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Plant and animal stem cells: conceptually similar, molecularly distinct?

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Animals and plants maintain small pools of stem cells that continuously provide the precursors of more-specialized cells to sustain growth or to replace tissues. A comparison of plant and animal stem cells can highlight core aspects of stem-cell biology. In both types of organism, stem cells are maintained by intercellular signals that are available only in defined regions (niches) in the tissues. Although plants use different signals and are more flexible at establishing stem-cell niches in new locations, recent evidence suggests that the mechanisms restricting cell fate in stem-cell progeny are similar in both kingdoms and might pre-date the evolution of multicellular organisms.

Stem cells have attracted much attention from the general public because of both their potential use in medicine and the ethical debate on the use of embryonic stem cells. The concept of stem cells is, however, neither recent nor restricted to medicine. It was in use at least 50 years ago, when cell lineage analysis suggested that tumour growth is sustained by a few immortal stem cells [1], and is central to our understanding of the development of multicellular organisms. Stem cells form a reserve of undifferentiated cells that maintains itself, while providing a steady supply of precursor cells to form differentiated tissues. By this definition, both plants and animals have stem cells; in fact, stem cells are especially prominent in plants, whose shoot and root apices produce new organs and tissues throughout the life cycle.

There are intriguing parallels in the way that stem cells function in animals and plants [2,3]. On the one hand, some of the similarities are clearly independent solutions to comparable developmental problems. On the other hand, recent evidence suggests that common mechanisms might control the shift from the undifferentiated state of stem cells to the defined cell fates in their early progeny. Here I discuss these parallels, mainly by reference to two examples of stem cells from animals, fly germline stem cells (GSCs) and mouse haematopoietic stem cells (HSCs), and two examples from plants, *Arabidopsis* shoot and root meristem stem cells.

The stem-cell niche

Stem cells are maintained in microenvironments called 'stem-cell niches' [4,5]. Typically, a few stem cells are maintained by short-range signals produced by a localized source. As the stem cells divide, some of the daughter cells are placed outside the reach of the signal and begin to differentiate. The source of signal and its effective range define the stem-cell niche (Figure 1).

An example of a structurally well-defined niche is found in the *Drosophila* germarium – a sac-like structure that functions as a production line for immature eggs [6] (Figure 2a). At the anterior end of the germarium are the cap cells, which are in direct contact with a few GSCs. Each GSC divides asymmetrically: one daughter cell stays in contact with the cap cells and remains a GSC, the other proceeds through the germarium, divides and differentiates to form the egg cyst. The GSC niche encompasses the cap cells and the region where GSCs are maintained. In addition, a second niche in the germarium maintains somatic stem cells (SSCs), which give rise to the follicle cells that surround the mature egg cyst. The maintenance signal for the SSCs is also thought to emanate from the cap cells, which are located several cell layers away.

Another well-studied type of stem cell is the HSC (Figure 2b). In the adult mammal, these cells are localized in the bone marrow. Recent evidence suggests that, in the bone marrow, a specific type of osteoblast termed an 'SNO' (for spindle-shaped, N-cadherin^{+} CD45^{-} osteoblast) might be the specialized cell that maintains the niche for slowly cycling, long-term HSCs [7,8]. The latter cells in turn give rise to multipotent progenitor cells that subsequently originate the different types of blood cell [9].

In plants, the two best-studied stem-cell niches are found in the shoot and root apical meristems in *Arabidopsis* [10,11]. In the shoot, stem cells are located in the central region of the meristem. Surrounding them, their immediate descendants divide more rapidly and provide the precursor cells to initiate new organs, such as leaves, in a regular pattern (Figure 2c). Maintaining the stem cells requires expression of the homeodomain protein WUSCHEL (WUS) in an underlying group of cells known as the 'organizing centre'.

In the root meristem, the signal that maintains the stem cells originates from a small group of cells, called the 'quiescent centre', at the centre of the root tip (Figure 2d). Stem cells surrounding the quiescent centre give rise to

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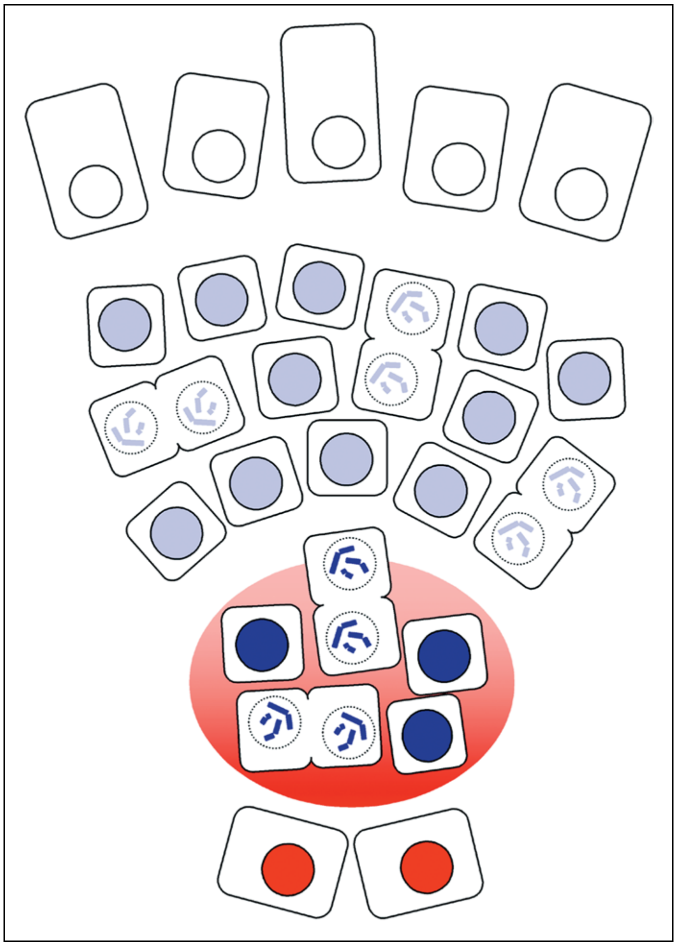

Figure 1. General features of a stem-cell niche. Specialized cells, with nuclei shown in red, produce an intercellular signal with a limited range (represented by the graded red area). Stem cells within the range of this signal, with nuclei shown in dark blue, remain undifferentiated and divide regularly (indicated by the twinned cells with condensed chromatin). The region in which the maintenance signal is effective, together with the source cells, is termed the 'stem-cell niche'. When cell division places a daughter cell outside the niche, the cell begins to differentiate. An increase in the rate of cell division frequently precedes or coincides with the early stages of differentiation: cells at this stage, with nuclei shown in light blue, are sometimes called 'transit-amplifying' cells. Terminally differentiated cells are indicated by white nuclei.

regular files of daughter cells. The daughter cells facing the root tip differentiate as the root cap, which protects the meristem and is constantly renewed as the root grows through the soil. The daughter cells facing away from the tip give rise to the concentric tissue types that form the root. Differentiation of these cells begins in the immediate descendants of the stem cells, which divide rapidly, and is completed in more distal parts of the root tip, where cells stop dividing and begin to elongate.

Thus, in both plants and animals, stem cells are maintained by an external signal and their daughter cells differentiate when displaced from this signal. Below, I discuss the nature of the signal and how cells shift from a stem-cell identity to a differentiated state.

Maintenance signals

In animals, stem cells are maintained by intercellular signals that are used in various developmental contexts and not only in the stem-cell niches. In mammals, the Wnt [12] and Notch [13] pathways control the self-renewal of not only HSCs [7,14], but also stem cells in the gut crypt, skin and nervous system [15–17]. In the fly germarium, decapentaplegic (Dpp), which is related to transforming growth factor-β [18], and Hedgehog (Hh) [12] maintain GSCs, whereas Hh signaling alone controls the proliferation of SSCs [6]. The signals seem to be interpreted according to the identity of the cell receiving them: in germaria depleted of GSCs, the cap cells continue to produce both Dpp and Hh, but when SSCs eventually occupy the empty GSC niche, they continue to behave as SSCs and proliferate specifically in response to Hh [19].

In plants, the signals that maintain stem cells are unknown, but their presence has been inferred from ablation experiments and from the non-cell-autonomous role of genes that promote stem-cell maintenance. In the *Arabidopsis* root, ablation of the quiescent centre causes differentiation of the stem cells, and a similar effect is caused by mutations in *SCARECROW* (*SCR*) or *SHORT ROOT* (*SHR*), which are required for development of the quiescent centre [20,21]. In the shoot, loss of stem cells can be caused by the mutation of regulatory genes (such as *WUS*) that are expressed in neighbouring cells [22,23]. However, *WUS* also functions non-cell-autonomously in ovule development in a pathway that does not seem to involve stem cells [24]. Similarly, *SCR* is not exclusively dedicated to establishing the root stem-cell niche, but it is more widely expressed and has a separate function in the differentiation of cortex and endodermis cells [21].

The overall picture is that, both in plants and animals, versatile signalling pathways are used as devices to communicate the position of stem cells relative to other cells, and stem-cell functions are connected to these pathways in a specific context.

Control of the location and number of stem cells

From the way in which the stem-cell niche is organized, it is expected that the position and size of the stem-cell population should depend on the source of the maintenance signal. Accordingly, in the fly germarium excess Dpp increases the number of GSCs, whereas in the bone marrow induction of extra SNO cells causes a corresponding increase in the HSC population [7,8,19]. Both for fly GSCs and for HSCs, production of the signal can persist when the niche is depleted of stem cells, facilitating subsequent recolonization of the niche [8,19].

In plants, control of the size and position of the stem-cell niche seems to be more dynamic. In the *Arabidopsis* shoot, there is two-way signalling between the organizing centre and stem cells: excessive stem-cell proliferation is prevented by a negative signal that diffuses from the stem cells to the organizing centre to repress *WUS*, thereby reducing the positive maintenance signal [25]. The small protein CLAVATA3 (CLV3) is part of the negative signal secreted by the stem cells [26,27]. Additional evidence that the domain of *WUS* expression is continuously set by intercellular communication has come from experiments in tomato, in which expression of *WUS* was found to re-establish quickly in adjacent cells after ablation of the organizing centre [28].

The dynamic control of the size and position of the shoot stem-cell niche might be an essential requirement for

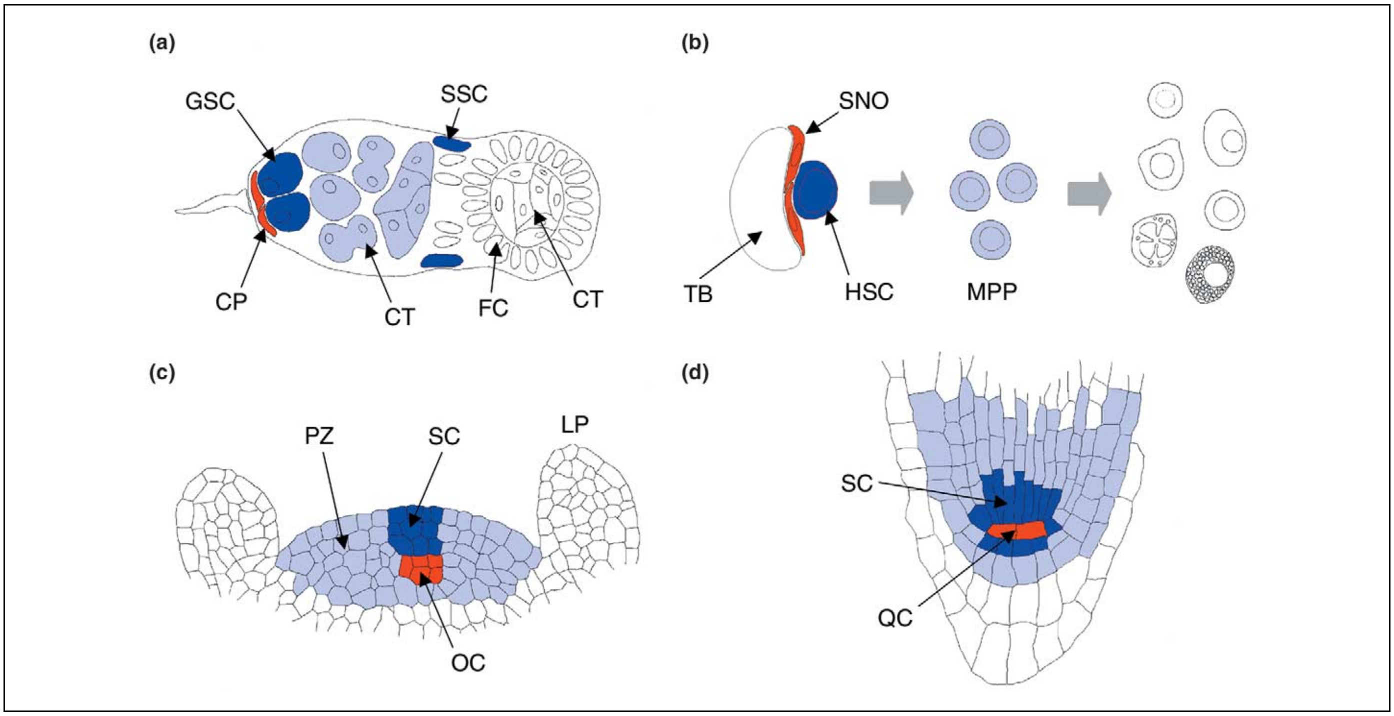

Figure 2. Four examples of stem-cell niches. The source of the maintenance signal is shown in red, stem cells are shown in dark blue and the early descendants of the stem cells are shown in light blue. **(a)** *Drosophila* germarium. Germline stem cells (GSCs) contact the cap cells (CPs). Descendants of the GSCs become cyst cells (CTs), which divide to form a mass of interconnected cells (termed the egg cyst). The mature egg cyst (furthest from the cap cells) is surrounded by follicle cells (FCs), which are produced by somatic stem cells (SSCs). **(b)** Haematopoietic stem cell (HSC) niche. HSCs contact the spindle-shaped, N-cadherin$^+$ CD45$^-$ osteoblastic (SNO) cells, which are attached to trabecular bone (TB) in the bone cavity. The early descendants of haematopoietic stem cells are multipotent progenitor (MPP) cells, which give rise to the various types of differentiated blood cell. **(c)** Shoot apical meristem. The cells of the organizing centre (OC) express the *WUSCHEL*(*WUS*) gene (red) and function non-cell-autonomously to maintain the overlying stem cells (SCs), which are surrounded by a transit-amplifying population of cells in the meristem peripheral zone (PZ), from which leaf primordia (LP) emerge. **(d)** Root meristem. The root stem cells (SCs) are maintained by a signal from the quiescent centre (QC). The early descendants of the stem cells proliferate and give rise to the root cap (which covers the root tip) and to the differentiated cells that form the root (not shown).

the way in which the shoot grows. Stem-cell daughters (at least those of stem cells in the deeper layers of the meristem, where the orientation of cell divisions is random) are expected to accumulate between the stem cells and the underlying organizing centre. To maintain apical growth, it might be necessary to correct the position of the organizing centre continuously. If early descendants of the stem cells become part of the organizing centre, small increases in the stem-cell population will be quickly amplified by a corresponding increase in the number of cells producing the maintenance signal. Thus, the CLV negative feedback loop might stabilize this otherwise inherently unstable mode of growth. Clonal analysis is required to test whether or not WUS-expressing cells descend from the overlying shoot stem cells, and whether or not cells that have stopped expressing WUS contribute to the tissues that form below the shoot tip.

There is also evidence for dynamic positioning of the niche in the root. The position of the quiescent centre seems to be specified by the overlap between *SCR* expression and high levels of the phytohormone auxin, whose distribution is determined by continuous directional transport to the root tip [21]. In contrast to the shoot stem cells, the root stem cells produce new daughter cells on the side facing away from the quiescent centre; thus, an increase in stem-cell activity is not expected to be amplified by a positive feedback loop. Perhaps not surprisingly, a negative feedback from the stem cells to the quiescent centre, similar to the CLV pathway, has not been found in the root. Recent work has shown that CLV3 and CLV3-like polypeptides causes a gradual loss of meristem when expressed in roots [29,30]; however, the primary effect of these peptides seems to be on the early descendants of the stem cells, rather than on the quiescent centre or on the stem cells themselves [30].

The examples above show how stem-cell populations are tightly controlled in both plants and animals by mechanisms based on maintenance signals external to the stem cells.

Leaving the niche and choosing a fate

In addition to stem-cell niches, animals and plants share the feature that when daughter cells exit the niche, they usually go through a period of rapid cell division (the transit-amplifying stage) before they complete differentiation. Although not obvious in the germarium, the transit-amplifying stage is prominent in other animal stem-cell systems such as those of the skin and intestinal epithelium [15,17]. The maintenance of a small reserve of slowly dividing stem cells whose progeny is amplified before differentiation might be a safeguard against accumulating somatic mutations [31].

In plants, protection from mutations is particularly important in the shoot apical meristem, where stem cells give rise not only to somatic tissues but also to the germ line. The more rapid division of the cells in the peripheral zone of the meristem, as compared with the central zone (where stem cells localize), has been recently confirmed directly in live meristems [32,33]. In *Arabidopsis*, the proliferation of shoot meristem cells to a sufficient number before their recruitment into organogenesis requires the homeodomain protein SHOOT MERISTEMLESS (STM), which is expressed throughout the meristem but is down-regulated in leaf primordia [34]. In addition to delaying differentiation in the peripheral zone of the meristem, STM renders the stem cells in the central zone responsive to the WUS-induced signal [35–37].

As the descendants of stem cells divide and distance themselves from the niche, they differentiate. The early stages of differentiation can be reversible [38], however, as has been recently shown for fly GSCs. These cells were induced to differentiate by pulsed expression of the gene *bag of marbles* (*bam*), which promotes egg cyst formation; as the pulse of *bam* wore off, however, their differentiation was eventually reversed and the cells that occupied the GSC niche became stem cells again [39].

In plants, the ability to reverse differentiation and to re-establish stem-cell activity is widespread [2]. For example, meristems can be re-established in differentiating tissues by the ectopic expression of *STM* and *WUS* [35–37]. Every time a lateral root is initiated in the root system, a new stem-cell niche is established from differentiated pericycle cells. The regeneration of whole plants from organ fragments or even from single cells is commonplace, although in this case it is not clear whether regeneration begins with a reversal to stem-cell identity. The ability to re-establish stem cells readily might be related to the dynamic nature of plant stem-cell niches, as discussed above.

In addition to the reversibility of differentiation, another important issue is the repertoire of cell fates that are possible for the descendants of stem cells. In animals, apart from embryonic stem (ES) cells (which can be established in tissue culture from pluripotent cells found in early embryos [40]), most stem cells give rise to a limited range of differentiated cell types. It is unclear to what extent this restriction is a property of the stem cells themselves and to what extent it is influenced by the surrounding tissues. In several cases, the observation that stem cells could acquire an unexpectedly wide range of fates after transplantation was later attributed to cell fusion events, rather than to true reprogramming of the stem cells [38]. Long-term HSCs transplanted to damaged heart differentiate as blood cells, indicating that HSCs have an intrinsically restricted developmental potential [41,42]. In plants, stem cells specified by ectopic WUS expression give rise to shoot tissues, even when they are embedded in the root meristem, showing that WUS establishes both stem-cell identity and shoot fates [43].

In summary, both plant and animal stem cells might have a wide but intrinsically limited range of possible fates, and the acquisition of a specific fate in daughter cells can be a gradual process.

Fate restriction and chromatin modification

It is not known how stem cells are maintained in a pluripotent state – in other words, with many differentiation choices open to their progeny. It has been proposed that stem cells simultaneously express low levels of regulators of several differentiation pathways [44]. A problem with this idea is that regulatory genes that control differentiation typically reinforce their own expression and antagonize alternative differentiation pathways, and thus a small change in the levels of different regulators might tip the balance towards one of the pathways. It is not clear how such an unstable balance could be maintained for a long time in stem cells and then broken in their progeny.

One way in which to restrict and to stabilize patterns of gene expression is by modifying chromatin structure. Of the many known regulators of chromatin modification, polycomb group (PcG) proteins have been directly implicated in modifying chromatin in stem cells and their immediate progeny. PcG proteins function in transmitting cell identity through cell division and establish a silent chromatin state by histone methylation and by recruiting proteins that deacetylate histones [45].

In mouse, the PcG proteins Ezh2 and Eed function together in a complex that is essential for establishing ES cells [46]. Additional links between PcG proteins, chromatin silencing and pluripotency have come from work on X-chromosome inactivation (X-inactivation). In female mammals, one of the X chromosomes is inactivated to maintain the same gene dosage as in XY males. This inactivation happens at the onset of differentiation during early embryogenesis and also occurs on the differentiation of ES cells*in vitro*. X-inactivation requires the Ezh2–Eed complex and involves methylation of histone H3 [47,48]. It has been recently shown that X-inactivation initially happens throughout the blastocyst but is transiently reversed in the pluripotent cells from which ES cells are derived. During this stage, the Ezh2–Eed complex is released from the previously inactive X chromosome [49]. These pluripotent cells also show a specific pattern of methylation of histone H3 that is abolished in mutants of Ezh2 and Eed, suggesting that these proteins establish a unique chromatin state in pluripotent cells [50].

The example of X-inactivation suggests that PcG proteins function at the onset of cell differentiation to restrict cell fate by selective chromatin silencing. There is also evidence, however, that PcG proteins maintain stem cells by repressing genes that are incompatible with self-renewal: the PcG group protein Bmi has been implicated in maintaining HSCs [9,51], at least in part, by repressing genes that promote apoptosis ($p19^{Arf}$) or inhibit cell division ($p16^{Ink4a}$). Whether Bmi is involved directly in repressing these genes has not been determined.

In plants, there is also evidence that loss of the pluripotent, meristematic state is associated with PcG-mediated gene silencing. In particular, the proteins FIE and CLF (homologues of Eed and Ezh2, respectively) are required to keep *STM* repressed during leaf development in *Arabidopsis* [52]. Thus, chromatin modification constitutes part of the mechanism that restricts cell fate in both animal and plant stem-cell niches.

Fate restriction and small RNAs

Further suggestions of molecular mechanisms shared in plant and animal stem cells come from the involvement of ARGONAUTE (AGO) proteins in stem-cell maintenance. Proteins of this family function in association both with short interfering RNAs (siRNAs) during RNA interference (RNAi) and with microRNAs (miRNAs) to control mRNA stability or translation [53–55] (Figure 3). Consistent with having a role associated with small RNAs, these proteins contain a domain that binds single-stranded RNA [56–58].

In *Drosophila* females, the AGO protein Piwi is required to maintain GSCs, functioning both in the cap cells to produce the maintenance signal and in the stem cells to increase their rate of division [59]. In *Arabidopsis*, the AGO protein ZWILLE (ZLL, also called PINHEAD) is required during embryogenesis to establish a stable population of shoot stem cells. In *zll* mutants, the meristem frequently terminates shortly after germination, producing one or a few organs [60]. This phenotype is not completely penetrant, probably because ZLL is partially redundant with its homologue AGO1. The *zll ago1* double mutant embryo arrests at an early stage and accumulates *STM* mRNA, but not protein, suggesting that ZLL has a role in the posttranscriptional control of *STM* [61]. Ectopic expression of ZLL, which induces extra cell divisions and ectopic meristems, also suggests that this protein has a role in establishing stem cells [62]. It remains unclear where the expression of *ZLL* is relevant for stem-cell maintenance. Whereas its role in STM translation suggests that it functions cell-autonomously, the ectopic expression experiments indicate that it has a non-cell-autonomous effect [62]. Clonal analysis is required to clarify this point.

In addition to its functional redundancy with *ZLL* in the embryo, *AGO1* is required for RNAi [63] and for the function and correct distribution of miRNAs that control early steps of shoot organogenesis [64,65]. The involvement of miRNAs in the early stages of differentiation has been also shown during haematopoiesis [66]. A role for miRNAs in mouse ES cells has been suggested by the isolation of miRNAs expressed specifically in the ES cells and their differentiating progeny, but these miRNAs have not been functionally characterized [67].

In addition to their role in controlling mRNA function, AGO proteins also participate in a mechanism in which small RNAs target homologous genomic sequences for histone methylation and silencing in both fission yeast [68] and *Arabidopsis* [69] (Figure 3). *piwi*itself has been implicated in RNAi and in heterochromatic silencing (which, in *Drosophila*, also requires PcG proteins) [70,71].

The above data suggest that small RNAs have a prominent role both in maintaining stem cells and in the early stages of differentiation in their descendants. This idea is attractive because the link between small RNAs and chromatin silencing also implies that there is a mechanism for stabilizing cell fates in the differentiating progeny. Currently, however, there are three caveats to this idea. First, it has not been shown that small RNAs are part of the mechanism by which Piwi and ZLL control stem-cell functions. Second, the link to chromatin silencing has been established for siRNAs, but not for miRNAs. Third, it is clear that miRNAs are not specialized for fate decisions in stem cells or their close progeny, but are part of a more widely used regulatory mechanism [54].

Concluding remarks

The independent origin of multicellularity in plants and animals makes their comparative study a unique opportunity to highlight the logic of multicellular development [72]. Stem-cell biology is a clear example. As discussed above, there are parallels between stem-cell behaviour in both kingdoms, including their maintenance by short-range intercellular signals and the relatively slow division of stem cells as compared with their immediate descendants. At the same time, the signalling molecules used in animal stem-cell niches have no obvious homologues in plants, and the signals in animals are varied and used in several developmental contexts.

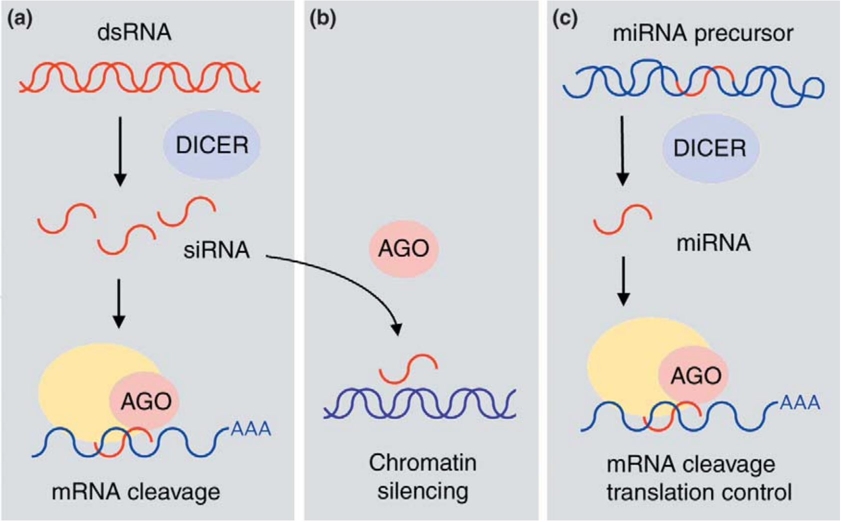

Figure 3. The interactions between small RNAs and ARGONAUTE (AGO) proteins. **(a)** RNA interference (RNAi) is initiated by double-stranded RNA (dsRNA), which is cut by the RNase DICER to produce short interfering RNAs (siRNAs). These are incorporated in an AGO-containing protein complex and used as a guide to locate and to cleave matching mRNAs. **(b)** In addition to mRNA degradation, siRNAs direct chromatin modification of matching genomic sequences in a process that requires AGO proteins. However, the details of how the siRNAs interact with DNA are unknown. **(c)** MicroRNAs (miRNAs) are comparable to siRNAs in length and chemical structure but they originate from precursors transcribed from specific genes. The precursor RNA forms double-stranded regions that are processed by DICER to a miRNA with a defined sequence (red). miRNAs are also incorporated in an AGO-containing complex that cleaves or controls the translation of matching mRNAs.

Thus, the presence of stem-cell niches in plants and animals is most probably the result of convergent evolution under similar constraints. To replace or to grow tissues in which cell specialization comes at the expense of the ability to self-renew, it is necessary to maintain a reserve of unspecialized cells. Confining these cells to a specific region is necessary if their uncommitted, self-renewing state is incompatible with patterning and differentiation signals required to form the tissues. Dependence on a restricted supply of external signals is an effective way by which to maintain a stable number of stem cells.

Despite the probably independent origin of stem-cell niches in plants and animals, the roles of AGO and PcG proteins suggest that there are some molecular similarities. AGO proteins and chromatin remodelling are part of an ancient mechanism for stabilizing cell fates that is also used in unicellular organisms. In *Schizosaccharomyces pombe*, for example, mating type loci are silenced by methylation of histone H3, and the establishment of this heterochromatic silencing involves AGO1 and the RNAi machinery [73]. Furthermore, in *Tetrahymena*, an AGO protein is involved gene deletion mediated by small RNAs (an extreme form of cell fate restriction) [74,75]. Thus, it seem that, even though the actual cell fates are completely different in animals and plants, a similar tool kit for limiting and stabilizing cell fates has been recruited in plant and animal stem-cell niches.

There are still many gaps in our knowledge of how stem cells, but not some of their descendants, are maintained in a pluripotent state and how external signals are connected to this mechanism. For plants, unresolved issues also include the nature of the maintenance signals and why plants seem to be more flexible towards re-establishing stem-cell identity. The similarities in stem-cell functions in animals and plants raise parallel questions in the two fields and make stem cells a unifying theme in developmental biology.

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