# Stemness as a cell default state

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The potential of stem cells to generate all cell types while retaining the ability to self-renew has attracted much attention; a degree of focus comparable to that of developmental biologists on the capacity of an egg to produce a full organism. This interest has been enhanced by the prospective biomedical applications of stem-cell-derived technologies. As such, efforts are under way to identify the factors responsible for stem-cell fate and for conferring stemness, but a clear and dedicated factor remains to be identified.

I suggest that stemness-promoting factors have proven so elusive because stemness is not a specific fate acquired by cells, but rather a default state intrinsic to non-differentiated cells. In passing from unicellular to multicellular organisms, cells acquired the capacity to differentiate, ultimately forming tissues and organs that require a supply of differentiated cells. In this context, some cells retain their multipotency or totipotency by escaping differentiation. In so doing, they retain a fundamental feature of primordial cells—the ability to divide and proliferate, which is at the origin of self-renewal. This strategy accounts for the origin of stemness. Accordingly, I propose that stem cells emerge not as a consequence of factors promoting stemness, but rather as a result of factors repressing differentiation pathways. Stem cells are of different types, and a tissue stem cell is more differentiated than a totipotent embryonic cell. Even so, compared to the rest of the tissue, tissue stem cells are still the least differentiated, precisely to keep them as stem cells. In the light of this hypothesis, I analyse the properties and features associated with stem cells. I apologize in advance to those colleagues whose work I could not cite in this article, owing to space constraints.

Many observations suggest that maintenance of the pluripotent state is dependent on the absence or inhibition of signals that stimulate differentiation. More precisely, there are cases in which self-renewal is enabled by elimination of an ERK-mediated differentiation signal [1], and even cases where the suppression of ERK signalling promotes pluripotency [2]. Extrinsic stimuli are also dispensable for the derivation, propagation and pluripotency of stem-cell cultures [1]. These observations have led to the 'ground state' hypothesis, which holds that stem cells in culture are not dependent on any signal, and that once established, their propagation is preserved by neutralizing inductive signals [1,3]. I extend the interpretation of these experiments by suggesting that inhibition of differentiation might be the cause of stemness.

However, the culture environment sometimes alters cells in ways that modify their developmental potential, and thus it is pertinent to analyse the relationship between differentiation and stemness *in vivo*. The best-characterized system is probably germ stem cells (GSCs) in the *Drosophila* ovary. Here, three principal requirements are essential to maintain GSC identity: transcriptional repression of the *bag-of-marbles* (*bam*) gene, involved in cystoblast differentiation; RNA translational repression of differentiation-promoting genes by the Pumilio (Pum) and Nanos (Nos) repressors; and expression of microRNAs that potentially silence targets that contribute to the differentiation programme [4]. Thus, *in vivo* observations are also compatible with stemness being established by repression of differentiation programmes. If so, a corollary of the 'stemness as a default' hypothesis is that differentiation-suppressing factors required for stemness are dispensable in the absence of factors triggering differentiation. The phenotype of *scrawny* (*scny*) mutant flies is consistent with this possibility. In *scny* mutant females, the number of GSCs within the germaria is reduced—a phenotype often associated with the premature activation of differentiation genes—and the abnormal GSCs often express *bam*. However, *scny* GSC-like cells are not lost and remain in the germarium if they are also mutant for *bam* [5].

We can also analyse the role of factors such as Oct4, Sox2 and Nanog. It should first be noted that these factors are not universal inducers of stemness [6]. More importantly, Oct4 can act as a dose-dependent differentiation factor [7] and Oct4 and Sox2 also direct stem cells towards lineage specification [8]. They have even recently been found to regulate germ-layer differentiation [9]. It could be argued that, as is often the case, the same factors contribute to different processes in distinct cell contexts, but I propose that Oct4 and Sox2 might be performing the same role in each case. Each factor promotes a given fate by repressing the alternative: Oct4 suppresses neural ectodermal differentiation and promotes mesendodermal differentiation, while Sox2 inhibits mesendodermal differentiation and promotes neural ectodermal differentiation [9]. When acting together, they repress all germ-layer differentiation and, in so doing, allow pluripotent stem-cell development.

The concept of a stem cell is closely associated with that of the niche, as a specialized local microenvironment where stem cells reside and that directly promotes their maintenance [10]. According to the 'stemness as a default' hypothesis, the niche will be that local environment where stem cells can escape the differentiating signals either because of a physical hindrance or because they are counteracted by factors repressing differentiation. Indeed, the key role of the niche as a means to prevent a given number of cells from entering differentiation would make it less relevant whether this is achieved by a strict cell asymmetrical self-renewal or by the asymmetrical self-renewal of a cell population.

Asymmetrical cell division is also often associated with stem cells, particularly in two scenarios consistent with my hypothesis. In one scenario, the importance of asymmetrical division relies on the axis of cell division as a mechanism to ensure that one cell remains in the niche as a stem cell while the other escapes it and thus receives a differentiation signal. This could be the case for *Drosophila* GSCs. In the other scenario, differentiation factors are already present in the mother cell, and asymmetrical division coupled with their uneven distribution ensures these factors are inherited by only one daughter cell. In both scenarios, one daughter cell does not enter differentiation and thus remain pluripotent.

 

Germ cells function as a special class of stem cell, as their potential to generate all somatic fates is postponed until fertilization. Phenomena such as parthenogenesis, however, show the full stemness potential of non-fertilized oocytes in some species. While retaining this potential, primordial germ cells also undergo their own differentiation to become fully functional oocytes or spermatocytes. As to the nature of germ cells as stem cells for all somatic fates, it precisely requires the repression of somatic differentiation, albeit through different mechanisms in different organisms. Importantly, mutations enabling somatic differentiation in germ cells compromise their viability. In all cases, germ cells are set aside from the embryonic somatic cells. One of the best-known cases is that of *Drosophila,* in which the germ cells are the first to cellularize and, in so doing, escape epithelial differentiation. Thus, specification of *Drosophila* germ cells requires transcriptional silencing and escape from apicobasal polarity and formation of cell junctions. In other words, specification of *Drosophila* germ cells as stem cells requires them to acquire mesenchymal-like features and inhibit an epithelial transition [11].

The data on the relationship between epithelial transitions and stemness are controversial. On the one hand, epithelial-to-mesenchymal transition (EMT) in mammary cells has been shown to generate cells with the properties of stem cells [12], specifically the generation of so called 'cancer stem cells' [13]. Indeed, it has recently been shown that the Bmi1 protein, which is required for stem-cell self-renewal in many cell lineages, is also responsible for inducing an EMT through the regulation of Twist1, a well-known EMT regulator [14]. However, other reports indicate that a mesenchymal-to-epithelial transition (MET) initiates and is required for somatic cell reprogramming into induced pluripotent stem cells (iPSCs) [15]. In these cases, the genetic shift associated with a cell transition might transiently alter the steady state of gene repression in these cells and thus facilitate global repression of differentiation and the generation of iPSCs. This interpretation would fit with the idea that MET is necessary for, but not sufficient to induce, pluripotency in mesenchymal cells such as fibroblasts [16]. Yet, as mentioned, the relationship between epithelial transitions and stemness is controversial and requires a deeper understanding of the mechanisms of generation of iPSCs.

The observation that a stem cell can take many differentiation pathways suggested that chromatin in these cells exists in an 'open' conformation, thus keeping various regulatory networks ready to become active. However, characterization of the genes required for stem-cell maintenance has shown that many of them function in gene silencing. Thus, the emerging picture is one of stem cells with 'closed' chromatin to prevent stem-cell differentiation. Interestingly, recent data indicate that there is no global increase in silenced genes during differentiation; instead, discrete local changes are detected. Consistently, the total number of active genes is roughly equal in stem cells and several differentiated cell types tested [17]. I suggest that silenced genes in stem cells might correspond to those responsible for alternative differentiation pathways. At the same time, active genes perform the functions associated with an undifferentiated cell—which might be shared in varying degrees with differentiated cells—and those involved in the control and effectors of cell division. According to this view, it would be easy to argue that self-renewal could exist as a default state in the absence of lineage-specific gene expression consolidation.

I have suggested an alternative view of stem cells: that stemness is a cell default state and that a stem cell is a stem cell because it has escaped differentiation. This does not mean that stem cells are not committed; indeed, they are committed to a given lineage and even differentiated accordingly. But a stem cell has many differentiation potentials in a given lineage, not because all these alternatives are open, but rather because they are all closed. Rather than considering what a cell requires to become a stem cell, our focus should be on what a cell needs to avoid to become a stem cell. Thus, less-differentiated tissue cells could be a better starting material than some rather artefactual iPSCs. My hypothesis does not provide an explanation for all the observations in the field, but it does suggest many experiments that might prove or disprove the assumptions I have put forward. In this regard, I hope this hypothesis will be helpful for the broad research community working in the field of stem cells and cell differentiation.

ACKNOWLEDGEMENTS

I am grateful to M. Averof, E. Batlle, M. Calleja, K. Campbell, A. Casali, N. Djabrayan, J. Font, M. Furriols, C. González, A. González-Reyes, O. Martorell, G. Morata, D. Shaye and E. Sánchez-Herrero for discussions and comments on the manuscript).

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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EMBO *reports* (2012) **13,** 396–397; published online 10 April 2012; doi:10.1038/embor.2012.47