STEM CELLS

Stem-cell niches: nursery rhymes across kingdoms

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Abstract | Despite the large evolutionary distance between the plant and animal kingdoms, stem cells in both reside in specialized cellular contexts called stem-cell niches. Although stem-cell-specification factors have been recruited from plant-specific gene families, maintenance factors that repress stem-cell differentiation are conserved between plants and animals. Recent evidence indicates that stem cells in multicellular organisms can be specified by kingdom-specific patterning mechanisms that connect to a related core of epigenetic stem-cell factors.

Stem-cell niche
The cellular microenvironment that provides the signals and physical support to maintain stem cells.

Asymmetrical divisions
Mitotic divisions that generate distinct fates in the two daughter cells; in the case of stem cells, one daughter preserves the stem-cell state.

Transit-amplifying cells
Dividing stem-cell daughters that are fated for differentiation and that can retain the ability to divide.

Totipotent
Cells that can produce progeny that can form an entire organism.

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Imagine the excitement among stem-cell biologists if a Pathfinder mission to Mars led to the discovery of green creatures with 2,000-year lifespans that continuously extend their body lengths with 2,000-year-old stem cells on the top of their heads. Everyone would want to understand how these stem cells are programmed and how their genetic controls differ from the ones that are now being unravelled in stem cells of earthly animals.

Fortunately, we do not have to hold back our excitement until the first encounters with such extraterrestrials, as these green creatures already exist on our planet. Distinct unicellular progenitors have evolved into a separate branch of multicellular life: the plant kingdom. Plants use stem cells to maintain growth and development, and in long-lived trees such as *Sequoia sempervirens* these cells can be active for over 2,000 years (FIG. 1). The best characterized plant stem cells are located at the tips of shoots and roots. Shoot stem cells continuously form leaves and stems but can also be reprogrammed to form alternative leaf-like structures such as flowers. Root stem cells form the underground root system. Last, a set of cambial stem cells in the trunk produce cells that continuously increase their girth and generate daughter cells that form wood.

The lack of cell migration in plants greatly facilitates the identification and analysis of stem cells because cell lineages can be easily tracked (BOX 1). Although the secrets of *S. sempervirens* stem cells remain to be unveiled, their relatives in the small weed *Arabidopsis thaliana* are the subject of intense investigation. In particular, gene networks that are involved in root and shoot stem-cell specification and maintenance have been identified. This review attempts to put emerging insights into the general context of stem-cell research. First, I compare the cellular organization of stem-cell pools in plants and animals, which reveals intriguing parallels. I then discuss the transcription-factor codes that specify plant stem cells and the patterning mechanisms that determine the domain of activity of these factors. This information will then be used to address two current issues in stem-cell research: plasticity of the stem-cell state and molecular mechanisms of stem-cell maintenance. Last, I present a model that proposes overlapping functions of chromatin-associated protein complexes in plant and animal stem-cell maintenance.

Niches that straddle the kingdoms of life

The idea that stem cells do not sit unattended but are maintained in a specialized microenvironment that is commonly known as the stem-cell niche originates from studies on mammalian haematopoietic stem cells[1]. A detailed description of a stem-cell compartment at the cellular level was first achieved in a more accessible system, the *Drosophila melanogaster* ovary, in which special 'organizing' cells were shown to maintain germline stem cells by short-range signals[2]. Since then, the concept of the stem-cell niche has been extended to include various stem-cell types, including the progenitors of mammalian gut and hair cells[3].

A prototypical stem-cell niche in the fruitfly. In the ovaries of the fruitfly, stem cells undergo asymmetrical divisions to produce cystoblasts that give rise to the oocyte and nurse cells (FIG. 2a). The cystoblasts undergo several cycles of cell division, and these amplify the cell pool prior to differentiation. Stem-cell daughters will traverse the transit-amplifying cell population to become a cluster of differentiated cells (one of these cells, the oocyte, is totipotent after fertilization).

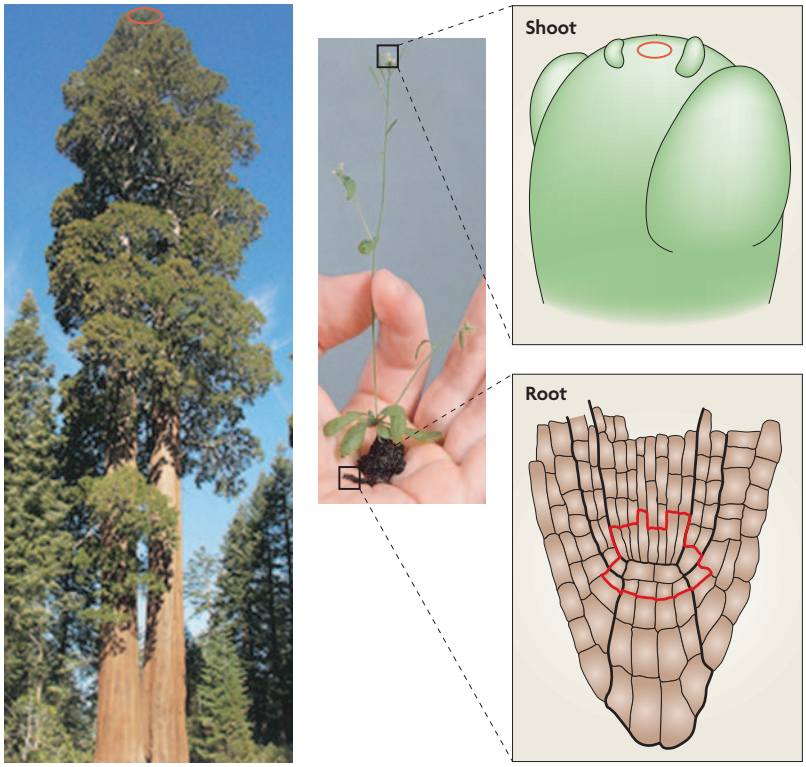

Figure 1 | **Stem cells in the plant kingdom.** The tallest 'green creature' on earth, *Sequoia sempervirens* (left), carries stem-cell reservoirs that can be active in a single individual for more than 2,000 years. All plants, including the small laboratory weed *Arabidopsis thaliana* (middle), carry small groups of stem cells at the growing tips of shoots and roots (right). Stem cells at shoots and roots are highlighted in red but they can also be found in the tips of all lateral branches. Shoot stem cells give rise to leaves and stem tissue. Root stem cells continuously extend the root.

The stem cells are maintained by the cap cells, which function as organizing cells in two ways. The organizer tethers stem cells by *Drosophila* epithelial (DE)-cadherin-mediated adhesion[4], which maintains stem cell–organizer contact. This tethering is important for the maintenance of neighbouring stem cells. In addition, signalling proteins that are produced by the organizer are perceived in the stem cells. These cues positively regulate factors that inhibit cell differentiation[5,6]. In the female, the germline stem cells are located on one side of the organizing cells, but in the male testis the organizer cells maintain stem cells all around[7].

***Stem-cell niches in* A. thaliana.** Remarkably, a similar stem-cell-niche organization is apparent in plant roots (FIG. 2b). In *A. thaliana* root tips, multipotent stem cells surround centrally located organizing cells, and laser ablation of organizer cells, as well as mutation of genes that are required for organizer identity, forces adjoining stem cells into differentiation[8,9]. Similar to the niche in the fly ovary, stem cells are maintained by signals from the organizer that inhibit differentiation. Tethering of stem cells to the organizer in plants does not require a separate system, but is facilitated by the shared polysaccharide cell wall that holds plant cells together.

The stem cells, also called initials, continuously produce daughter cells by asymmetrical divisions, and these move away from the niche and differentiate. Two pools of stem cells can be distinguished. The proximal stem-cell daughters form a transit-amplifying cell population in which extra rounds of cell division take place. This dividing-cell group is called the meristem. Proximal stem cells will give rise to epidermal, ground tissue and vascular cell types. During differentiation, these cells strongly expand along the main axis and therefore carry the stem-cell niche forwards along with the tip of the root. The distal stem-cell daughters do not divide again, but they differentiate into starch-containing cap cells that ultimately detach from the organ. The organizer remains at the same distance from the tip of the organ because the outermost layer of distal cap cells detaches after the formation of each new layer (FIG. 2c). Despite apparent lineage restrictions, which are due to precise control of cell divisions, stem-cell daughters can adopt different fates depending on their position[10].

A different stem-cell niche is present in shoot tips. The activity of the shoot niche has to be considered in three dimensions as it produces lateral organs and a growing stem (FIG. 1). Rather than operating at the single-cell level by control of asymmetrical divisions, the shoot stem cells are maintained as a pool. Three layers of distal stem cells are maintained by an organizing centre that can be marked by visualizing the expression of the homeodomain transcription factor *WUSCHEL* (*WUS*)[11,12]. Multipotent stem-cell daughters at the shoot tip become displaced and can be incorporated in organ primordia that form leaves or flowers; if they are not included in primordia, they form part of the plant stem that separates the leaves. Cells at the proximal surface of the organizing centre also contribute to the stem, and their expansion carries the niche along with the growing tip of the shoot. It is noteworthy that, during shoot growth, the *WUS*-expression domain is continuously respecified because cells appear to continuously leave the *WUS*-expression domain. So, there are no permanent 'organizing' cells in the shoot apical meristem, and the stem-cell niche seems to be maintained by continuous positional signalling.

Combinatorial stem-cell patterning

Recent whole-genome transcription-factor-binding studies on murine and human embryonic stem cells have revealed a paradigm for vertebrate stem-cell specification. The transcription factors OCT4 (octamer-binding transcription factor-4), NANOG and SOX2 (SRY-related high mobility group (HMG)-box protein-2) have distinct functions that specify the inner cell mass of the mouse embryo, where pluripotent stem cells reside. These transcription factors are required to derive embryonic stem cells from early embryos — indicating a role for these factors in stem-cell maintenance[13–15]. High-resolution chromatin-immunoprecipitation studies showed that these factors jointly occupy the promoters of many transcription factors that are expressed in differentiating cells and keep them repressed in the stem cells. However, OCT4, NANOG and SOX2

Multipotent
Cells that can produce progeny that can form all cell types of a tissue.

Homeodomain
Conserved DNA-binding domain in both plant and animal transcription factors.

Pluripotent
Cells that can produce progeny that can form all cell lineages.

Box 1 | **Defining stem cells**

Definitions of stem cells combine two criteria: (**a**) when a stem cell divides it should yield at least one daughter cell that is also a stem cell, and (**b**) the stem cell must be able to give rise to progeny that can differentiate into at least one specialized cell type. Stem cells thereby provide long-term resources of undifferentiated cells that remain capable of embarking on specialized paths during normal development or during regeneration of tissues. Several methods (listed below) have been used to assess stem-cell characteristics.

- A straightforward criterion to pinpoint stem-cell activity is to monitor stem-cell divisions and progeny over time. This can be achieved by clonal analysis, which has been done both in animals and in plants.
- Plant systems have the experimental advantage that secreted cell-wall polymers link neighbouring cells together, which allows tracing of lineages on the basis of the position of cells in tissue sections.
- The potential of cells to continuously yield progeny, including differentiated cells, can be tested *in vitro* or after reintroduction in animals.
- Dormant stem cells can be identified by a negative criterion, namely their withdrawal from the cell cycle, by detecting a lack of incorporation of labelled precursors in DNA.

also occupy the promoters of genes that are actively expressed in stem cells[16,17]. Therefore, at least three transcription factors with overlapping expression patterns can sustain stem-cell renewal in the mouse embryo, and suppression of differentiation is an important mechanism in this process.

Mechanisms of root stem-cell specification. Combinatorial activity of transcription factors is also an important event in the root stem-cell niche of *A. thaliana*, which indicates that transcription-factor circuitry is important for both plant and animal stem cells. Notably, the transcription factors that are involved in stem-cell maintenance are members of two plant-specific families, which indicates that their role in stem-cell maintenance is not ancestral but evolved after the separation of plant and animal kingdoms[18,19]. Furthermore, the patterning mechanisms that are used to position the overlapping domains of these transcription factors are beginning to be understood; these mechanisms are also plant specific and bear no resemblance at the molecular level to animal patterning mechanisms.

A combination of radial- and apical–basal-patterning input allows positioning of the stem-cell niche (FIG. 3). Directional transport of the small indolic plant-growth regulator auxin through polarly localized transmembrane proteins of the PIN family contributes to auxin accumulation in the stem-cell-niche area[20]. Auxin determines the expression domain of the double-AP2-domain transcription factors PLETHORA1 (PLT1) and PLT2, which provide transcriptional input for stem-cell specification during and after embryogenesis[21]. Furthermore, the provascular expression of the GRAS-family transcription factor *SHORTROOT* (*SHR*) initiates movement of the SHR protein to the surrounding cell layer[22,23]. Regulated protein movement leads to nuclear activity of SHR and its target, *SCARECROW* (*SCR*), in a single layer that passes through the *PLT1*- and *PLT2*-expression domain[23–25] (FIG. 3). The overlap between the highest level of *PLT1* and *PLT2* expression and the SHR and SCR protein expression domain defines the stem-cell niche at cellular resolution. SCR, through its cell-autonomous function, defines organizer identity. In addition, *SCR* expression outside the organizer contributes to the size of the transit-amplifying cell population in a non-cell-autonomous manner[9]. Separate functions of PLT genes in organizer-cell specification, stem-cell maintenance and transit-amplifying cell divisions have not been reported yet.

Although PLT1 and PLT2 as well as SHR and SCR are all crucial for stem-cell maintenance, these two groups of transcription factors control different enhancer-trap markers for the organizing cells and the loss of each group affects the maintenance of the stem-cell pool at different rates[9,21]. This difference indicates that the PLT and SHR–SCR pathways do not fully converge on the same set of target genes. It will be informative to comprehensively compare all the targets of the four transcription factors in the stem-cell niche to gather insights into the mechanisms of root stem-cell specification.

The shoot stem-cell niche. Two separate pathways contribute to stem-cell maintenance in the shoot apical meristem. Radial- and bilateral-patterning information is combined to position the stem-cell niche and the large transit-amplifying cell population that surrounds the niche (FIG. 4). The main transcription factors that are required for stem-cell maintenance are the homeodomain transcription factors WUS and SHOOT MERISTEMLESS (STM).

In the WUS pathway, *WUS* expression in the stem-cell organizing centre maintains the overlying stem cells[12]. The initiation of *WUS* expression seems to depend on continuous positional information, as a new organizing centre can be re-established after laser ablations[26]. Two activities are known to restrict the domain of *WUS* expression at early embryonic stages: polar auxin transport by PIN transporters, including PIN1, and the activity of the HANABA TARANU GATA transcription factor[20,27]. However, positive spatial cues for the initiation of *WUS* expression have not yet been reported.

The STM pathway for stem-cell maintenance is defined by the STM homeodomain transcription factor, which suppresses cell differentiation in the stem-cell area and in the surrounding transit-amplifying cells[28]. Among the positional cues for the initiation of *STM* expression are the CUP-SHAPED COTYLEDON (CUC)

Auxin
Plant hormone that is implicated in a range of developmental processes, including cell-fate specification, cell division and cell expansion. In most plants, indole-3-acetic acid is the main active form.

PIN family
Transmembrane proteins that mediate auxin efflux, named after the founding member, PIN-FORMED1.

AP2 domain
A plant specific DNA-binding domain that was first found in the floral homeotic protein APETALA2. Members of a distinctive subclade of the AP2-domain family, which includes both *APETALA2* and the *PLETHORA* genes, contain two AP2 domains.

GRAS family
A plant-specific transcription-factor family named after the first three members cloned: GIBBERELLIN-INSENSITIVE, RGA and SCARECROW.

GATA
Transcription-factor family in both plants and animals with zinc-finger DNA-binding domains.

NAC domain
A plant-specific DNA-binding domain first found in *Petunia* NO APICAL MERISTEM and *Arabidopsis* ATAF1 and CUP-SHAPED COTYLEDON2.

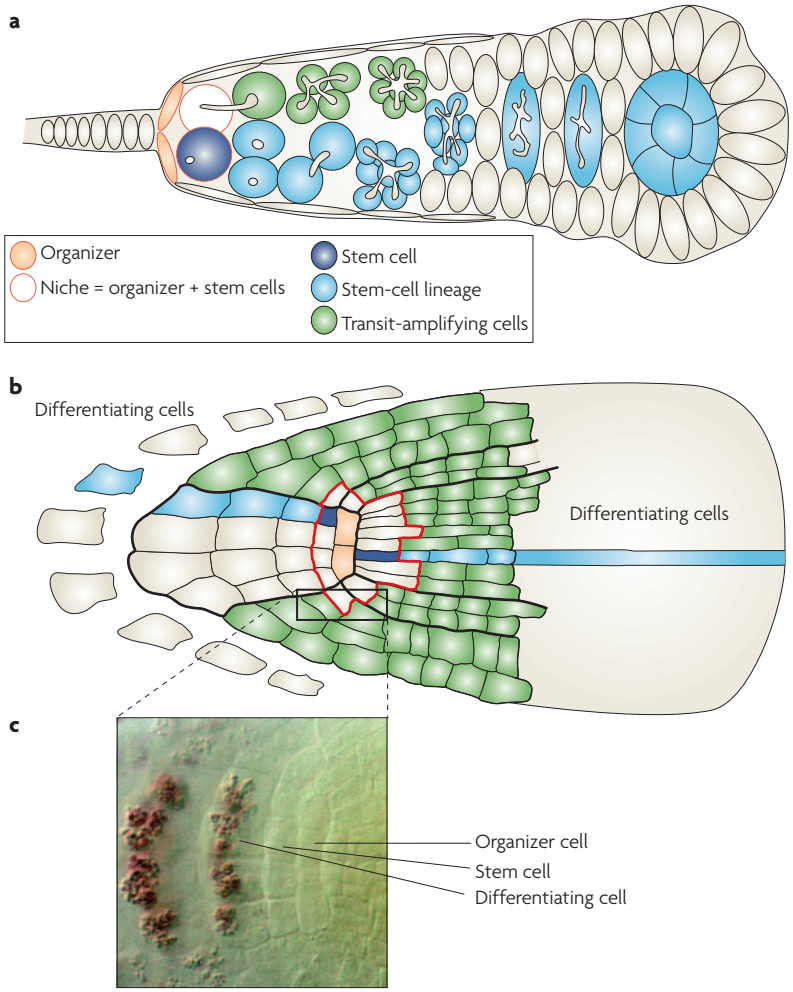

Figure 2 | **Stem-cell-niche organization in animals and plants. a** | A stem-cell niche in an ovary of *Drosophila melanogaster*. The niche consists of stem cells and organizing cap cells. Organizing cells are the source of short-range signalling molecules and are required for stem-cell maintenance. Stem-cell daughters undergo four rounds of cell division (transit-amplifying cells) to give rise to a connected group of one oocyte and nurse cells (the lineage of one stem cell is shown). **b** | Stem-cell niche in *Arabidopsis thaliana* root. The niche contains stem cells and organizing cells (same colour code as in **a**) that are required for stem-cell maintenance. Stem-cell daughters at the distal end immediately differentiate and ultimately detach from the organ (blue lineage projecting to the left). Proximal stem-cell daughters undergo around four rounds of cell division (transit-amplifying cells) and form differentiated cells (blue lineage projecting to the right). **c** | Nomarski optics image of a transparent root tip with anatomically distinct stem and organizer cells; the brown starch granules mark the differentiation of distal stem cells.

RNA-induced silencing complex
A complex that binds double-stranded RNA and targets it to homologous sequences to induce silencing.

Arabidopsis response regulators
(ARR). Transcription factors that are activated by phosphorelay in cytokinin signal transduction.

transcription factors, which are members of the plant-specific NAC-domain family[29]. In the embryo, these factors are restricted to a lateral subdomain through polar-auxin-transport regulators[30,31] (FIG. 4a). Polar auxin transport therefore seems to restrict the stem-cell promoting activity of CUC and STM transcription factors to a lateral domain on the top half of the early embryo. Another positional cue for *STM* expression might be generated from provascular tissue by the ZWILLE (ZLL) protein, which is required for the proper establishment of *STM* expression[32–34] (FIG. 4a). *ZLL* is homologous to *D. melanogaster Piwi*, which is also implicated in non-cell-autonomous stem-cell maintenance[5], and both encode RNA-induced silencing complex (RISC) components, suggesting that roles of RISC complexes in the generation of small RNAs are crucial in stem-cell niches.

Although the role of WUS appears to be confined to promoting stem-cell-pool maintenance, STM is also active in stem-cell daughters to define the size of the transit-amplifying cell population and to maintain embryonic leaf boundaries[35] (FIG. 4b,c). Despite their different roles, both WUS and STM are required to keep stem cells undifferentiated.

An important question is whether maintenance of stem cells by the WUS and STM pathways involves control of the same or different target genes. Repression of cell differentiation and the maintenance of cell-division potential are important functions of stem cells and a comparison of targets of both transcription factors should reveal whether there is an overlap in effector genes. STM suppresses the activity of factors that promote differentiation, such as the Myb transcription factor AS1 (REF. 36) — much in line with the mentioned roles for animal stem-cell regulators. This suppression involves both STM and redundant STM homologues[37]. However, it is unclear whether the regulatory interaction between STM and AS1 occurs in all cells in the *STM*-expression domain or whether this interaction is only important at the boundary of the transit-amplifying cell population (FIG. 4c,d). There is also some evidence for the regulation of the cell cycle because both STM and WUS regulate *Arabidopsis* response regulators (ARR) genes, which are involved in the response to the plant hormone cytokinin[38–40]. Cytokinins can stimulate cell division and can rapidly regulate the expression levels of D-cyclins, which are involved in cell-cycle progression[41].

Feedback loops for plant stem cells

Hints for dynamic programming of plant stem-cell niches and their associated transit-amplifying cell populations come from a consideration of the mutual interactions between factors that set up the initial stem-cell domain. The root stem-cell niche, for example, is strongly dependent on the activity of PIN proteins in the embryo, which leads to basal accumulation of auxin and the subsequent high expression of *PLT1* and *PLT2* in cells in which the auxin levels reach a maximum concentration (FIG. 3). However, expression of several PIN genes is in turn dependent on PLT activity[20], which suggests the possibility that auxin transport, and thereby auxin accumulation and stem-cell niche positioning, can be self-regulated by PLT gene activity.

In the shoot apical meristem, several clues hint at a gene network for stem-cell programming that is extremely rich in feedback loops. Once the organizing centre is in place, the overlying stem cells and their immediate daughters express the small secreted protein CLAVATA3 (CLV3)[42]. *CLV3* expression depends on WUS[43,44], but STM can also have a role in the maintenance of *CLV3* expression post-embryonically[45]. CLV3 is detected by receptor-kinase complexes[46–49], which negatively regulate *WUS* expression to restrict the size of the organizer[42–44] (FIG. 4d). Acute CLV3 reduction by

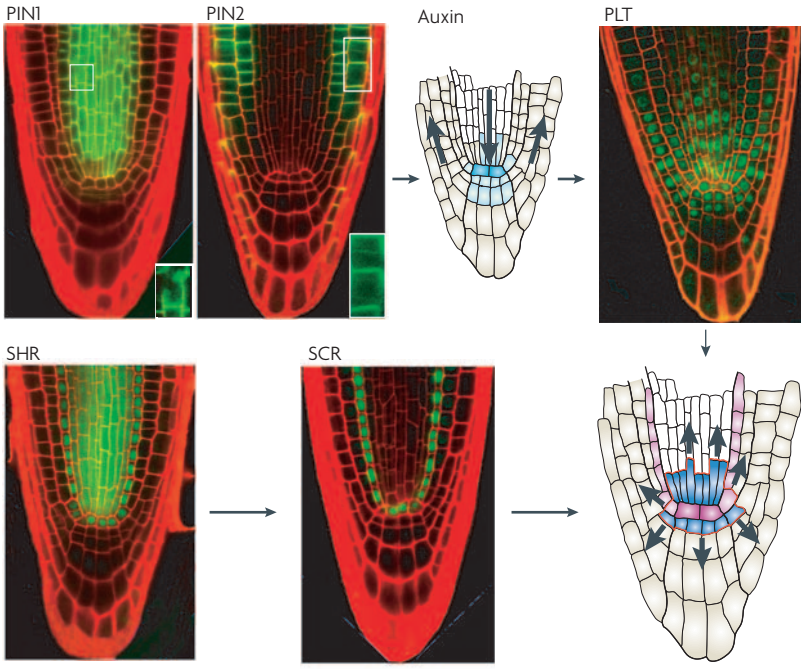

Figure 3 | **Stem-cell specification in the root apex.** *Arabidopsis thaliana* polar-auxin-transport facilitators PIN1 and PIN2 determine the direction of auxin flow by polar membrane localization. Insets show polar localization of PIN1 and PIN2. The upper schematic diagram indicates how the direction of auxin transport (arrows) promotes auxin accumulation at the stem-cell area (dark and light blue), which leads to expression of the *PLETHORA1* (*PLT1*) and *PLT2* transcription factors (top right panel). The *SHORTROOT* (*SHR*) gene is expressed in the central tissues, but SHR protein cannot efficiently translocate into the nucleus. SHR moves to peripheral cells presumably through cytoplasmic connections between cells (the plasmodesmata), after which it accumulates in the nucleus and activates SCARECROW (SCR). The lower schematic indicates how, together with the PLT proteins (dark blue), the SHR and SCR transcription factors (pink) specify the stem-cell niche (red outline, organizer in purple) at cellular resolution. The stem cells produce daughter cells in the direction of the arrows. The confocal images of roots visualize functional green fluorescent protein (GFP) fusions of indicated proteins (green) with counterstained cell walls (red).

RNA interference (RNAi) shows that CLV3 rapidly restricts its own expression domain, possibly owing to the CLV3-mediated regulation of the *WUS*-expression domain[50]. Accordingly, an acute increase in CLV3 levels leads to rapid repression of *WUS* and endogenous *CLV3* expression[51]. Furthermore, CLV3 non-autonomously suppresses proliferation in neighbouring transit-amplifying cells, perhaps explaining previously noted interactions between CLV genes and *STM*[50–52]. The regulatory loop between CLV3 and WUS therefore has the potential to stabilize stem-cell pool size, aided by more recently identified transcription factors that restrict the *WUS*-expression domain[27,53]. Also, STM and WUS mutually maintain each other's expression levels, which indicates that regulatory interactions between these two factors might have a role in dynamic stem-cell programming[11]. Last, the boundary between the transit-amplifying cells and developing cotyledon primordia is dependent on the activity of the CUC proteins, which first help to initiate *STM* expression but then become partly dependent on STM for proper expression at boundaries[29].

Cytokinin
Purine derivates that function as a plant hormone. Cytokinin is implicated in the control of cell division, but also in the determination of shoot identity.

Developmental plasticity of stem cells

The 'fixed' organization of stem-cell niches in, for example, fruitfly germaria can give the false impression that the properties of stem cells are irreversibly lost once cells embark on differentiation pathways. However, in the *D. melanogaster* ovary, the niche can reprogramme partially differentiated cells to the stem-cell state[54]. Plasticity in mammalian adult stem cells has also led to a reconsideration of the 'irreversibility' of differentiation pathways[55,56].

Stem-cell reprogramming in plants. The versatility of stem-cell descendents to regain pluripotency after manipulation is also evident in plants. First, spontaneous division-plane alterations in stem-cell lineages lead to position-dependent changes in cell identity[57]. Second, in both roots and shoots, the entire niche can be reprogrammed after laser ablation[10,26]. In roots, this reprogramming requires the activity of the same patterning genes that determine the position of the stem-cell niche in the embryo[58]. Therefore, embryonic patterning programmes appear to be used to re-establish the stem-cell niche in the root tip upon damage. Third, ectopic induction of *WUS* and *STM* expression can revert differentiating cells to transient stem-cell niches[59]. In extreme cases, *WUS* expression in the root tip can non-autonomously induce *CLV3* expression and shoot outgrowth, indicating that the shoot stem-cell niche might be transiently re-established even in a root context[60].

More natural examples of stem-cell reprogramming are provided by intrinsic programmes in plants that recreate stem-cell niches in a context of differentiating cells. The positions and patterns of activation of these new stem-cell niches determine the architecture of the plant (FIG. 5). Plant embryos initially generate only one root and one shoot stem-cell population at opposite ends. During post-embryonic development, daughter cells of the stem cells are set apart and form new stem-cell niches.

In the root, the pericycle is an internal cell layer in which the cell cycle is suspended. Cells in this layer can re-initiate cell division and create lateral root primordia with new stem-cell niches. Re-establishment of lateral root primordia involves polar transport and detection of the plant hormone auxin, which forms new local response maxima in the pericycle[61]. This re-emergence of auxin-response maxima correlates with renewed expression of *PLT* genes[21] followed by *SCR* expression[62] (FIG. 5). Regulation of auxin response is required for primordium outgrowth[63–66], and polar auxin transport has been proposed to initiate this process[61,67]. The mechanism that selects pericycle cells in a regular pattern and allows for reprogramming of stem-cell niches probably involves a response to periodic variations in polar auxin transport and response[68].

In the shoot, a subset of cells in the axils of leaf primordia form new stem-cell niches to create 'buds', lateral shoots that can either grow out or remain dormant. So, the positioning of new stem-cell centres during shoot development is connected to the specification of leaf

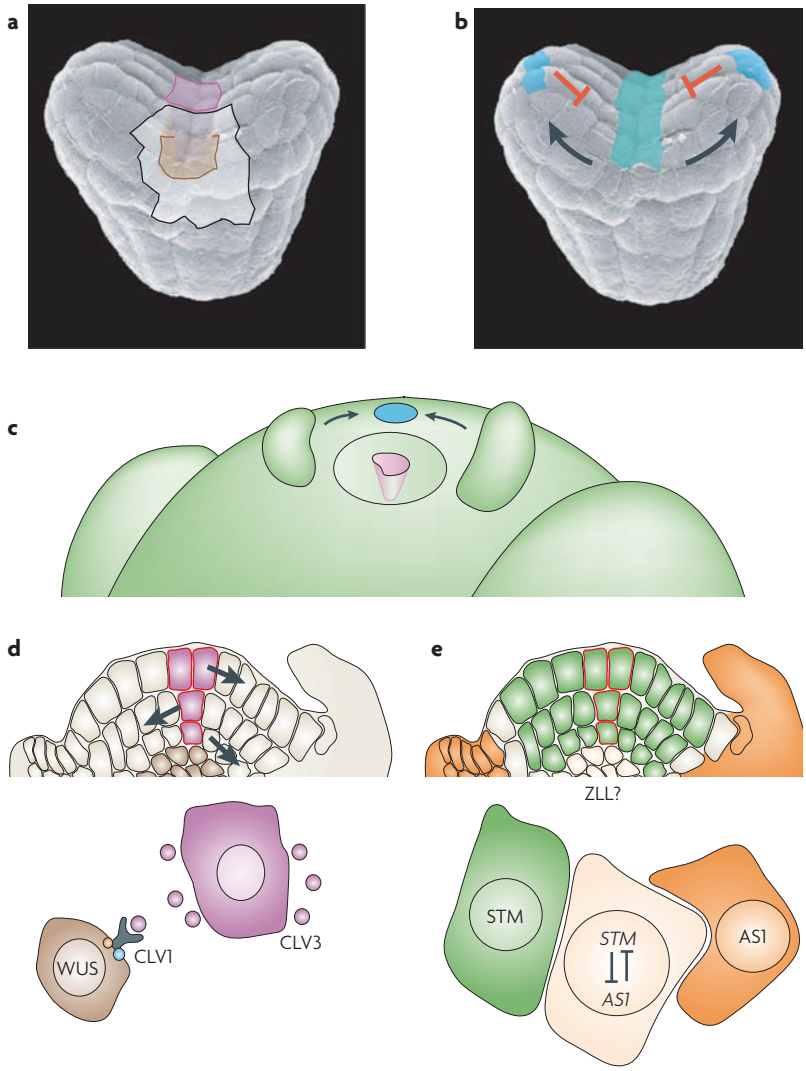

Figure 4 | **Stem-cell specification in the shoot apex.** The three layers of stem cells at the apex are maintained by two mechanisms in *Arabidopsis thaliana*. **a** | Scanning electron micrograph of a heart stage embryo; stem cells are marked with CLAVATA3 (CLV3; pink). *CLV3* expression is maintained by signalling from the underlying organizer centre that expresses *WUSCHEL* (*WUS*) (brown, indicated lighter shaded area of embryo is transparent). **b** | A heart stage embryo in which polar auxin transport (arrows) leads to auxin accumulation (blue), which represses (red) expression of the *CUP-SHAPED COTYLEDON* (*CUC*) and *SHOOT MERISTEMLESS* (*STM*) genes (green). **c** | Post-embryonic shoot apex with CLV3 in stem cells and STM in stem cells and transit-amplifying cells (circle). Outside the *STM*-expression domain, new leaf primordia (blue) are specified in positions that depend on polar auxin transport (direction of transport indicated with arrows). Stem cells and transit-amplifying cells are maintained by STM and by the underlying *WUS*-expressing organizing cells. **d** | Transverse section through apex visualizes *WUS*- and *CLV3*-expression domains in stem and organizer cells, respectively; stem cells produce daughters in the direction of the arrows; higher magnification depicts CLV signalling repressing *WUS* transcription. Secreted CLV3 protein is shown as pink balls and CLV1 receptor and associated proteins are marked. **e** | Transverse section through apex visualizing *STM* (green) and *AS1* (orange) expression in meristem and organ primordia. Higher magnification depicts mutual repression between STM and AS1. Red outlines show stem cells. ZLL, ZWILLE. Images for parts **a** and **b** courtesy of M. Aida, NAIST, Nara, Japan.

primordia (FIG. 5). The pattern of initiation of leaf primordia is tightly linked to polar-auxin-transport patterns in the shoot tip[69] and correlates with the downregulation of *STM* expression[70].

Towards understanding dynamic regulatory networks. Theoretical modelling approaches are beginning to be used to investigate how feedback loops can maintain a stem-cell domain[71] and how leaf primordia can be positioned by an interplay between auxin concentration and auxin transport[72–74]. Once primordia are specified, buds form at the adaxial position of the leaf primordium. Bud formation depends on the redundant activity of homeodomain and the Leu-zipper-motif-containing transcription factors PHABULOSA, PHAVOLUTA and REVOLUTA; the main function of these factors is to distinguish abaxial and adaxial domains[75–78]. These factors also seem to govern dynamic interplay with stem-cell-niche factors, as their joint inactivation not only leads to loss of leaf polarity but also to the loss of stem cells.

In the incipient niche, the specification of new stem-cell areas and the re-initiation of *WUS*- and *STM*-expression domains involves GRAS and Myb transcription factors with highly specific expression domains[79–81]. CUC genes, which are already expressed in the incipient bud region at the shoot apex, have been implicated in this process on the basis of their ability to promote adventitious shoot formation upon ectopic expression[82]. Last, *WUS*-expression domains are set up in flowers, which are lateral organs with short-term stem-cell reservoirs. Intriguingly, floral stem cells are maintained until WUS activity initiates a feedforward circuit to terminate its own expression[83,84].

Taken together, dynamic regulatory networks appear to define and redefine the activity of stem-cell-niche regulators in a regular pattern. In these networks, the dynamic function of regulators cannot be deduced from terminal phenotypes after genetic perturbations. Therefore, a combination of novel imaging techniques[85], inducible gene manipulations[50] and computer modelling[72–74] will be essential to fully understand stem-cell dynamics in plants.

Epigenetic controls in stem cells

Recent exciting work on mammalian stem cells indicates that stem-cell chromatin represses differentiation genes while simultaneously allowing for the activation of stem-cell regulators and proliferation factors. Nearly all of the regulatory regions of repressed genes occupied by the stem-cell factors OCT4, SOX2 and NANOG are also occupied by subunits of the mammalian Polycomb group (PcG) protein complexes PRC2 and PRC1 (FIG. 6)[86,87]. PRC-complex-binding sites in stem cells also harbour trimethylated histone H3 Lys27 (H3K27met)[88,89], which can be catalysed by PRC2 and bound by PRC1 to consolidate silencing[90,91].

Intriguingly, PcG proteins repress many differentiation-promoting transcription factors in stem cells; however, stem cells should differentiate under appropriate conditions in order to maintain pluripotency. So, repression should be reversible. Accordingly, key developmental genes that are bound by PcG proteins in stem cells do not only harbour repressive marks, but also activating histone H3 Lys9 methylation (H3K9met) marks, which have been proposed to poise these genes for activation

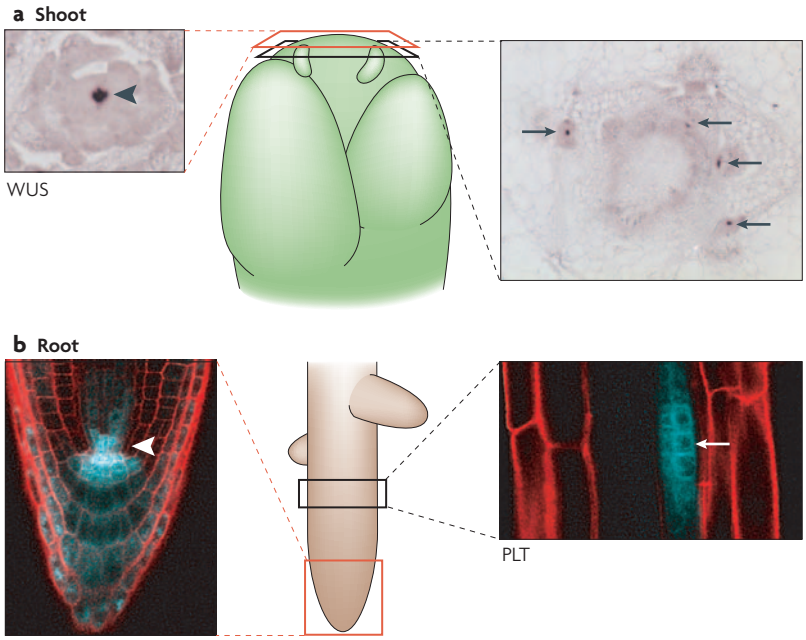

Figure 5 | **Secondary stem-cell niches for plant architecture. a** | In the shoot apex, the distal stem-cell niche (red rectangle) is marked by *WUSCHEL* (*WUS*) expression (arrowhead). Cells in the axils of leaf primordia develop an associated new stem-cell niche (black rectangle) that can support a new lateral shoot. Such new stem-cell niches can be marked by *WUS* expression (brown staining indicated with arrows). **b** | In the growing root, an internal cell layer, the pericycle (black rectangle), remains competent for cell division and re-initiates new stem-cell niches marked by *PLETHORA* (*PLT*) expression (blue staining indicated with arrow), which will support new lateral roots. The pattern of activation of these new stem-cell niches determines the three-dimensional architecture of the plant. The red rectangle shows the distal stem-cell niche marked by high *PLT* expression. Images for part **a** courtesy of P. Doerner, University of Edinburgh, UK.

upon differentiation[92]. An important remaining question is how chromatin is marked and how PcG complexes are positioned in vertebrate stem cells. Is OCT4-, SOX2- and NANOG-mediated transcriptional repression merely maintained in stem cells by epigenetic mechanisms, making the epigenetic regulators slaves of the primary transcription factors? Or do epigenetic marks, or global differences in chromatin structure[93], influence target-site selection of OCT4, SOX2 and NANOG?

Plant perspectives on epigenetic control. Given the prominent role of transcriptional control in cellular differentiation in plants, it is reasonable to assume that transcription factors that initiate cell differentiation are also stably repressed in pluripotent plant stem cells. Could PcG complexes repress differentiation-inducing factors in plants? Several distinct plant PRC2 complexes have specialized functions, such as the regulation of vernalization, flowering time and maternal control of embryonic growth[94,95] (FIG. 6). Although a PRC2 complex suppresses the shoot stem-cell regulator STM in differentiated cells, probably by direct binding to the STM promoter[96,97], no role for PRC2 complexes in the stem-cell niche has been reported so far. However, it is possible that the functions of the PRC2 complexes in stem-cell niches have been missed owing to redundancy[98,99] or to pleiotropic phenotypes of non-redundant factors[100]. It is interesting to note that PRC1 homologues are not present in plants. Despite the absence of structural homologues, PRC1 analogous functions can be performed by different chromatin-modifying complexes. Recent evidence implicates the LIKE HETEROCHROMATIN PROTEIN1 (LHP1) in the maintenance of PRC2-initiated silencing of at least one euchromatic gene[101,102].

Possible models for epigenetic stem-cell control. Although there is no proof yet that PcG proteins contribute to the stem-cell state in plants, other factors that preserve epigenetic marks have been implicated in stem-cell maintenance. Mutations in subunits of the plant chromatin-assembly factor-1 (CAF1) affect the expression of stem-cell regulatory genes, stem-cell pool size[103] and the stable maintenance of epigenetic marks[104]. Another potential link between stem-cell regulation and chromatin modification in plants is the plant RETINOBLASTOMA-RELATED (RBR) protein. Local reduction of *RBR* gene activity dose-dependently expands the root stem-cell pool, and increased gene activity eliminates stem-cell renewal, indicating that RBR regulates stem-cell maintenance[105]. Intriguingly, cell-cycle progression seems to be unaltered in the expanding columella stem-cell population when RBR is reduced, which indicates that RBR manipulation in stem cells primarily affects cell differentiation and not cell cycle. Localized induction of RBR activity in the shoot apex has recently been shown to also induce differentiation in the stem-cell area[106]. Further evidence is necessary to establish whether RBR function in plant stem-cell niches is truly stem-cell intrinsic.

Although the retinoblastoma (RB) protein was first identified as a regulator of the G1–S transition during cell-cycle progression (reviewed in REF. 107), it is now evident that RB can promote cell differentiation by forming complexes with distinct differentiation factors[108,109]. Upstream factors in the RB pathway have been implicated in animal stem-cell maintenance, but a recent report reveals that RB deletion (without simultaneous deletion of the homologous p107 and p130 proteins) in mouse haematopoietic stem cells does not affect stem-cell maintenance[110]. Notably, RB associates with and modulates the activity of chromatin-modifying enzymes[111], and the RB-binding protein Musashi-1 (MSI1; RbAp46/48 in yeast and p55 *in D. melanogaster*) is a member of the PRC2 complex in plants, as well as a component of the CAF complex[103,112,113] (FIG. 6). A recent report establishes that RB is, at least in one case, important to target PRC2 to the promoter of a cell-cycle control gene, suggesting the *in vivo* significance of the molecular relationships between RB and PRC2 complexes[114]. These findings indicate the possibility that, in plants, RBR mediates stem-cell control through an epigenetic silencing mechanism that is connected to self-renewal mechanisms found in animal stem cells.

The ability to detect stem-cell functions in plants as well as in animals is influenced by redundancy and by the technical difficulties that investigators face when

Polycomb group
A class of genes and proteins that was originally identified in *D. melanogaster* through mutations that are unable to maintain the repression of homeotic genes.

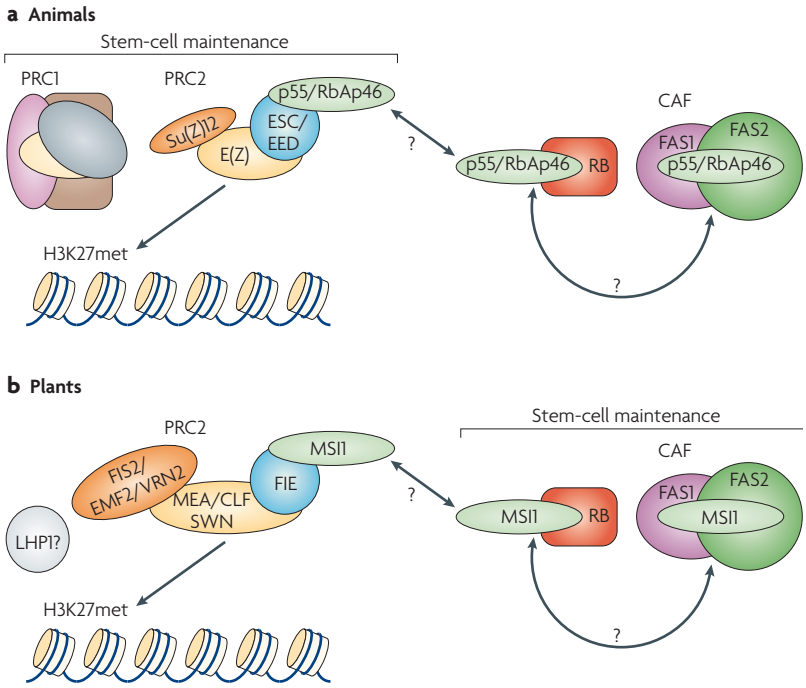

Figure 6 | **Stem cells and chromatin modification.** Several complexes implicated in epigenetic control of stem-cell maintenance are conserved between plant and animal kingdoms. In mammalian stem cells, the Polycomb complexes PRC1 and PRC2 have a role in embryonic stem-cell maintenance through chromatin modifications; for example, the PRC2-mediated trimethylation of histone H3 Lys27 (H3K27met). The PRC2 complex members Su(Z)12, E(Z), ESC (extra sex combs; the human EED) and p55 (the yeast RbAp46) are all conserved between animal and plant stem cells. The retinoblastoma (RB) protein, which can bind to p55, is implicated in the maintenance of certain animal stem cells. PRC1 homologues have not been found in plants, but unrelated factors with similar functions might be present (LIKE HETEROCHROMATIN PROTEIN1 (LHP1) is shown as an example). In plants, RB levels and the CAF complex, which consists of FAS1, FAS2 and the p55 homologue Musashi-1 (MSI1), are important for stem-cell maintenance. p55/RbAp46/MSI1 can bind to RB and the CAF and PRC2 complexes in animals and plants. It is not known whether p55/RbAp46/ MSI1 can bring the PRC2, RB and CAF complexes together (indicated by question marks). CAF, chromatin-assembly factor; CLF, CURLY LEAF; EMF2, EMBRYONIC FLOWER2; FIE, FERTILIZATION-INDEPENDENT ENDOSPERM; FIS2, FERTILIZATION-INDEPENDENT SEED2; MEA, MEDEA; SWN, SWINGER; VRN2, VERNALIZATION RESPONSE2.

they try to separate the functions of essential factors. The current evidence might indicate that the related complexes discussed here are two sides of a similar stem-cell-maintenance mechanism in both kingdoms that has not yet been fully exposed owing to experimental constraints. On the other hand, it is too early to exclude the possibility that different chromatin-modification complexes have been recruited in both kingdoms to preserve the stem-cell state.

Conclusions and future perspectives

Stem cells are generally maintained by specialized organizing cells in their vicinity, and the cellular organization of the resulting niche reveals striking similarities in animals and plants. This similarity in cellular organization is probably due to convergent evolution because both kingdoms evolved from different ancestral unicellular organisms. In support of independent evolution of animal and plant stem-cell niches, many of the transcription factors that specify stem cells and the patterning mechanisms that activate these factors, and so position stem-cell niches, are plant specific.

Regulatory interactions between plant stem-cell-patterning factors create a dynamic picture of stem-cell programming in which reversibility is the rule rather than the exception. Flexibility in stem-cell dynamics allows the plant to determine its final architecture in response to environmental factors, a pivotal element for a sessile lifestyle in which adverse conditions often have to be dealt with by modulation of developmental programmes. In these features, plant and animal stem cells seemed to differ at first, but a static view on animal stem cells has been challenged over the past years and stem cells in animal phyla in which regeneration has an important role can display a flexibility that resembles the situation in plants.

Recent evidence indicates an important role for epigenetic control mechanisms in stem cells. In the animal field, PcG proteins have been at the forefront of investigation, whereas in the plant field, potential roles for an RB-related protein and for the CAF complex in stem-cell maintenance have caught attention. Further experiments in both kingdoms are needed to test whether these findings uncover a general theme. Does stem-cell maintenance involve conserved cross-talk between RB, CAF1 and PRC complexes? Or are these complexes used in different ways in plant and animal stem cells and are the factors they share merely coincidental?

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Competing interests statement
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