Plant and animal stem cells: similar yet different

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Abstract | The astonishingly long lives of plants and their regeneration capacity depend on the activity of plant stem cells. As in animals, stem cells reside in stem cell niches, which produce signals that regulate the balance between self-renewal and the generation of daughter cells that differentiate into new tissues. Plant stem cell niches are located within the meristems, which are organized structures that are responsible for most post-embryonic development. The continuous organ production that is characteristic of plant growth requires a robust regulatory network to keep the balance between pluripotent stem cells and differentiating progeny. Components of this network have now been elucidated and provide a unique opportunity for comparing strategies that were developed in the animal and plant kingdoms, which underlie the logic of stem cell behaviour.

Stem cell research is a rapidly developing field, and it is enjoying an unprecedented level of public interest owing to its therapeutic potential, such as in human tissue replacement and drug discovery. Plant stem cells, as in animals, are defined by their ability to both renew themselves and to generate daughter cells to produce new tissues. They share several common features as they are both maintained in specialized microenvironments, which are known as stem cell niches, where local signals from an organizer act to maintain the adjacent stem cells[1,2]. This structural similarity, together with the observation that plant stem cell niches can have staggering longevity and a unique regeneration capacity, has stimulated a wide interest in plant stem cell research to uncover whether molecular similarities underlie such conceptual communalities.

Plant stem cell niches are specified during embryogenesis (BOX 1), and, post-embryonically, they are maintained within an organized group of dividing cells known as the meristem (FIG. 1). Dividing stem cell progeny in the meristem are equivalent to the animal transit-amplifying cells. The activity of meristems enables plants to continuously generate new organs and structures throughout their lifetime, thus determining plant architecture. This contrasts with animal development, as the animal body plan is mostly defined during embryonic development; adults generally lack pluripotent stem cells and multipotent stem cells mainly function to maintain tissue homeostasis and in tissue repair.

In meristems, two opposite processes occur: stem cell self-renewal is stimulated to maintain the population of stem cells, and cells are recruited from the meristem into developing organs. Remarkably, the number of stem cells and their dividing daughter cells in the meristem remains constant, despite continuous displacement of differentiating cells into new organs. This indicates that the formation of new cells and cell differentiation are dynamically and almost perfectly balanced.

In this Review we focus our attention on two main meristems in the model plant *Arabidopsis thaliana*, which are responsible for almost all the growth that occurs post-embryonically: the shoot apical meristem and the root meristem, which generate above-ground and below-ground structures, respectively (FIG. 2). We describe the molecular mechanisms that underlie shoot and root stem cell niche positioning and maintenance during organogenesis. We also analyse differences and similarities between animal and plant stem cell niches, and highlight how plant stem cell research can aid and complement research in the animal field, while leading to crucial discoveries for the understanding of plant growth and behaviour.

The shoot and root stem cell niches

The organization of the shoot and the root stem cell niches is remarkably similar to that of animal stem cell niches (FIG. 1). In both cases stem cells are maintained by short-range signals that arise from specialized organizer cells that act cell non-autonomously to sustain the self-renewal capacity of stem cells[3–5]. In addition, their daughter cells undergo several rounds of divisions, which generate transit-amplifying cells that eventually differentiate at a distance (FIG. 1). It is of note that whereas

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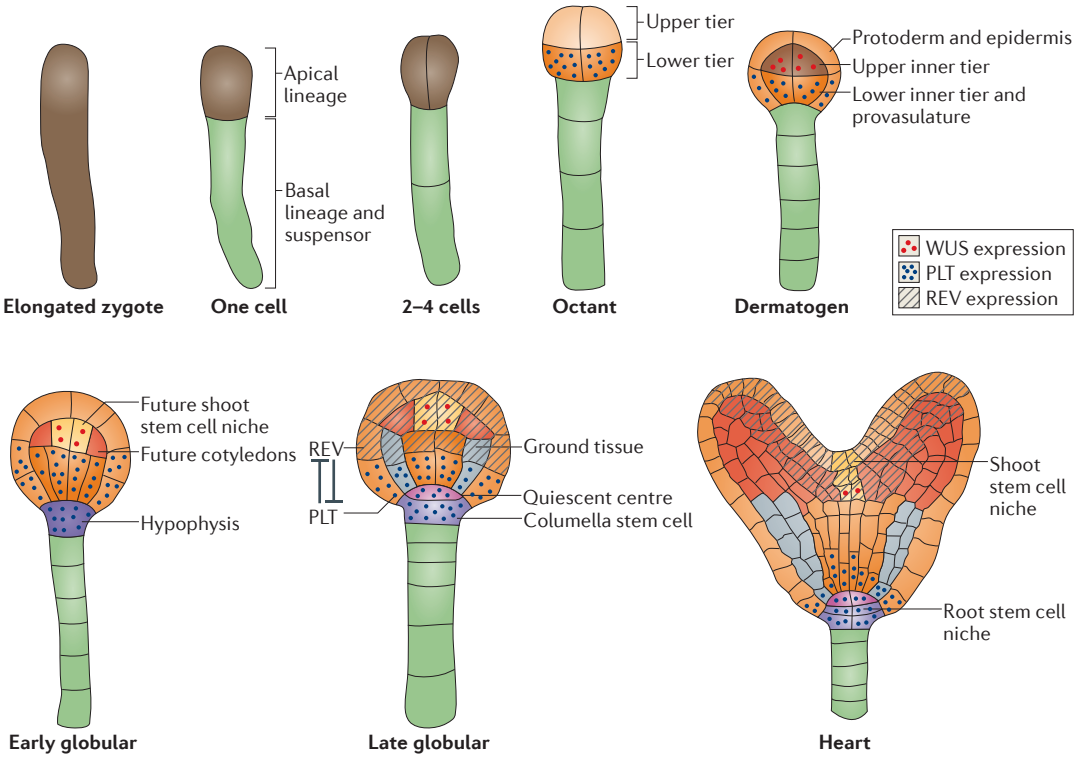
Box 1 | **Embryonic origin of plant stem cell niches**

Plant embryogenesis is different from animal embryogenesis, which involves complex cell movement and migration, as it is characterized by almost invariant cell division patterns that eventually generate the embryo body[149]. In this way, shoot and root stem cell niche positioning and specification can be traced back to cellular decisions that have been made during early embryogenesis, which provides the unique opportunity to establish a direct link between pattern formation and niche gene expression. In the model plant *Arabidopsis thaliana* the position of the future shoot and root stem cell niche can be nearly completely deduced soon after the first asymmetric zygote division. Several pathways have been linked to early asymmetric divisions, including a kinase signalling cascade[150–154], cell-specific transcription factors[155] and the plant hormone auxin[156,157]. Soon after zygote asymmetric division and after further formative divisions that characterize different stages of embryonic development (see the figure), the shoot and the root stem cell niche can be morphologically identified. Their activity is marked by specific transcription factors such as *WUSCHEL* (*WUS*)[16,17] and the CLASS III HOMEODOMAIN-Leu ZIPPER (HD-ZIP III) family member *REVOLUTA* (*REV*)[158] for the shoot stem cell niche and PLETHORA (PLT) genes for the root stem cell niche[107]. These genes provide crucial positional cues for stem cell niche specification and self-organizing properties. Ectopic expression of REV in the embryo root pole is sufficient to convert the root into a second shoot structure by antagonizing *PLT* activity[159]. Conversely, ectopic expression of *PLT* in the shoot pole causes a shoot to root transition[107,159]. Thus, the intersection of several shoot- and root-specific positional cues drives a series of formative cell divisions from the asymmetric division of the zygote onwards, which are pivotal to position and to specify the shoot and root stem cell niche during embryogenesis. How these inputs are coordinated is still not clear. Recent advances in transcriptomics approaches and imaging of the early *A. thaliana* embryo will be of great help to improve our understanding of these early embryonic events[160–162].



post-embryonic animal stem cells are used sparingly — with a few exceptions, such as in the haematopoietic system and in the intestine — plant stem cells are in a state of perpetual action, continuously producing a differentiating cell progeny. Furthermore, whereas animal stem cells are in general responsible for maintaining a specific tissue, plant stem cells generate complete organs, and as such resemble animal pluripotent embryonic stem (ES) cells.

Shoot stem cells are maintained on the basis of their position relative to the organizer, which is known as the organizing centre (FIG. 2a). The shoot meristem can be subdivided into a central zone that consists of three clonally distinct stem cell layers (L1–L3) and the underlying organizing centre, a surrounding peripheral zone of more rapidly dividing cells that differentiate to form lateral organs and an underlying rib zone that adds cells to the elongating stem. Whereas the L1 and L2 layers consist of anticlinally dividing cells that form a sheet of epidermal and subepidermal tissue, respectively, cells of the L3 layer divide in all directions to give rise to the internal tissues[6,7]. The progeny of shoot stem cells form all the above-ground tissues, which indicates stem cell multipotency.

In contrast to the shoot meristem, the root meristem contains tissue-specific stem cells. That is, stem cells that are committed to generating specific tissues of the growing root: stele, ground tissue (endodermis

Anticlinally dividing cells
Cells in which the division plane is perpendicular to the surface of the organ, thus maintaining a single cell layer.

Stele
In vascular plants, the stele is the central part of the root or stem that contains the vascular tissues.

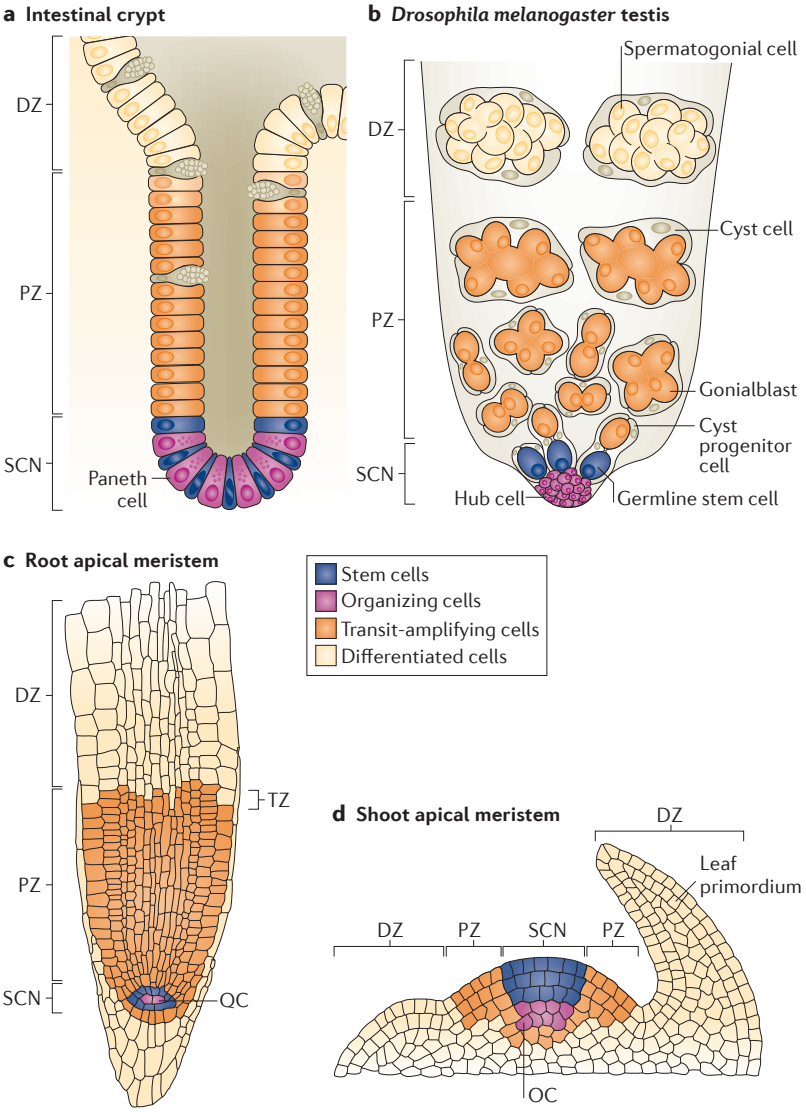


Figure 1 | **Comparing stem cell niches in animals and plants.** In all cases, stem cells (purple) are maintained by short-range signals that arise from specialized organizing cells (pink) that act cell non-autonomously. Stem cell daughter cells undergo additional divisions to generate transit-amplifying cells (in the proliferation zone (PZ); shown in orange), which eventually differentiate at a distance (in the differentiation zone (DZ)). Whereas post-embryonic animal stem cell niches (SCNs) are in general responsible for the maintenance of a specific tissue, plant stem cell niches generate complete organs. **a** | The mouse intestinal crypt. Paneth cells function to maintain the self-renewal potential of adjacent intestinal stem cells. Stem cells generate transit-amplifying cells that differentiate into the cells that constitute the intestinal epithelial layer. **b** | The *Drosophila melanogaster* testis. Hub cells signal to maintain the germline stem cells from which gonialblasts originate. The gonialblasts divide several times to form spermatogonia, which ultimately develop into sperm. **c** | The *Arabidopsis thaliana* root meristem. The quiescent centre (QC) signals to the surrounding stem cells to prevent their differentiation. Stem cell daughters divide in the PZ or meristem before reaching the transition zone (TZ), where they stop dividing and begin to differentiate. **d** | The *A. thaliana* shoot apical meristem. The organizing centre (OC) signals to maintain the overlying stem cells, which generate transit-amplifying cells that will eventually differentiate and give rise to entire organs, for example a leaf.

Columella
Specialized root cells that are involved in gravity-sensing mechanisms.

and cortex), epidermis, lateral root cap and columella (FIG. 2b). Nevertheless, despite their apparent tissue specificity, laser ablation studies have shown that stem cells can switch fate in response to positional signals from maturing cells[8]. Stem cells surround the mitotically less active quiescent centre, a group of four cells that act as an organizer to prevent their differentiation[9]. Each stem cell that is adjacent to the quiescent centre divides asymmetrically to renew itself and to produce a daughter cell that divides a number of times in the meristematic zone before exiting the cell cycle in the transition zone. Subsequently, cells elongate and acquire a specific differentiation status (FIGS 1,2b). Stem cells that are distal to the quiescent centre produce daughter cells that differentiate, without further rounds of division, into gravity-sensing columella cells that are ultimately shed by the growing root.

The function of the organizing centre and quiescent centre resembles that of the hub cell in the *Drosophila melanogaster* male germline stem cell niche[10] and that of the Paneth cell in the mouse intestinal epithelium[4] (FIG. 1). Although much remains to be uncovered about the signals that are produced by the organizing centre and quiescent centre, a growing number of genes and pathways that underlie organizing centre and quiescent centre function are involved in shoot and root stem cell maintenance.

Regulating stem cell numbers

Maintaining stem cell homeostasis in the shoot and root stem cell niches is essential to ensure that an equal number of new cells are generated to replace those that are displaced from the niche, to differentiate and to enable the growth and formation of new tissues and organs. Remarkably, the RETINOBLASTOMA-RELATED (RBR) protein, the plant homologue of the RB tumour suppressor protein, has a crucial role in both niches[3,11]. As in animals, RBR inhibits cell cycle progression by interacting with an E2F transcription factor homologue[12]. Moreover, reduced levels of RBR result in an increase in stem cell numbers, and increased RBR levels lead to stem cell differentiation, which indicates a prominent role for RBR in stem cell maintenance[13–15]. At present, RBR is the only known protein involved in stem cell function that is conserved between the animal and plant kingdoms.

Maintaining shoot stem cells. Maintenance of a stable shoot stem cell pool mainly involves a feedback loop between the homeodomain protein WUSCHEL (WUS) and a ligand–receptor signalling cascade, which is collectively known as the CLAVATA (CLV) pathway. Expression of the *WUS* gene defines the organizing centre and the WUS protein acts as a non-autonomous signal to maintain stem cells and, at least in meristems, is sufficient to promote stem cell identity[16–21]. Restricting WUS movement results in premature loss of stem cells, which indicates that its movement is important for stem cell maintenance[22]. WUS binds to and activates the promoter of *CLV3*, which encodes a signal peptide that is expressed in stem cells and, in turn, CLV3 signalling represses *WUS* transcription[20,22–27] (FIG. 3a). However, although *CLV3* is a marker for stem cells, *clv3* mutants retain a stem cell population, which suggests that the CLV3 protein is not essential for stem cell specification,

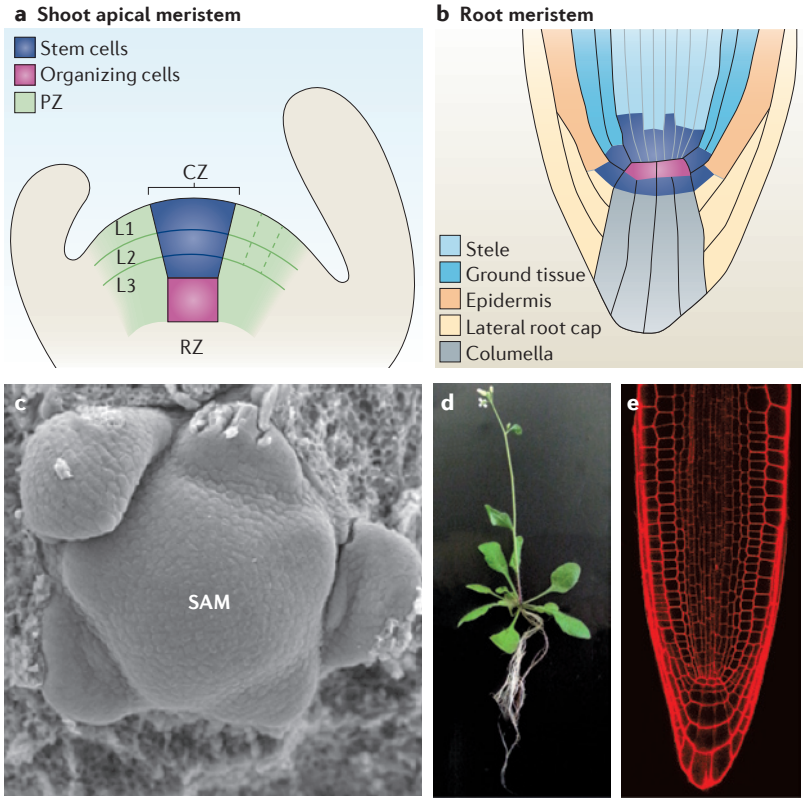


Figure 2 | **Organization of the shoot and root stem cell niches.** **a** | Shoot stem cell niche. The central zone (CZ) of the shoot apical meristem contains the organizing centre (pink) and the overlying stem cells (purple), which generate daughter cells that divide while traversing the meristem peripheral zone (PZ) before differentiation and incorporation into lateral organs such as leaves. The rib zone (RZ) lies below the CZ and PZ; division and elongation of rib meristem cells give rise to the stem of the plant. Lines indicate lineage relationship in the different tissues with anticlinally dividing stem cells in layer 1 (L1) and L2 (the dashed lines indicate the division planes). Stem cells in L3 divide into all directions and their progeny are also incorporated into the growing stem. **b** | Root stem cell niche. The root meristem is comprised of concentrically arranged tissue layers (shown in different colours). The quiescent centre (pink) is surrounded by tissue-specific stem cells (purple), the progeny of which sustain root growth. Lines indicate the lineage relationship of stem cells and their progeny. **c** | Scanning electron micrograph of an *Arabidopsis thaliana* shoot apical meristem (SAM), which is flanked by leaf primordia. **d** | Adult *A. thaliana* plant. **e** | Confocal laser micrograph of an *A. thaliana* root meristem which was treated with propidium iodide (red fluorescence). Part **c** from REF. 179, Nature Publishing Group.

Hub cell
Specialized cell of the *Drosophila melanogaster* testes that is necessary to maintain the adjacent stem cell.

Paneth cell
Specialized cell of the intestinal epithelium, which secretes factors that sustain the self-renewal capacity of the contacting stem cell.

E2F transcription factor
Member of a family of transcription factors that, by interacting with other proteins, control cell cycle progression.

although it has a signalling role[24]. The mature CLV3 ligand, which is generated by post-translational cleavage and modification[28–31], is perceived by three main receptor complexes that are present in the organizing centre and in surrounding cells[32–37] (FIG. 3a). The phosphatases POLTERGEIST (POL) and POLTERGEIST-LIKE 1 (PLL1) are crucial intermediates between CLV receptors and *WUS* expression[38].

Analysis of the *A. thaliana* shoot meristem transcriptome that is differentially regulated by WUS has shown that most WUS-activated genes are specific to stem cells and the organizing centre region, whereas WUS represses a large group of genes that is expressed in differentiating cells of the peripheral zone[39–41]. WUS-binding sites of different affinity have been identified and, interestingly, WUS was found to directly bind to similar conserved motifs to both activate (for example, *CLV3*) and repress (for example, *KAN1*) gene expression[22,39,40,42]. Together, these results suggest that WUS recruits local co-activators and co-repressors to target promoters, and, consistent with WUS forming a protein concentration gradient, it may regulate gene expression in a concentration-dependent manner. The communication between stem cells and the organizing centre is buffered against environmental fluctuations to maintain a stable stem cell pool. Transient increases in CLV3 levels are tolerated without causing changes in meristem size[43]. Such phenotypic stability results from WUS also directly repressing *CLV1*, which encodes a receptor-like protein kinase (that transduces the CLV3 signal), thus reducing the effect of CLV3 signalling. This suggests an autoregulatory loop whereby WUS sustains its own expression in the organizing centre[40] (FIG. 3a). Moreover, treatment with the plant hormone cytokinin (which promotes cell proliferation) induced an expansion of the *WUS* expression domain, while *CLV3* expression remained relatively stable[44,45]. ERECTA (ER) family receptor kinases were shown to redundantly play a part in buffering cytokinin effects downstream of WUS (FIG. 3a). When treated with cytokinin, *er erl1 erl2* triple mutant seedlings showed, in addition to increased *WUS* expression, greatly upregulated levels of *CLV3* expression[45]. Furthermore, cytokinin induced a striking increase in meristem size in *clv3* mutants compared to treated wild-type plants, which indicates that the CLV3 signalling pathway also acts to buffer cytokinin effects[46].

Recently, the basic helix–loop–helix (bHLH) transcription factor HEC1 was found to specifically stimulate stem cell proliferation[47]. *HEC1* is negatively regulated by WUS and the absence of HEC1 from the organizer is essential for stem cell maintenance. HEC1 has the ability to uncouple the WUS–CLV feedback loop and to skew the stem cell to organizer ratio, which indicates that it has a function in balancing proliferation versus differentiation[47].

Root stem cell maintenance — differentially similar. Similar to the situation in the shoot niche, *WUSCHEL-RELATED HOMEOBOX 5* (*WOX5*), a homologue of *WUS*, marks the quiescent centre from inception onwards. Loss of *WOX5* function specifically results in differentiation of the distal columella stem cells without altering root growth and meristem size[48]. Nevertheless, mutant analyses indicate WOX5 redundantly controls proximal stem cell maintenance[48]. Recently, a model has been proposed in which CLAVATA3/ESR-RELATED 40 (CLE40) peptide signalling counteracts WOX5-mediated stem cell-promoting effects to enable distal stem cell differentiation (FIG. 3b). The absence of *WOX5* enabled CLE40 to induce quiescent centre differentiation towards columella cell fate[49]. CLE40 is a homologue of the CLV3-signalling peptide that is mentioned above and acts through the CRINKLY 4 and Leu-RICH REPEAT (LRR) family receptor-like kinases ACR4 and CLV1, with CLV1 also being involved in the CLV3-mediated shoot stem cell regulation. CLE40 expression from differentiated columella cells promotes distal stem cell differentiation[49,50].

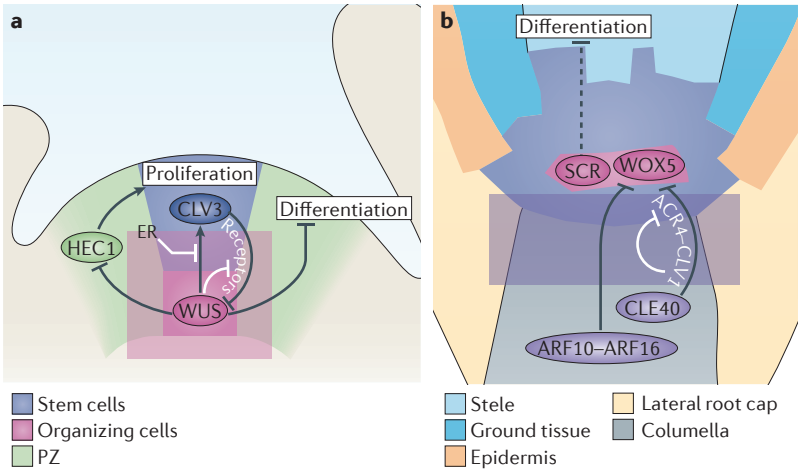


Figure 3 | **Maintenance of the stem cell niche.** **a** | In the shoot stem cell niche, WUSCHEL (WUS) is expressed in the organizing centre (pink) and moves to induce CLAVATA 3 (CLV3) expression in the stem cells (purple). In turn, CLV3 signals to repress *WUS* expression through its receptors that are expressed in an organizing centre encompassing region (pink shading). The WUS–CLV3 feedback loop is further influenced by the action of ERECTA (ER) family receptors to buffer the effects of WUS signalling, whereas WUS represses receptor gene expression and sustaining its own expression (white inhibitory arrows). WUS also inhibits *HEC1* in the organizing centre, which induces stem cell proliferation and WUS represses differentiation-promoting genes at a distance in the meristem. **b** | In the root stem cell niche, WUSCHEL-RELATED HOMEOBOX 5 (WOX5) is expressed in the quiescent centre (pink) and inhibits distal stem cell (purple) differentiation. The CLAVATA3/ESR-RELATED 40 (CLE40) peptide from differentiated columella cells signals through its receptors (ACR4–CLV1) that are expressed below the quiescent centre (purple rectangle) to counteract the WOX5 activity and to promote cell differentiation. The CLE40 activity is buffered by the effect of CLV1 on ACR4-mediated signalling (white inhibitory arrow). Stem cell fate is also restricted by AUXIN RESPONSE FACTOR 10 (ARF10)–ARF16 signalling. The *SCARECROW* (*SCR*) gene acts cell autonomously for the quiescent centre function and cell non-autonomously to control transit-amplifying cell differentiation (dashed inhibitory arrow).

Conversely, additional columella stem cells form distal to the quiescent centre in *cle40*, *acr4* and *clv1* mutants, which is correlated with an expanded *WOX5* expression domain[49–51]. Surprisingly, CLV1 signalling seems to decrease ACR4-dependent signalling (FIG. 3b). This, together with the finding that receptor complexes localize at plasmodesmata led to the hypothesis of a ligand–receptor complex, which functions to physically restrict the exchange of 'stemness information' beyond adjoining quiescent centre and stem cells[50,52].

WOX5 and *WUS* are functionally interchangeable in shoot and root stem cell maintenance and CLE40 can substitute CLV3 in shoot signalling[48,53], which provides a link between shoot and root stem cell regulation. However, the effect of the CLE40–ACR4–CLV1 module on *WOX5* expression may be indirect, and no feedback from *WOX5* towards *CLE40* is known. Whether WOX5 moves, like WUS, to locally prevent stem cell differentiation is not known.

Interestingly, CLE40 signalling by differentiated columella cells to control the self-renewal capacity of their stem cell progenitors resembles what has been observed in several animal stem cell niches. Recent studies suggest that committed stem cell progeny provide versatile feedback signals to their stem cell parents, thus becoming an indispensable component of the niche[54]. For example, in the *D. melanogaster* haematopoietic niche, signals from differentiating haemocytes were found to regulate haematopoietic progenitor quiescence[55].

Another pathway that has been found to restrict distal stem cell daughter fate and to promote columella cell differentiation relies on auxin. The root cap-specific expression of the redundantly acting *AUXIN RESPONSE FACTOR 10* (*ARF10*) and *ARF16* is dependent on auxin and the microRNA (miRNA) miR160, which act independently[56]. Distal stem cell daughters fail to differentiate, and they retain quiescent centre identity in *arf10 arf16* double mutant roots, whereas increased auxin levels and ARF16 activity reduce *WOX5* expression and increase distal stem cell differentiation[56,57]. Given their non-overlapping expression patterns, ARF10 and ARF16 may restrict the *WOX5* domain, which might involve CLE40 or related peptide signalling to regulate stem cell maintenance from a distance (FIG. 3b).

Finally, the SCARECROW (SCR) transcription factor, which is required for quiescent centre specification (see below), is involved in both stem cell maintenance and the differentiation of their progeny[58,59]. In the quiescent centre, SCR directly represses the expression of the differentiation promoting cytokinin-response transcription factor ARR1 (REFS 59,60). Ectopic ARR1 accumulation in *scr* mutants results in higher auxin levels and auxin responses in the quiescent centre, because it activates auxin biosynthesis genes, leading to stem cell loss[59]. These findings are in agreement with data suggesting that high levels of auxin promote stem cell differentiation[57]. Importantly, this local fine-tuning of auxin production in turn enables SCR to control non-autonomously, from the quiescent centre, *ARR1* levels in the distal meristem transition zone where ARR1 promotes differentiation commitment, thereby restricting meristem size[59,60]. Such an effect from a distance was also observed in the shoot meristem after experimentally-induced downregulation of *WUS* expression: the area of the primordial auxin response maximum was broadened, extending towards the meristem centre and thus leading to enlarged organ primordia. This suggests a long-range WUS-dependent control of auxin signalling and/or auxin-mediated patterning[21]. Hence, the root and shoot stem cell niche organizers not only control the activity of surrounding stem cells but also regulate differentiation of distant transit-amplifying cells.

In the root meristem transition zone, ARR1 controls transit-amplifying cell differentiation by regulating auxin signalling and distribution[60]. Changes of auxin distribution in the transition zone could affect the establishment of the auxin maximum in the root quiescent centre, which is crucial for stem cell niche function[61–70]. If so, this would be another example of how stem cell progeny feed back to their stem cell progenitors to control their activity.

Reversible stem cell daughter differentiation. Damaged stem cells in the root can be replaced by symmetric division of an adjacent stem cell that subsequently adopts the tissue fate according to positional signals[8], as is also

Plasmodesmata
Microscopic channels that traverse the cell wall of plant cells, which enables transport and communication between them.

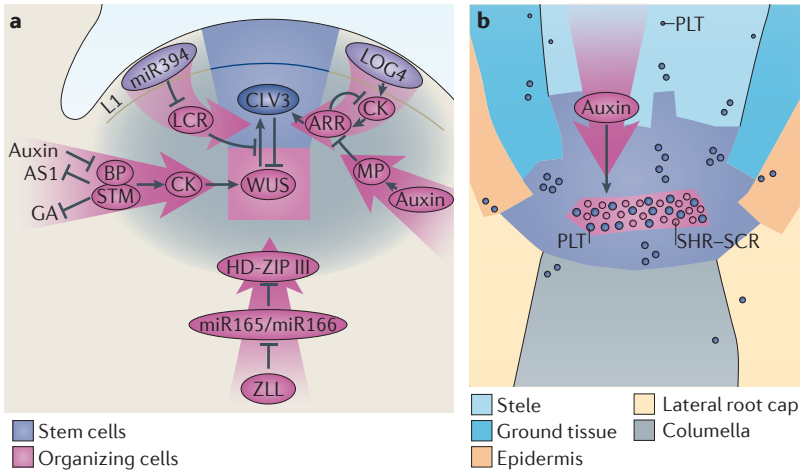


Figure 4 | **Positioning the stem cell niche.** **a** | In the shoot stem cell niche, SHOOT MERISTEMLESS (STM) inhibits the activity of the differentiation factors AS1 and GIBBERELLIC ACID (GA) in the meristem (grey), and induces cytokinin (CK) biosynthesis genes. Provascular ZWILLE (ZLL) sequesters microRNA 165 (miR165) and miR166 to enable the expression of CLASS III HOMEODOMAIN-Leu ZIPPER (HD-ZIP III) genes in the meristem. Both *STM* and HD-ZIP III genes are required for meristem activity and stem cell maintenance. The enzymatic activity of LOG4, which is expressed in layer 1 (L1), produces CK, which in turn signals through its receptors that overlap with the organizing centre to activate *WUSCHEL* (*WUS*) expression, thereby acting as a positional cue. CK effects are regulated by the induction of inhibitory ARR genes that generate a negative feedback loop, whereas WUS inhibits these ARR genes to reinforce its expression. Auxin signalling through MONOPTEROS (MP) inhibits the expression of ARR genes to exclude them from the periphery and to localize the stem cells, through ARR stimulated *CLAVATA 3* (*CLV3*) expression, above the organizing centre. miR394 moves from L1 to inhibit the differentiation factor LEAF CURLING RESPONSIVENESS (LCR) and to maintain WUS-directed stem cell activity, thereby providing positional information for stem cell competence. **b** | In the root stem cell niche, auxin accumulation in the quiescent centre acts a positional cue to localize high *PLETHORA* (*PLT*) and *BABY BOOM* (*BBM*) (*PLT/BBM*) expression. PLT/BBM proteins accumulate in the quiescent centre to form an instructive gradient. In parallel, SHORT ROOT (SHR) and SCARECROW (SCR) form a complex to specify the quiescent centre. Expression of SHR and SCR outside of the quiescent centre is omitted for clarity. Pink arrows indicate the influence of various pathways on niche positioning.

observed in several animal stem cell niches, for example in the *D. melanogaster* germarium[71]. Dedifferentiation of stem cell progeny provides a second mechanism for stem cell replacement. In the shoot meristem, respecification of differentiating daughter cells to stem cell fate was observed by cell tracking after sustained inducible WUS activation, leading to an enlarged shoot stem cell pool[21,72]. Similarly, induced *WOX5* overexpression prevented differentiation of distal root stem cell daughters, leading to the formation of multiple stem cell-like layers[48], although it was not reported whether dedifferentiation was involved. Dedifferentiation of transit-amplifying cells in animal systems is similarly governed by signals from the niche. For example, after loss of germline stem cells in the fly testis, the niche can be repopulated by dedifferentiating transit-amplifying spermatogonia[71,73]. Moreover, increased signalling from hub cells results in an expanded stem cell population[74,75]. Dedifferentiation may therefore be an essential mechanism to maintain tissue homeostasis in both plants and animals.

Integrin
Transmembrane protein that mediates attachment between an animal cell and its surroundings, such as cells or the extracellular matrix.

Maintaining the stem cell niche position

An important question in both plants and animals is how the position of the functional stem cell niche is maintained within a dynamic structure. For example, anterior positioning of the *D. melanogaster* testis hub involves both ligand–receptor signalling, which prevents posterior niche differentiation[76], and integrin-mediated adhesion to prevent hub cell migration[77]. Also, several positional cues have been identified in plants, in which the position of a stem cell niche can be observed with cellular resolution from early embryonic stages onwards (BOX 1).

The shoot case. In addition to the importance of WUS in niche maintenance, the homeodomain transcription factor SHOOT MERISTEMLESS (STM) is required as a meristem-promoting factor. *STM* is expressed throughout the meristem and is downregulated in developing organ primordia, which are specified by localized auxin accumulation and subsequent activation of organ-specific transcription factors such as *AS1* (REFS 78–81). STM prevents stem cell differentiation by suppressing the activity of the differentiation factor *AS1* in the meristem centre. In turn, AS1 and auxin repress *STM*-related factors, such as *BREVIPEDICELLUS* (*BP;* also known as *KNAT1*), in the lateral organ primordia[80,82,83] (FIG. 4a). These double repressive interactions contribute to keeping meristem cells and organ founder cells separate. Moreover, STM activates the cytokinin biosynthetic gene *IPT7* (*ADENYLATE ISOPENTENYLTRANSFERASE 7*) and cytokinin activates CYCLIN D3 (CYCD3*)* family genes, which provides a direct link to cell division regulation[84–86]. Furthermore, STM directly downregulates the biosynthesis of the hormone GIBBERELLIC ACID, while upregulating its degradation, possibly to prevent the differentiation promoting effects that are caused by GIBBERELLIC ACID movement from lateral organs into the meristem[85,87,88] (FIG. 4a). Cytokinin also has an important function in positioning the shoot stem cell niches. As in other plant species, the *A. thaliana* cytokinin biosynthetic enzyme LOG4 was found to be expressed in the epidermal layer of the shoot meristem, and computer-based modelling, backed up by experimental evidence, indicated that the local synthesis of cytokinin in the meristem acts as a positional cue for the organizing centre within the stem cell niche[46,89,90] (FIG. 4a). A cytokinin signalling response maximum and expression of the cytokinin receptor His KINASE 4 (HK4; encoded by *AHK4*) both localize to the organizing centre. Moreover, HK4 and its homologue HK2 are required for cytokinin-induced expression of *WUS*[44,46]. In addition, WUS represses type-A *ARR* cytokinin signalling inhibitors (such as, *ARR7* and *ARR15*), thereby increasing cytokinin signalling, which may in turn reinforce WUS expression. Expression of a constitutively active form of ARR7 mimics the *wus* phenotype[91], which indicates that cytokinin signalling is important for WUS function in the organizing centre. It was proposed that WUS controls cytokinin biosynthesis in the epidermis, thereby creating a negative feedback loop[46]. Furthermore, the cytokinin degrading enzyme

CYTOKININ OXIDASE 3 (CKX3) is expressed in the organizing centre[41,92], and *ckx3 ckx5* double mutants exhibit increased *WUS* expression and larger shoot meristems[92]. Therefore, cytokinin signalling within the organizing centre seems to be spatially controlled by fine-tuned biosynthesis, perception and degradation. Interestingly, expression of *ARR7* and *ARR15* spatially and temporally overlaps with that of *CLV3* and is required to maintain high levels of *CLV3* expression in stem cells independently of *WUS*. Downregulation of *ARR7* and *ARR15* increased the size of the shoot meristem, probably owing to both reduced CLV3 signalling and increased cytokinin signalling, which promote *WUS* expression and enlarge the stem cell pool. Additional auxin signalling through MONOPTEROS (MP; also known as ARF5), which accumulates in the peripheral zone and mediates direct repression of *ARR7* and *ARR15* promoters, should prevent their expression in the shoot meristem periphery and localize stem cells above the organizing centre[93] (FIG. 4a).

miRNA signalling in the niche. In addition to cytokinin, miR394 was identified as a signal emanating from the epidermis and acting as a positional cue for stem cell competence[94]. A mutation in the *MIR394B* gene was identified in a sensitized enhancer screen that uses a weak stem cell mutant. This enhancer mutant showed compromised stem cell activity that is associated with reduced *CLV3* expression, although *WUS* expression remained unaffected, which indicates that miR394 has a role in stem cell maintenance downstream of WUS signalling[94]. miR394 targets the mRNA that encodes the F-box protein LEAF CURLING RESPONSIVENESS (LCR), which is also involved in leaf morphogenesis[95]. LCR was suggested to target proteins that are involved in WUS function and/or movement[94]. Whereas *MIR394B* expression is restricted to the epidermal layer in the shoot apical meristem, miR394 movement into underlying layers is required to repress LCR and to maintain stem cell activity and *CLV3* expression[94] (FIG. 4b). Repression of differentiation-promoting transcripts in stem cells by local miRNAs to promote self-renewal is also observed in animal systems[96,97], which provides another mechanistic analogy between plant and animal stem cell regulation.

In contrast to miR394, which acts as a positive stem cell cue, the accumulation of miR165 and miR166 (miR165/miR166), which target the same mRNAs, needs to be prevented to maintain the shoot meristem stem cell activity[98,99]. The ARGONAUTE (AGO) protein ZWILLE (ZLL; also known as AGO10) has the unusual function of sequestering miR165/miR166 in the provasculature that surrounds the shoot meristem[100]. This interaction prevents the degradation of miR165/miR166 targets, which are the mRNAs of CLASS III HOMEODOMAIN-Leu ZIPPER (HD-ZIP III) family genes that are expressed in the shoot meristem and that function to maintain it[94,98,101–103] (FIG. 4a). Interestingly, sequestration of miR165/miR166 by ZLL is essential for shoot meristem maintenance in the *Arabidopsis* accession *Landsberg erecta* (*Ler*) and generally correlates with higher expression of the AGO1 cofactor SQUINT (SQN; also known as CYP40) compared to other accessions[104,105]. Absence of the ZLL protein would thereby promote HD-ZIP III mRNA degradation, which is mediated by SQN-activated AGO1 and which leads to stem cell exhaustion. However, changing *SQN* expression did not result in identical phenotypes of *zll* alleles between accessions, which indicates the effect of additional modifiers[105].

Thus, maintaining the position of stem cells at the shoot apical meristem involves several inputs from surrounding tissues besides the organizing centre (WUS), including the epidermis (cytokinin and miR394), the peripheral zone (auxin) and the provasculature (ZLL) (FIG. 4a). How epidermal-derived expression of miR394 and ZLL activity in the provasculature converge in the maintenance of shoot stem cells is unclear.

The root case. In the root, the plant hormone auxin is the main positional cue to maintain stem cell niche position. The combined action of local auxin biosynthesis and directional auxin transporters such as PIN proteins maintain an auxin concentration and response maximum in the quiescent centre, which is crucial for stem cell niche function[61–70]. Auxin readout depends, at least in part, on four partly functionally redundant double APETALA2-domain PLETHORA (PLT) and BABY BOOM (BBM) (PLT/BBM) transcription factors, which accumulate along gradients and have the highest protein levels in the stem cell niche that coincide with the auxin maximum[62,106]. PLTs can regulate PIN gene family expression[61,106], which suggests a feedforward loop to maintain high auxin and PLT levels in the stem cell niche. Expression of *PLT*/*BBM* genes in the stem cell niche guarantees quiescent centre specification and stem cell maintenance[106,107] (FIG. 4b). Interestingly, inducible overexpression of *PLT1* or *PLT2* is sufficient for the formation of ectopic root stem cell niches[106,107], which indicates that the auxin–PLT/BBM readout provides a mechanism for concentration-based specification and maintenance of root stem cells (BOX 2).

How auxin controls *PLT*/*BBM* expression and how these genes interpret auxin concentrations is still not clear. *PLT*/*BBM* are relatively late auxin response genes, which indicates that other upstream regulators of auxin signalling are involved[107,108]. In addition, auxin-induced TYROSYLPROTEIN SULFOTRANSFERASE (TPST) and the Tyr-sulphated ROOT MERISTEM GROWTH FACTOR (RGF) peptides were proposed to link the auxin pathway to transcriptional and post-transcriptional regulation of PLT/BBM in the root stem cell niche[109,110].

The SHORT ROOT (SHR) and SCR transcription factors are required in parallel to PLT/BBM to specify quiescent centre function and to maintain surrounding stem cells (FIG. 4b). SHR, which is expressed in the stele, moves to the surrounding cells, including the quiescent centre where it activates *SCR*[111,112]. SCR cell-autonomously specifies the quiescent centre, but it requires the SHR protein for its function[58,113]. Indeed, it has been shown that SHR and SCR proteins interact to form a complex[114].

Box 2 | **Epigenetic maintenance of plant stem cells**

The maintenance of a stem cell status in plants, as for animal stem cells, is likely to involve epigenetic control. Mutant analyses have shown that chromatin factors affect key plant stem cell regulators, such as *WUSCHEL* (*WUS*), *WUSCHEL-RELATED HOMEOBOX 5* (*WOX5*) and *PLETHORA* (*PLT*) genes[163–169]. Studies of human embryonic stem cells revealed that their differentiation is accompanied by a gradual deposition of repressive histone marks and progressive chromatin compaction[170–172]. Animal stem cell fate can be induced by overexpressing a small set of transcription factors (POU5F1 (POU domain, class 5, transcription factor 1), SOX2, KLF4 (Krueppel-like factor 4) and MYC) in committed differentiated cells that, as a result, revert to the pluripotent state. This reversion is accompanied with a reversion of the chromatin state[140] (reviewed in REF. 173). Ectopic expression of plant stem cell regulators (such as, WUS, PLT and BABY BOOM (BBM)) also results in the formation of ectopic stem cell niches and in some cases even somatic embryos[106,107,174,175] (see the figure). This reversion may also involve changes of the chromatin status in the reprogrammed cells. Mapping of chromatin modifications in individual plant cell populations that traverse from stem cell status towards differentiation, by means of ground breaking tissue-specific profiling methods[41,65,176,177,178], will help to reveal whether a stem cell-specific chromatin state is required to maintain a flexible stem cell status.

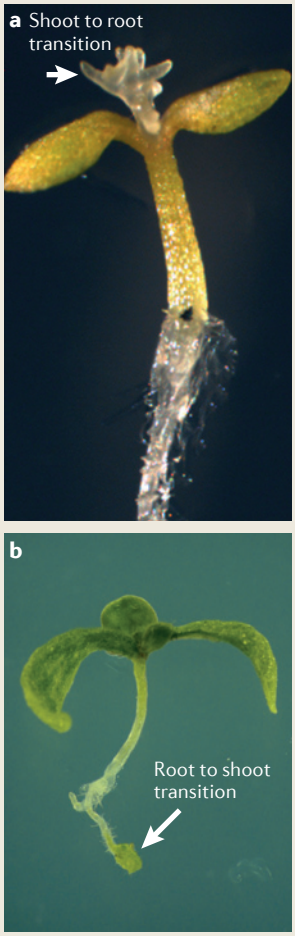


Image in part **b** courtesy of R. Sablowski, Department of Cell and Developmental Biology, John Innes Centre, Norwich, UK.

Plant organizers — apparently different

Division of quiescent centre cells does not necessarily correlate with stem cell differentiation, and the biological importance of their quiescence had remained speculative until recently. Initially defined as infrequently dividing cells, quiescent centre cells divide twofold less frequently than surrounding stem cells and fourfold less compared to meristem cells[115,116]. Lineage analyses suggested that quiescent centre cells can replace all stem cells in the *A. thaliana* root[117], although the results were hampered by the low division frequency of quiescent centre cells. New data show that under normal conditions this may not be the case, as quiescent centre daughter cells only populate the clonally related distal columella[115]. Quiescent centre-specific knockout or reduction of RBR function indicated that RBR controls quiescent centre division in a cell-autonomous manner[115,118]. This function of RBR depends on its capacity to bind LXCXE (X indicates any amino acid) domain proteins[115], which is similar to RB-mediated quiescence in animal cells[119]. SCR contains an LXCXE motif and the expression of a mutated SCR^{AXCXA} version results in additional asymmetric quiescent centre divisions, which indicates that the RBR–SCR complex inhibits quiescent centre cell division[115]. Such activity is reminiscent of the role that RBR has to inhibit asymmetric division in the mature endodermal ground tissue layer by repressing SCR activity[120]. To enable asymmetric division of the ground tissue stem cell daughter into endodermis and cortex, SCR–RBR interactions are disrupted by a cyclin-dependent kinase (CDK)–CYCLIN D6;1 (CYCD6;1) complex that phosphorylates RBR. *CYCD6;1* is a target of SHR–SCR, which creates a feedforward loop[120,121]. However, high levels of auxin in and around the quiescent centre confine *CYCD6;1* expression and thereby the occurrence of the asymmetric divisions to the stem cell niche. Protein turnover after asymmetric ground tissue division is proposed to reset the system[120]. The observations that the absence of CYCD6;1 does not completely abolish asymmetric ground tissue division and that CYCD6;1 is not expressed in the quiescent centre suggest that additional cyclins regulate asymmetric divisions that involve RBR (REFS 115,121,122). Such a mechanism could explain how in plants the specificity of asymmetric division is guided for stem cells of different tissues within the niche. If the mechanistic similarity between plant and animal stem cell niches holds true, similar mechanisms could act in multipotent animal stem cells.

These results do not exclude the possibility that the quiescent centre acts as a stem cell reservoir, for example to replace damaged stem cells[117,123]. To address this question, transient treatments with DNA-damaging agents were carried out, which resulted in the death of quiescent centre-neighbouring stem cells[115,124,125]. Slowly-dividing quiescent centre cells were more resistant, which correlates with relatively higher expression levels of DNA damage repair genes compared to surrounding stem cells[124]. Consistent with this, treating roots in which quiescent centre cells divided more frequently, owing to reduced RBR levels, resulted in quiescent centre cell death and subsequent exaggerated loss of meristem activity compared to wild-type roots[115]. Quiescent centre division is also prevented by the APC/C (anaphase-promoting complex; also known as the cyclosome), which regulates cell cycle progression through degradation of mitotic cyclins[126]. Mutating the *CCS52A2* (also known as *FZR1*) gene induces quiescent centre division[127]. This gene is expressed in the stem cell niche and encodes a WD40 protein that activates the APC/C through its binding to specific substrates. Recently, it has been shown that CCS52A2 interacts with the ERF115 transcription factor[124], which belongs to the ETHYLENE RESPONSE FACTOR (ERF) family that that is known to mediate stress responses[128]. Efficient stem cell niche regeneration after treatment with DNA-damaging agents was reported to occur by displacement of damaged cells through quiescent centre expansion by division that requires ERF115 family members[124]. Supposedly, cells of the expanded quiescent centre would acquire stem cell identity to restore the stem cell niche wild-type organization, although this was not investigated. Thus, quiescent centre mitotic quiescence correlates with insensitivity to stress conditions and functions to replace damaged stem cells, thereby contributing to stem cell niche longevity. It is unknown how the APC/C and the RBR pathway functionally interact to ensure a low mitotic state in the quiescent centre.

APC/C
(Anaphase-promoting complex; also known as the cyclosome). An E3 ubiquitin ligase protein complex that targets cell cycle proteins for degradation by the 26S proteasome, thus enabling cell cycle progression.

The ability of the quiescent centre to replace (damaged) stem cells indicates a 'reserve' function that is able to restore the stem cell niche, which is similar to the plasticity observed during regeneration of the crypt base columnar stem cell compartment in the mammalian intestine or similar to the reactivation of quiescent stem cells in the bone marrow after injury (reviewed in REFS 4,129). However, whereas in animal systems lost stem cells can be replaced by the reversion of a differentiating cell to a stem cell or by symmetric self-renewal of a surviving stem cell, in plants it seems that the (quiescent) stem cell function is a property of organizing cells within the niche.

Stem cells are also hypersensitive to DNA damage in the shoot meristem, in which CCS52A2 also seems to be essential for meristem maintenance[130], which suggests a common mechanism that involves ERF115-related factors in stem cell recovery. Nonetheless, laser ablation of the stem cells in tomato shoot meristems did not seem to affect organizer size, which is determined by the *WUS* expression domain, and a functional meristem was readily recovered[131]. It was not determined whether stem cells were replenished by division of the organizer or by recruitment of cells that are adjacent to the ablated region. After ablation of stem cells and organizer, respecification of organizer fate was quickly observed in adjacent cells, which was followed by the recuperation of meristem activity[131]. This property of dynamic shoot organizer respecification may be crucial to maintain the growing shoot under normal conditions. Clonal analysis indicates that the shoot is derived from only a few stem cells in three apical cell layers[132–135]. Inherent to the position of the organizer below the stem cells and given the random division pattern in the L3 stem cell layer, cells that are displaced downward need to be incorporated and respecified as organizer in order for the organizing centre to maintain its position and to ensure its function in maintaining the three apical stem cell layers. At the same time, to maintain organizer size, cells at the basal position in the organizing centre should lose their organizer fate and are incorporated into the growing stem. The signalling pathways that are described above ensure the position of the stem cell niche and the balance between organizer and stem cell numbers, but this also implies that the organizer consists of a set of transit-amplifying cells that transiently adopt the organizer fate as part of their normal differentiation trajectory towards inner tissues. Hence, the shoot organizing centre seems to be a special case with respect to the way it needs to be maintained.

Conclusions and perspectives

Most studies of plant stem cells have been conducted on the *A. thaliana* root and shoot stem cell niches, which are responsible for all post-embryonic plant growth. Multiple signals were identified as necessary to position and to maintain the shoot and root stem cell niches. Given that within the plant kingdom, roots have evolved later than shoots[136], it is interesting to note conserved mechanisms in the shoot and root stem cell niche function, which indicates that their evolution may have predated the formation of these distinct meristems.

These studies have also revealed parallels between animal and plant stem cell niche organization and behaviour. Multicellularity in plants and animals arose independently[137], thus the presence of stem cell niches in both kingdoms and their similarity suggests that they are the result of convergent evolution under similar constraints[2]. Indeed, besides the RBR case, genes and proteins that are involved in animal and plant stem cell function seem purely plant- or animal-specific. Nevertheless, it is clear that general mechanisms are conserved. In both cases stem cells are slowly dividing cells that are maintained by short-range signals that arise from specialized cells. Some of these signals are known in the animal field, such as the interleukin-like cytokine Unpaired, which is secreted by hub cells in *D. melanogaster* testis[10], or WNT3, pro-epidermal growth factor (EGF), transforming growth factor-α (TGFα) and the Delta-like protein 4, which are secreted by the Paneth cells in the intestinal crypt[138]. In plants, the nature of these signals is still not completely clear. Recently, WUS that specifies the organizing centre has been found to act as a signal itself, which moves from the shoot organizing centre to control the above stem cells[21]. The signals that inhibit differentiation, which are generated by the quiescent centre, in the root to control the surrounding stem cells are yet to be discovered. Interestingly, both organizers seem to have a role in controlling differentiation of distal transit-amplifying cells[21,59]. Such a mechanism could have developed to coordinate stem cell division with differentiation of its progeny, hence guaranteeing coherent organ growth. Besides signals emanating from the organizing centre, it is clear that in both animal and plant stem cell niches important signals that affect stem cell behaviour are also generated by stem cell progeny. This phenomenon has been observed in the *D. melanogaster* haematopoietic niche[55], the epithelial hair follicle and the mouse intestinal crypt stem cell niche[54]. In the *A. thaliana* root stem cell niche, the CLE40 peptide feeds back to columella stem cells to promote their differentiation[49,50], whereas shoot stem cell niche activity also depends on unknown leaf-derived signals[102,139]. Again, generation of these feedback signals from differentiated progeny to regulate stem cell behaviour could be a robust strategy to control tissue homeostasis in animals and to guarantee coherent organ growth in plants. Rather than being a specialized cell type with stem cell-specific gene expression patterns, it seems that stem cells much resemble their dividing and differentiating daughters, as they are under the influence of many surrounding inputs, with the mere difference that they are kept in a developmentally arrested state owing to their position in the niche.

It is noteworthy that besides the complexity of stem cell behaviour in both kingdoms their specification can be related to a small set of transcription factors; for example, POU5F1 (POU domain, class 5, transcription factor 1; also known as OCT3 and OCT4), SOX2, KLF4 (Krueppel-like factor 4) and MYC (commonly referred to as OSKM factors) for mouse or human embryonic stem cells[140,141], and WUS and PLT/BBM for *A. thaliana* (BOX 2). Furthermore, it is important to note that although dedifferentiation of committed cells was previously

thought to be a plant-specific wonder, this phenomenon is now also being observed in the animal kingdom.

In both plants and animals, hormones control every stage of stem cell life, including specification, maintenance and differentiation. The effects can be cell autonomous or non-autonomous through the niche. For example, the plant hormones cytokinin and auxin provide important positional cues to control both shoot and root stem cell niche function and specification[46,59,61] but can also provide cell non-autonomous signals to control stem cell differentiation[59]. Similarly, the thyroid hormones control mouse intestinal morphogenesis that affect crypt stem cell niche development and epithelial stem cell proliferation[142].

With the insights gained into the molecular mechanisms that are involved in stem cell function, it is evident that these are highly dynamic and wired in feedback circuits, which has made their detailed mode of action too complex to understand intuitively. Computational modelling has become a key factor in the understanding of biological systems[143]: physico-mathematical models help to investigate relations between local morphogenetic processes and global patterns, as well as the interaction between genes, hormones and growth, inspiring the development of new mathematical formalisms and applications. A proof of concept that complex regulatory networks within meristems can be understood through the interplay of computational modelling and experiments has already been provided in the plant field[144–146]. Such models will be improved by incorporating advances in dynamic three-dimensional meristem imaging and mechanical modelling[147,148]. These new approaches will undoubtedly facilitate our knowledge of the logic of plant stem cell function and might help us to understand unsolved issues, such as: which are the short-range signals from the organizer to maintain the surrounding stem cells? How are asymmetric stem cell division and renewal achieved? Which are the molecular mechanisms that underlie stem cell progeny differentiation? These questions are enough to keep the field busy in the coming years.

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Competing interests statement
The authors declare no competing interests.