# Molecule mechanism of stem cells in *Arabidopsis thaliana*

**Wenjin Zhang**[1], **Rongming Yu**[1,2]

[1]*Biotechnological Institute of Chinese Materia Medica,* [2]*College of Pharmacy, Jinan University, Guangzhou, China*

Submitted: 11-02-2014 Revised: 27-03-2014 Published: 10-06-2014

## ABSTRACT

Plants possess the ability to continually produce new tissues and organs throughout their life. Unlike animals, plants are exposed to extreme variations in environmental conditions over the course of their lives. The vitality of plants is so powerful that they can survive several hundreds of years or even more making it an amazing miracle that comes from plant stem cells. The stem cells continue to divide to renew themselves and provide cells for the formation of leaves, stems, and flowers. Stem cells are not only quiescent but also immortal, pluripotent and homeostatic. Stem cells are the magic cells that repair tissues and regenerate organs. During the past decade, scholars around the world have paid more and more attention toward plant stem cells. At present, the major challenge is in relating molecule action mechanism to root apical meristem, shoot apical meristem and vascular system. The coordination between stem cells maintenance and differentiation is critical for normal plant growth and development. Elements such as phytohormones, transcription factors and some other known or unknown genes cooperate to balance this process. In this review, *Arabidopsis thaliana* as a pioneer system, we highlight recent developments in molecule modulating, illustrating how plant stem cells generate new mechanistic insights into the regulation of plants growth and development.

**Key words:** *Arabidopsis thaliana*, molecule mechanism, stem cells

## INTRODUCTION

Higher plants form organs throughout their life via the repetitive organ formation and cell proliferation embedded in specialized structures called meristem. The meristem contains a pool of pluripotent stem cells in special microenvironments; the homeostasis in these stem cells is regulated through a dynamic balance during meristem regulation of gene expression. The major challenge is in relating molecule action mechanism to root apical meristem (RAM), shoot apical meristem (SAM) and vascular system. Plants of the meristematic tissue do not undergo cellular repositioning or redeployment during development and do not disseminate because of the closed and discontinuous nature of the meristem channels.[1] Stem cells reside at the growing points of a plant, vascular system and the tips of roots and shoots. The vascular system of plants which is composed of xylem and phloem has evolved into connecting plant organs and transports various molecules between them. SAM and RAM are situated at the main domain and lateral apical of shoots and roots, which generate cells for the lateral organ formation and tip growth. Some investigations demonstrated that extensive communication was modulated by signal molecules in these domains although some key enzymes between them have ambiguously clarified. The signaling mechanisms employed comprise diffusible peptides, directional transport of plant hormones, as well as complex interactions between transcription factors.[2] In this study, we have reviewed recent developments on signaling molecule regulation of cell-to-cell communication processes with an emphasis on how signals are modulated over periods of time in the plant. We have also provided a brief overview of how the activities of hormones in plants mediate stem cells with local factors.

**Address for correspondence:**
Dr. Rongmin Yu,
Biotechnological Institute of Chinese Materia Medica,
Jinan University, Guangzhou 510632, China.
E-mail: tyrm@jnu.edu.cn

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Quick Response Code:

**Website:**
www.phcogrev.com

**DOI:**
10.4103/0973-7847.134243

## SHOOT APICAL MERISTEM

In *Arabidopsis*, stem cells of shoot are located at the apex of SAM and their function is the key to give rise to all the aerial parts of the plant skeleton and the development of complete plants. The progeny of shoot stem cells recruited which become organs and tissues where they differentiate and adopt specific functional roles, maintain main regulators of above ground plant developments, and format the postembryonic aerial organs. SAM has specific functions in shoot growth and development and is situated in a microenvironment. This meristem contains an amount of stem cells residing in a niche at the apex of the dome-shaped meristem.

The differentiation of the stem cells is suppressed by signals, which in the surrounding cells, and an amount of peripheral cells are used in the formation of lateral organs such as leaves, which can be regarded as a transient amplifying population.[3] The stem cells divisions of the progeny remain located in the niche and continue to maintain an undifferentiated state, whereas any progeny has two types of fate. The stem cells remain in the center called progeny, while others have continuously taken the place of the center outward toward the periphery or downward into the interior layers. These cells displaced from the niche are bound to undergo differentiation in response to positional signals. The stem cells divide at a speed and faster than the stem cells in the center and provide the founder cells for the formation of either lateral organs or the main stem. The stem cells in the heart, which are the ultimate source of all cells in SAM not only divide rarely in the majority of plant species and do not immediately take part in organogenesis but also the lateral cells divided producing progeny cells which are displaced by the peripheral cells and can be served as founder cells. The number of stem cells remains constant despite the continuous departure of their progeny into initiating lateral organs, indicating that the recruitment of cells into new organs is precisely balanced by the formation of new stem cell derivatives.[4,5] The balance between organ formation and meristem maintenance must be carefully controlled in order to preserve the potential for indeterminate growth. Lateral organs such as leaves are primitively from the meristem tissue of flank in a pattern termed as phyllotaxis, which is founded in the periphery of SAM by auxin in accumulation.[6] The signaling of the intrinsic intercellular, instead of genetically predetermined cell differentiation, controls the organization and the SAM activity, which is established by classic physiological and modern molecular experiments.[7]

Changing the cell plane of division from anticlinal to periclinal and producing a protuberance from within SAM is one of the first critical steps in the prime of a new lateral organ primordium. Cell division rates are rapid in the developing primordium, and the cells undergo exclusively mitotic cell cycles with very little change in cell size. As the primordium enlarges, the distal tip cells stop dividing and begin to differentiate and expand to cell types specifically. SAM and their differentiating progeny cells maintain endocellular homeostatic balance despite lack of cell migration, programmed cell death and cell divisions in characteristic physical asymmetric. The homeostatic process included coordination of incidents such as stem cells differentiation, self-renewing in stem cells, cell divisions and the behavior of specialized stem cell.[8]

In the shoot meristem of the model plant *Arabidopsis*, the studies have elaborated that SAM has three different zones. Stem cells are preserved in the central zone (CZ) and compresses slowly dividing cells situated at the tip of the dome. The descendants of CZ cells are replaced by the peripheral zone (PZ) and divide at a faster rate where they are recruited into new primordial organs at fixed periods and differentiate in a specific spatial and temporal sequence to come into being lateral organs such as leaves or flowers.[9] Shoot meristem tissue is expressed in both the PZ and the CZ, which maintains cell division and delays differentiation. The rib meristem (RM) is located beneath the CZ. The stem cells of progeny are replaced by downwards toward the RM also differentiate and come to being a portion of the stem. Cells in RM referred to as the organizing center (OC), WUSCHEL (WUS) expresses here, which have been established to provide cues for the specification of stem cells and keep the stem cells in the CZ and therefore they can be termed as niche. Stem cells located in different parts of the SAM exhibit distinct cell behaviors and express different genes although the mechanism of intracellular signal is not clearly elaborated.[10]

The SAM of *Arabidopsis* has three kinds of stem cells that could be divided owing to each part of the three layers at the tip of the meristem. Cells in the outermost called L1 in anticlinal divide and come to being the epidermal layer. The innermost called L3 comprises multiple cell layers which helps in the formation of the internal tissues, mesophyll and vascular. The intermediate between L1 and L3 also produce a single layer that gives rise mostly to mesophyll tissue.[11]

Two molecules in *Arabidopsis* play a key role in the maintenance of the numbers of the SAM. CLAVATA3 (CLV3) is a signaling polypeptide secreted from CZ. WUS is a member of WUS-related homeobox (WOX) family and a homeodomain transcription factor expressed in stem cells of the OC. Shoot meristem tissue was kept in the feedback loop of CLV-WUS to come true. WUS positively regulates CLV3 expression in overlying cells of the CZ through a non-cell autonomous mechanism, whereas CLV3 is expressed in stem cells of the CZ and delimits the stem cell domain by repressing WUS by binding to CLV1 or CRN/CLV2 receptor-like kinases.[12,13] CLV2 which lacks the kinase domain has shown to contribute to the stability of CLV1. CLV3 binds affinity to receptor CLV1 and mediates CLV1 internalization, but not requires CLV2. Some proposal has been given about other roles for CLV2 in mediating CLV1 signaling.[14] The molecular activities of WUS and CLV3 explain the expansion of WUS domain in the apical layers of CLV3 mutants, which eventually leads to massive enlargement of SAM and the size of the SAM, and the stem cell domain is determined by the combination of the regulation feedback loops between CLV3 and WUS.[15]

In the parallel level of the WUS–CLV signaling pathway, class I KNOTTED1- LIKEHOMEODOMAIN (KNOX) proteins are a number of other critical stem cell homeostasis regulators in the shoot. Class I KNOX genes not only partly achieved by the coordination of phytohormone and offer an environment in which stem cells can be sustained by the CLV-WUS feedback loop but also encode homeodomain transcription factors which use to stop from cellular differentiation in the SAM.[16]

The related class I KNOX genes, KNAT1, KNAT2, KNAT6, and STM, have overlapping and redundant functions in SAM and incarnate the activity of redundant of their diversity in some degree in terms of maintenance and organogenesis in stem cell.[17] In *Arabidopsis*, both the establishment and maintenance of the embryonic SAM through postembryonic development require STM.

It proposed that the STM could induce the formation of ectopic shoot meristem to renew.[18] STM expressed in the stem cells and the surrounding cells in the PZ, but is excluded from those peripheral cells which will form a new lateral organ.[19] Thus, ectopic expression of KNOX genes is not sufficient to prevent the initiation of organs from the meristem flank. Conversely, loss of STM expression from the SAM leads to the precocious formation of leaves from all regions of the SAM, including the stem cells. Therefore, STM plays a key role in preventing the stem cells from rightly taking part in organ formation, and losing it leads to regulating the initiation of organ model. The leaf founder cells are downregulated by the expression of all KNOX genes and in *Arabidopsis,* particularly it remains inhibited throughout organ formation. The genes have been implicated in maintaining proliferation of stem cells and amplifying cells in the SAM and/or stopping from their differentiation.[20]

Activities of KNOX genes in the homeostasis of stem cell are closely accompanied with several plant hormone pathways. The defects of STM mutants can be inhibited by the level of cytokinin increased through application of exogenous hormone or overexpression of isopentenyltransferase (IPT) genes which encode an enzyme in the cytokinin biosynthetic pathway,[21] suggesting that positively regulated IPT7 and ectopic expression of KNOX give rise to accumulation of cytokinin. Gibberelic acid (GA) signaling molecule confronts with cytokinin signaling molecule, KNOX genes which encode a critical GA biosynthetic enzyme in the SAM and further facilitate the cytokinin pathway by negatively regulating the expression of GA-20 oxidase.[22,23]

KNOX genes partly dedicate to the activity of stem cell by promoting cytokinin accumulation in the meristem tissue. However, the various types of the SAM may well respond differently to cytokinin. Whether local differences in cytokinin signaling dedicate to segmentation of the meristem tissue in regions including the stem cells, the organizing center, or amplifying cells should be further investigated.[24] Regulation of the KNOX gene expression is itself controlled by multiple factors including chromatin modification. Chromatin modification plays an important role in restricting KNOX gene expression to appropriate cell types during development, ensuring appropriate differentiation can appear in lateral organs whereas allowing the indeterminate maintenance of meristem tissue.

## ROOT APICAL MERISTEM

As a result of the plants being accustomed to transform environmental demands, RAM is supposed to be evolved from SAM probably. Therefore, the key regulation mechanisms present in the shoot are also expected and found important in the development and regulation of the primary root meristem, such as plant hormones and their receptors as well as transcription factors. But at a cellular level, the organization and operation of the root and the shoot meristem stem cells is in the fully incomplete way in *Arabidopsis*. All cell layers are organized in a simple radial pattern. The root meristem tissue can be composed of three main regions: The meristematic zone, the elongation zone and the differentiation zone. The stem cell niche is included in the meristematic zone, consisting the infrequently partitioning of the quiescent center (QC) cells, which control the surrounding stem cells. After division of an initial cell, the descendant cells still in contact with the QC keep its stem cell fate, whereas the other cells become a transit-amplifying cell and after further divisions and expansion in the elongation zone, it acquires its destined cell fate in the differentiation zone.[25,26] As a consequence, the balance between two competing processes which includes cell division and cell differentiation maintain the size of root meristem.

The stem cells in the root meristem surround a small group of infrequently dividing cells, the quiescent center (QC) cells, whose function resembles those of the OC in the shoot meristem. In that it provides signals to prevent the stem cells from undergoing differentiation. The QC of RAM gives rise to distal columella, lateral root cap and epidermis and proximal cortex, endodermis and stele. Self-renew stem cells of the root meristem tissue are regulated at the single cell level. Stem cells seem that it greatly outweighs their differences and each individual cell division producing descendant is asymmetric. Stem cells, leading to these keep in touch with the QC and remains undifferentiated, whereas descendant that is disconnected with the QCdifferentiate cells.[27]

The molecular skeleton regulating development of the root stem cells appears to be different from that of the shoot. Correct pattern and specification of the RAM carry out by means of plant hormone such as auxin and several transcription factors. Regulating the cell division and differentiation balance in roots via the effect of cytokinin and auxin.[28] Auxin response regulators, the AUX/IAA-ARF pair BODENLOS (BDL) and MONOPTEROS (MP), regulate auxin signaling and provide correct pattern information by means of activating the PLETHORA (PLT) genes.[29] GRAS-family are crucial to radial patterning and stem-cell renewal in the Arabidopsis root including transcriptional regulators SHORT-ROOT (SHR) and SCARECROW (SCR). The PLT transcription factors subsequently function in concert with the GRAS family members SHR, which moves from the stele above the QC into the QC cells, and SCARECROW (SCR), which is activated by SHR, to specify QC identity. Auxin in the proximal meristem tissue mediates degradation of the SHY2 protein, promoting cell division. Auxin is supposed to be synthesized in the aerial organs and transported acropetally in the stele to the root meristem, resulting in an auxin at peak discharge in the tip of the root. Researchers have clarified that cytokinin and auxin regulate the size of root meristem tissue by means of the effect on expression of short hypocotyls 2 (SHY2). SHY2 as an IAA-class inhibitor of auxin signaling suppresses the expression of PINFORMED (PIN) auxin transport facilitator genes which is inducible by auxin. SHY2 encodes indole-3-acetic acid (IAA) which is an inducible protein 3 (IAA3).[30,31] The PIN genes are indispensable for the development of an appropriate auxin maximum. As a result, it is necessary to regulate and control cell division in the root meristem.[32] Polar auxin transport is made possible largely through the action of PIN proteins, auxin efflux carriers that are polarly localized to plant membranes. The localization depends on the cell type and decides the direction of

auxin flow. Five members of PIN family have been extensively characterized and dedicated to the development of embryonic root pattern and tropic growth movement.[33] Cytokinin's main function is to promote cells in meristem differentiation. In the transition zone, cytokinin activates SHY2 transcription factor by means of acytokinin-dependent transcription factor, *Arabidopsis* response regulator 1 (ARR1). SHY2, an IAA-class inhibitor of auxin signaling, mediates between cytokinin and auxin as crosstalk.[34] ARR1, a primary cytokinin response factor, was shown to bind the SHY2 promoter and activate SHY2 transcription, which then inhibit PIN1 expression, causing auxin redistribution. Then auxin induces SHY2 degradation and thereby stabilizes PIN expression levels in return, suggesting that RAM is like a capacitor that efficiently stores auxin. The root maintains the maxima and the meristem, through the PIN-directed auxin flux in turn. The auxin maxima is maintained and stable in the QC cells but dynamic around the elongation zone and/or meristematic zone border, allowing for different levels of growth and expansion in the root. Capacitance is dependent on the PIN layout within the root, and only drastic changes in the layout can alter the auxin distribution.[35]

In *Arabidopsis*, RAM has multiple signal molecule regulation mechanisms, such as the regulatory interactions sustained among the GRAS family transcription factors SHR, SCR, etc. The interplay between the redundant transcription factors PLETHORA (PLT1, PLT2); baby boom (BBM) and the auxin signaling pathway; the CLAVATALIKE40 (CLE40) and WUSCHELRELATEDHOMEOBOX5 (WOX5).[36-38]

The above-mentioned transcription factors, SCR, SHR, PLT1, and PLT2, have essential roles in QC establishment and stem cell maintenance. Inputs of both the PLT and SHR/SCR pathways are demanded in QC specification and appear to act in parallel. SHR is made in the pro-vascular tissue, but SHR protein moves out of the adjacent cells of endodermis and QC where it gets the nucleus to activate SCR transcription. It seems that SCR suppresses SHR from moving further outwards to adjacent layers in sequence.[39] SCR can affect by means of cellular automation because the expression in the QC cells is recognized by QC identity, but the expression of SCR in the QC region could not rescue the root meristem defects of SHR mutant seedlings, suggesting that the role of SHR is not limited to SCR activation. Whereas, the observation that the precise nuclear localization of SHR losses in SCR mutants, together with the ability to bind SHR, indicated a role for SCR in restricting SHR movement. Presentating that both SHR and SCR protein in the QC is required to maintain it, both SHR and SCR proteins are also expressed in the entire endodermis and exogenous auxin can transform these cells into QC with adjacent stem cells.[40]

Auxin-inducible PLT1 and PLT2 of *Arabidopsis*, encoding AP2 type transcription factors, were identified as effectors of the instructive auxin maximum into root stem cell formation and maintain the activity of RAM. The expression patterns of PLT1 and PLT2 are first in the basal region of the early embryo and later in the distal root meristem. Double mutants of PLT1 and PLT2 slightly suffer from a size reduction of cortex cells and QC markers that failed to express, giving rise to the terminating roots post embryonic, but the reason has not very clear,[41] suggesting that PLT appear to be short of a functional QC and revealing the differentiation of stem cells. Apart from this, PLT members lost with overlapping expression bring about rootless PLT1, PLT2, and PLT3 triple mutants. Seedlings without a complete root and hypocotyl, relating copy of BBM (the fourth member of the PLT family) is removed. Constitutive expression of BBM or PLT2 can both induce formation of ectopic root structures, together with the function lost, giving us data indicating a role for PLT genes as master regulators for root development.[42,43] PLT protein levels show a distinct graded pattern in the root meristem, with maximum levels specifying the root stem cells. Ectopic promoter and complementation experiments clarify that mitotic activity is regulated by intermediate expression levels, while PLT levels drop below a certain threshold. More importantly than all of that, QC marker genes were activated with a lack of prior accumulation of auxin at new positions, evaluating meristem cell fates.[44]

Identification of the CLE40-WOX5 feedback loop in the RAM regulate through intermediates in the signaling cascade methods. CLE40 roots in the closest homolog of the stem-cell-defining signal CLV3 and is expressed in specific root tissues, indicating the character in controlling root cell fates. CLE40 is consecutively limited to the basal regions of the embryo which forms the root meristem and the vasculature in the entire globular stage embryo. After germination, CLE40 remains expressed only in the differentiation zone of the stele that forms the inner layers of the root and differentiate the columella cells.[45] CLE signaling pathway in the root controls the size of a cell population and is regulated at the individual cell level in the root.

WOX5, a transcription factor, is expressed exclusively in the QC cells and maintains distal stem cell fate. WOX5 and WUS are close homologs and can substitute for each other if expressed in the shoot or root niche, respectively.[46] CLE40 can reduce WOX5 expression. WOX5 acts from the QC to maintain the distal stem cell population and its function can replace WUS. Similarly, CLE40 can replace CLV3 if expressed from the shoot stem cell domain. Together, it suggests that pathways controlling stem cell in shoot and root are at least partially conserved at the molecular level. A negative feedback loop between CLE40 and WOX5 and a positive self-regulation of WOX5 likely occurs via the auxin signaling pathway. Concerning this respect has been demonstrated ambiguously.

## VASCULAR MERISTEM

Vascular meristem compared to many regulatory mechanisms controlling the development and function of root and SAM is less revealed and the knowledge of lateral meristem in vascular cambium is still limited, whereas development of lateral meristem, such as pro-cambium and cambium, is still relatively incomplete. We try our best to elaborate modulation of vascular meristem by the way of molecular mechanism and genetic regulation.

The plant vascular meristems are elaborated to be in the formation of concentric rings of xylem, cambium and phloem, evolved to connect plant organs and transport various molecules between them. Vascular stem cells which are commonly derived from pro-cambium and cambium are in the formation of vascular systems. The primary growth in the apical direction mainly increases the plant biomass and the secondary growth locates in the lateral direction. Cambium are derived from pro-cambium and the cells around them, which are present in the stem and the root of the plants where they promote the secondary growth.[47]

Vascular systems correlate all the plant skeletons by their conductive tissue, from the root tip to the all kinds of organs in the shoot; pro-cambium and cambium refer to the intervening pluripotent cells that are able to become the two tissue types based on asymmetric periclinal cell divisions. Primary xylem and phloem dated from intervening pro-cambium in the course of the primary growth, with secondary development in stem and root of the plants, the vascular pattern is further contained to ultimately generate concentric rings of xylem, cambium and phloem with interacting among the pro-cambial pluripotent cells. Then comes the conductive tissues; xylem tissue mainly offers physical strength to plant skeletons and transport water and other nutrient substances required for the function of growth and defense, while phloem distributes photosynthetic products and various signaling molecules.[48]

The methods of mutant analyses have provided with vascular pattern and the various signaling molecules controlling vascular meristem development, but the molecular control of vascular pattern is still relatively poor compared with other plant tissues.[49] *Arabidopsis thaliana* as a pioneer system, the initiation of vascular meristem during embryogenesis is well characterized. Vascular meristem tissue is first generated during the stage of embryo formation. Vascular meristem tissue serves as an undifferentiated pro-cambial tissue in the innermost domain of the plant embryo which is enclosed by the epidermal and ground tissue layers.[50] Cell proliferation initially originates from the primary cells of the pro-cambial tissue. Cells gradually start to divide in the pro-cambial tissue between the phloem and xylem after differentiation of the phloem and xylem within this domain, and then the undifferentiated cells come into being lateral meristem.[51,52] At the beginning of *Arabidopsis* embryos, the cells inside the protoderm divide into distinct layers, the precursor of ground tissue and the initiation of vascular stem cells.[53] At the late period of globular stage, four pro-cambial cells bring about the pericycle and vascular stem cells. The number of pro-cambial cells continues to increase by further periclinal cell divisions, as a result of the radial vascular pattern identical to that of a postembryonic development primary root.[54-56]

Over the recent years, plenty of cell genetics research has clarified some genes involved in the vascular meristem in the period of *Arabidopsis* embryogenesis, though several of those are still indistinctly elaborated. Plant hormones play a key role in the vascular meristem development, in particular, auxin has been shown to trigger and maintain cambial activity and the formation of vascular meristem. It has been suggested that the formation of vascular meristem tissue is required for polar auxin transport and in order to the develop pro-cambial cells.[48,57,58] An auxin-responsive transcription factor, MP/auxin response factor 5 (ARF5) which in the specification of vascular stem cells express in the pro-cambial cells. The expression of ARF5 is upregulated by auxin at a high rate of speed during the early period of embryogenesis. In MPKNOCKOUT, mutant embryos reveal that pro-cambial cells absolutely fail to form and display irregular development of the weak alleles in vascular tissue.[59,60] It seems that it is related to the defect of the polar auxin transportthat became deficient during the formation of the embryo. The proliferation of vascular meristemin tissue is regulated by downstream of the auxin signal molecule. In the pro-cambial cells, *Arabidopsis thaliana* HOMEOBOX8 (ATHB8), an auxin inducible homeobox gene in the class III homeodomain leucine zipper (HD-ZIP III) transcription factor family gene, is expressed in the pro-cambial cells of the newly forming vascular strands.[61] The expression of PIN1 was shown to precede pro-cambium formation and ATHB8 expresses during leaf vein pattern. During early embryogenesis, PIN1 proteins, major auxin efflux carriers, are localized in the inner cells of pre-embryo prior to them turn into pro-cambial cells. The expression of PIN1 is sharply decreased in a dysfunction of mutation and suggesting that an MP might regulate its transcription with a critical role.[62] PIN1 is an auxin efflux carrier protein as the function of polar auxin transport, located in vascular stem cell initiation. And the survival of PIN1 is strongly supporting the auxin canalization theory.

PIN proteins circulate between plasma membranes and endosomes via the activity of ARF–GEF protein called GNOM. Researchers measured that SCARFACE (SFC)/VAN3 generates dense induced vein of the leaves and cotyledons in fragment through the mutation of the gene's location in PIN proteins. This experiment is further evident that the polar auxin transport in vein makes a critical role. SFC encodes an ADP ribosylation factor GTPase-activating protein (ARF-GAP), a modulator of ARF-GEF, involved in the vesicle trafficking. SFC might influence the abnormity of intracellular endosomal trafficking in PIN1.[63,64]

*Arabidopsis* genome contains two genes encoded xylogen, AtXYP1 and AtXYP2, polarly localized in the cell walls and which function as an intracellular signal molecule. OCTOPUS encodes phloem continuity which is situated in the membrane-associated protein of the phloem.[65]

Vascular meristem is different from stem cells in SAM and RAM, because vascular meristem tissue in the developmental leaves contrast to those produced in the vein networks on the contrary. As a result, the same molecular processes involved in the vascular meristem tissue are essential to the appropriate growth of the vein. How the signal of the pro-cambial cells from one to another to form continuous vascular strands become a main step. A hypothesis is accordingly provided that the positive feedback regulation of auxin transport promotes the development of the vein. The mechanism

shows that the auxin maximum value was established in the pro-cambial cells and improved the auxin transportation in a polar manner. The adjacent cells subsequently perceive a higher level of auxin and turn it into the pro-cambial cells. Then the adjacent cells repeat the same process to the next neighbor cell.[66]

The adaxial/abaxial polarity of lateral organs and the shoot vascular tissue are established by the antagonistic activity of two gene families, including the plant-specific class III homeodomain leucine zipper containing (HD-ZIPIII) transcription factors [phabulosa (PHB), revoluta (REV), phavoluta (PHV), corona (AtHB15) and AtHB8] and the KANADI [KAN1-3] GARP transcription regulators.[67-69] MP directly modulates the transcription factor ATHB8. Then ATHB8 directs the formation of prepro-cambial cells in subsequent and induces the expression of PIN1.[70] These processes come to being a positive feedback regulatory loop (auxin-MP-ATHB8-PIN1) in the primary of vascular meristem tissue. The positive feedback maintains the spatial specification of pro-cambial cells and the expression domain using a pro-cambium marker (J1721) expand via the mutant of ATHB8.[71]

Xylem differentiation and patterning likely result from various combinations of HD-ZIPIII protein activities and the regulation of their expression by miRNA 165/166. The JBA-1D phenotypes are caused by overexpression of miR166g, which targets class III homeodomain leucine zipper (AtHD-ZIP) family genes. Overexpression of miR166g results in downregulation of the ATHB-9/PHV, ATHB-14/PHB, and ATHB-15mRNAs, and concomitantly enhances in vascular development.[72] Mutations in the auxin-regulated gene REV resulted in the absence of interfascicular fibers in the stem and disrupted development of xylem fibers and vessel elements. Only a few genes have been identified that directly regulate in vascular meristem. ALTEREDPHLOEMDEVELOPMENT (APL) is a MYB transcription factor which has been identified as a critical modulator of phloem generation. Two NAC domain genes, VND6 and VND7, seem to control xylem cell identity since those overexpression and result in the development of ectopic TRACHEARY elements of protoxylem (VND7) or metaxylem (VND6).[73,74]

As described above, the vascular cambial meristem is located between the xylem and the phloem cells. They are different from the neighboring cells in their ability to periclinally divide. Secondary vascular development in *Arabidopsis* can be divided into two phases, differentiating cell types from the proliferated cambial meristem. Only vessel elements and parenchymatous cells have been produced during the early phase of secondary vascular development. In the later phase, the cambium starts to produce vessel elements and lignified fiber cells. Compared to the secondary phloem development, the secondary xylem development is excessively generated.[75] Very few know about the molecular mechanism in the secondary vascular development compared with the primary vascular development. A loss of function mutation in REV gives rise to disrupt the development of lignified fiber cells and vessel elements in *Arabidopsis*, suggesting that REV plays a critical role during the secondary phase of vascular development. The two principal tissue types, xylem and phloem, proliferate from undifferentiated cells, which suggests that the first committed phloem and xylem mother cells originate from the undifferentiated central zone. Because periclinal divisions produce new phloem or xylem cell, the functional definition of the cambial initial states that they are the only cell type in each radial file that are able to produce both phloem and xylem derivatives and to initiate new cell file by radial divisions.[76,77]

Parts of molecular mechanism are in favor of the compartmentalization of the cambial zone and several distinct layers identified in the poplar cambia. The solution is still ambiguous, elaborating the differentiation of molecular events at the level of a single cell layer. The main obstacle to resolving this question is the lack of reliable markers to confirm the layer of cambial meristem.[78,79]

## CONCLUSION

Understanding the principles of stem cell maintenance and cellular differentiation and the distinctive features of the cell cycle of plant stem cells are still elusive. In comparison with other plant tissue types or organs, vascular development is still poorly characterized. Even the anatomy and development of lateral meristems, pro-cambium or vascular cambium are not completely described at the moment compared with the SAM and RAM; even less is known about their genetic regulation. In the RAM and SAM, the elucidation of the organization and regulatory interactions of the stem cells was based on a combination of anatomical analysis and functionally defined molecular markers. As the details of differentiation in the cambial zone remain unclear, molecular markers would also assist in defining the phases of cambial development, such as pro-cambium vs. vascular cambium or the early and late phases of secondary vascular development. Several plant hormones, in particular auxin, cytokinin, and gibberellins are known to regulate cambial development. However, knowledge of how the hormonal signaling pathways regulate lateral growth is still relatively scarce and it remains to be seen whether the hormonal regulation of the cambium is acting through similar pathways to those found in the SAM or RAM. In a number of cases, studies in plants demand further new discoveries, demonstrating the power of plants as a system to study cell cycle regulation during development.

## ACKNOWLEDGMENTS

This research work was financially supported by National Natural Science Foundation of China (No. 81073004, 81102771, and 81274045) and Natural Science Foundation of Guangdong Province (No. S2011040003028).

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**How to cite this Article:** Zhang W, Yu R. Molecule mechanism of stem cells in *Arabidopsis thaliana*. Phcog Rev 2014;8:105-12.

**Source of Support:** Nil, **Conflict of Interest:** None declared