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Plant stem cells carve their own niche

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Stem cells are the precursors of differentiated cells and are, thus, indispensable for growth and development in plants and animals. Stem cells from both types of organisms share the fundamental features of a capacity for self-renewal and an ability to generate differentiated cells. The maintenance of stem cells in both systems is dependent upon reciprocal signalling between stem cells and the specialized tissue microenvironment known as the niche, which provides intercellular signals for stem cell regulation. One significantly underexplored facet of plant stem cells is the nature of their intrinsic transcriptional programme. A potentially rich avenue for addressing this deficiency is to combine laser-assisted microdissection and genome-wide transcriptional profiling to unravel the molecular road map controlling plant stem cells and their niches.

Stem cells

Current awareness of the potential of stem cells in regenerative medicine has radically altered the way the general public views disease, biological injury and repair, medicine and cellular regeneration [1,2]. Stem cells are discussed in almost every media around the globe, with much attention focused on the ethics of the therapeutic applications of embryonic stem cells. Thus, stem cells are now a deeply ingrained component of not just the scientific landscape, but popular culture as well. So what are stem cells?

Stem cells in both animals and plants are defined by their remarkable ability to replenish themselves through self-renewal as well as the potential to generate differentiated cells [3–5]. Each new cell generated by the division of a stem cell can either stay as a stem cell or differentiate into a specialized cell. Differentiation implies phenotypic specialization that is underpinned by altered gene expression patterns that eventually leads to functional divergence [6]. An intermediate population of rapidly dividing committed progenitors, the transit amplifying cells (TA cells), usually forms between the stem cell and its terminally differentiated progeny. TA cells have reduced proliferative capacity and restricted differentiation potential. As their name implies, these intermediate cells serve to amplify the number of cells produced from the division of a single stem cell. Another defining feature of stem cells is the asymmetric outcomes of cell divisions: given that the division of a stem cell results in two kinds of progeny, these divisions can be considered to be intrinsically asymmetric in nature [7,8]. Two general strategies underlie the asymmetric outcomes [9,10]. The first strategy involves divisional or invariant asymmetry where a stem cell division produces strictly one daughter cell that retains the stem cell fate and one daughter that either differentiates or acquires a TA cell fate. The acquisition of differentiated developmental potential by the daughter cells can result from either an unequal partitioning of cell fate determinants or differentiated signalling from their surroundings. In the second strategy, the stem cell identity is maintained by a population-based mechanism where any individual stem cell can give rise to two stem cells, or to two cells that proceed onto differentiation, or to one stem cell and one cell that proceeds onto differentiation [6,9,10]. Populational asymmetry relies upon extrinsic controls to restrict the stem cell population and regulates the potential of stem cell daughters to maintain a stem cell fate [10,11].

The notion that stem cells are located within specialized protective microenvironment niches arose nearly 30 years ago [12]. The niches are composed of stem cells, and the surrounding differentiated cell types release signals that manifest the unique intrinsic characteristics of stem cells. The niche theory implies that the special properties of stem cell maintenance are regulated by a combination of the interaction between the intrinsic potential and signals emanating from the surrounding microenvironment [11,13–15]. Benjamin Ohlstein *et al.* [16] define the stem cell niche as 'a specific location in a tissue where stem cells can reside for an indefinite period of time and produce progeny cells while self-renewing'.

Similar to animal stem cell niches, the key features of plant stem cell niches are specialized organ location and neighbouring cells that release signals required for stem cell regulation. Plant stem cell niches are located within meristems, the specialized structures present at root and shoot tips. Besides structural similarities, examples of similarities in gene circuitries that regulate stem cells are becoming apparent. Some of the genes required for stem cell regulation in plants are closely related to animal genes with a related function. For example *ZWILLE/PINHEAD*, a gene that plays a key role in shoot apical meristem (SAM) stem cell regulation in plants is closely related to *PIWI*, which is essential for stem cell maintenance in animals [5,17,18]. An *Arabidopsis RETINOBLASTOMA-RELATED* (*RBR*) gene crucial for root apical meristem (RAM) stem cell maintenance [19] is the plant counterpart of the RB protein that was first identified in mammals [20]. These observations are remarkable, particularly in view of the independent evolution of multi-cellularity in plants and animals [21]. Another

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recent advance is the recognition of microRNA-modulated gene regulation in stem cell maintenance in both types of organisms [22,23]. Despite notable progress over the past few years, particularly for animal systems, some fundamental questions regarding stem cell biology still remain unanswered. A key question in both animal and plant stem cell biology is the nature of the repertoire of the intrinsic transcriptional regulatory circuitry controlling self-replenishment behaviour. Genes that are crucial for the intrinsic 'stemness' of stem cells still remain elusive. Additional pathways that might be important in intercellular signalling between stem cells and their neighbouring cells in a niche remain to be discovered. In this review, we emphasize the nature of the similarities between animal and plant stem cell niches and highlight some of the many unanswered questions concerning the molecular basis of 'stemness' of plant stem cells and present perspectives for using innovative genomic tools for advancing our knowledge in this exciting area.

Animal stem cells and their niches

Stem cells in animal systems can be either totipotent, pluripotent or multipotent [6,7]. Totipotent signifies cells having the (total) potential to produce a complete new organism with all types of body cells, a property that is best displayed by the fertilized egg, the zygote. Mammalian embryonic cells appear to lose their totipotent character from the eight-cell stage onwards and subsequent post-zygotic embryo development proceeds to a stage where it comprises an undifferentiated mass of pluripotent stem cells that gives rise to an organism containing a diverse array of specialized cell types [24]. The differentiation ability of the descendants of these previously totipotent embryonic stem cells gradually becomes limited during further development, culminating in multipotent somatic stem cells that can differentiate into a limited range of cell types or in unipotent somatic stem cells that can differentiate into only one type of cells. Multipotent somatic stem cells are exemplified by tissue-specific haematopoietic stem cells (HSCs) present in the bone marrow that can give rise to blood cells of all types [25]. For example, skin epidermis and hair follicles rely on multipotent stem cells residing in the hair follicle to provide new cells for hair shaft growth and epithelial turnover [26,27]. Such tissue-specific stem cells were initially recognized by their persistence in mature tissues and their slow cycling behaviour, as reflected by their ability to retain H^3-thymidine and BrdU labels [28,29]. These somatic stem cells display some of the fundamental features of embryonic stem cells, such as the capability for self-replenishment.

The first animal stem cell niche to be delineated at the structural and functional levels was that of germ-line stem cells (GSCs) in *Drosophila* ovaries [15,30]. The adult ovary of *Drosophila* comprises ~15 ovarioles, each of which contains a specialized structure known as the germarium on its anterior tip (Figure 1c). Each germarium contains two or three GSCs that are surrounded by diverse somatic cells: cap cells, inner germarial sheath cells and terminal cells. The GSCs remain in direct contact with cap cells via E-cadherin-mediated cell adhesion [15,30]. This adhesion to cap cells is essential for maintaining GSCs, as is the expression of certain genes in cap cells [30]. Each GSC divides into two daughter cells: the cell that stays in contact with the cap cell remains a GSC; the other cell loses contact with the cap cell and proceeds onto differentiation and the initiation of oogenesis.

The GSC niche of the *Drosophila* testis has a similar architecture (Figure 1a), in which male GSCs stay in direct contact with the hub comprising somatic cells at the apical tip of the testis. Between seven and 15 stem cells reside in each *Drosophila* testis niche [14–16]. Asymmetric division of a male GSC produces one stem cell that maintains its stem cell character by remaining in contact with the hub, the other cell (the gonialblast) dissociates from the hub to proceed onto a differentiation pathway leading to it becoming a spermatogonium. The bone marrow HSC niche has been well characterized in vertebrate systems [14,15]. The HSCs traverse the inner bone surface, which is lined with osteoblasts. A subset of osteoblasts termed SNO cells (N-cadherin$^+$CD45$^-$) have been proposed as specialized cells to which HSCs physically attach in the bone marrow (Figure 1d). The maturation of HSCs is timed by the loss of physical contact with specialized osteoblasts that leads to proliferation and the ultimate move into blood vessels via the central bone marrow cavity.

The epithelial stem cells in the skin are located in the hair follicles [14,15,26,27,31]. The multipotent epithelial stem cells reside in the bulge, which is the region that functions as a niche (Figure 1b). The progeny of these epithelial stem cells exits the bulge niche and either migrate in an upward direction into the basal layers of the epidermis or in a downward direction to the hair matrix, resulting in two populations of TA cells in the skin [14,15,26,27,31]. Other animal stem cell niches that have been well characterized are those of the intestine and neural cells of mice [14–16]. The stem cells in animal stem cell niches are maintained by reciprocal intercellular signalling between stem cells and the local microenvironment. The same signalling pathways that coordinate normal embryonic developmental pathways appear to have been recruited to regulate stem cell proliferation and fate specification. For example, in mammals, the Notch and wnt pathways maintain not only HSCs, but also stem cells in the skin, gut crypt and nervous system [24–26].

Plant stem cells and their niches

The concept of the plasticity of cellular state and totipotency was established long before animal stem cells were described. The term 'totipotency' was introduced in 1902 by the Austrian botanist Gottlieb Haberlandt to describe the ability of a cell to develop into a different type of cell [32]. It was proposed that individual cells of plants retain the ability to give rise to a complete plant. This concept was practically demonstrated in 1958 when a carrot plant was cloned from carrot cells that had been cultured *in vitro* [33]. The ensuing literature is replete with reports of cell- and tissue-culture-based regeneration of complete plants belonging to numerous genera. The ease with which tissue culture conditions can induce de-differentiation of

at least certain types of differentiated cells followed by speciali zation into entirely different cell types questions the validity of the stem cell concept in plants. Thomas Laux [34] provided a convincing argument that cultured totip otent cells do not exhibit the normal stem cell functions of self-renewal and asymmetric division.

So what are true stem cells in the context of an intact plant? Plant cells that show stem cell properties of asymmetric divisions coupled with self-renewal are localized in a specialized meristem structure [5], and the continuous development of plants throughout their life cycle is dependent on this reservoir of stem cells [35].

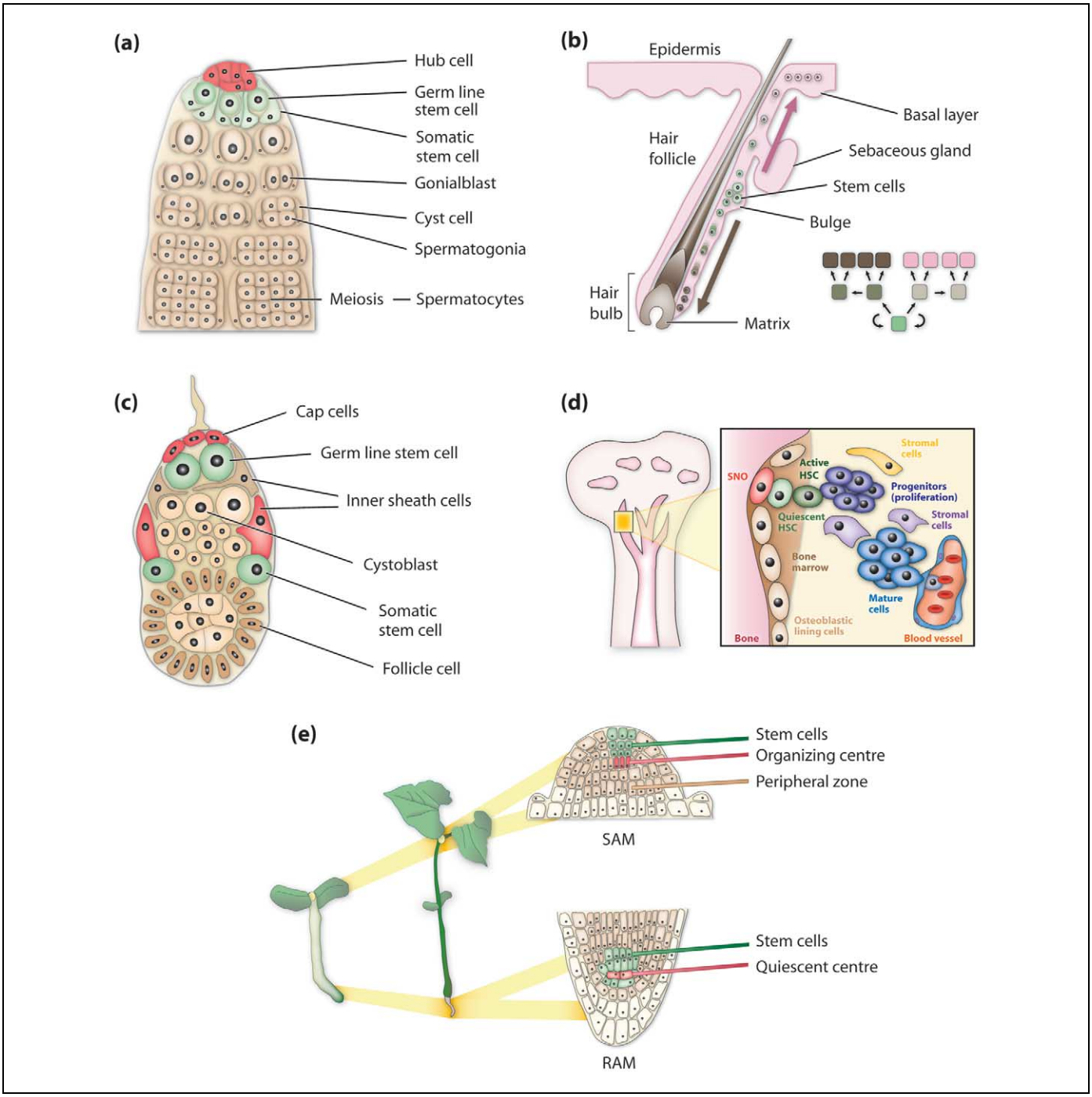

Figure 1. Examples of animal and plant stem cell (SC) niches. The SCs are shown in green and the source cells for maintenance signals are highlighted in red. **(a)** SC niche at the apical tip of the *Drosophila* testis depicting the locations of germ-line SCs (GSCs) and somatic stem cells (SSCs). Hub cells (in red) at the apical tip of the testis form the niches for both types of stem cells. **(b)** Mouse epidermal stem cells. Stem cells reside in the bulge region of the hair follicle located below the sebaceous gland. Upon activation, stem cells undergo division to give rise to transit amplifying (TA) matrix cells, which migrate downwards to become hair-matrix progenitors responsible for hair regeneration. In damaged skin and in neonatal mice, stem cells can also migrate upwards and convert to epidermal progenitors that replenish lost or damaged epidermis. **(c)** Cross section of *Drosophila* germarium depicting the location of GSCs and SSCs. Cap cells are the source of maintenance signals for GSCs. A cytoblast is produced when division of a GSC gives rise to a cell that loses contact with the cap cell. These TA cells divide and then differentiate to produce cysts (in gold). **(d)** Haematopoietic SC (HSC) niche (adapted from [15]). HSCs contact the spindle-shaped, N-cadherin^{+} osteoblastic (SNO) cells that line the inner surface of the trabecular bone (TB). The early descendants of HSCs give rise to the various types of mature blood cells that migrate and infiltrate blood vessels. **(e)** Plant shoot apical meristem (SAM) and root apical meristem (RAM) SC niches (adapted from [38]). The organizing-centre cells provide signals for maintenance of the overlying SCs, which are surrounded by a TA population of cells in the meristem peripheral zone (PZ). In the RAM, the quiescent centre (QC) is the source of SC maintenance signals.

Similar to animal cells, the plant stem cells present in meristems are undifferentiated cells capable of self-renewal, proliferation and generation of a large number of differentiated progeny that gives rise to diverse tissues and organs. The specialized structures present in the meristems of plants parallel the stem cell niches in animal systems.

The cells surrounding plant stem cell niches located in the meristems provide signals to maintain the stem cells in an undifferentiated state [5,10,35–38]. The post-embryonic development of a plant is dependent on the maintenance of shoot, root and vascular meristems containing tissue-specific stem cells. Stem cells in the SAM provide the cells for the continuous development of new organs such as the internal tissues of the stem, leaves, flowers, fruits and roots; stem cells in the RAM provide the cells for the formation and continuous development of the root system (Figure 1e). The SAMs of seed plants share a basic architecture, being organized into radial domains comprising discrete cell layers and concentric zones. The central zone of the SAM contains stem cells, and the surrounding domains display cell differentiation and organ initiation. The SAM stem cells were initially identified by their slow cell cycle. The *Arabidopsis* SAM comprises ~100 cells and is ~100 μm in diameter [5].

In *Arabidopsis*, stem cells are maintained by two-way signalling between stem cells and the organizing centre (OC), which is a group of cells immediately beneath the stem cells (reviewed in [39]). The OC cells express WUS, which encodes a homeodomain transcription factor [10,40,41] and must be essential for maintaining the stem cell pool [42] given that no self-maintaining stem cells are initiated and maintained in loss-of-function *wus* mutants. The *WUS* itself is repressed by negative signals originating from stem cells. SAM stem cells express the CLV3 gene, and the secreted *CLV3* protein counteracts *WUS* activity. CLV3 itself acts together with CLV1 and CLV2 genes. These three CLV genes act together in a feedback loop to restrict stem cell proliferation [43]. Thus, the dynamic balance regulated by crosstalk between stem cells and the cells of the OC strictly controls the number of stem cells in the SAM niche. A recent study by Andrea Leibfried *et al.* found that *WUS* acts directly on a series of *ARABIDOPSIS RESPONSE REGULATOR* genes (*ARR5*, *ARR6*, *ARR7* and *ARR15*) that had previously being shown to act in the negative feedback loop of cytokinin signalling [44]. Leibfried *et al.* propose that repression of these signalling protein genes is necessary for correct meristem function.

The RAM niche regulates the maintenance of root stem cells [45]. The stem cells that give rise to all the tissues of the root reside in the meristematic zone present at the root tip. The signal that maintains these stem cells originates from a small group of cells that reside in the quiescent centre (QC) at the centre of the root tip. QC cells define the stem cell niche; the stem cells (also known as initial cells) adjoining the QC produce longitudinal rows of daughter cells. The daughter cells produced in the direction of the root tip give rise to the root cap that protects the RAM. The daughter cells above the QC facing away from the tip give rise to concentric cylinders of cells that constitute the structure of a typical root. The role of the initial cells of the root is equivalent to CLV-3-expressing stem cells in the SAM, and QC cells act like WUS-expressing cells in the OC [10,46]. Whereas stem cell specification in the SAM is regulated at the population level, the RAM stem cells appear to be regulated individually by short-range signalling from the QC cells.

The positional information needed for QC specification is provided by the combined action of two pathways [46,47]. The *SHORTROOT/SCARECROW* pathway involving two transcription factors belonging to the GRAS family provides radial information [48–50] and the polar transport of the hormone auxin focuses the expression of two AP2-class *PLETHORA* transcription factors (*PLT1* and *PLT2*) at the root apex [47]. Overlap of the *PLT1/PLT2* and *SCARECROW* expression domains leads to the specification of the QC, the root stem cell niche. Laser-ablation studies have shown that the QC maintains the stem cell status of surrounding cells by inhibiting their differentiation [51]. Marjolein Wildwater *et al.* [19] recently reported that a gene named *RETINOBLASTOMA-RELATED* (*RBR*) is crucial for the regulation of RAM stem cell maintenance in plants. The RBR protein is the plant homologue of the RB tumour-suppressor protein in retinoblastoma, which was first identified in mammals [20]. RB prevents cell division by repressing the E2F/DP family of transcription factors that regulate the expression of genes involved in cell proliferation and survival. Wildwater *et al.* [19] have shown that RBR acts downstream of SCR in regulating the size of the RAM stem cell population. The similar roles played by RB in animals and by RBR in plants in stem cell regulation highlights common stem cell maintenance pathways in the two systems.

Common features of plant and animal stem cell niches

The features shared by various animal stem cell niches are a specialized tissue location, the requirement for physical contact mediated by adhesion molecules such as cadherin and integrins in the extracellular matrix [52,53], the generation of signalling molecules involved in the regulation of stem cell maintenance [17,54,55] and an asymmetric structure to facilitate the exit of one of each of the two daughter cells. The common feature of plant SAM and RAM stem cell niches is also a specialized organ location neighbouring cells that release signals required for stem cell regulation [56–59]. Recently, Robert Sablowski [60] suggested that the presence of stem cell niches in both plants and animals probably resulted from convergent evolution brought about by their shared necessity to maintain a reservoir of undifferentiated cells that are capable of self-renewal. The niche is vital for ensuring the self-replenishing undifferentiated nature of the occupant stem cells in plant and animal systems. The crucial role of the stem cell niche in regulating stem cell fate has been established experimentally using a mouse melanocyte stem cell system, in which substitution of stem cells in the niche with externally added cells resulted in the introduced cells acquiring the features of stem cells [61,62]. Because plant cells are enclosed in thicker cell

walls, such transplantation of single cells to foreign stem cells niches has not yet been achieved.

Conclusions and future perspectives

What are the mechanisms that maintain cells in a niche in an uncommitted state and refractory to differentiation signals that modulate the fate of surrounding cells? In other words, what are the molecular processes underlying 'stemness'? Is stemness a default state in the stem cell niche that is protected from intercellular signals that induce differentiation? Is the absence of external signals sufficient to maintain stem cells in an uncommitted state or is there any intrinsic regulation at the transcriptional and post-transcriptional levels? Are there any stem-cell-specific genes that confer stemness? The availability of whole-genome microarrays has led to attempts to identify features common to all animal stem cells. It has been proposed that the defining characteristics of stem cells are underpinned by a genetic programme common to stem cells of all origins, and that stem cells therefore share a conserved molecular signature [63]. Gene expression profiling of murine embryonic stem cells, HSCs and neurospheres in two independent studies [64–68] revealed only six common stem cell genes; analogous studies have not revealed a conserved stem cell molecular signature [63]. These observations have led to suggestions that 'stemness' represents a state rather than a defined entity [4,69]. Furthermore, it has been proposed that the combined effect of small, quantitative differences in many different genes can account for the variations in establishing a stem cell state [63,68]. Although conserved stem cell signatures remain elusive, gene expression studies have identified several gene networks and signalling pathways that appear to regulate different types of stem cells [6,69]. Cell-type-specific transcriptional profiling thus represents a powerful tool for analysing gene networks of plant stem cells and the surrounding cells that support them. A potential hurdle in such analyses is the difficulty of isolating sufficient pure cells because of their location within complex tissues. Kenneth Birnbaum *et al.* [70] reported a novel method to isolate hundreds of thousands of plant cells of a specific cell type that was based on the use of reporter lines expressing green fluorescent protein (GFP). The method involves GFP-positive cells being isolated using a fluorescence-activated cell sorter after protoplasts are isolated. RNA isolated from purified cells is then labelled and used for microarray studies. This cell-sorting strategy has been used to obtain a transcriptional profile of the *Arabidopsis* root QC [71]. However, this elegant strategy is not applicable to all cells because the requirement for transgenic lines expressing fluorescent markers and the isolation of different cell types from the same sample requires plants expressing multiple fluorescent marker proteins. These shortcomings can be avoided by using laser-assisted microdissection (LAM) for isolating specific cell types from complex tissues [72]. LAM employs a laser beam to excise specific microscopic domains from tissue sections, with the RNA isolated from these domains being used to probe microarrays. LAM was recently used to analyse gene expression during *Arabidopsis* embryo development [73]. That study found differential expression of genes belonging to various functional categories, and the microarray results could be verified by promoter:GUS analysis of six representative genes. The use of LAM to excise cells selectively from intact tissues along with specialized plant gene microarrays represent new and exciting tools for enhancing our understanding of plant stem cells and their remarkable niches.

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