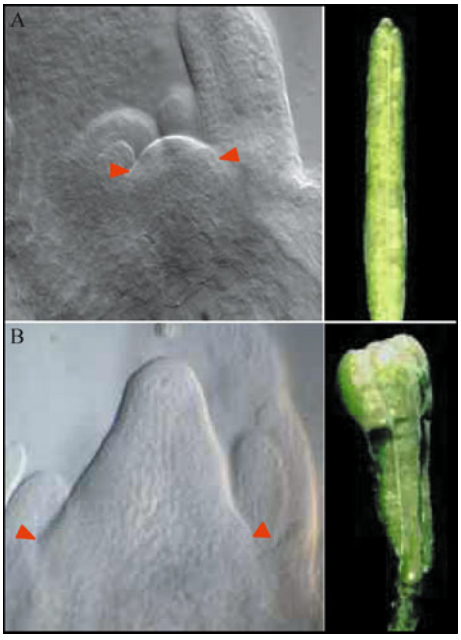

Fig. 2 The phenotypes of the *clavata* (*clv*) mutants. The photos show the size of SAMs (left) and the shape of siliques (right) of wild type (A) and *clv3* mutant (B). Note the greatly enlarged SAM and increased carpel number in *clv3*.

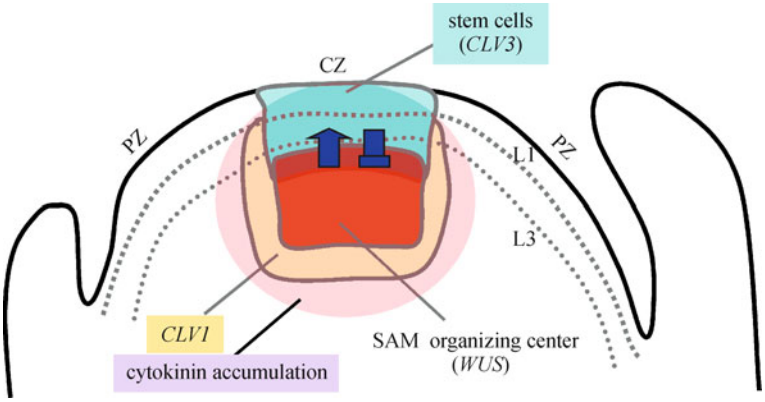

Fig. 3 Schematic representation of the stem cell regulation machinery in shoot apical meristem. Stem cells (cyan) are marked by the expression of *CLV3*, while stem cell organizing center is marked by the expression of *WUS* (red). The mobile peptide ligand CLV3 produced by stem cells provides a stem cell-restricting signal that is perceived by CLV1 receptor kinases in cells below. The signal is then delivered to over-lapping stem cell organizing center cells to repress the expression of *WUS*. WUS is a stem cell-promoting signal that enhances the expression of *CLV3*. In such, CLV and WUS together build a negative feedback regulation loop to maintain the proper number of stem cells in SAM. A high level of cytokinin (pink) located in the center of SAM may provide a position signal to maintain the WUS domain.

(Fletcher et al., 1999; Lenhard and Laux, 2003). Another CLE member, CLE40, shares little sequence identity to CLV3 except CLE motif (Hobe et al., 2003). The peptide identity was verified firstly through the *in vitro* function assay using a 14-AA synthetic peptide (CLV3p) corresponding to the conserved CLE motif among CLV3, CLE19 and CLE40 (Fiers et al., 2005). In Arabidopsis genome, there are at least 34 CLE members that share a common feature of small proteins less than 10 kD, with a signal peptide at their N-terminals and a conserved 14-AA CLE box at their C-terminals (Cock and McCormick, 2001). *In vivo* complementation experiments showed that the non-conserved central domain and C-terminal tails can be deleted without affecting CLV3 functions (Fiers et al., 2006). Although the endogenous CLV3 peptide has never been isolated from wild type plants, using *CLV3* overexpression cell lines, Kondo et al. (2006) identified a 12-AA peptide corresponding to CLV3p, with two proline residues modified by hydroxylation. Later, another group proposed that the endogenous CLV3 has 13-AA, with three arabinoses covalently linked to one of two hydoxylated prolines (Ohyama et al., 2009) (Fig. 4). Since the unmodified peptides are functional, it is likely that these modifications may enhance the stability of the peptides. CLV3 peptides bind directly to the extracellular domain of CLV1 (Ogawa et al., 2008). Mutation of the *CLV3* ortholog of rice, *FON4*, leads to an increased number of floral organ, suggesting a functional conservation between dicots and monocots (Chu et al., 2006). The suppressor screen using *RCH1::CLE19* overexpression plants also identified a *SOL1* locus that encodes a Zn-dependent caboxylpeptidase (Casamitjana-Martínez et al., 2003). Most likely, SOL1 is involved in removing the C-terminal AAs from CLV3 and CLE19 (Fig. 4).

3.2 The WUS transcription factor and regulatory loops

In balance with SC limiting factor of CLV3-CLV1/CLV2/CRN, the SC-promoting signals are required to maintain SC identity and consequently the size of SAM, which are produced by a group of underlying SCOCs that express a homeodomain transcription factor *WUS*. The *wus* mutant has no SAM (Laux et al., 1996; Mayer et al., 1998), while overexpression of *WUS* leads to overproliferation of SCs in the SAM, a phenotype resembling *clavata* mutants (Schoof et al., 2000). During plant growth and development, the SCOC needs to be renewed continuously. The sharply defined domain of *WUS* expression suggests that a precisely regulated mechanism is present to define this SCOC in SAM. Most likely the negative signal comes directly from CLV3 peptides produced by SCs, which are able to defuse to the underlying cell layers where CLV1, CLV2 and CRN receptor kinases are expressed, thereby to repress the expression of *WUS*, then restricting the SCOC domain (Gross-Hardt and Laux, 2003; Fiers et al., 2007) (Fig. 3). A mathematic simulation establishes a dynamic model to elucidate the feedback regulatory machinery between SC and SCOC in the SAM (Jönsson et al., 2005; Geier et al., 2008).

4 Cytokinin is the upstream signal defining the SCOC

Cytokinin is an adenine-derived plant hormone that plays an essential role in embryonic and postembryonic development (Hwang and Sheen, 2001; Riefler et al., 2006; Müller and Sheen, 2008). It has been known that cytokinin promotes *in vitro* shoot regeneration in most plant species

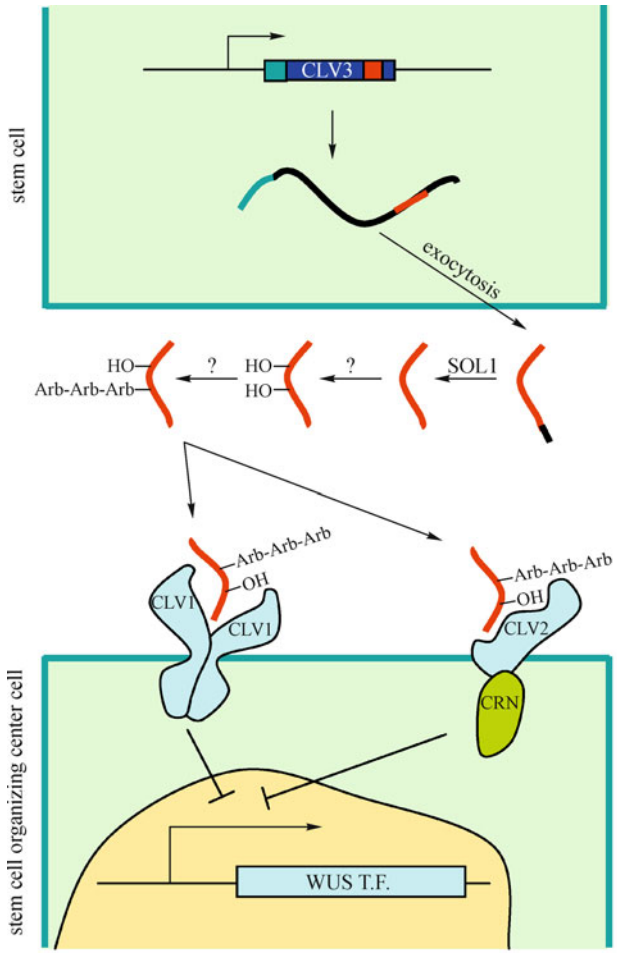

Fig. 4 The CLV3-WUS signal transduction pathway. CLV3 preproproteins produced by stem cells are secreted to intercellular spaces and then cleaved to a small peptide, further processed by SOL1 carboxylpeptidases, modified by hydroxylation and glycosylation. The peptide signal is most likely perceived by either a CLV1 homodimer and a CLV2-CRN heterodimer or a multimeric complex containing CLV1, CLV2 and CRN, which then repress the expression of *WUS* in stem cell organizing center cells.

(Skoog and Miller, 1957), suggesting the critical role of cytokinin in re-establishment of SAM (Kurakawa et al., 2007). In plants, cytokinin is accumulated in the central zone of the SAM, and the hormonal signal is perceived by a family of two-component histidine kinase receptors (Nishimura et al., 2004), which then transmit the signal to two types of transcription factors: type A and type B Arabidopsis response regulators (ARRs) (Sakai et al., 2000; Hwang and Sheen, 2001; To et al., 2004; Leibfried et al., 2005). *WUS* directly represses the transcription of *ARR5*, *ARR6*, *ARR7* and *ARR15*, which act in a negative-feedback loop of cytokinin signalling (Leibfried et al., 2005). These *ARR* genes negatively regulate meristem size in SAM and their repression by *WUS* might be necessary for the maintenance of the meristem identity (Lindsay et al., 2006). It seems that cytokinin and WUS are able to reinforce each other to maintain the identity of SCOC in SAM through multiple feedback regulation loops.

5 Auxin polar transport defines the position of organ primordia

It was shown over 60 years ago that, in SAM, the existing leaf primordia determine the emerging sites of newly formed organs in a distal region of the SAM (Steeves and Sussex, 1989). This laid a foundation for further elucidation of the mechanism that controls plant phyllotaxy. Using *in vitro* cultured zygotic embryos, Liu et al. (1993) demonstrated that auxin polar transport defines the shaped and the position of cotyledons. With molecular markers in combination with surgical experiments, Reinhardt et al. (2000, 2003) revealed that auxin is transported from existing leaf primordia towards the region where the new leaf primordia are to be formed in the epidermal layer, creating a heterogeneous distribution of auxin in SAM. Such a local auxin accumulation occurs only at certain distances from existing leaf primordia and thus defines the positions of future leaves (Reinhardt et al., 2000, 2003; Benková, 2003; Heisler et al., 2005).

6 Conclusions and prospectives

The most striking features of SCs in plants, as compared to that in animals, are the flexibility and longevity, which allow many plant species to grow in principle for an indefinite period. Given the sessile and light-dependent life style, the construction of plant body relies largely on regulation of SC activities in SAM and RAM. Recent work in SAM has established a working model, which comprises several precisely defined feedback regulatory loops involving peptide ligand, receptor kinase, homeodomain transcription factors and cytokinin, to ensure the presence of sufficient numbers of SCs and coordinated organ formation in SAMs (Fig. 3). Although advances have been made in understanding the SC regulation in SAM, the molecular mechanisms underlying such regulation are still elusive (Fig. 4). Furthermore, unlike animal SCs, the self-renewal and differentiation of plant SCs are greatly influenced by positional and environmental cues, which confer on plants the plasticity to adapt to ever-changed environment and adjust their development schedules, but how these cues are integrated into SC regulation is unknown. In addition, although basic cell cycle regulation is conserved between plant and animal, critical genes such as homeodomain transcription factors, peptide ligands and receptor kinases involved in SC regulation in plants do not have their counterparts in the animal kingdom, and *verse versa*, suggesting that SC regulations in plants and animals are evolved independently. The study of SC regulations in plants is also necessary to understand developmental

mechanisms on evolutionary basis between the two kingdoms.

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