Stem Cells in Plant Development

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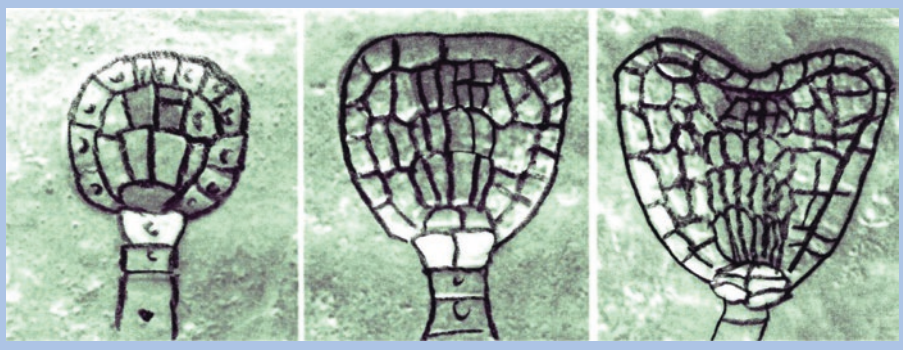

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What Will You Learn in This Chapter?

The aim of this chapter is to give you a basic understanding of how stem cells function in plant development. Plants and animals lead quite different lives. Plants, for one, do not often move and cannot escape adverse environmental conditions. The most important consequence of their sessile mode of life is that plant development is not restricted to embryogenesis. Instead, it continues throughout the plant's life, as it continues to grow and produce new organs. We will briefly discuss the implications of plant immobility on their developmental strategies, and how plant stem cell activity contributes to their indeterminate growth mode. Next, we will discuss the concept of plant meristems. These are highly specialized tissues that contain the plant stem cells and control both their maintenance and the production of new organs and tissues. Two main meristems are responsible for most of the growth in plants, the shoot and root apical meristems, and will be the focus of this chapter. We begin by reviewing the organization of apical meristems. Next, we discuss their embryonic origin, and we explore in finer detail the key signalling pathways involved in both the specification and maintenance of stem cell identity and activity. We will highlight the mechanisms that underlie the coordination of cell proliferation and differentiation. Hopefully, the concepts exposed in this chapter will provide you with a base from which to further explore stem cell activity and maintenance in plants, but also with the tools to draw interesting comparisons between animal and plant stem cells.

7.1 Overview of Plant Development and Stem Cells

As you have learned in previous chapters, stem cells are undifferentiated cells with an unlimited capacity to self-renew and generate the different cell types that compose an organism. In animals, they act during embryogenesis to produce all the tissues required for organogenesis, but also during adult life, repairing and replenishing certain adult tissues. Plant stem cells are also initiated in the embryo, however, unlike in animal development, they remain inactive throughout embryogenesis. In plants, unlike animals, the product of embryogenesis in plants is not a complete body that will grow into maturity, but a minimal body plan with a main axis and two poles, the shoot and the root. Upon germination, after embryonic development ceases, stem cells are activated to divide and form the mature plant body, with organs like the leaves and flowers. Hence, plant development differs from animal development in that it is not restricted to the embryonic stages, but it occurs throughout a plant's life with the continuous production of new organs such as leaves, roots and flowers.

Plants are sessile organisms for the most part. This means they cannot escape unsuitable abiotic conditions or biotic threats. Instead, they must constantly adapt, shaping their body plans and architecture to the surrounding environment. The post-embryonic development strategy is the answer of plants to the constraints imposed by their sessile lifestyles. It provides plants with an enormous capacity to adapt by generating new structures or replacing damaged ones. It also underlies their indeterminate growth and record-breaking longevity of some plant species.

Plant stem cells, therefore, arise in a variety of contexts and are maintained throughout the plant's life. Developmental stem cells are contained in specialized tissues called meristems. Plants have two main meristems initiated during embryogenesis and localized at the two tips of the main body axis, called the apical meristems. The shoot apical meristem (SAM) localized at the apex of the main stem produces all

aerial organs, such as leaves, flowers and primary vascular tissues. The root apical meristem (RAM) localized at the tip of the main root axis gives rise to the network of underground tissues. Together, the SAM and RAM contribute to growth along the primary plant body plan or axis. In some species, another type of meristem, called cambium, produces the secondary vascular tissues, xylem and phloem, contributing to growth along the lateral axis. Outside of the primary meristems and cambium, plant stem cells can also be regenerated by de-differentiation of some tissues to generate new axes of growth in the shoot and root, or in response to wounding.

The capacity to produce unlimited new organs and repair wounded ones over the whole life of the organism poses the problem of maintaining and regenerating stem cells over a long span of time and in multiple cellular contexts. In plants, this is possible due to a great capacity to de-differentiate and change cell fate depending on context and positional cues. Despite the diversity of contexts in which plant stem cells are active there is an overarching theme to the way they are organized into meristems, their specification and the maintenance of a stable pool of undifferentiated and proliferating cells. Whether in the shoots, roots or regenerating organs, a conserved set of transcriptional and hormonal signalling pathways interact to regulate meristem function.

Note on Tissue Coordination in Plants Plant cells are surrounded by cell walls that constrain their movement, fixing them in the position they arise via division. An emerging property of this constraint is that cell–cell communication plays an important role in cell fate specification. This is in contrast with animal development where lineage-specific fate is a major stem cell specification mechanism and cells can migrate to their final niche. The importance of cell–cell communication in plants is highlighted by the many non-cell autonomous actors in cell fate specification. In addition, coordination of developmental and cellular events at a tissue and organ level requires mid to longer range signals. In plants, hormones (phytohormones) often play this role, bridging the cellular and tissue scales.

In this chapter, we will focus on the mechanisms of stem cell initiation and maintenance that have been highlighted in the plant model species *Arabidopsis thaliana*. Elegant work in other species such as tomato, petunia, rice and maize has brought to light deeper conservation of these mechanisms at an evolutionary scale.

7.2 Primary Meristems Organization

As mentioned briefly, plant stem cells arise in different contexts. Here and in the following sections, we will concentrate on the two main stem cell populations which are localized at strategic places in the plant called the apical meristems (Fig. 7.1). The meristem is a specialized tissue that coordinates cell proliferation and organ or tissue differentiation. It contains the stem cells proper, additional proliferating cells and the mitotically less active niche cells that induce and maintain stem cell identity in a non-cell autonomous way.

The terms ‘meristematic cells’ and ‘stem cells’ are sometimes used interchangeably. While meristematic cells may include the broader range of cell types in a meristem, here we will use ‘stem cells’ to designate specifically the cells that remain undifferentiated and proliferating at the centre of the meristem.

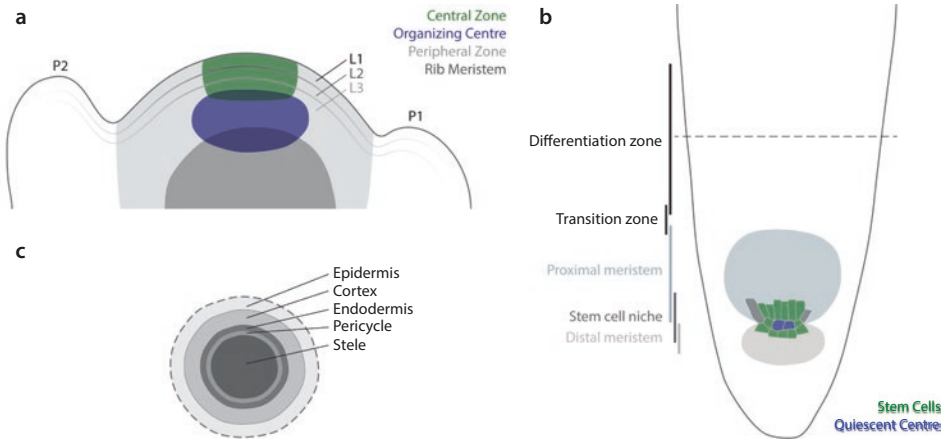

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Fig. 7.1 Schematic representation of *Arabidopsis thaliana* apical meristems. **a** Depiction of the shoot apical meristem. The central bulge contains the meristem proper which can be divided into the central zone (CZ, green), organizing centre (OC, blue), peripheral zone (PZ, light grey) and rib meristem (RM, darker grey). The three distinguishable cell layers are indicated (L1–L3), as well as two developing organ primordia (P1, P2) which are separated from the meristem by a dip indicating the boundary zone. **b** Depiction of the root apical meristem and radial organization of root tissues. The meristem is divided into stem cell niche, distal and proximal meristems. The stem cell niche is further decomposed into the quiescent centre (blue) and stem cells or initials (green). At its proximal end, the meristem is flanked by the transition zone. In the differentiation zone, the cell files acquire their mature identities and the concentric organization of tissues can be seen. **c** Radial organization of root tissues at the level of the dashed line in **c**. From the outside, the root is organized into epidermis, cortex, endodermis, pericycle and the stele where vascular tissues differentiate. More detailed views of **a** and **b** can be found in Figs. 7.3 and 7.4, respectively

7.2.1 Organization of the Shoot Apical Meristem

The shoot apical meristem contributes the cells that will produce shoot growth along the main body axis, but also all cells in aerial lateral organs such as leaves, lateral shoots (via axillary meristems) and flowers (via inflorescence and floral meristems).

From an anatomical point of view, the shoot apical meristem can be described by two well-defined cell layers, called the L1 and L2, covering a third cell layer, L3, and a deeper mass of less organized inner cells (Fig. 7.1a). The L1 gives rise to the epidermis in one continuous layer of cells across all aerial tissues. The L2 and L3 produce the ground internal tissues and the primary vascular tissue.

Conceptually, the SAM can be further organized into four zones that have been identified based on the properties of the cells within, or the expression of certain molecular markers [1, 2]. At the centre of the apex and spanning all three layers, the *central zone* (CZ) contains the pool of undifferentiated stem cells, as defined by the expression of plant stem cell marker, *CLAVATA3*. Below the CZ sits the *organizing centre* (OC) which transmits positional cues to the cells above it to induce and maintain their stem cell identity. Surrounding the OC and CZ, and creating a transition zone between the meristem and organ initiation, is the *peripheral zone* (PZ). Underlying these zones, deeper within the apex, is the rib zone or *rib meristem* (RM) where proliferating cells contribute to main shoot growth.

The stem cells contained in the CZ divide slowly. As they divide, some of the daughter cells stay in the CZ replenishing the stem cell pool while others are pushed into the peripheral zone. In the PZ, cells divide more quickly amplifying the initial population. After a few rounds of division, cells reach the periphery of the meristem where they are incorporated into organ initiation as organ primordia [2]. As we will see in future sections, the integrity of the meristem depends on a strong coordination of all these cellular events.

7.2.2 Organization of the Root Apical Meristem

The root apical meristem contains the pool of stem cells that give rise to the underground tissues. Along with the SAM, it contributes to the main growth axis of the plant. Additional growth axes also occur in underground tissues and can be formed by root branching. This, however, is not a direct consequence of RAM activity but of de-differentiation of pericycle cells in the mature root.

Although analogous structures can be found in the SAM and RAM, these are organized in different ways. Localized at the tip of the primary root, the RAM is composed of the stem cell niche, the distal meristem and the proximal meristem (◘ Fig. 7.1b). The *stem cell niche* comprises the *quiescent centre* (QC) and the surrounding *stem cells or initials* [3]. Like the OC in the SAM, the mitotically inactive QC provides the positional cues that specify stem cell identity and suppress differentiation [4]. However, while in the SAM stem cells make up a mass localized above the OC, in the root, stem cells are found in a single layer directly around the QC, thus ensuring that all stem cells maintain cell to cell contact with the instructing niche.

Root stem cells are also called initials as they are the first, or initial, cells in the files that give rise to the different root tissues in a stereotypical root organization. The initials below the QC belong to the *distal meristem* region and will produce the columella and lateral root cap cells. The initials to the sides and above the QC will give rise to the epidermis, cortex, endodermis, pericycle and vascular tissues and belong to the *proximal meristem* zone. The proximal meristem connects to the *transition zone* just above it, where cells proliferate and after a few rounds of cell division exit the meristem entering the *differentiation zone* where they elongate and acquire a fate.

7.3 Stem Cell Initiation

The shoot and root apical meristems, containing the two main populations of plant stem cells, are initiated during embryogenesis, as the embryo is patterned into the apical and basal poles, by the action of interacting hormone signalling pathways and transcriptional networks.

Although the first signs of the shoot and root apical meristems and can be seen at mid-embryo stages, the precise series of cell division patterns that eventually gives rise to these cell populations can be traced to the first asymmetric division of the single-cell zygote (◘ Fig. 7.2) (reviewed in [5, 6]). This first mitosis splits the zygote into a small apical cell that will give rise to most embryo tissues, and a large basal cell that will produce the extra-embryonic suspensor and the basal-most embryonic

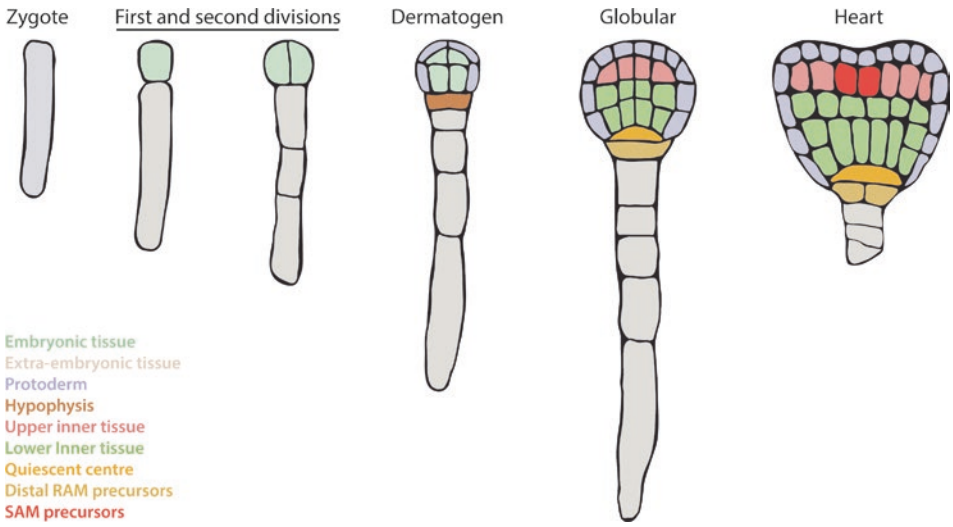


Fig. 7.2 Schematic representation of key stages in *Arabidopsis thaliana* embryo development. All stages are represented in longitudinal section. The first division produces the small apical cell (light green) and the larger basal cell (light grey). Both apical and basal cells go on to divide. The apical cell produces most of the embryonic tissues. By the dermatogen stage, the apical region splits into an outer layer, called the protoderm (light purple), and an inner mass of cells. The inner cells further divide into upper tissue (pink) and lower tissue (green). By early heart stage, the two cotyledon bulges can be seen in the apical region of the embryo, and between them the precursors of the SAM start to express meristem markers. The basal cell produces the extra-embryonic tissue called suspensor, as well as the basalmost embryo tissues. At the dermatogen stage, the top cell of the suspensor divides asymmetrically producing the hypophysis (brown). This cell further divides to give rise to the quiescent centre precursor (yellow) and the precursors to the distal RAM

tissues, including the root stem cell niche. As the apical cell divides, the embryo transitions to the dermatogen stage where the future L1 (protoderm) and the inner tissues become individualized. At the globular stage, the inner tissues further subdivide. The lower inner tissues acquire vascular precursor identity while the upper inner tissues will contribute to the shoot meristem niche which starts to be visible by the heart stage embryo, between the two cotyledon bulges. Meanwhile, at the dermatogen stage, the uppermost cell of the suspensor called the hypophysis divides asymmetrically forming the precursors to the root quiescent centre and the distal meristem cells.

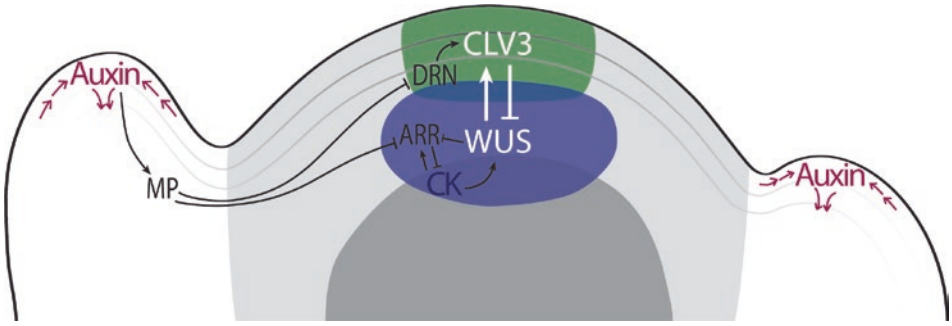
The patterning of the embryo into apical and basal regions involves several transcriptional and signalling pathways. Among those, the phytohormones auxin and cytokinin (CK) play crucial roles in the specification of the shoot and root identities. This was elegantly demonstrated by Skoog and Miller [7] who experimented with different ratios of auxin and CK in growth media. They showed that a high CK/auxin ratio promotes shoot formation and a low ratio promotes root differentiation. More recent experiments confirmed the role of auxin and CK in embryo patterning into apical and basal poles [8] and detailed how their signalling pathways interact with transcription factors to initiate meristems. Below we will explore the mechanisms and molecular pathways involved in the specification of stem cell identity at the apical and basal poles.

7.3.1 Shoot Apical Meristem Initiation

Our understanding of shoot meristem maintenance predates the efforts to unveil the mechanisms of its origin. Hence, candidates for the initiation of shoot meristem stem cells during embryogenesis have been borrowed from the extensively researched field of mature SAM organization.

At the centre of mature SAM maintenance and activity is a feedback loop between shoot stem cell marker *CLAVATA3* (*CLV3*) and stem cell-inducing factor *WUSCHEL* (*WUS*) (◘ Fig. 7.3 detailed in ► Sect. 7.4.1). In the embryo, *WUS* expression in the presumptive OC precedes the first signs of shoot apical meristem initiation and the concomitant expression of *CLV3* [9, 10]. However, despite sustaining *CLV3* expression in the mature plant WUS is not required for stem cell initiation in the embryo [11]. Instead, the WUSCHEL-RELATED HOMEOBOX (WOX) transcription factors in the WOX2 module (comprised of WOX2 and the closely related WOX1, WOX3 and WOX5) contribute to shoot stem cell initiation and embryo patterning by promoting the expression of *CLV3* and shoot patterning genes of the class III HD-ZIP (Homeodomain Leucine Zipper) family of transcription factors [12]. The WOX2-module roles in embryo patterning and shoot meristem specification are partially achieved by regulating the auxin/cytokinin balance in this tissue, promoting cytokinin signalling and repressing auxin transport in the presumptive shoot meristem [12, 13]. How cytokinin signalling helps promote shoot meristem identity is still largely unknown. However, seedlings with impaired cytokinin signalling in *ARABIDOPSIS RESPONSE REGULATOR* (*ARR*) overexpressing lines have arrested meristem phenotypes, highlighting its importance in meristem development [14].

Another early regulator of shoot meristem activity is the class II KNOX (KNOTTED-LIKE HOMEOBOX) homeodomain transcription factor *SHOOT MERISTEMLESS* (STM). Loss of function *stm* mutants lack meristem formation [15]. Weak *stm* alleles, however, form a meristem that arrests shortly after initiation



◘ **Fig. 7.3** Schematic representation of the *Arabidopsis thaliana* shoot apical meristem. The CZ (green) contains the CLAVATA3 expressing stem cells. CLV3 moves to the OC below it (blue) and inhibits the WUSCHEL transcription factor. WUS in turn moves to the cells above it, promoting stem cell identity maintenance and activity via the expression of *CLV3*. The two side bulges represent organ primordia, where cells enter differentiation programs. Phytohormone signalling and transcriptional network-mediated cross-talk between the meristem and the organ primordia ensures that a stable pool of stem cells is maintained, and meristem size remains constant. ARR, ARABIDOPSIS RESPONSE REGULATOR. CK, cytokinin. DRN, DORNROSCHEN. MP, MONOPTEROS. See main text for details

[16] suggesting that STM may not be required for stem cell specification in the embryo but rather to maintain meristematic activity levels at later stages. Accordingly, *CLV3* expression is not completely lost in *stm* mutants [11].

7.3.2 Root Apical Meristem Initiation

The root apical meristem is first evident at the mid-globular stage with the asymmetric division of the hypophysis which produces QC precursor cells, expressing the future QC marker *WOX5* [17]. Upstream of *WOX5* expression, an intricate network of transcription factors and auxin-dependent signalling, specifies the root stem cell niche.

WOX5 expression is mediated by auxin signalling via the AUXIN RESPONSE FACTORS, ARF10 and ARF16 [18]. Asymmetric distribution of auxin efflux carrier PIN-FORMED1 (PIN1) creates an auxin flow that results in a response maximum in the uppermost cell of the suspensor as detected by the auxin response reporter DR5 [8]. Concomitantly, the auxin response factor MONOPTEROS (MP or ARF5) mediates auxin signalling in the basal embryo region, inducing the expression of *PLETHORA* (*PLT*) genes [19]. *PLT* genes encode APETALA2-type transcription factors that feedback on PIN1 distribution to reinforce the auxin distribution pattern [20]. In the incipient embryonic root region, PLT transcription factors specify root stem cell identity [19] and pattern the meristem zones through protein gradients that inhibit differentiation and promote cell proliferation [21].

In a parallel pathway, the GRAS-type transcription factors SHORTROOT (SHR) and SCARECROW (SCR) help pattern the root radial organization specifying QC centre activity and stem cell identity [22, 23]. SHR and SCR induce expression of *WOX5* in the QC [18] which may partially account for their roles in the specification of both QC activity and, non-cell autonomously, stem cell identity in the surrounding cells (Fig. 7.4) [23].

A third set of genes may play a role in root meristem specification by regulating early embryo patterning. Studies of *POLTERGEIST* (*POL*) and *POLTERGEIST-LIKE1* (*PLL1*) double mutants show a role in early embryo cell division and apical-

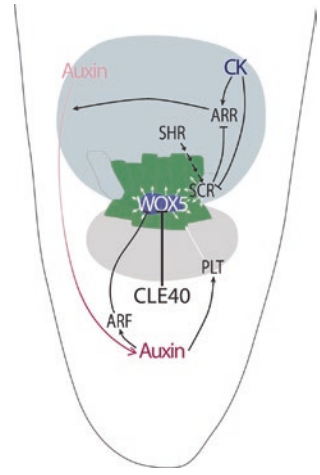


Fig. 7.4 Schematic representation of *Arabidopsis thaliana* root apical meristem (RAM) depicting some of the signalling pathways involved in its function. Stem cells (green) are maintained undifferentiated and proliferating by positional cues from the quiescent centre (blue). The stem cell niche receives signals from both proximal and distal meristem regions (grey areas), and differentiating tissues, and feeds back to them to coordinate proliferation and differentiation. ARF, AUXIN RESPONSE FACTOR. ARR, ARABIDOPSIS RESPONSE REGULATOR. CK, cytokinin. PLT, PLETHORA. SCR, SCARECROW. SHR, SHORTROOT. See main text for details

basal axis patterning [24]. POL and PLL1 activity is required for *WOX5* expression and loss of meristem in *pol pll1* mutants is accompanied by the loss of *SCR* and *SHR* expression but not *PLT* [24].

7.4 Stem Cell Maintenance

Although stem cells are constantly dividing to produce new organs and tissues, meristems remain at a near-constant size throughout plant development. To achieve meristem maintenance, plants must coordinate cell proliferation in the meristem niche with cell differentiation outside of it. By coordinating these two events, plants ensure a balance between the cells that remain in the meristem niche and those that leave to join organ and tissue formation.

Two main pathways are known to regulate the balance between stem cell maintenance and differentiation across meristems in plants. Central to stem cell specification and maintenance is a transcriptional and signalling feedback loop between members of the CLE (CLAVATA3/EMBRYO SURROUNDING REGION-RELATED) and WOX families. Coordinating stem cell proliferation at the centre of the meristem and cell differentiation at its periphery involves the action of phytohormones auxin and cytokinin. Crosstalk between transcriptional regulators and hormone signalling pathways integrates all the information into one coherent network.

7.4.1 Stem Cell Maintenance in the Shoot Apical Meristem

As mentioned above, the CLE–WOX feedback loop, represented here by *CLV3* and *WUS*, is a key part of the regulatory mechanisms involved in the maintenance of a stable pool of stem cells in the shoot apical meristem (Fig. 7.3). Mutations in *WUS* and *CLV3* signalling demonstrate how this dynamic regulatory loop maintains a stable pool of proliferating stem cells. *wus* mutants have a strong reduction or complete loss of the stem cell pool, illustrating WUS requirement for the maintenance of stem cell identity [25]. *clv3* mutants have an enlarged stem cell pool, suggesting that CLV3 regulates stem cell proliferation promoting differentiation [26]. *WUS* and *CLV3* have non-overlapping expression domains but their activities affect the expression of one another which led to the proposal of a non-cell autonomous feedback mechanism [9]. The details of this loop have been worked out over the last two decades and are briefly detailed below.

WUS encodes a homeodomain transcription factor that is expressed in the OC [9] and migrates to the CZ above it via cell–cell connections called plasmodesmata [27, 28]. In the CZ, the non-cell autonomous WUS signal induces the expression of *CLV3* and promotes the maintenance of an undifferentiated proliferative state [9, 29].

Under the influence of WUS, *CLV3* is exclusively expressed in the CZ where it produces a short mobile peptide that acts non-cell autonomously, diffusing away from the stem cells and into the OC below [10]. In the OC, the CLV3 peptide is perceived by a combination of receptor-like kinases triggering a signalling cascade that results in the repression of WUS [29–31]. Thus, with two elements, WUS promotion of CLV3 and CLV3 repression of WUS, the CLV3-WUS loop provides a self-regulatory mechanism for meristem size maintenance.

The signalling cascade triggered by CLV3 signalling has not been fully clarified but several of the leucine-rich-repeat (LRR) receptors and receptor-like kinases (RLK) involved are known. Among the better characterized receptors involved in CLV3 signalling is CLV1, a LRR-RLK [10, 32, 33] and the LRR/pseudo-kinase dimer CLV2/CORYNE (CLV2/CRN) [34–36]. Other members of the CLV1 family of RLK such as BARELY ANY MERISTEM1-3 (BAM1-3) and the more distantly related RECEPTOR-LIKE PROTEIN KINASE 2 (RPK2) also play a role in meristem activity and size maintenance [37–39].

One reason for our incomplete understanding of this intricate network of receptors is that the relationships between the different components are complex. For example, CLV1 and BAM1 directly bind CLV3 but the CLV2/CRN dimer and RPK2, although capable of integrating CLV3 signalling, do not [40, 41]. In addition, genetic redundancy and feedback mechanisms that add to the robustness of this system can also mask the functions of individual actors. For instance, CLV3 binding to CLV1 regulates this receptor availability at the membrane inducing its trafficking and degradation [33], while CLV1 activity negatively regulates *BAM* expression [42, 43].

The signalling cascade downstream of CLV3 that results in the reduction of WUS also remains poorly understood with only a few identified elements, like the protein phosphatases POL and PLL1. While POL and PLL1 are required for *WUS* expression, CLV1 signalling inhibits POL and PLL1 activity, thus providing one explanation for how CLV signalling might regulate WUS [44]. Additional factors contributing to the activity of WUS in meristem maintenance and activity are the KNOX transcription factor STM and the GRAS transcription factors HAIRY MERISTEM (HAM). STM contributes to meristem maintenance by suppressing differentiation and inducing cell proliferation [45]. Both WUS and STM can ectopically induce meristematic identity, including cell proliferation and eventual organ initiation [46]. While WUS and STM have similar roles in suppressing cell differentiation, STM has a broader effect maintaining an undifferentiated state of cells in the peripheral zone as well as the central zone [47]. HAM transcription factors act in parallel with WUS and STM to promote the maintenance of undifferentiated cells in the CZ [48, 49]. Despite having an effect in stem cell maintenance in the CZ, HAM transcription factors are expressed in the deeper layers of the meristem, likely acting in a non-cell autonomous way like WUS [49]. Indeed, WUS and HAM share a number of targets with roles in cell division and stem cell identity maintenance [50].

The events downstream of WUS activity in the OC and CZ are not entirely elucidated. Nevertheless, two genome-wide studies of transcriptional targets provide insight into the mechanisms of WUS regulatory activity. Notably, they show that WUS works as a repressor, limiting the expression of differentiation factors like *ASYMMETRIC LEAVES 2* and *KANADI*, but also promoting the expression of shoot stem cell specification factors such as *TOPLESS* [51, 52].

7.4.2 Stem Cell Maintenance in the Root Apical Meristem

Stem cell maintenance in the RAM reiterates the basic elements of the CLE–WOX feedback loop found in the shoot, while also echoing some of the stem cell initiation mechanisms reviewed in ► Sect. 7.3.2.

In the root, CLE–WOX clades are represented by *WOX5* and *CLE40* (Fig. 7.4). Like WUS, WOX5 marks the niche that induces stem cells, which in the root consists of the mitotically inactive quiescent centre. Similarly, WOX5 acts non-cell autonomously moving to the adjacent cells to promote the maintenance of an undifferentiated state [18]. On the other side of the loop, the CLE-related mobile peptide CLE40 is produced in differentiated root cap cells. CLE40 is perceived by CLV1 and ARABIDOPSIS CRINKLY 4 (ACR4) in both the initials and the differentiated cells in the distal meristem, restricting *WOX5* expression to the QC [53]. The CLV2/CRN complex is also expressed in the root and mediates CLE40 signalling in the proximal meristem [36]. Likewise, BAM1 and RPK2 mediate CLE signalling in the root to regulate cell proliferation [54].

One regulator of *WOX5* expression is REPRESSOR OF WUSCHEL 1 (ROW1), a plant homeodomain protein involved in transcriptional regulation via chromatin modification [55]. ROW1 was first identified in the shoot apical meristem where it is required to repress WUS [56]. In the root, ROW1 restricts *WOX5* expression to the QC, repressing it in the proximal meristem [55].

In parallel to the CLE40-WOX5 loop, root stem cells are maintained by the PLT and SHR/SCR pathways initiated during embryonic development. The auxin response maximum at the root tip induces the expression of *PLT* in the QC [20]. PLT proteins diffuse via cell–cell connections and form a gradient that tails towards the transition zone and patterns the meristem in a dose-dependent manner [57]. High PLT levels maintain stem cell activity while lower levels allow cell differentiation [21]. The two pathways are linked by WOX5, which upregulates *PLT* expression in distal stem cell activity maintenance linking the two pathways [58].

Recapitulating its role in the embryo, in the mature root, *SHR* is expressed in the stele and moves to the QC inducing the expression of *SCR* [22, 59]. SCR in turn prevents SHR from moving to additional cell layers by sequestering it in the endodermis initials [60]. SHR and SCR maintain stem cell activity non-cell autonomously, partly by regulating cell-cycle genes [23, 61]. Downstream of SHR/SCR, the zinc-finger proteins MAGPIE and JACKDAW help maintain *SHR* expression domains and preserve asymmetric cell division in the stem cell niche [62].

7.4.3 The Role of Hormones in Stem Cell Maintenance in Plant Meristems

In the SAM, the CLV3-WUS network interacts with cytokinin signalling to regulate meristem activity. Cytokinin acts at the centre of the meristem inhibiting cell differentiation, and promoting cell proliferation and meristematic maintenance of stem cells via WUS activation [63, 64]. WUS in turn represses the expression of *ARR* family members [14]. ARR proteins are negative regulators of cytokinin signalling, thus producing a positive feedback on *WUS*' own expression. The combinatorial effects of cytokinin and CLV3-WUS signalling in the apex are sufficient to explain the WUS domain position in the shoot apical meristem in a minimal computational model [65]. Auxin acts oppositely at the periphery of the meristem, inducing organ primordia initiation and cell differentiation [66]. Like the cross-talk in the CZ and OC mediated by CLV3 and WUS, the central zone and peripheral zone hormone

signalling pathways also feedback on each other. Notably, MP-mediated auxin signalling represses ARR members, promoting cytokinin response and *WUS* expression [64]. In addition, MP-mediated auxin signalling directly represses the expression of APETALA2 type factor *DORNROSCHEN* (*DRN*) [67]. *DRN* is expressed in the central zone where it promotes *CLV3* expression and stem cell maintenance [67, 68].

In the RAM, the auxin/cytokinin balance also plays a role in maintaining a balance between cell division and cell differentiation. However, in the RAM their roles are reversed, with auxin inducing stem cell positioning and cytokinin promoting cell differentiation [69]. Auxin polar transport and biosynthesis in the root creates a response maximum in the distal meristem and a gradient that tails towards the differentiation zone. The auxin response maximum is required for QC maintenance [20], while ARF-mediated signalling in the distal meristem promotes differentiation of root tip cells and limits *WOX5* expression to the QC [58]. The auxin gradient helps guide root patterning [70] with the auxin minimum defining the boundary between proliferation and differentiation zones [71]. Cytokinin accumulation in the transition zone promotes cell differentiation [72] and inhibits stem cell identity genes *SCR* and *WOX5* [73]. Cytokinin and auxin interact in a context-dependent manner to achieve a dual balance. In the proximal meristem, SCR inhibits cytokinin signalling factor ARR1. ARR1 in turn stimulates auxin biosynthesis in the stem cell niche, tipping the balance towards auxin activity and stem cell maintenance [74]. In the transition zone, however, *ARR1* expression in turn represses auxin accumulation via the PIN1 transporter, tipping the balance towards cytokinin [74].

Other phytohormone groups like gibberellins and brassinosteroids have also been shown to promote root meristem activity and root growth by regulating cell proliferation in the stem cell niche, but their mechanisms in this context are still poorly known [75, 76].

7.4.4 Conservation of Meristem Maintenance Mechanisms in Plants

There are a number of elements that suggest the fundamental mechanisms that regulate stem cell activity in plants are conserved. In addition to being present in both apical meristems, the CLE and WOX family members share deeper functional homologies. WUS and WOX5, for example, can replace each other's functions in shoot and root stem cell maintenance [18], while CLE40 can substitute for CLV3 in the shoot meristem and CLV3 can promote distal meristem differentiation in the root [53, 77]. Furthermore, the same overlapping network of receptor-like kinases is active in CLV signalling in both the shoot and the root meristems [36, 42]. The CLE–WOX module is further conserved in the maintenance and differentiation of the vascular meristem (cambium). Like WUS, WOX4 maintains cell proliferation in the cambium. However, unlike CLV3 and CLE40, CLE41/44 signalling induces the expression of *WOX4* rather than repressing it [78]. In addition, CLE41 and CLE44 signal through a novel LRR-RLK called PHLOEM INTERCALATED WITH XYLEM (PXY) [79]. Like other LRR-RLKs, PXY mediates *WOX4* expression through an as yet unknown mechanism [79].

The same two hormonal pathways are also involved in the specification and maintenance of meristem activity in different contexts with interesting implications. While

auxin and cytokinin signalling have opposing effects in the meristem context, their roles are reversed from one meristem to the other. Although not yet understood, this reversal of roles could be part of a long-distance communication mechanism between the two main meristems. Meristem activity in root and shoot could thus be coordinated in response to overall auxin/cytokinin levels. Such a mechanism could allow a balanced growth of aerial and underground tissues and a quick integrated response to environmental changes.

Take-Home Message

- Plant development occurs mostly post-embryonically in an indeterminate fashion, with nearly constant growth and production of new organs.
- The indeterminate growth mode of plants is possible due to the continuous activity of plant stem cells which remain active throughout the plant's life.
- Plant stem cells are organized into specialized cell niches called the meristems.
- Meristems are responsible for maintaining a stable pool of stem cells, coordinating cell proliferation and cell differentiation.
- Two key signalling pathways are involved in meristem patterning and activity, including the specification of stem cell identity and the promotion of cell proliferation.
- The feedback loop between CLE and WOX family members ensures communication between stem cells and the organizing or quiescent centre, creating a self-regulatory mechanism that maintains a stable pool of stem cells.
- The phytohormones auxin and cytokinin keep a balance between cell proliferation in the centre of the meristems and cell differentiation at the periphery.

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