The Stem Cell Concept in Plants: A Matter of Debate

Minireview

Thomas Laux*
Institute of Biology III
University of Freiburg
79104 Freiburg
Germany

Throughout their life, which can last for over a thousand years, plants have the fascinating ability to give rise to new organs from founder cells in their apical meristems. Whether these founder cells are equivalent to the pluripotent stem cells in animals has been a long-standing controversy amongst plant scientists. Here, this controversy will be addressed in light of classical observations and recent findings.

What Is a Stem Cell?

The current definition of stem cells is that they are clonogenic precursors whose daughters can either remain stem cells or undergo differentiation. To be sure to have a stem cell in hand, these clonogenic properties need to be demonstrated, which has been done in two ways. First, single cells can be genetically marked and their descendants identified by the spread of this label. This approach has provided genetic evidence for the existence of blood forming stem cells (for review, see Weissman et al., 2001), and has similarly been applied to plants (see below). In the second approach, cells are purified based, for example, on the presence of cell surface molecules and are then tested for their clonogenic properties in an appropriate host. This is not possible in plants, but has been successfully applied to identify single animal stem cells (Osawa et al., 1996).

Properties of the Shoot Meristem

The shoot meristem is the ultimate source of all cells from which leaves, stems, and flowers are derived during postembryonic development. Clonal studies of genetically marked cells have allowed determining the number, potency, proliferative activity, and life span of cells in the shoot meristem. They demonstrated that all postembryonically formed shoot organs are ultimately derived from as little as 6–9 founder cells (Stewart and Dermen, 1970). These cells are organized in several layers that give rise to the different tissues of plant organs, such as epidermis and central tissue. Each individual founder cell is pluripotent in that it gives rise to all cell types within its layer and in case one of its daughters is displaced into a different layer by an aberrant cell division, also to the cell types of the invaded cell layer. Based on geometrical arguments, the founder cells are located in the apical layers of the central zone (CZ) of the shoot meristem (Stewart and Dermen, 1970). Their proliferation results in the tip growing away, leaving behind cells that differentiate and form new organs (Figure 1).

Thus, the founder cells of the shoot are able to self-renew and to give rise to differentiating progeny, and they therefore fulfill the definition of a stem cell. General

*Correspondence: laux@biologie.uni-freiburg.de

differences between plant and animal development have, however, raised the question of whether the stem cell concept is applicable to plants.

Are Stem Cells Different from Other Meristem Cells?

A longstanding question in meristem biology is whether the shoot meristem stem cells represent a specific subset of cells within the meristem, or whether all meristem cells are equivalent. Consistent with the latter, upon ablation of cells in the meristem center, new meristems are readily induced from more peripheral cells of the original meristem (for review: Steeves and Sussex, 1989). One interpretation of these results is that most shoot meristem cells have stem cell properties, but that the peripheral ones are rapidly pushed out of the meristem and undergo differentiation, whereas only the apical ones are long-lived simply because they reside at a privileged position.

Cells in different meristem regions exhibit clear histological and molecular differences. First, cells in the CZ (Figure 1) divide less rapidly and are slightly more vacuolated than cells in the peripheral zone. Thus, the stem cells actually look slightly more differentiated than their rapidly proliferating peripheral daughter cells. (Having said that, stem cells are clearly undifferentiated relative to their descendants in organs that are massively enlarged and have developed a prominent central vacuole.) Furthermore, cells in the apical three layers of the CZ express the *CLAVATA3* (*CLV3*) gene, which encodes a putative signal peptide involved in regulating the size of the stem cell pool in *Arabidopsis* (Fletcher et al., 1999; see below) and which is not expressed in any other cell. This expression domain coincides with the predicted position of the stem cells and in addition, *wuschel* (*wus*) mutant embryos that fail to form stem cells do not express *CLV3* (Brand et al., 2002). Therefore, *CLV3* expression appears suitable as an operational stem cell marker for the shoot meristem. Cells outside the CZ not only discontinue *CLV3* expression, but also initiate expression of differentiation related genes. Still further down from the tip, the gene expression patterns change again and organ primordia emerge. Thus, even though it is difficult to relate the expression of molecular markers to cell fates, these findings suggest that the shoot meristem is comprised of a series of distinct cell states with the stem cells at the very tip and gradually differentiating cells in more proximal positions. The induction of new meristems from the flank of a previous one after ablation of central cells would then suggest that peripheral meristem cells that have just left the stem cell state can readily revert to it, and that they are normally prevented from doing so by an inhibitory function of the stem cells in the central zone.

This situation is very similar to what has been found for animal stem cells. There, stem cells often express specific properties rather than being "primitive" (for review: Fuchs and Segre, 2000), and recent daughter cells that normally would differentiate appear to be able to revert to the stem cell state (Doetsch et al., 2002).
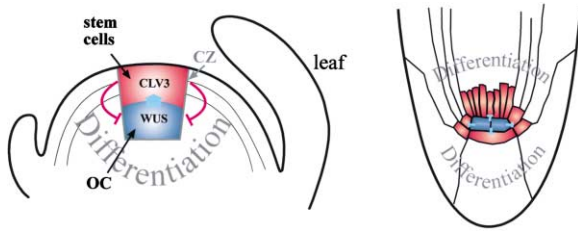
Figure 1. Organization of the *Arabidopsis* Shoot and Root Meristems

The shoot meristem (left) consists of a central zone (CZ), that presumably harbors stem cells and *WUS*-expressing organizing center (OC), and surrounding differentiating cells. The stem cells are organized into three layers due to anticlinal (normal to the meristem surface) cell divisions in the outer two layers. Signaling from the OC (blue arrow) confers a stem cell state upon the apical cells, which in turn restrict the size of the OC via CLV3 signaling (red T-bars). In the root meristem (right), signaling from the quiescent center (blue) inhibits differentiation of the surrounding stem cells (red). For details, see text.

The Role of Positional Information

Traditionally, a major perceived difference between animals and plants is that plant stem cells are regulated by positional information, whereas animal stem cells have been viewed as cell lineages of a fixed fate. For the shoot meristem stem cells this view is illustrated by Newman's conclusion some decades ago that the stem cells (there named initial cells) are only "the temporary occupants of a permanent office" which parallels Schofield's niche concept, in which stem cells are located in microenvironments that provide signals to maintain their undifferentiated cell state (Newman, 1965; Schofield, 1978).

Clonal experiments suggested that self-renewal of the shoot meristem stem cell pool is regulated at the population level: cells that stay inside the niche remain undifferentiated, whereas cells displaced outside the niche undergo differentiation, irrespective of their individual origin (Stewart and Dermen, 1970). Recent studies in *Arabidopsis* have provided a genetic and molecular framework for the niche concept in the shoot meristem. Maintenance of the stem cell state requires expression of the *WUS* homeobox gene in the underlying organizing center (OC) (Figure 1), indicating that the OC provides signals to the stem cells (Mayer et al., 1998; Schoof et al., 2000). The stem cells in turn express CLV3 which presumably acts as a signal ligand to restrict the size of the OC (for review, see Clark, 2001). This negative feedback mechanism between the OC and the stem cells establishes a self regulatory system that enables the plant to dynamically control the size of the stem cell pool (Brand et al., 2000; Schoof et al., 2000).

At the other end of the plant, the root meristem displays a similar organization as the shoot meristem (Figure 1): here, the stem cells surround a small group of cells, the quiescent center (QC), whose function resembles that of the OC in the shoot meristem in that it provides signals to prevent the stem cells from undergoing differentiation (van den Berg et al., 1997). In contrast to the shoot meristem, however, self-renewal of the root meristem stem cells is regulated at the single cell level: each individual stem cell division is asymmetric in pro-

ducing one daughter cell that stays in contact with the QC and remains a stem cell and one daughter cell that is separated from the QC and will differentiate.

Since Schofield proposed his niche concept for hematopoietic stem cells, it has been demonstrated in many cases that also animal stem cells are not cell lineages of a fixed fate, but that maintaining the stem cell state requires signals from surrounding cells (for review, see Spradling et al., 2001). Similar to plants, the stem cell pool can either be regulated at a population or at the single cell level. Thus, the emerging picture is that the stem cell state in both plants and animals is maintained by positional information.

Do Plants Have the Better Stem Cells?

A major difference between plant and animal stem cells is that plant stem cells provide cells for complete organs and thus serve a much broader developmental program than their animal (adult) counterparts, which regenerate cells restricted to one tissue type. However, numerous findings indicate that the developmental capacity of the descendants of stem cells in both kingdoms is not determined by the stem cell, but is dictated by the environment the daughters are exposed to, and can be dramatically expanded if this environment is altered (for review, see Morrison, 2001). Thus, the fact that complete organs are derived from plant stem cells appears to reflect the remarkable ability of plants to provide an environment to stem cell daughters for organ formation outside the embryo, rather than an inherently larger potency of plant stem cells compared to their animal counterparts.

Is Every Plant Cell Like a Stem Cell?

Another striking difference between plants and animals is the ease with which stem cells can be formed de novo from differentiated parts of a plant. The formation of lateral root meristems from differentiated pericycle cells of the main root is one example in normal plants where previously differentiated cells de-differentiate and give rise to multiple cell fates (Malamy and Benfey, 1997). In tissue culture and transgenic plants, this ability has been demonstrated for many tissues by relatively simple manipulations. These observations have led to the concept of totipotency of most, if not all (living) differentiated plant cell types (for review, see Steeves and Sussex, 1989). They have also raised the question of whether the stem cell concept is useful in plants, if every cell has stem cell properties. Using the stem cell definition given above, stem cell function in a normal plant is clearly restricted to the meristems. In addition, the operational stem cell marker for the shoot meristem, *CLV3* is not expressed in differentiated cells, thus expression has to be reactivated during de novo meristem formation. These observations suggest that differentiated plant cells have not retained stem cell properties but need to actively re-enter the stem cell state.

In contrast to plants, differentiated animal cells are more rigidly locked in their state, and with a few exceptions, no strategies have been identified yet to overcome this block. Thus, the different regenerative potencies of plants and animals appear not to be due to their stem cells, but to the ease with which the differentiated descendants can revert to a stem cell state.

Common Principles in Stem Cell Regulation

In conclusion, the similarities between plant and animal stem cells appear to vastly outweigh their differences.

In both kingdoms, stem cells are defined by their clonogenic properties and are maintained by signals from the surrounding cells. Since plants and animals are thought to have originated from different single cell ancestors, they appear to have independently evolved similar strategies for maintaining sets of cells at specific locations in a relatively undifferentiated state as a reservoir for the daily demand for new cells. Are there also molecular similarities? Given the heterogeneity of different types of stem cells even within one organism this may be too much to ask for, but modern molecular profiling approaches may provide a more solid answer in the near future.

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