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Deconstructing stemness

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Stem cells are unique in their capacity to self-renew and generate differentiated progeny to maintain tissues throughout life. A common molecular program for stem cells has remained elusive. We discuss what the molecular logic of stemness may be. We suggest that it may not be coupled to distinct cellular properties such as self-renewal or multipotency, but rather to the stable suspension at a specific developmental stage. In this view, the stem cell niche allows a cell to maintain a transcriptional accessibility enabling the generation of specific differentiated progeny.

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Introduction

Stem cells form tissues during embryogenesis and maintain them in the adult, placing them at center stage both in developmental biology and regenerative medicine. But what, at the molecular level, makes them unique compared to other cells? The understanding of what makes a cell a stem cell promises to teach us much about developmental biology, and even raises the prospect of converting other cells into stem cells.

In pursuit of a transcriptional code for stem cells

The development of tools for global gene expression analyses allowed characterization of the transcriptomes of different stem cell populations in search for a common molecular profile. In 2002, the first studies comparing the transcription profiles of embryonic, neural and hematopoietic stem cells were received with great enthusiasm (Ivanova *et al*, 2002; Ramalho-Santos *et al*, 2002). Neither study could, however, provide a compelling unique transcriptional profile. Instead, one mutually observed phenomenon was that most of the

genes enriched in stem cells were not exclusive to these cells, indicating that the combination of many genes rather than a few individual genes might provide the stem cell its characteristics. Moreover, quite surprisingly, the two reported common expression profiles for different stem cells showed minimal overlap. Shortly after, other studies likewise failed to uncover a unique genetic fingerprint for stem cells and it was suggested that the limited overlap between the profiles identified in these studies was due to somewhat different experimental approaches (Evsikov and Solter, 2003; Fortunel *et al*, 2003).

Even though differences in the selected cells, microarrays, culture methods and computational analyses likely affected the number of overlapping genes for each stem cell type and all stem cells combined, it appears unlikely that these differences could account for the limited overlap between the studies. If specific stemness genes contributing to the characteristic features of stem cells exist, the comparison of the expression profiles of widely different stem cell populations should have revealed a common genetic signature. This is supported by the observation that *Oct4* and *Nanog* were identified as embryonic stem cell-specific genes in almost every single profiling experiment, regardless of culture methods and species origin (Ivanova *et al*, 2002; Ramalho-Santos *et al*, 2002; Fortunel *et al*, 2003; Sato *et al*, 2003; Sperger *et al*, 2003; Bhattacharya *et al*, 2004; Ginis *et al*, 2004). However, the overall outcome of the different stem cell transcriptome analyses suggests that there is not a single common genetic program controlling the properties of different stem cell.

What is the unique character of a stem cell?

The apparent difficulties in finding a common molecular fingerprint for different stem cells raises the question whether stem cells have shared properties that are unique compared to other cells. The most commonly used definition of a stem cell is a cell that can give rise to multiple differentiated cell types, that is, multipotency, and has the ability to self-renew (Potten and Loeffler, 1990).

Both these criteria are, however, relative. There are unipotent self-renewing cells, most notably germline stem cells, which most scientists would argue are obvious stem cells. Hence, the existence of such unipotent stem cells disqualifies multipotency as an obligate stem cell criterion. Neither is the feature self-renewal absolute. Some stem cells may undergo self-renewal during a short window of time, whereas others may do so for the lifespan of the organism. A limited period of self-renewal is characteristic for transitory developmental stages, for example for inner cell mass cells in the blastocyst. These cells can be propagated indefinitely *in vitro* as embryonic stem cells, indicating that the limited self-renewal *in vivo* is not due to a cell intrinsic constrain, but rather to the loss of an environment that can support sustained self-renewal.

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In contrast to the inner cell mass cells, adult stem cells reside in stable niches in the mature tissue and may self-renew over the lifespan of the organism.

The problem with multipotency and self-renewal as defining characters of stem cells is not limited to these criteria being relative. Perhaps even more troublesome is that neither multipotency nor self-renewal is a feature unique to stem cells. First, stem cells often give rise to multipotent progenitor cells that have no or limited self-renewal. What distinguishes the multipotency of stem cells from that of progenitor cells? Second, unipotent, bipotent and multipotent progenitor cells can often self-renew for quite a number of times (Back *et al*, 2004; Trentin *et al*, 2004). Even differentiated cells, such as B and T lymphocytes in response to antigenic stimuli (Fearon *et al*, 2001) as well as pancreatic β -cells (Dor *et al*, 2004), can renew themselves.

Since self-renewal and multipotency are not unique features of stem cells, it is unlikely that understanding these processes will suffice to delineate the molecular machinery that makes a stem cell.

The stem cell is halted along the line of specialization

Since it is difficult to delineate distinct features that are specific to stem cells, a slightly different view than focusing on specific cellular processes such as multipotency and self-renewal may be helpful when considering a molecular logic for stemness. The development of a cell along a certain lineage can be pictured as a linear process with a gradual specialization through determination, commitment and final differentiation (Figure 1A). Most cells in the mature body will be at the very end of a differentiation line. However, at certain steps in the progression, some cells will halt their succession at an intermediate position along this line. In this view, a cell that stands still somewhere along this line of specialization and divides to give rise to more of its own type and of cells that progress along the line is a stem cell (Figure 1B).

Let us compare this view with the classical definition of a stem cell as a multipotent self-renewing cell. In the novel model, the multipotency and self-renewal features are not specific. For example, transit amplifying progenitor cells may be multipotent and self-renew, but they are not halted at an intermediate position, as they inevitably progress toward terminal differentiation. The unique feature of a stem cell in this view is the resistance to progress along the line of specialization, which cannot be reduced to the features multipotency or self-renewal capacity.

This view of stem cells is not informative when searching for stem cells *in vivo*, since the criteria to be halted along the specialization line cannot be tested experimentally. However, it may aid in the conceptualization of mechanisms that make stem cells unique. When entertaining this view, the question of what makes a cell a stem cell can be rephrased: How can a cell settle somewhere along the line of specialization and is there a common molecular program for this?

The stem cell is retained in the niche

A key feature in the maintenance of the stem cell state is the interaction with the immediate environment, forming the so-called stem cell niche (Spradling *et al*, 2001). Stem cells

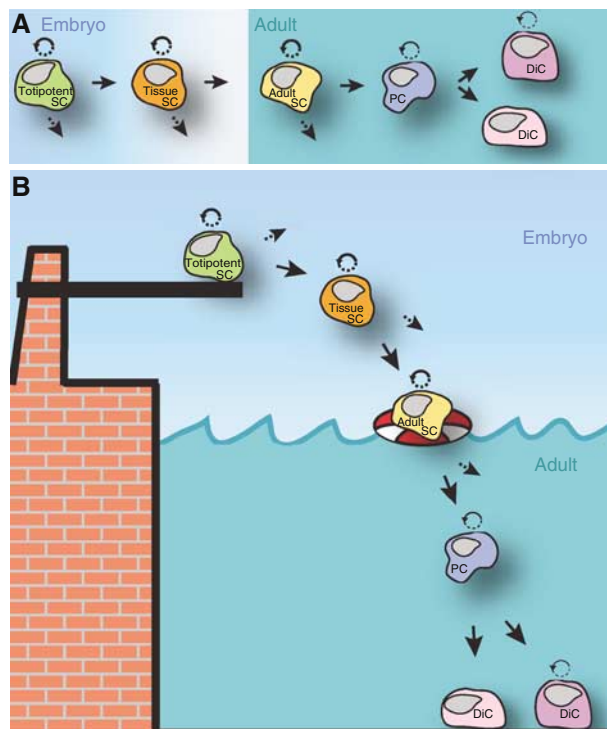


Figure 1 Stem cells are halted along the line of differentiation. **(A)** The development of a cellular lineage can be depicted as a linear progression with a gradual increase in specialization and loss of options. Cells at different stages may have self-renewal capacity (indicated by circular arrows) and the potential to give rise to multiple different types of progeny (branch points in the lineage). Self-renewal capacity and multipotency are commonly used criteria for stem cells, but it is apparent from the schematic image that these features are not unique to stem cells. **(B)** A different view on stem cells focuses on their stable position along the line of differentiation. The stem cell is maintained in a certain lineage position by the niche, here depicted as a life preserver that keeps the stem cell floating on the surface. The unique feature of the stem cell in this view is their stable position at a certain developmental stage. Many cells may display self-renewal capacity and multipotency, but cells that are not halted inevitably plummet through the lineage. SC, stem cell; PC, progenitor cell; DiC, differentiated cell.

and niches seem to go hand in hand. Even tissues, for which the suspected stem cells have not been identified, are expected to contain stem cell niches. It has been proposed that the niche provides stem cells with the appropriate signals to lock into a quiescent state (Chen *et al*, 2000; Heissig *et al*, 2002). In some experimental settings, the niche even appears to be able to induce progenitor cells to revert to a stem cell state (Marshman *et al*, 2002; Brawley and Matunis, 2004; Kai and Spradling, 2004). The position of stem cells along the line of specialization thus appears to be imposed by the environment rather than being controlled strictly cell autonomously. A simplistic model of stem cells and their environment is that the niche serves as a time capsule to maintain a developmental stage, and thereby halt the progression along the line of specialization.

The tight interdependence between a stem cell and its immediate environment indicates that the key to the stem cell state may be found in the signals that are provided by the niche. Different niches could potentially share signals that maintain the stem cell's state preventing its further differentiation and allowing asymmetrical cell divisions to

self-renew and generate progenitor cells in response to cues induced by a deregulated tissue homeostasis. Several extracellular signaling pathways such as members of the WNT, hedgehog, bone morphogenic protein (BMP) and NOTCH families indeed have important functions in multiple stem cell lineages (Fuchs *et al*, 2004).

It is, however, clear that the niche does not provide the full answer. A striking example is provided by the intestinal stem cell niche. In the crypts of the small intestine, stem cells reside adjacent to their progeny Paneth cells, which are terminally differentiated (Sancho *et al*, 2004). The stem cells and Paneth cells must be exposed to very similar, if not identical, extracellular cues, but yet have completely different properties. WNTs are essential for the function of intestinal stem cells and Paneth cells, but impose different programs on these neighboring cells (van de Wetering *et al*, 2002; Sancho *et al*, 2004; van Es *et al*, 2005). Thus, local factors are critical for stem cell function and maintenance, but the influence of such cues is dependent on the cellular state, and not any cell introduced into the niche will act as a stem cell.

The stem cell state is open minded

During the progression through a lineage, a cell's differentiation options are gradually restricted. At the transcriptional level, this will be reflected in reduced expression of genes associated with other lineages. The transcriptional accessibility to different genes is regulated through the chromatin status by epigenetic means. The condensed nature of chromatin makes DNA inaccessible for transcription factors and the activation of gene transcription.

The regulated accessibility of genes appears to be a critical feature in the stem cell state. The so-called 'multilineage priming' model suggests that stem cells express low levels of a large number of genes that are highly expressed in its different committed progeny (Cross and Enver, 1997). Along the course of differentiation, the number of cell fate options available to the differentiating cell becomes more and more restricted as genes attributing to the selected lineage are activated and genes associated with other lineage are shut down (Figure 2). Comparison of the expression profiles of hematopoietic cells at different stages of differentiation demonstrated that stem cells express a large number of genes highly expressed by different specific hematopoietic lineages, whereas committed precursors only express the proportion of the genes that are related to their lineage (Akashi *et al*, 2003). In addition, hematopoietic stem cells express a relatively large number of non-hematopoiesis-associated genes, in particular neuronal genes. Although most of these neuronal genes have not been linked to the induction of neuronal differentiation, and their neuronal exclusiveness remains elusive, it illustrates the wide variety of genes expressed in hematopoietic stem cells. A similar situation seems to hold for embryonic stem cells. Embryonic stem cells, which have the tendency to acquire a neural identity, express several neuronal lineage-specific genes under normal culture conditions (Tropepe *et al*, 2001).

Thus, it appears that differentiation is accompanied by a successive restriction in the repertoire of genes that can be expressed, and that the stem cell state is characterized by accessibility to genes for downstream fates. This implies that

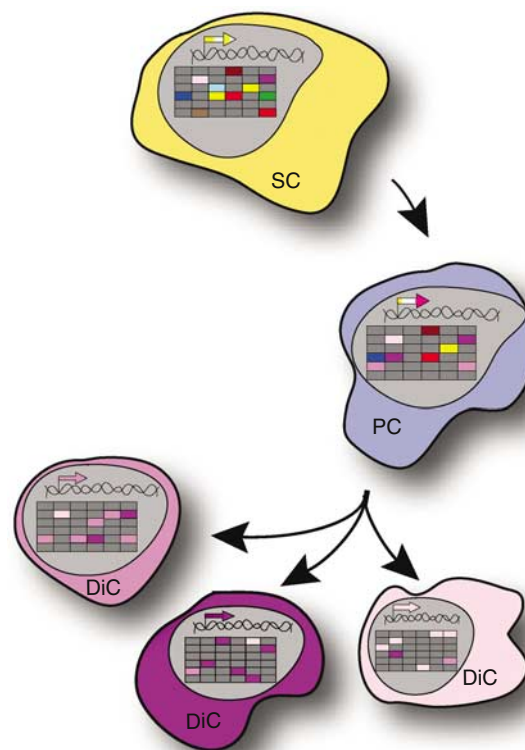


Figure 2 The molecular map of an open mind. Stem cells express many genes abundant in cells committed to a certain lineage. The expression of multiple differentiation genes allows the stem cell to generate progeny belonging to every lineage that normally expresses these genes. The further a cell has entered a differentiation program, the more choices have been shut down through loss of access to genes belonging to other lineages and a gain in the number of genes expressed by cells in the entered lineage. Colored boxes indicate expression of lineage-specific genes (every lineage has a different color), whereas the dark boxes correspond to the expression of housekeeping genes.

there is a close relation between differentiation potential and chromatin accessibility, and that multipotency at the transcriptional level is dependent on having access to several differentiation programs.

Chromatin modifications maintain chromatin status through DNA replication and mitosis. In particular, methylation of the lysine residues in histone 3 appears to play a role in epigenetic memory. These histone modifications recruit polycomb or trithorax group protein complexes to maintain the silencing or activation status of genes, respectively (for review, see Ringrose and Paro, 2004). Since stem cells self-renew without losing any of their features, they should inherit the same epigenetic status in order to maintain their characteristics. From this viewpoint, it is not unexpected that polycomb group members, such as *Bmi1*, play a role in the maintenance of several stem cell populations and affect self-renewal (Park *et al*, 2003) and stem cell memory (Iwama *et al*, 2004).

A common code for the stem cell-specific expression of factors involved in the epigenetic modifications and/or transcription silencing or activation has not been described. This may not be very surprising, as all cells, including differentiated cells, need a solid epigenetic memory to maintain their inherited transcription profile in order to prevent cell death or acquiring an altered cell fate.

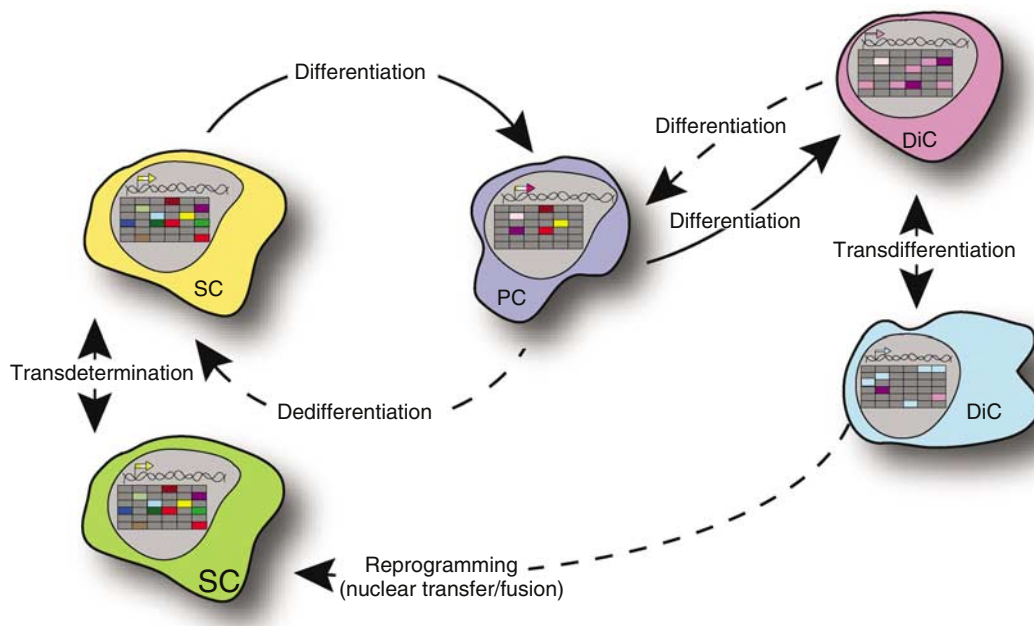


Figure 3 Extreme lineage makeover. The figure is a schematic depiction of possible ways of lineage switching.

Nature versus nurture

A stem cell may thus be defined by its chromatin status and the environment it is positioned in, but what is the relative importance of these two parameters? The original view on silencing maintenance was that the access of transcriptional activators and RNA synthesis was prohibited once a locus was locked into a silent state through binding of the polycomb group complex. This would mean that a transcription profile and the cellular state are irreversible. Recent discoveries have demonstrated that silencing is not written in stone, and the cloning of mammals from differentiated cells is perhaps the most dramatic example of this (Hochedlinger and Jaenisch, 2003). Gene silencing rather appears to be the result of a *status quo* in the dynamic competition between activator and repressor complexes, maintaining not only the transcription status of cells, but also allowing switching of their transcription profiles.

Although there often are clear limitations in the power of the milieu over the cellular state, there are several examples of a remarkable reprogramming activity of the environment. One striking example is that of ectoderm-derived cranial neural crest cells delaminating from the neural tube, which are induced by local cues to form myocytes, cartilage and bone cells, indistinguishable from such cells derived from mesoderm (Trainor *et al.*, 2002).

Cell transplantation experiments during development and in adults have delineated the determination of cells to specific lineages and the possibility of an altered environment to influence this. An alternative approach to investigate the relative role of the environment and the cell state is to change the molecular signals in a niche. A recent study demonstrated that an experimentally overactive WNT pathway induced progenitor cells in the lung to take on an intestinal phenotype (Okubo and Hogan, 2004).

In the last years, numerous reports have reported lineage plasticity of stem and progenitor cells (Lakshminpathy and

Verfaillie, 2005) (Figure 3). However, the mechanisms responsible for the observed plastic behavior have remained a controversial issue. It appears that, dependent on the tissue and experimental settings, de- or transdifferentiation as well as cell fusion can explain the occurrence of transdetermination of stem cells (Frisé, 2002; Weimann *et al.*, 2003; Wurmser *et al.*, 2004). On the basis of the 'multilineage priming' model, transdetermination would be by far simpler at an early stage of differentiation, when numerous genes belonging to several differentiation programs are readily available, than later on in terminally differentiated cells.

In summary, much like personality traits, in which it is difficult to clearly separate the relative importance of nature and nurture, stemness appears to be orchestrated by a complex interplay between cellular state and the signals the cell receives from its environment.

Conclusion

Stem cells are uniquely capable of maintaining and regenerating tissues, but to find a specific molecular code for stemness has proved difficult. The capacity to self-renew and give rise to multiple types of differentiated progeny is often central to their function. However, these properties are not exclusive to stem cells and unveiling the molecular programs for such features is unlikely to provide the full picture of what makes a cell a stem cell.

A more unique feature of stem cells may be that they are halted in their progression toward differentiation. This is not a strictly cell autonomous property but rather imposed and maintained in interaction with the niche where the stem cell resides. The stem cell state appears to be linked to an accessible chromatin structure. Such a chromatin organization creates accessibility for transcription factors and allows expression of a large number of genes involved in distinct

differentiation programs. Consequently, the common logic for stem cells will be having access to several differentiation programs. However, as the difference between distinct stem cells is their capacity to give rise to divergent cell types, they will have access to different differentiation programs. The molecular logic for stem cells is then rather quantitative, with access to several genetic programs, than qualitative with one shared transcriptional code.

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