

Stem cell identity: *life is plastic, it's fantastic!*

A gospel truth of developmental biology has been that cells are fated to stay in the germ layer from which they arise. Endoderm makes endoderm but not mesoderm or ectoderm. Precursor cells give rise to a limited repertoire of progeny or a restricted lineage, depending on the tissue and position from which they arise. These basic rules, taught for generations in developmental biology classrooms, are now being questioned by the recent spate of research in stem cell biology. The long-held view in development has been that the lineage derived from a stem cell is restricted to its tissue of origin. A stem cell's position was thought to be a key factor in determining its fate. However, transplantation studies with adult stem cells have shown reprogramming of the stem cell in response to external signals in its new environment, to produce unexpected derivatives.

The bone marrow (BM) has long been a favourite source of stem cells as these are easily accessible and identifiable compared to stem cells in other tissues. The first indication that stem cells may be able to generate unexpected derivatives, dictated by their environment, came from studies on BM-derived stem cells. Ferrari *et al* (1998) transplanted a reporter gene-expressing BM stem cell in non-transgenic mice and detected reporter expression in myotubes of recipient mice, suggesting that the transplanted stem cell had differentiated in the host tissue to give a novel derivative. Similar results were obtained in later studies using male donor BM cells transplanted into female muscular dystrophy mice (Gussoni *et al* 1999), or using purified hematopoietic stem cells (HSCs) for transplantation (Orlic *et al* 2001). In all cases donor-derived myotubes were detected. Conversely, muscle stem cells were shown to reconstitute all hematopoietic lineages (Jackson *et al* 1999). BM-derived stem cells have been shown to produce hepatocytes, adipocytes, osteocytes, chondrocytes and possibly neurons.

The long held dogma that mammalian neurons are born only in embryonic life or during early postnatal development was proved wrong by the demonstration of adult neural stem cells (NSC) (reviewed in Gross 2000). In 1999 Bjornson *et al* showed that clonal populations of NSC, identified as such on the basis of markers, could produce blood cells. However, this demonstration of multipotentiality was not new. As early as 1982, Bartlett's group had shown that mouse brain cells could give rise to blood cells, suggesting the presence of an ectodermal (brain) stem cell capable of giving ectodermal (neural) or mesodermal (hematopoietic) derivatives. However, the lack of sufficient markers to unambiguously identify NSCs undermined the importance of this result. Later studies also demonstrated the myogenic potential of neural stem cells. Finally Jonas Frisen's group (Clarke *et al* 2000) clearly showed the multipotentiality of neural stem cells both *in vitro* and *in vivo*.

In most experiments, the response to extrinsic differentiation signals was monitored after exposing a stem cell to an environment it would not normally encounter. These studies demonstrate that the seemingly limited differentiation potential of adult stem cells may simply reflect the inductive or inhibitory properties of their neighbours. Several questions arise from these stimulating results. First of all, how do we define and identify a stem cell? Would one be able to tell a stem cell by its cover i.e. by the expression profile it displays? Do these new derivatives actually arise during normal development? And finally, are adult stem cells more plastic than embryonic stem cells?

Unlike embryonic stem cells, adult stem cells make up a small fraction of the tissue that they reside in and in most cases cannot be identified by morphology or gene expression profile. Blood stem cells are the most well-characterized of stem cells and hence, a variety of marker combinations is available, to differentiate among the various kinds of blood stem cells. On the other hand, NSC are identified

mostly in retrospect, depending on the progeny that they produce. Studies showing myotube or hepatocyte formation from BM transplants have been done using male to female transplants and the Y chromosome as a tracer for the donor cells. However these do not conclusively prove that the cells used were stem cells alone. One cannot rule out the possibility that circulating stem cells from different tissues may contaminate the experiment.

The differentiation of muscle precursors to blood is probably from a common progenitor for the two lineages that was unknown before (Seale *et al* 2000) and has a hematopoietic as well as myogenic potential, rather than by a transdifferentiation event. However, Krause *et al* (2001) injected a single hematopoietic stem cell, selected on the basis of its marker profile and ability to home to the bone marrow, intravenously and found that it could give epithelial progeny in the lung, liver, kidney, intestine and skin. This was a convincing demonstration that a true stem cell was used.

While the debate rages over whether embryonic or adult stem cells should be used for research and therapy, some researchers are looking more closely at the means used to identify a stem cell. Heterogeneity in a population is one of the biggest caveats of stem cell biology. Stem cells cannot be identified only on the basis of morphology and/or expression of specific antigenic proteins. What is a “true” stem cell? Stem cells have been attributed the property of indefinite division. However it is not easy to say whether a given cell is a stem cell or not. The ability to divide indefinitely is generally not tested *in vivo* for a given cell. Even in *in vitro* experiments done on freshly isolated populations there are problems in ensuring that one is working with a true stem cell. The isolated cells are generally used right away for experimentation or allowed a limited number of divisions. It is impossible to tell whether all the cells in a population would have divided indefinitely and would behave as true stem cells. At present one cannot distinguish between truly multipotent cells and lineage-restricted precursors. Before we realize the full potential of stem cells and use them in treatments, we have to elucidate their defining features to be able to isolate these cells.

Could there exist stem-cell specific genes that would not express in any other cell type? The groups of Weissman and Lemischka (Terskikh *et al* 2001; Phillips *et al* 2000) have undertaken detailed molecular analyses of the stem cell, to generate a panel of markers resulting in an identification tag to differentiate each cell used. The investigators used subtractive cDNA libraries and cDNAs from highly homogeneous cell populations to generate microarrays and examine gene-expression profiles of HSC (Phillips *et al* 2000) or NSC (Terskikh *et al* 2001). Obtaining a homogeneous population of the rare stem cells is extremely critical for such sensitive studies, as a majority of the expression data would otherwise represent non-stem cells. Comparison of the HSC and NSC gene-expression profiles revealed non-identical but overlapping profiles. This suggests the existence of common genetic programs that help keep a stem cell undifferentiated and a unique combination of other expressed genes that would give the stem cell its identity. However this identity seems to be easily switched depending on environmental cues, resulting in plasticity.

It would be wonderful if a stem cell from any source could be directed to make any cell type desired. How far can we go with transdifferentiation? If the cell we commanded could actually make everything, would it be a wise choice for therapy? Most genes are expressed in embryonic stem cells and possibly adult stem cells and are shut off as differentiation proceeds. Some genes may be reactivated in development, often in abnormal situations such as tumours. The best cited example Wnt1, is involved in several cell fate decisions in normal development and is also the cause of tumors when aberrantly expressed. The plasticity of adult stem cells is apparent only when they are moved from their tissue of residence, an event that may not occur during normal life. Unlike therapeutic drugs that are inactivated in the body within a few hours or days, a stem cell entering the body would be effectively eternal. One of the defining properties of a stem cell is that it can self-renew as well as give differentiated progeny. Unless one could control the consequences of a stem cell injection all through the life of the stem cell and its progeny, it would be difficult to devise a therapeutic use. Even if one were to utilize only the differentiated derivatives from culture, one does not know at present if and to what extent they may de-differentiate and possibly generate an unexpected cell type from the donor cells. Clearly a lot more remains to be done on establishing stem cell identity and on ways to ensure that the regulatory controls stay in place during therapy. Unravelling the secret of the fantastic plasticity of the stem cells is the order of the day.

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