# Regulatory Role of Phytohormones in Maintaining Stem Cells and Boundaries of Stem Cell Niches

**Aqib Syed, Anwar Hussain, Waheed Murad, and Badshah Islam**

## Abstract

Plants are multicellular organism composed of different types of cells. These all kinds of cells are formed from pluripotent stem cells present at different positions in plant called stem cell niches. All these stem cell niches and their boundaries are maintained by complex regulatory mechanism at molecular level involving different genes, cofactors, and phytohormones. In this chapter, we discussed the regulatory mechanism and models of stem cell maintenance, specifying their boundaries at different stem cell niches.

**Key words** Stem cell niches, *Arabidopsis*, Cytokinin, Auxin and jasmonic acid

## 1 Shoot Stem Cell Niches

Multicellular mode of life begins with a single cell which develops into multicellular body via multiple rounds of cell divisions. Plants are among the multicellular organisms living thousands of years. During their long journey of life, they pass through repeated cycles of developmental stages and witness endless mechanical injuries which contribute to their amazing power of regeneration and recovery of dead cells by producing new cells. This ability is due to the stem cells which remain undifferentiated and undergo several rounds of divisions. Locations of these stem cells within the plants are called stem cell niches. In plants, the stem cell niches are called meristems which foster the survival of stem cells and also the production of the progeny cells fated for differentiation. The meristem mostly lies at the tips of the plants, including SAM and RAM. Two additional meristems called vascular cambium and cork cambium are the lateral meristems of plant body, which are involved in secondary growth. The ring-shaped vascular cambium produces xylem and phloem, while cork cambium's responsibility is the replenishment of the regularly shedding outer layer called bark. Besides other functions, all kinds of meristems have two basic

Muhammad Naseem and Thomas Dandekar (eds.), *Plant Stem Cells: Methods and Protocols*, Methods in Molecular Biology, vol. 2094, https://doi.org/10.1007/978-1-0716-0183-9_1, 

functions: the production of new cells and the initiation of organ formation [1]. The mechanism of the production of new cells has been demonstrated by Satina and coworkers in 1940 by cell-tracking experiments in the shoot meristem. Through the treatment of colchicine, they produced polyploidy in the single cell of *Datura* and observed that the shoot meristem is comprise of clonally separated three layers of cells named as L1 (epidermal layer), L2 (subepidermal layer), and L3 (inner layer) [2]. Three layers of the shoot meristem is typical characteristic of dicots, but this varies in other groups of plants, e.g., in monocot, it consists of two layers; gymnosperm, only one layer; and, in lower plants (bryophytes and ferns), these layers are absent and all of their cells are from single apical cell. Later on, in 1970, Stewart and Dermen found that one-third of each layer is originated from one single cell which indicates the presences of three stem cells in one layer [3]. Stem cells can produce new cells and remain undifferentiated when they remain in a location having specific environment. Interestingly, the fate of the daughter stem cells does not depend on their parental cell but on the environment where they locate, and their displacement among different zones modulates their behavior according to their new destination, e.g., when L1 cells are displaced to the periphery of L2, they change to L2-type cells. This interesting phenomenon suggests the existence of some kind of chemical signals that maintain the ability of division, and cells moving away from the influence of these signals are bound to differentiation [1].

Plants survive for many years with continuous growth and replace old and dead cells by cell division throughout their life span. Here, question arrived that how the plant stem cell deals with mutation created during replication and survives for hundreds of years without accumulating mutation. Possible answer of this question is the infrequent division of the stem cell and the finite number of division of the stem cell daughter cells before their displacement. These two reasons reduce the chance of mutation produced during DNA replication [3, 4]. Besides that, DNA present in the root and shoot meristem cells is supersensitive to DNA damage resulting in death of the cell's damaged DNA, keeping stem cell system clean from the compromised DNA [5].

### 1.1 Structure of Shoot Apical Meristem

The shoot apical meristem (SAM) of *Arabidopsis* is differentiated into three distinct zones including the peripheral zone (PZ), central zone (CZ), and rib zone (RZ). Stem cells of the peripheral zones undergo rapid divisions as compared to that of the central zone. These rapidly dividing stem cells found in the peripheral zone are responsible for and are source of the lateral organs in mature shoot. In the rib zone, the cells start to assume a flattened shape to initiate the differentiation toward the central stem tissue (Fig. 1). The individual divisions of the shoot meristem stem cells produce two cells among which one acts as the stem cell, while the other

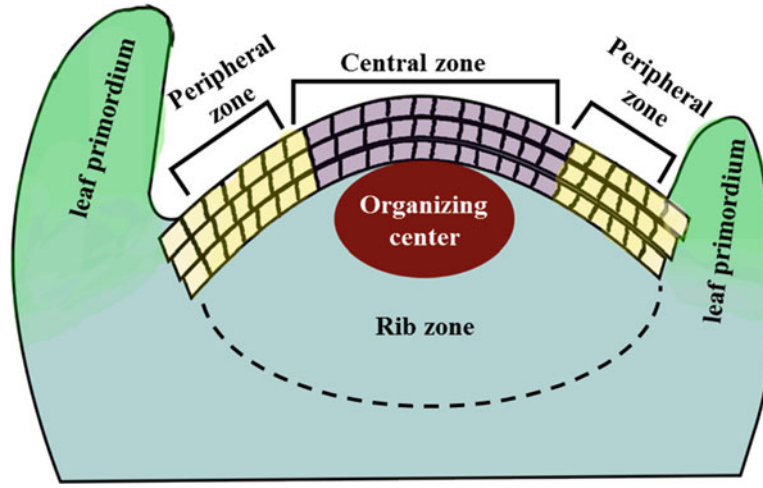


**Fig. 1** Graphical presentation of the shoot apical meristem showing different zones

undergoes differentiation depending on their positions [6, 7]. How stem cells maintain their identity and how the boundaries of stem cell niches are maintained in the meristem zones are difficult questions to answer. Investigation of the past decade provides adequate knowledge to understand the basic molecular mechanism of stem cell regulation.

### 1.2 STM Maintains the Shoot Meristem by Regulating Cytokinin and Gibberellic Acid

The first gene (*KN*) known to be involved in plant stem cell regulation was identified in maize [8]. This gene is a member of *KNOTTED1-like homeobox* (KNOX) gene family. Normally, *KN* gene is expressed in undifferentiated cell in meristematic region but absent in leaf anlagen cells indicating its key role in maintaining the stem cells undifferentiated [9]. In *Arabidopsis*, *SHOOTMERISTEMLESS (STM)* shows similar pattern of expression, and mutation in this gene leads to the absence of the shoot meristem [10] and fused cotyledons. Mutant of the *STM* also failed to initiate the meristem at postembryonic stage. Study of *STM* mutant suggests that *STM* gene has two functions; the first is to prevent the cell differentiation in the meristematic region, and the second is the repression of cell division between the lateral organs to keep them separated [10–12]. STM gene regulates cytokinin (CK) biosynthesis by activating the transcription of IPT7 gene, known for its crucial role in meristem maintenance. By applying exogenous CKs, the phenotype of *SMT* mutant can be reversed, and overexpression of SMT induces the biosynthesis of CKs and results in the formation of ectopic meristem [13–15]. Another gene of the KNOX gene family known as KNAT1/BREVIPEDICELLUS (BP) also regulates CK biosynthesis suggesting an active role for this gene in maintaining the meristem [14, 16]. Besides, SMT

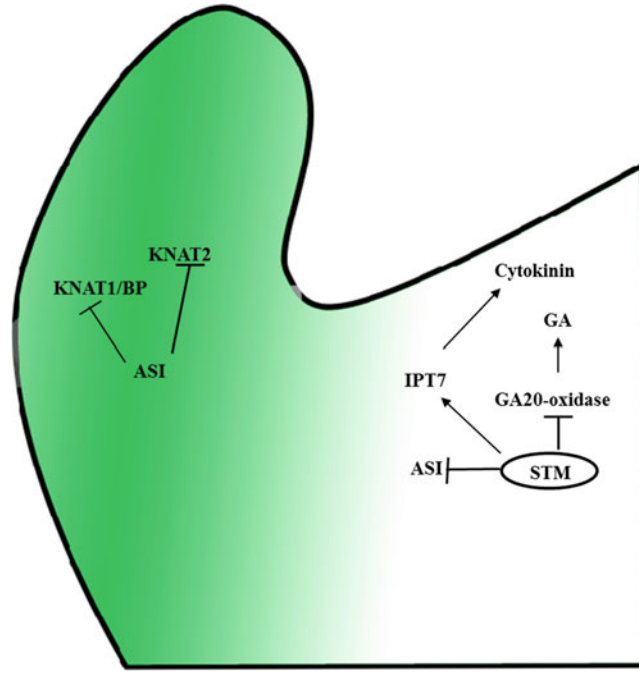


**Fig. 2** Regulation of meristem boundaries by STM. STM promote CK biosynthesis and its activities by upregulating the IPT7 and downregulating AS1 which in association with auxins represses KNOX gene activities responsible for promoting the meristem. The STM is also known for its suppression of CKs and Gas in the region of leaf primordial

also represses the meristem-promoting activity of two factors including KNAT1/BP and KNAT2 in primordia by suppressing the expression of ASI gene in the shoot meristem [17]. Furthermore, STM downregulates the production of cell differentiation promoter gibberellic acid (GA) in the stem cell by suppressing GA 20-oxidase gene responsible for the biosynthesis of GA and upregulates GA 2-oxidase gene involved in degradation of active gibberellin (Fig. 2) [14, 18, 19].

### *1.3 WUS and ZLL Are Key Genes to Maintain the Stem Cell Meristem*

An important gene known as *wuschel (WUS)* codes for a plant-specific homeodomain protein and is required for shoot meristem maintenance. WUS belongs to WUSCHEL-RELATED HOMEOBOX (WOX) gene family. Members of this family regulate diverse aspects of development [20]. Mutation in WUS gene leads to the absence of the meristem and partial differentiation of stem cells showing its importance in maintaining the stem cell undifferentiated [21, 22]. Overexpression of the WUS enlarges meristem, showing that this gene promotes stem cell identity [13, 23–25]. WUS gene is expressed in the organization center of the shoot meristem located underneath the three stem cell layers and maintains stem cells in two ways including its translocation

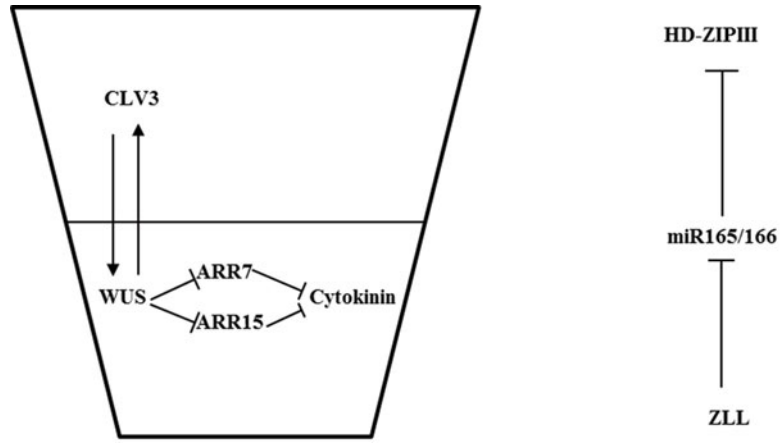


**Fig. 3** Negative feedback loop of WUS/CLV3 maintains stem cell activity. WUS regulates ARR7/ARR15 expression to inhibit intracellular CK response and maintain stem cells. Expression of ZLL in the vasculature promotes the activities of HD-ZIPIII genes in the shoot primordium by sequestering miR165/miR166

(through plasmodesmata) from OC to the CZ where it binds to the promoter of CLV3 to initiate transcription. Decrease in the amount of the WUS leads to the loss of the shoot meristem suggesting that maintenance of stem cells is dependent on the translocation of WUS [9, 25, 26]. The WUS contribute to stem cell maintenance by regulating the expression of two type A *ARABIDOPSIS RESPONSE REGULATOR* genes ARR7 and ARR15 which encode the inhibitor of the intercellular response to CKs (Fig. 3). It suggests that the presence of WUS in both OC and stem cells is important to preserve the undifferentiated status of the stem cells. The results of chromatin immunoprecipitation and transcription profiling assay revealed that WUS act on vast majority of genes that regulate the meristem and control cell division and phytohormonal pathways [27, 28].

ZWILLE/PINHEAD/AGO10 (*ZLL*) encode a protein of 988 amino acids called ARGONAUTE (AGO) expressed in the vascular primordium playing a key role in the maintenance of meristem cells (Fig. 3). Mutation in this gene results in the differentiation of apical meristem stem cells [29, 30]. In *ZLL* mutant, *WUS* gene expressed normally in the OC, but the expression of CLV3 was not maintained. Overexpression of *WAS* gene in *ZLL* mutant also failed to accumulate stem cells in the meristem [31]. All these findings suggest that *ZLL* enhance *WUS*-dependent cell signaling. AGO proteins are the repressor of microRNA 165 and microRNA 166 which are the cleaver of the mRNA of *HOMEODOMAIN-LEUCINE ZIPPER III* (*HD-ZIPIII*) genes *ATHB-9/PHV*, *ATHB-14/PHB*, and *ATHB-15*. Mutation in the AGO-coding gene *ZLL* leads to the accumulation of microRNA 165 and microRNA 166 resulting in the loss of *HD-ZIPIII* gene

products [1, 32]. How *HD-ZIPIII* gene products maintain the stem cell is not clear. AGO1 is the close homolog of ZLL and shows high similarity in PAZ and MID domains which help in binding to small RNAs and PIWI domain responsible for target mRNA cleavage in AGO1. The N terminal sequences of both genes are different and do not show sequence similarities. The dual role of AGO1 is clear from literature. For instance, this gene is responsible for overlapping and antagonistic effect on gene development and silencing [33]. Biochemical evidence demonstrates that the binding affinity of ZLL to the miR165 and miR166 is higher than the AGO1, but its HD-ZIPIII mRNA degradation efficiency is lower than the AGO1. These biochemical results suggest that ZLL sequesters miR165 and miR166 form the AGO1 to upregulate the expression of HD-ZIPIII [34].

### 1.4 Clavata and Its Receptors Contribute to OC Stability

Stem cell maintains their size, shapes, and internal organization, but how do they control the boundaries of meristem? Answer of this question became possible after observing extended stem pool and production of comparatively more organs in the *Clavata* (*clv*) mutant as compared to wild-type plants [35]. One of the family members of 32 small proteins (CLE family) called the CLV3 protein is important for intercellular talk [36]. The cause of the expanded meristem in the CLV3 mutant was the enlarged WAS domain [24]. Contrary, the overexpression of CLV3 represses the expression of WUS and shows phenocopy of *WUS* mutant [37]. The CLV3 expressed in the wedge-shaped domain coexist with stem cells. A WUS gene mutant *Arabidopsis* is characterized by the downregulation of CLV3, while its expression is upregulated in plants overexpressing WUS. This positive regulation of CLV3 by WUS constitutes a negative feedback loop where the CLV3 expression in the stem cells depends on the WAS expression in OC [24]. Thus, the stability and maintenance of stem cells in the SAM rest on this negative feedback loop (Fig. 3). For the homeostasis of stem cells in the SAM, perception of CLV3p by a receptor protein is a key step. Among these receptors, LRR receptor kinase encoded by CLV1 expresses in the central zone of SAM. Another gene, CLV2, encodes for a protein homolog of LRR receptor kinase that lakes intercellular kinase domain. This LRR receptor-like protein interacts with CORYNE (CRN)/SUPPRESSOR OF LLP1 2 (COL2) which has kinase domain but lakes the receptor domain. The third identified receptor in the CLV3p perception is RECEPTOR-LIKE PROTEIN KINASE 2 (RPK2)/TOADSTOOL2 (TOAD2) [38, 39]. Triple mutant *clv1 clv2 rpk2 Arabidopsis* shows phenocopy of *clv3* mutant which suggests that these three receptors are involved in main pathways of CLV3p perception. CLV3 repress WUS at the distal and lateral boundaries of the organizing center (OC), and its overexpression in L1 layer leads to complete suppression of WUS [37]. The binding of the receptors

to the CLV3p in overlying cells limits its spreading from the stem cell in lateral direction and maintains OC stability in the meristem [37]. Schoof et al. proposed that graded signal emanated from the stem cell promotes the expression of WUS keeping the stem cell niches at the tip of the plant [24]. Some observation suggests the involvement of CKs in this process [40].

## 2 Root Stem Cell Niches

The root tip has stem cells which are responsible for root growth toward the gravity. Central region of the root tip is called quiescent center (QC) which is made of relatively mitotically inactive cells. QC is surrounded by the stem cells and by a division which gives rise to different files of the root such as the epidermis, stele, endoderm, cortex, root cap, and columella (Fig. 4). An asymmetrical initial division of the root stem cell produces two cells, one of which remains in the QC and the other one undergoes several rounds of mitotic division and subsequent differentiation. Columella stem cells (CSCs) are located at the distal side of QC, and its daughter cells differentiate into starch-containing graviperceptive columella cells. Every stem cell of the root meristem has limited potential of differentiating into only one kind of tissue. Contrary to the lineage-based differentiation of the daughters of shoot stem cells, signals received from the already differentiated cells play a vital role in the differentiation of root stem cell daughters [1].

### 2.1 Organization of Stem Cell Niche in the Root

QC play a key role in controlling root stem cell function. The ablation of QC leads to the blockage of CSC proliferation and its differentiation to the starch-containing columella cells [41]. The QC signals are of short range as only the adjacent stem cells are maintained, and the nonadjacent stem cells differentiated. RETINOBLASTOMARELATED (RBR) also play a key role in maintaining stem cells as several layers of the cells remain undifferentiated around the QC in *Arabidopsis* where the expression of RBR has been downregulated by RNAi [42]. The undifferentiated state of these cell layers changes into differentiated cells when the QC was ablated which indicates that the stem cell-promoting signals were from QC and can work within some diameter, but normally they are counteracted by those cells which are not in direct contact with QC. The signals from the QC maintaining root stem cells are not yet discovered, but some important pathways are identified in recent works [1].

### 2.2 WOX5 Gene as Stem Cell Stabilizer

WOX5 is a homolog of WUS and expressed in the QC. The loss of the WOX5 function leads to the differentiation of CSCs same as in the result of ablation of QC. Overexpression of WOX5 represses the process of differentiation in the columella cells and makes stem

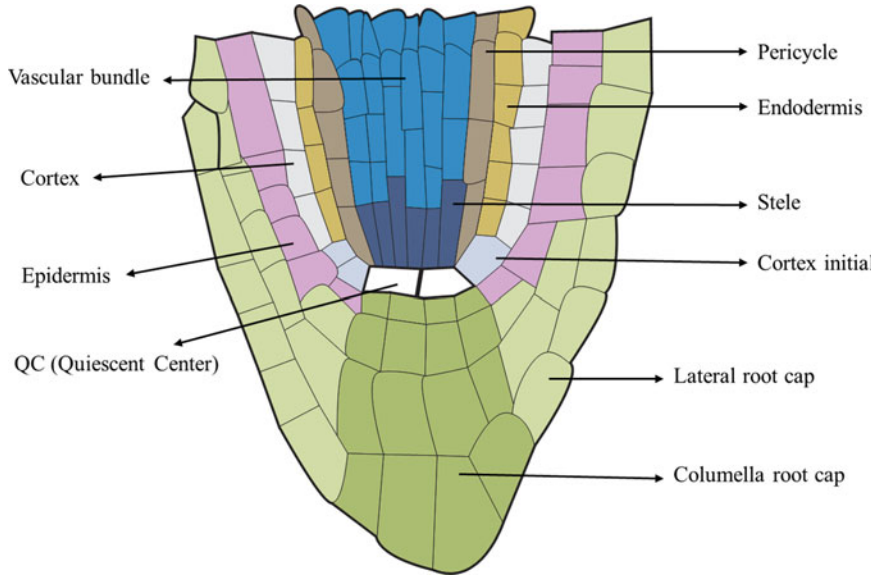


**Fig. 4** Diagrammatic view of *Arabidopsis* root longitudinal section showing different regions of the root apical meristem in different colors

cell-like cells. The ablation of QC does not suppress the WOX5 overexpression effect on columella cells which indicates that there are no other signals except WOX5 from the QC which maintain the root stem cell in undifferentiated state [43].

Some CLE peptides play important role in differentiation and maintenance of shape and size of cells in the root meristem like CLE40 which is expressed in differentiated columella cells (Fig. 4) [36, 44]. CLE40 together with ACR4, which is expressed in the columella stem cells and first layer of differentiated columella cells, promote the cell differentiation of the columella cells by counteracting the stem cell-promoting signal from the QC cells [44].

### *2.3 SHR Signals From the Stele Are Important for QC Function*

SHR is a GRAS (GAI, RGA, SCR) transcription factor and is expressed in the stele region. From the stele region, it moves to the neighbor cells including QC where they activate the expression of SCR gene which facilitates the nuclear localization of SHR (Fig. 5) [26]. Mutation in both genes, SHR and SCR, leads to the irregular morphology of root stem cell niches and downregulation of QC-specific markers and finally results in the collapse of the root meristem. In *shr* mutant, the QC-specific expression of SCR failed to rescue the QC defect of *shr* indicating that the presence of the products of these two genes is necessary in the QC for its stem cell maintenance activity [45–47].

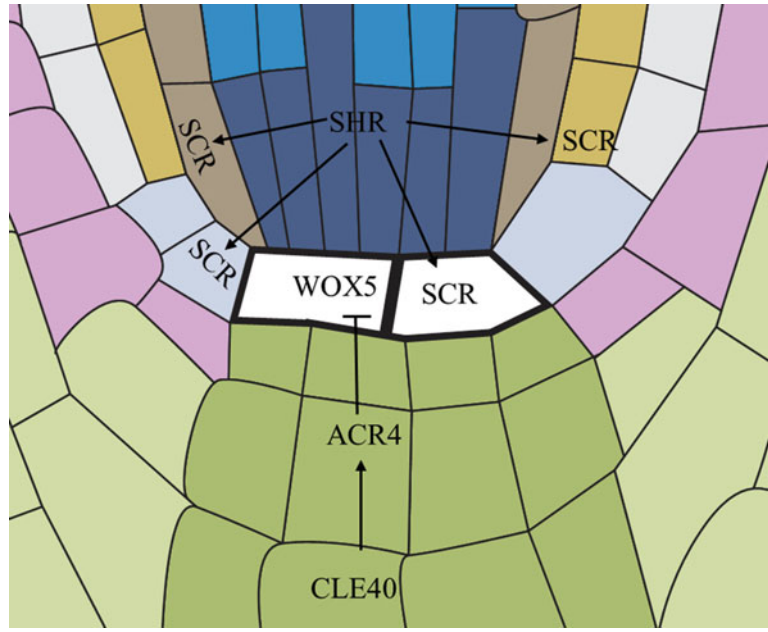


**Fig. 5** The transcription factor SHR moves out of the stele region (expression spot) to the adjacent cells and QC where the expression of SCR is promoted resulting in nuclear localization of SHR. The stem cell-maintaining signals from QC are suppressed by CLE40 (expressed in differentiated columella cells) and ACR4 (expressed in the first layer of columella stem cells)

### *2.4 Stem Cell Maintenance of Auxin via PLT*

Auxin accumulation in the QC is achieved by shootward auxin transport in the epidermis and lateral root cap and rootward auxin transport in the vascular tissues [48]. With the removal of the root tip, auxin starts to accumulate in epic cells and establish auxin maximum which leads to the formation of new root tip and new stem cell niches [49]. This indicates that auxin maximum and stem cell niches are functionally linked. Auxin maintains the stem cell niches via *PLT* transcription factors [50]. The activity of PLT in the cells depends on its expression level indicating its dose-dependent function. High level of PLT expression in the QC promotes stem cell niches, intermediate level of expression in the proximal meristem promotes mitotic cell division, and low level of expression initiates differentiation. Auxin is indirectly linked with PLT expression by tyrosylprotein sulfotransferase (TPST) and root growth factor (RGF). Auxin enhances the expression of TPST and RGF which results in TPST sulfate, the RGF proteins which upregulate the expression of PLT by an unknown mechanism. PLT proteins enhance the expression of PIN, creating a positive feedback loop for the stabilization of auxin maximum at root tips [51, 52]. The function of the auxin in the root meristem depends on cell texture, e.g., in QC, auxin helps in the maintenance of stem cells, while, in the columella, it promotes cell differentiation. This cell texture-

dependent readout of auxin is due to auxin response factor 10 and auxin response factor 16 (ARF10, ARF16). Auxin activates the transcription of ARF10 and ARF16 which restrict the activity of WOX5 by suppressing its transcription, restrict it to the QC, and promote differentiation in CSC daughter cells [53, 54]. In short, auxin not only promotes root stem cell niches but also restricts it in the CSC daughter cells to initiate differentiation.

## 3 Vascular Stem Cell Niches

The vasculature in plants is the main path of mineral and nutrient transport. Through the xylem, minerals in the water are absorbed by the root from the rhizosphere and transported to the upper part of the plant for utilization. The phloem transports organic compounds synthesized in green parts of the plant which are transported downward to other parts of the plant. Besides that, the vasculature also provides mechanical support to the stem. All vascular cells are almost of same type but vary in architecture depending on its position. In *Arabidopsis* root, the centrally located metaxylem is sorrounded by protoxylem. On both sides of the xylem, the phloem is located at perpendicular axis to the xylem. The region between the xylem and phloem is occupied by the procambium which is consist of pluripotent stem cells. The xylem, procambium, and phloem are surrounded by pericycle and form the vasculature (Fig. 6a). In the stem, vascular bundles present in ring form where the procambium (fascicular cambium) presents at central position, the xylem on the inside, and the phloem on the outside [1]. During the secondary growth, the procambium is connected by the interfascicular cambium and forms a closed ring.

### 3.1 Stem Cell Maintenance in the Procambium

Similar to the shoot meristem, cambium stem cells are maintained by many key regulator genes, most of which are identified and show similarities to the apical meristem. Tracheary element differentiation inhibitory factor (*TDIF*) encodes CLE peptide protein, promotes cell proliferation, and represses differentiation in xylem cells (Fig. 7) [36]. *CLE41* and *CLE44* are homologs of *Arabidopsis TDIF* (tracheary element differentiation inhibitory factor) gene, are expressed in the phloem cells, and induce proliferation in the neighboring procambial cells of hypocotyl and shoot. PHLOEM INTERCALATED WITH XYLEM/TDIF RECEPTOR (PXY/TDR) receptor-like kinase are CLVI-like proteins, perceive CLE41 and CLE44 protein in the stem cells, and promote cell division. Lake of PXY/TDR results in reduced number of procambial calls, loss of procambium cell division orientation, and interspersed xylem and phloem [55, 56]. It indicates that differentiating phloem daughter cells maintain stem cell stability through stem cell-promoting signals and behave like niche cells similar to the OC and QC in the root and shoot meristem, respectively.

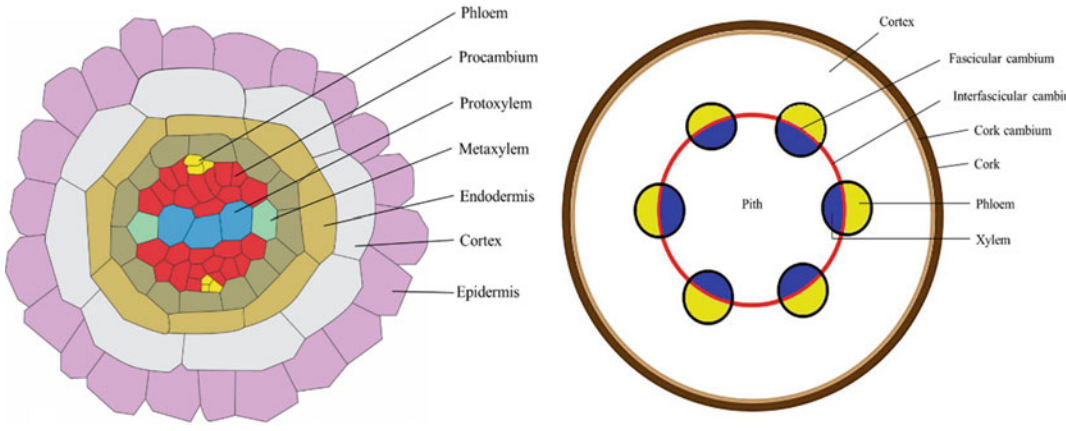


**Fig. 6** Vascular patterning and tissue organization in *Arabidopsis* root (**a**) and stem (**b**)

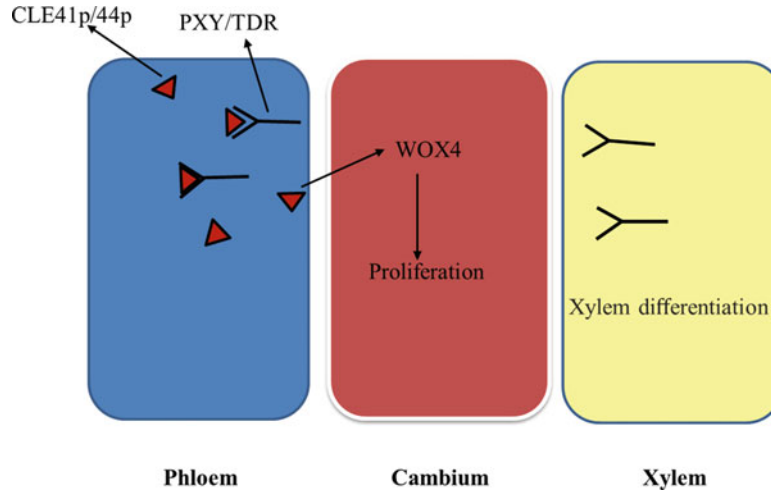


**Fig. 7** The CLE–PXY/TDR–WOX4 pathway responsible for the proliferation of stem cells driving orientation of the cell division

Ubiquitous overexpression of CLE41/CLE44 represses xylem differentiation and accumulates calls in vascular bundles and interfascicular region, but the specific overexpression of CLE41 in phloem cells and ubiquitous overexpression of PXY/TDR cannot repress the differentiation of xylem cells. From this, it can be concluded that some other unknown factors limit the range of CLE41p by an unknown mechanism. Therefore, CLE41p-PXY/TDR module defines the boundaries between vascular cell types and also regulates the number of stem cells. CLE41 ubiquitous and xylem-specific expressions induce disorientated procambial cell division, but its expression in the phloem cells induces normal cell division. This indicates that the position of the stem cell's relative cells producing CLE peptide correlates with the orientation of stem cell division [55].

WOX4 is another gene in the procambium which expression is regulated by CLE41 and CLE44. CLE41/CLE44 promote cell division in the procambium by promoting the expression WOX4. Unlike pxy/*tdr*, loss of WOX4 does not show complete loss of intervening procambial cell layer and does not suppress discontinuous xylem stand formation and TDIF application [57]. So, WOX4 mediates only stem cell division regulated by PXY/TDR, and some unidentified pathway must mediate repression of xylem differentiation by PXY/TDR (Fig. 7).

The application of the CLE41p/CLE44p with other CLE peptides to the plant results in the proliferation in the vasculature [58]. Two receptor-like kinases MOLI and RULI also affect the activity of cambium. MOLI regulate negatively, while RULI positively regulate cambium activity [59]. Furthermore, the result of transcript profiling of *Arabidopsis* and *Populus* indicates that two shoot meristem regulators CLV1 and STM are also important for the maintenance of vascular stem cells, but the mechanism on how they regulate vascular stem cells is not clear [60, 61].

### 3.2 Auxin and Cytokinin Signalings Control the Boundaries of Vascular Stem Cell Niches

Cytokinins (CKs) translocate from the shoot to the root via symplastic connection of the phloem and maintain the status of stem cells in the procambium. Any reduction in the CKs or its signaling results in the reduced number of cells in the vasculature [1]. The site of active CK signaling lies in the procambial cells adjacent to the xylem axis which affect the localization of PIN3 (expressed in pericycle) and PIN7 (expressed in phloem and procambium) [62]. This bisymmetrical localization of PIN channels auxin to central axis of xylem where auxins induce the expression of ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6) which blocks protoxylem formation by repressing CK signaling in the protoxylem position [62]. Thus, the mutational impeding interaction of CKs and auxins determines the boundaries between protoxylem and procambium stem cells (Fig. 8).

ZIPIII protein promotes xylem differentiation in procambial cells [63]. The transcription factors SCR and SHR activate the expression of miR156a/miR166b genes in the endoderm. From the endosperm, miR156a/miR166b move to the stele center where they suppress the expression of HD-ZIPIII genes *PHB*, *PHV*, *REV*, *CORONA (CNA)*, and *ARABIDOPSIS HOMEOBOX* GENE by degrading their transcripts. The concentration gradient of HD-ZIPIII protein determines development of xylem in a concentration-dependent way. High level of this protein produces xylem, while lower level produces protoxylem [64]. High level of this protein produce xylem while lower level produce protoxylem [64]. All these findings conclude that neighboring cells along the xylem axis are important because their positional signals decide the fate of the cells in their vicinity.

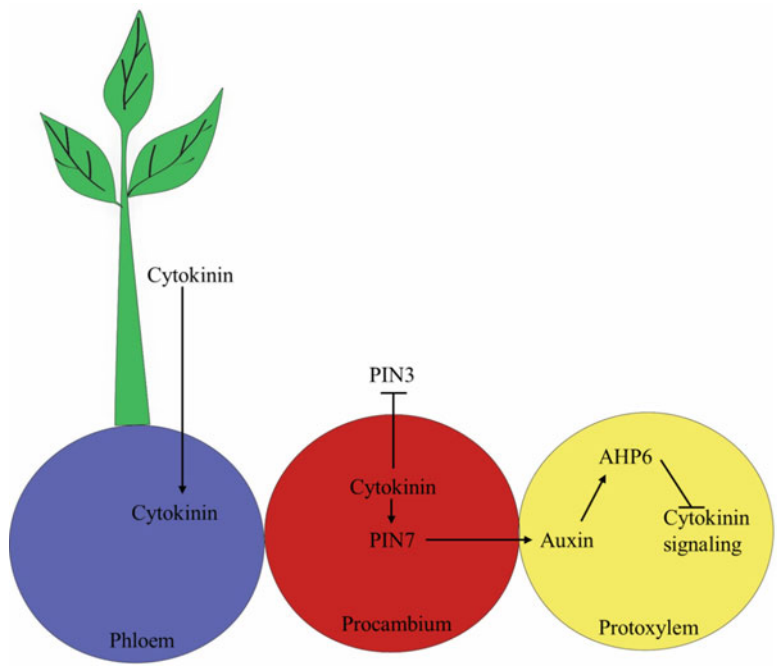


**Fig. 8** Auxin and cytokinin signalings control the boundaries of vascular stem cell niches

### *3.3 JA Signaling Stimulates Interfascicular Cambium Initiation*

During plant development, the plant gains weight specifically by producing green canopy. To bear increasing weight, the plant starts secondary growth in response to give strength to the stem. It has been proven from the experimental work that loading artificial weight on the immature plant tip induces IC formation, possibly through auxin signaling. But recent studies did not find any direct correlation between plant weight and IC initiation [65]. Recent studies demonstrate that JA signaling is involved in secondary growth. *JAZ10* is a touch-inducible JA signaling gene and is expressed in the xylem and IC of the basal stem. The intra-tissue tension which develops either as a result of cell division in the fascicular cambium or pushing of cambium outward due to the generation of xylem is thought to be important in the activation of JA signaling and subsequent initiation of IC formation. Hence, the intra-tissue tension and body weight are supposed to be involved in the stem cell niches of cambium [66].

## References


1. Aichinger E et al (2012) Plant stem cell niches. Annu Rev Plant Biol 63:615–636
2. Satina S, Blakeslee AF, Avery AG (1940) Demonstration of the three germ layers in the shoot apex of Datura by means of induced polyploidy in periclinal chimeras. Am J Bot 27 (10):895–905
3. Stewart R, Dermen H (1970) Determination of number and mitotic activity of shoot apical initial cells by analysis of mericlinal chimeras. Am J Bot:816–826
4. Lyndon RF (1998) The shoot apical meristem: its growth and development. Cambridge University Press, Cambridge
5. Fulcher N, Sablowski R (2009) Hypersensitivity to DNA damage in plant stem cell niches. Proc Natl Acad Sci 106(49):20984–20988
6. Laux T (2003) The stem cell concept in plants: a matter of debate. Cell 113(3):281–283
7. Spradling A, Drummond-Barbosa D, Kai T (2001) Stem cells find their niche. Nature 414(6859):98
8. Hake S, Vollbrecht E, Freeling M (1989) Cloning Knotted, the dominant morphological mutant in maize using Ds2 as a transposon tag. EMBO J 8(1):15–22
9. Smith LG et al (1992) A dominant mutation in the maize homeobox gene, Knotted-1, causes its ectopic expression in leaf cells with altered fates. Development 116(1):21–30
10. Long JA et al (1996) A member of the KNOTTED class of homeodomain proteins encoded by the STM gene of Arabidopsis. Nature 379(6560):66
11. Endrizzi K et al (1996) The SHOOT MERISTEMLESS gene is required for maintenance of undifferentiated cells in Arabidopsis shoot and floral meristems and acts at a different regulatory level than the meristem genes WUSCHEL and ZWILLE. Plant J 10 (6):967–979
12. Long JA, Barton MK (1998) The development of apical embryonic pattern in Arabidopsis. Development 125(16):3027–3035
13. Brand U et al (2002) Regulation of CLV3 expression by two homeobox genes in Arabidopsis. Plant Physiol 129(2):565–575
14. Jasinski S et al (2005) KNOX action in Arabidopsis is mediated by coordinate regulation of cytokinin and gibberellin activities. Curr Biol 15(17):1560–1565
15. Yanai O et al (2005) Arabidopsis KNOXI proteins activate cytokinin biosynthesis. Curr Biol 15(17):1566–1571
16. Frugis G et al (2001) Overexpression of KNAT1 in lettuce shifts leaf determinate growth to a shoot-like indeterminate growth associated with an accumulation of isopentenyl-type cytokinins. Plant Physiol 126 (4):1370–1380
17. Byrne ME et al (2000) Asymmetric leaves1 mediates leaf patterning and stem cell function in Arabidopsis. Nature 408(6815):967
18. Chen H, Banerjee AK, Hannapel DJ (2004) The tandem complex of BEL and KNOX partners is required for transcriptional repression of ga20ox1. Plant J 38(2):276–284
19. Sakamoto T et al (2001) KNOX homeodomain protein directly suppresses the expression of a gibberellin biosynthetic gene in the tobacco shoot apical meristem. Genes Dev 15 (5):581–590
20. van der Graaff E, Laux T, Rensing SA (2009) The WUS homeobox-containing (WOX) protein family. Genome Biol 10(12):248
21. Laux T et al (1996) The WUSCHEL gene is required for shoot and floral meristem integrity in Arabidopsis. Development 122(1):87–96
22. Mayer KF et al (1998) Role of WUSCHEL in regulating stem cell fate in the Arabidopsis shoot meristem. Cell 95(6):805–815
23. Lenhard M, Jürgens G, Laux T (2002) The WUSCHEL and SHOOTMERISTEMLESS genes fulfil complementary roles in Arabidopsis shoot meristem regulation. Development 129 (13):3195–3206
24. Schoof H et al (2000) The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. Cell 100(6):635–644
25. Yadav RK, Tavakkoli M, Reddy GV (2010) WUSCHEL mediates stem cell homeostasis by regulating stem cell number and patterns of cell division and differentiation of stem cell progenitors. Development 137 (21):3581–3589
26. Nakajima K et al (2001) Intercellular movement of the putative transcription factor SHR in root patterning. Nature 413(6853):307
27. Leibfried A et al (2005) WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. Nature 438(7071):1172
28. Busch W et al (2010) Transcriptional control of a plant stem cell niche. Dev Cell 18 (5):841–853
29. McConnell JR, Barton MK (1995) Effect of mutations in the PINHEAD gene of

Arabidopsis on the formation of shoot apical meristems. Genesis 16(4):358–366

30. Moussian B et al (1998) Role of the ZWILLE gene in the regulation of central shoot meristem cell fate during Arabidopsis embryogenesis. EMBO J 17(6):1799–1809
31. Tucker MR et al (2008) Vascular signalling mediated by ZWILLE potentiates WUSCHEL function during shoot meristem stem cell development in the Arabidopsis embryo. Development 135(17):2839–2843
32. Liu Q et al (2009) The ARGONAUTE10 gene modulates shoot apical meristem maintenance and establishment of leaf polarity by repressing miR165/166 in Arabidopsis. Plant J 58 (1):27–40
33. Mallory AC et al (2009) Redundant and specific roles of the ARGONAUTE proteins AGO1 and ZLL in development and small RNA-directed gene silencing. PLoS Genet 5 (9):e1000646
34. Zhu H et al (2011) Arabidopsis Argonaute10 specifically sequesters miR166/165 to regulate shoot apical meristem development. Cell 145 (2):242–256
35. Clark SE, Running MP, Meyerowitz EM (1995) CLAVATA3 is a specific regulator of shoot and floral meristem development affecting the same processes as CLAVATA1. Development 121(7):2057–2067
36. Ito Y et al (2006) Dodeca-CLE peptides as suppressors of plant stem cell differentiation. Science 313(5788):842–845
37. Lenhard M, Laux T (2003) Stem cell homeostasis in the Arabidopsis shoot meristem is regulated by intercellular movement of CLAVATA3 and its sequestration by CLAVATA1. Development 130(14):3163–3173
38. Katsir L et al (2011) Peptide signaling in plant development. Curr Biol 21(9):R356–R364
39. Kinoshita A et al (2010) RPK2 is an essential receptor-like kinase that transmits the CLV3 signal in Arabidopsis. Development 137 (22):3911–3920
40. Yoshida S, Mandel T, Kuhlemeier C (2011) Stem cell activation by light guides plant organogenesis. Genes Dev 25(13):1439–1450
41. van den Berg C et al (1997) Short-range control of cell differentiation in the Arabidopsis root meristem. Nature 390(6657):287
42. Wildwater M et al (2005) The RETINOBLASTOMA-RELATED gene regulates stem cell maintenance in Arabidopsis roots. Cell 123(7):1337–1349
43. Sarkar AK et al (2007) Conserved factors regulate signalling in Arabidopsis thaliana shoot and root stem cell organizers. Nature 446 (7137):811
44. Stahl Y et al (2009) A signaling module controlling the stem cell niche in Arabidopsis root meristems. Curr Biol 19(11):909–914
45. Sabatini S et al (2003) SCARECROW is involved in positioning the stem cell niche in the Arabidopsis root meristem. Genes Dev 17 (3):354–358
46. Levesque MP et al (2006) Whole-genome analysis of the SHORT-ROOT developmental pathway in Arabidopsis. PLoS Biol 4(5):e143
47. Cui H et al (2007) An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. Science 316(5823):421–425
48. Barton M (2010) Twenty years on: the inner workings of the shoot apical meristem, a developmental dynamo. Dev Biol 341(1):95–113
49. Wiśniewska J et al (2006) Polar PIN localization directs auxin flow in plants. Science 312 (5775):883–883
50. Galinha C et al (2007) PLETHORA proteins as dose-dependent master regulators of Arabidopsis root development. Nature 449 (7165):1053
51. Matsuzaki Y et al (2010) Secreted peptide signals required for maintenance of root stem cell niche in Arabidopsis. Science 329 (5995):1065–1067
52. Blilou I et al (2005) The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature 433 (7021):39
53. Wang J-W et al (2005) Control of root cap formation by microRNA-targeted auxin response factors in Arabidopsis. Plant Cell 17 (8):2204–2216
54. Ding Z, Friml J (2010) Auxin regulates distal stem cell differentiation in Arabidopsis roots. Proc Natl Acad Sci 107(26):12046–12051
55. Etchells JP, Turner SR (2010) The PXY-CLE41 receptor ligand pair defines a multifunctional pathway that controls the rate and orientation of vascular cell division. Development 137(5):767–774
56. Hirakawa Y et al (2008) Non-cell-autonomous control of vascular stem cell fate by a CLE peptide/receptor system. Proc Natl Acad Sci 105(39):15208–15213
57. Hirakawa Y, Kondo Y, Fukuda H (2010) TDIF peptide signaling regulates vascular stem cell proliferation via the WOX4 homeobox gene in Arabidopsis. Plant Cell 22(8):2618–2629
58. Whitford R et al (2008) Plant CLE peptides from two distinct functional classes

synergistically induce division of vascular cells. Proc Natl Acad Sci 105(47):18625–18630

59. Agusti J et al (2011) Characterization of transcriptome remodeling during cambium formation identifies MOL1 and RUL1 as opposing regulators of secondary growth. PLoS Genet 7 (2):e1001312
60. Zhao C et al (2005) The xylem and phloem transcriptomes from secondary tissues of the Arabidopsis root-hypocotyl. Plant Physiol 138 (2):803–818
61. Schrader J et al (2004) Cambial meristem dormancy in trees involves extensive remodelling of the transcriptome. Plant J 40(2):173–187
62. Bishopp A et al (2011) A mutually inhibitory interaction between auxin and cytokinin specifies vascular pattern in roots. Curr Biol 21 (11):917–926
63. Ilegems M et al (2010) Interplay of auxin, KANADI and class III HD-ZIP transcription factors in vascular tissue formation. Development 137(6):975–984
64. Carlsbecker A et al (2010) Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. Nature 465(7296):316
65. Ko J-H et al (2004) Plant body weight-induced secondary growth in Arabidopsis and its transcription phenotype revealed by whole-transcriptome profiling. Plant Physiol 135 (2):1069–1083
66. Sehr EM et al (2010) Analysis of secondary growth in the Arabidopsis shoot reveals a positive role of jasmonate signalling in cambium formation. Plant J 63(5):811–822