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ADULT STEM CELL PLASTICITY: Fact or Artifact?

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■ **Abstract** There has been unprecedented recent interest in stem cells, mainly because of the hope they offer for cell therapy. Adult stem cells are an attractive source of cells for therapy, especially in view of the recent claims that they are remarkably plastic in their developmental potential when exposed to new environments. Some of these claims have been either difficult to reproduce or shown to be misinterpretations, leaving the phenomenon of adult stem cell plasticity under a cloud. There are, however, other examples of plasticity where differentiated cells or their precursors can be reprogrammed by extracellular cues to alter their character in ways that could have important implications for cell therapy and other forms of regenerative treatment.

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INTRODUCTION

The current obsession with stem cells comes largely from the hope that they will revolutionize the treatment of injuries and diseases where cells die. Three high profile publications in 1998 seem to have triggered the obsession, although the cloning of Dolly in 1997 no doubt prepared the ground (Wilmut et al. 1997). First, Thomson and colleagues published the first isolation of human embryonic stem (ES) cells (Thomson et al. 1998). Mouse ES cells had been isolated in 1981 (Evans & Kaufman 1981, Martin 1981), and numerous studies had shown that they could be propagated indefinitely in culture and maintain their ability to produce all of the cell types found in a mouse (Rossant 2001, Smith 2001). Second, Gearhart and colleagues published the first isolation of human embryonic germ (EG) cells (Shamblott et al. 1998). Mouse EG cells had been isolated in 1991 (Matsui et al. 1992, Resnick et al. 1992) and shown to have many of the same remarkable properties as mouse ES cells (Donovan & Gearhart 2001, Labosky et al. 1994). Third, and most relevant for this review, one of the first of a number of reports of adult stem cell plasticity was published with substantial fanfare (Ferrari et al. 1998), challenging the long-held belief that adult mammalian stem cells are lineage restricted. In most of these plasticity studies, genetically marked cells from one organ of an adult mouse apparently gave rise to cell types characteristic of other organs following transplantation, suggesting that the cells were more plastic in their developmental potential than previously thought. These plasticity studies also provided ammunition to those who object to the production of human ES cells on the grounds that it involves the destruction of human embryos. The use of adult stem cells for therapy would avoid this ethical problem and would also have two additional advantages: (*a*) Because the cells could be isolated from the patient requiring treatment, it would avoid the problem of immunological rejection, which would complicate the use of allogeneic ES or EG cells; (*b*) it would reduce the risk of tumor formation, which occurs with high frequency when mouse ES or EG cells are transplanted into histocompatible adult mice (Martin 1980, Smith 2001).

These claims for adult stem cell plasticity are concerned with cell fate switching induced by a change in the extracellular environment. It is important to distinguish them from the demonstrations of cell fate switching induced either by nuclear transfer to a new cytoplasm or by direct alteration of gene expression, by the insertion of a transgene, for example, or the inactivation of an endogenous gene. This review concerns only the claims for environment-induced cell fate change, although we shall see that some of these claims have turned out to be misinterpretations in that fate changes were actually forms of nuclear reprogramming resulting from spontaneous cell fusion (Vassilopoulos et al. 2003, Wang et al. 2003), which is functionally equivalent to nuclear transfer.

This is a difficult time to write a review of this subject because there are still many unresolved controversies and uncertainties. Some of the claims for adult stem cell plasticity have been disproved, and it is unclear which of the others will survive. Before discussing the claims and counterclaims, however, I need to consider some terminology and definitions.

TERMINOLOGY

Stem Cells, Precursor Cells, and Progenitor Cells

There are inconsistencies in the way these terms are used. A stem cell, by definition, is an undifferentiated cell that can produce daughter cells that can either remain a stem cell (a process called self-renewal) or commit to a pathway leading to differentiation. The pathway to differentiation usually involves the daughter becoming a precursor cell, which proliferates before it differentiates. Because the proliferation amplifies the number of differentiated cells eventually produced, the precursors are often called transit amplifying cells (Figure 1). The terms precursor cell and progenitor cell are usually used interchangeably, but some use progenitor cell to refer to a cell with greater developmental potential than a precursor cell.

The mechanism(s) that determines whether a daughter of a stem cell remains a stem cell or commits to differentiation is usually not known but, in principle, it can depend on the inheritance of cell-fate determinants from the mother cell, on environmental factors, or on both (Spradling et al. 2001, Watt & Hogan 2000). Whereas the ability to self-renew is basic to the definition of a stem cell, there is inconsistency about how sustained the self-renewal must be. Some definitions require that stem cells be able to self-renew indefinitely, or at least for the lifetime of the organism, whereas others do not. Many stem cells can produce a variety of differentiated cell types, but this is not a required part of the definition: spermatogonial stem cells in the testis, for example, produce only spermatozoa (Meachem et al. 2001).

The distinction between a stem cell and a precursor cell is not always clear, especially in rodents. In both humans and rodents, stem cells usually maintain telomerase activity (Forsyth et al. 2002). Many human precursor cells, however, turn off telomerase activity. Thus even if they are maintained in an undifferentiated state in culture, they can divide only a limited number of times before one or more telomeres shorten and/or uncap sufficiently to cause a permanent cell-cycle arrest, a process called replicative cell senescence (Cong et al. 2002). Many rodent precursor cells, by contrast, maintain telomerase activity and can apparently divide indefinitely, at least in the right culture conditions (Mathon et al. 2001, Tang et al. 2001); in sub-optimal culture conditions, however, they eventually undergo a telomere-independent cell-cycle arrest, a process that has been called culture shock (Wright & Shay 2000). As discussed below, some rodent precursor cells can be induced by extracellular signals to convert to a state that resembles that of

stem cells. If precursor cells can also revert to stem cells in vivo, as may occur in intestinal crypts following irradiation-induced stem cell ablation (Marshman et al. 2002) and in the adult brain following prolonged EGF infusion (Doetsch et al. 2002), then it may not always be necessary for at least one daughter of a stem cell division to remain a stem cell in order to maintain the stem cell pool, as is often assumed. Even some differentiated cells may be able to revert to stem cells in vivo: In chickens, for example, differentiated supporting cells in the auditory epithelium can reenter the cell cycle and produce new hair cells when hair cells are destroyed (Stone et al. 1998).

Pluripotency, Multipotency, and Unipotency

One can operationally classify mammalian stem cells according to their developmental potential. ES cells (produced in culture from the epiblast cells of a blastocyst) and EG cells (produced in culture from primordial germ cells of an early embryo) are said to be pluripotent because they can produce all the cell types of the embryo proper, including germ cells. Because they cannot produce all of the extraembryonic tissues required for mammalian development, they are not considered to be totipotent. By contrast, some differentiated plant cells are totipotent, in that they can dedifferentiate and proliferate and give rise to an entire plant, starting from a single cell (Vasil & Hildebrandt 1965). The stem cells in animal organs are said to be multipotent if they produce more than one cell type and unipotent if they produce only one cell type. They ultimately arise progressively from epiblast cells in the embryo and are variably referred to as tissue-specific, organ-specific, or adult stem cells. In cell suspensions of tissues analyzed by fluorescence-activated cell sorting (FACS), the stem cells are often found among a small "side population" that is only weakly fluorescent after staining with a fluorescent dye (Goodell et al. 1996, Spangrude & Johnson 1990, Wolf et al. 1993); these cells express a high level of a specific ABC transporter that actively pumps the dye out of the cells (Zhou et al. 2001). Although operationally useful, the biological significance of this property of stem cells is unknown.

ADULT STEM CELLS

It was originally thought that adult mammalian stem cells were only present in organs such as blood, skin, gut, testis, and the respiratory tract that have high cell turnover rates. It now appears, however, that most, if not all, adult organs contain stem cells, or at least can produce stem cells in culture. Of the many types of adult stem cells, I consider here only those that have been involved in plasticity experiments.

Hematopoietic Stem Cells (HSCs)

Most of what is known about adult mammalian stem cells has come from studies of hematopoietic stem cells (HSCs). They were the first stem cells to be characterized

and isolated, and they were the first to be used clinically. Moreover, most adult stem cell plasticity experiments have used either HSCs or bone marrow (BM), which contains most of the HSCs in an adult, but also contains other types of stem cells (discussed below).

The atomic bombing of Hiroshima and Nagasaki dramatically demonstrated that exposure to a high dose of irradiation can destroy the blood system and thereby kill the victim. In the 1950s, it was shown that an infusion of bone marrow cells could reconstitute the blood system and save lethally irradiated mice (Ford et al. 1956, Nowell et al. 1956). In the 1960s, Till & McCulloch and their colleagues showed that soon after such a BM infusion the spleen contains macroscopic cell colonies, which often contain a mixture of red blood cells and various kinds of white blood cells (Till & McCulloch 1961). They used X-ray-induced chromosome marking of the donor cells to demonstrate that each spleen colony arises from a single cell, indicating for the first time that some of the infused BM cells are multipotential (Becker et al. 1963). They also showed that some of the colonies contain cells that are able to form new mixed spleen colonies (Wu et al. 1967), which indicates that at least some of the multipotent BM cells are capable of self-renewal, which became the defining characteristic of all stem cells.

On the basis of these and other findings, a model for hematopoiesis was proposed in which rare, slowly dividing stem cells produce rapidly dividing precursor cells, most of which eventually stop dividing and terminally differentiate (Till & McCulloch 1980). The hematopoietic precursor cells have a limited capacity for self-renewal and become progressively more restricted in their developmental potential. There is now abundant evidence supporting this model, not only for hematopoiesis, but also for many mammalian organs, and probably for many cancers (Reya et al. 2001). It would be difficult to overestimate how important the hematopoietic model has been for understanding how mammalian tissues develop and maintain themselves.

There have been many advances in understanding hematopoiesis since the pioneering studies of Till, McCulloch, and their colleagues. Mouse (Spangrude et al. 1988) and human (Baum et al. 1992) HSCs have been purified to near homogeneity using FACS and a variety of monoclonal antibodies that recognize cell-surface antigens. At least two classes of mouse HSCs can be distinguished on the basis of cell-surface antigens and potential for self-renewal: Long-term (LT) HSCs can reconstitute the blood system of an irradiated mouse for a lifetime, whereas short-term (ST) HSCs can do so for only about six weeks (Morrison & Weissman 1994). The details of the hematopoietic cell hierarchy have been filled in: LT-HSCs give rise to ST-HSCs, which produce common myeloid precursors and common lymphoid precursors; these, in turn, produce more restricted precursors that generate the various differentiated cells of the blood and immune systems, respectively (Weissman et al. 2001). Many of the cytokines and growth factors that promote the survival and proliferation of the different precursor cells have been identified (Moore 2002), and some are widely used clinically. G-CSF, for example, helps mobilize HSCs from the BM into the blood where they can be harvested and

enriched for the treatment of cancer patients who have had their own blood-forming cells destroyed by chemotherapy, irradiation, or both (Lapidot & Petit 2002).

It is still uncertain what determines whether HSCs self-renew or commit to differentiation or what controls the types of cells that HSCs or multipotent hematopoietic precursors develop into. Moreover, it remains a problem to expand normal HSCs in culture.

CNS Neural Stem Cells (NSCs)

The existence of stem cells in the adult mammalian central nervous system (CNS) was originally inferred by the finding that some neurons are produced throughout life in specific regions of the CNS, including the olfactory bulb and hippocampus (Altman & Das 1966). It was only in the 1990s, however, that multipotent adult neural stem cells (NSCs) were demonstrated in culture (Gritti et al. 1996, Lois & Alvarez-Buylla 1993, Morshead et al. 1994, Palmer et al. 1997, Reynolds & Weiss 1992, Richards et al. 1992).They are often identified as cells that form floating cell aggregates, or neurospheres, when cultured in serum-free medium on a non-adherent surface in the presence of EGF and/or FGF-2 (Gritti et al. 1996, Reynolds & Weiss 1992), although they can also proliferate as adherent cells (Gage et al. 1995, Johe et al. 1996, Richards et al. 1992). Adult NSCs in culture can self-renew and are multipotential, producing various unipotent and multipotent precursor cells that, in turn, differentiate into neurons, astrocytes, or oligodendrocytes, or some combination of these cell types (Cameron & McKay 1998, Gage 2000, Temple & Alvarez-Buylla 1999). Although NSCs can be cultured from many regions of the adult CNS (Palmer et al. 1999, Weiss et al. 1996), the major location in the adult brain is the subventricular zone (SVZ). Here, stem cells mainly generate precursor cells that give rise to neuroblasts, which migrate to the olfactory bulb where they differentiate into inhibitory interneurons (Alvarez-Buylla & Garcia-Verdugo 2002). Most NSC plasticity studies have used cultured brain neurosphere cells, although one has used freshly isolated, FACS-enriched NSCs from adult mouse brain (Rietze et al. 2001). NSCs cultured from different parts of the CNS are behaviorly and molecularly distinct, but they are adaptable: When transplanted to new CNS locations, they can adopt some of the characteristics appropriate to the new environment (Gage 2000, Hitoshi et al. 2002). The identity of NSCs in vivo is still controversial (Barres 1999, Laywell et al. 2000), and the ability to culture neurospheres from regions of the adult brain that do not normally undergo self-renewal (Palmer et al. 1995, Shihabuddin et al. 2000, Weiss et al. 1996) raises the possibility that neurosphere-forming stem cells can arise in culture from precursor or differentiated cells (Anderson 2001) (discussed below).

Skeletal Muscle Stem Cells

Adult skeletal muscle stem cells are among the few adult stem cells that can be identified prospectively in vivo. They are satellite cells, situated between the plasma membrane of the multinucleated muscle cell and the basal lamina that surrounds

each muscle cell (Bailey et al. 2001, Goldring et al. 2002, Mauro 1961). They are normally quiescent but proliferate in response to muscle injury, producing myoblasts that can either form new satellite cells or fuse with one another or pre-existing multinucleated muscle cells to help repair the muscle.

Mesenchymal Stem Cells (MSCs)

Adult mesenchymal stem cells (MSCs) are widely distributed in the connective tissues of the body, where they are found among the heterogeneous collection of cells collectively called fibroblasts. Depending on the extracellular matrix and signal molecules in their environment, they can develop into smooth muscle cells, adipocytes, or chondrocytes (Minguell et al. 2001, Pittenger et al. 1999). A common source of MSCs is BM, which contains both MSCs and HSCs. As with HSCs and NSCs, MSCs in culture are heterogeneous and vary in their properties, depending on the organ they are obtained from (Conrad et al. 1977).

Multipotent Adult Progenitor Cells (MPACs)

It has recently been reported that when MSCs from adult rodent or human BM are cultured at low density, a population of cells can emerge after many passages with properties similar to those of ES cells (Jiang et al. 2002a). These so-called multipotent adult progenitor cells (MAPCs) can also be obtained from adult muscle and brain (Jiang et al. 2002b). They resemble ES cells in their remarkable multipotentiality in culture and in vivo. When a genetically marked single MAPC was injected into a mouse blastocyst, for example, 33% of the mice, assessed four weeks after birth, were chimaeric, and, in some of these, the MAPCs had contributed to most organs (Jiang et al. 2002a). Also like ES cells, mouse MAPCs require leukemia inhibitory factor for expansion in culture, whereas human MAPCs do not (Smith 2001). Unlike ES cells, however, MAPCs seem not to form tumors when injected into immunodeficient adult mice. It remains unclear whether MAPCs preexist at very low frequency in adult organs or whether some MSCs gradually acquire ES-cell-like properties in culture. In either case, they could be an invaluable source of cells for cell therapy.

A possibly related population of adult stem cells can be cultured from the dermis of adult mouse or human skin. Like MAPCs and ES and EG cells, these skin-derived precursors (SKPs) can produce cell types that normally derive from more than one germ layer during embryogenesis, including neurons, glial cells, smooth muscle cells, and adipocytes, depending on the culture conditions (Toma et al. 2001).

CLAIMS FOR ADULT STEM CELL PLASTICITY

It has long been believed that adult mammalian stem cells are restricted to forming the cell types normally found in the organ in which they reside. Classical experiments showed that when fragments of an organ or a tissue are transplanted to a new site, the transplanted tissue maintains its original character. Similarly, when

dissociated cells from an organ or tissue are cultured, they also tend to maintain their original character; although they may lose some of their differentiated properties, they usually do not acquire differentiated characteristics of a different cell lineage. As discussed below, however, there are well-known exceptions, where one type of cell or tissue turns into another, processes called metaplasia, cell plasticity, lineage switching, or transdifferentiation (Tosh & Slack 2002).

Nonetheless, it was a shock when Ferrari et al. reported in 1998 that mouse BM cells could give rise to skeletal muscle cells when transplanted into a mouse muscle that had been damaged by an injection of a muscle toxin (Ferrari et al. 1998). Earlier, Eglitis & Mezey reported that transplanted mouse BM cells could give rise to brain astrocytes in adult mice, but this report received relatively little attention (Eglitis & Mezey 1997). These papers, however, were just the beginning. It was soon reported that transplanted BM cells could produce hepatocytes (Petersen et al. 1999, Theise et al. 2000a), endothelial and myocardial cells (Lin et al. 2000, Orlic et al. 2001), and CNS neurons and glial cells (Brazelton et al. 2000, Mezey et al. 2000, Priller et al. 2001). Moreover, it was reported that enriched HSCs could produce cardiac myocytes and endothelial cells (Jackson et al. 2001); purified HSCs could produce functional hepatocytes (Lagasse et al. 2000); and single HSCs could produce epithelial cells of the liver, gut, lung, and skin (Krause et al. 2001). It was also reported that BM stromal cells (MSCs) could produce brain astrocytes (Kopen et al. 1999), and cells enriched for stem cells from adult mouse skeletal muscle could produce blood cells (Gussoni et al. 1999, Jackson et al. 1999, Pang 2000). Perhaps most remarkable of all, it was reported that NSCs from adult mouse brain produce blood cells and immune cells (Bjornson et al. 1999), skeletal muscle cells (Galli et al. 2000), and, when injected into a blastocyst, a wide variety of embryonic cell types (Clarke et al. 2000). These forms of apparent plasticity were beyond anything seen before in mammals. It was especially surprising to see claims that cells derived from one germ layer could give rise to cell types that normally derive from another germ layer–mesoderm derivatives (BM) to endoderm derivatives (liver and gut) and to ectoderm derivatives (skin, neurons, and astrocytes), for example, and ectoderm derivates (NSCs) to cells of all three germ layers in an embryo.

Why had these dramatic forms of apparent plasticity not been seen before? The reason is almost certainly related to the methods used. In earlier experiments, organ or tissue fragments were usually transplanted and so the donor cells continued to have neighbors of the same tissue type. In the recent experiments, cell suspensions were usually transplanted so that individual donor cells could end up surrounded by cells of a different tissue type. Moreover, the new experiments all involved marking the donor cells genetically so that even rare cells expressing donor-cell genes could be identified in tissue sections. Two types of genetic markers have been used in mouse experiments: (*a*) Y chomosome DNA sequences to detect male donor-derived cells in female hosts by in situ hybridization and (*b*) transgenes encoding either bacterial β-galactosidase (β-gal) or green fluorescent protein (GFP), which can be detected in host tissues by X-Gal histochemistry (or immunohistochemistry)

or fluorescence, respectively. Sex chromosome markers have also been used to detect apparent plasticity in human liver or BM transplant patients, where blood cells were reported to give rise to either hepatocytes (Alison et al. 2000, Korbling et al. 2002, Theise et al. 2000b) or epithelial cells in skin and gut (Korbling et al. 2002).

PROBLEMS AND CONTROVERSIES

The adult stem cell plasticity experiments have caused great excitement, as they challenged developmental biologists' belief in lineage restriction and provided hope for the use of adult stem cells in cell therapy. They have also generated great controversy, beginning in 2001 (Anderson et al. 2001, Morrison 2001), gathering strength in 2002 (Castro et al. 2002, Frisen 2002, Joshi & Enver 2002, Morshead et al. 2002, Wagers et al. 2002), and showing little sign of abating in 2003. There are serious problems with many of the claims for adult stem cell plasticity, and I deal specifically with four, in increasing order of probable importance.

Characterization of Switched Cells in Host Tissues

In most reported cases, the phenotype of the donor-derived cells that apparently switched their normal fate was assessed by morphology and antibody staining, but rarely by function. Thus the cells may have acquired only a few of the characteristics of the new cell type but not any new functions. In some cases, such as Purkinje neurons in the cerebellum, cell-type identification by morphology alone can be unambiguous (Priller et al. 2001). In other cases, however, cell-type identification has been far less clear. Few morphologies or antigens are truly cell-type specific. In addition, it is easy to be misled when assessing whether an individual cell in a tissue section that expresses GFP or β-Gal also expresses a particular antigen, even in a confocal microscope. Rotation of the confocal images of an apparently double-labeled cell can reveal that the two labels are not in the same cell (Eisch 2002, Kornack & Rakic 2001; see also http://www3.utsouthwestern.edu/eisch/Images/Pitfalls.mov) (although it takes a long time to download this movie).

Identification of the Switching Cells

In most cases, a mixed population of donor cells was used, and thus the nature of the cells that apparently switched lineage is unknown. An example of how this problem can lead to misinterpretation is provided by the initial reports that skeletal muscle stem cells (satellite cells) could produce blood cells (Gussoni et al. 1999, Jackson et al. 1999, Pang 2000). When the blood-forming cells from muscle were better characterized, they turned out to be HSCs present in muscle, rather than satellite cells (Asakura et al. 2002, McKinney-Freeman et al. 2002, Seale et al. 2000). HSCs circulate in the blood in significant numbers and are therefore probably present in most organs (Wright et al. 2001). Even where purified or clonally

derived donor stem cells are transplanted, it is not known whether the stem cells themselves or the precursor cells or differentiated cells derived from them switched fates (or fused with host cells—see below). Thus even if fate-switching is unambiguously established in a transplantation experiment using purified, clonally derived, or single stem cells, it may still be misleading to refer to the process as adult stem cell plasticity.

The problem of identifying the switching cells is accentuated by the apparent low efficiency of the process in most cases. Usually, large numbers of donor cells are transplanted, but relatively few switched cells are detected in the recipient. It is unclear whether this is because only rare cells in the donor population have the capacity to switch (or produce cells that switch) or whether many cells have this ability but have a low probability of doing so. The low frequency of apparent switching raises the possibility that rare fusion events or contamination of the donor population by rare ES-like cells (such as MAPCs—discussed above) may account for some of the results.

Failures to Reproduce Results

In 2002, a number of papers appeared that reported failures to reproduce some of the original plasticity results. Brain-to-blood switching is one example. There have been three reports that transplanted brain-derived cells could produce blood cells. In the first, unfractionated adult mouse brain cells apparently produced blood-forming spleen colonies in irradiated mice (Bartlett 1982). In the second, mouse embryonic and clonally derived adult brain neurosphere cells apparently produced blood cells in irradiated recipients (Bjornson et al. 1999). In the third, human fetal brain neurosphere cells apparently produced blood cells in immunodeficient SCID mice carrying fragments of human BM and thymus under the kidney capsule; in this study, the apparent brain-to-blood switching required the microenvironment of the human BM fragments (Shih et al. 2001). However, one recent study failed to find any contribution to blood cell formation when more than 100 million clonally derived mouse embryonic brain neurosphere cells from four independent lines of NSCs were injected intravenously into 104 irradiated mice; BM-derived blood-forming colonies were examined four to seven weeks later for donor-derived colonies and none were found (Morshead et al. 2002). Although there were significant methodological differences in the way the positive and negative experiments were done, the explanation for the different results remains unclear. The potential problem of contaminating HSCs seems unlikely to be the explanation in at least two of the positive studies: In one (Bjornson et al. 1999), cloned NSCs were transplanted; in the other (Shih et al. 2001), careful controls were done in attempt to exclude such contamination. Spontaneous cell fusion (see below) has not been excluded as the explanation for the apparent lineage switching in any of the positive studies, but even if fusion were the explanation, it would remain unclear why fusion occurred in the positive studies but not in the negative ones. One possible explanation for the different results is that the NSCs transplanted were different. As mentioned above, brain NSCs are heterogeneous (Hitoshi et al. 2002) and can

also change in culture (Morshead et al. 2002). Thus compared with the transplanted cells in the negative experiments, those in the positive experiments may have contained or given rise to cells that either more readily formed blood cells or more readily fused with host hematopoietic cells. Heterogeneity of NSCs may also explain why adult mouse CNS neurospheres injected into mouse blastocysts can apparently contribute to a variety of embryonic organs, but do so only rarely (Clarke et al. 2000), whereas primitive NSCs formed rapidly in low-density cultures of ES cells can apparently do so with much greater frequency (Tropepe et al. 2001), although it is difficult to exclude the problem of contaminating ES cells in the latter experiments.

Most of the adult stem cell plasticity experiments have involved transplanted BM cells or HSCs. Two recent studies failed to reproduce some of the original findings (Castro et al. 2002, Wagers et al. 2002). In one (Wagers et al. 2002), lethally irradiated mice were reconstituted with genetically marked single HSCs, and then multiple tissues were examined after 4 and 9 months for donor-marked, non-hematopoietic cells expressing appropriate tissue-specific markers. In most organs, no such cells could be detected, although one cell (a Purkinje cell) was seen in the cerebellum and several were seen in the liver, all of which could have resulted from cell fusion (see below). No such cells were seen in the gut, even when the gut had been damaged by irradiation. Similar findings were obtained when chimeric mice were produced by parabiosis. In the second study (Castro et al. 2002), no evidence was found for HSCs producing neurons or macroglial cells in the CNS, despite earlier reports to the contrary (Brazelton et al. 2000, Kopen et al. 1999, Mezey et al. 2000, Priller et al. 2001).

Spontaneous Cell Fusion Masquerading as Cell Plasticity

Perhaps the most important weakness in many of the studies reporting adult stem cell plasticity has been the failure to exclude cell fusion as the explanation for the findings. Surprisingly, this concern was raised only recently, when two independent studies showed that NSCs (Ying et al. 2002) and BM cells (Terada et al. 2002), respectively, spontaneously fuse at low frequency with ES cells in culture. In both cases, fusion produced tetraploid hybrid cells that maintained the properties of ES cells, while expressing the genetic markers of the non-ES cells. In the case of the BM cells, the rate of fusion was not greater when enriched HSCs were used instead of unfractionated BM cells, suggesting that HSCs were not the main cell type in BM involved in the fusion. The possibility of cell fusion confounding the interpretation of plasticity experiments is especially great when HSCs are transplanted into irradiated mice and produce a substantial proportion of the blood cells in the host. Here, many millions of donor white blood cells would be potential fusion partners that could donate their marker genes (or sex chromosomes) to a host cell. Monocytes and macrophages may be the most likely donor cell type to fuse, as macrophages have been shown to fuse spontaneously in culture (Parwaresch et al. 1986).

Crucially, cell fusion has turned out to be the explanation for what was widely regarded as the most convincing example of HSC lineage switching. Lagasse

et al. (Lagasse et al. 2000) originally reported the results of transplanting a small number of purified, male HSCs expressing a *β-gal* transgene into lethally irradiated female mice with a fatal genetic liver disease (tyrosinemia) caused by a mutation in the *fumaryl-acetoacetate hydrolase* (*FAH*) gene. The transplanted HSCs not only reconstituted the blood system of the recipients but also apparently produced donor-type hepatocytes that rescued the liver deficiency and saved the mice. This study was noteworthy because the HSCs were purified, the donor-type hepatocytes (expressing Y chromosome sequences, the *β-gal* transgene, and wild-type *FAH*) were functional and constituted up to 50% of the liver. The donor-type hepatocytes, however, initially appeared as a small number of nodules in the recipient liver only many weeks after the recipient blood system had been reconstituted with donor cells, suggesting that the apparent conversion was a rare event (Wang et al. 2002). Now, two independent groups, including one of the groups responsible for the original findings, have used Southern blotting and cytogenetic analyses to show that the donor-type hepatocytes arise by cell fusion rather than by the differentiation of HSCs or their progeny into hepatocytes (Vassilopoulos et al. 2003, Wang et al. 2003). These findings cast doubt on all the reported examples of apparent HSC plasticity, as well as on some other examples of apparent plasticity where cell fusion could, in principle, explain the findings. They also, however, open up novel strategies for the treatment of genetic diseases. Whereas it is unlikely that cell fusion will turn out to be the explanation for all of the recent claims for adult stem cell plasticity, fusion must now be actively excluded in each case. In a recent example of plasticity, where unfractionated BM cells gave rise to skeletal muscle cells, fusion seems to have been excluded (LaBarge & Blau 2002), but the nature of the BM cells that produced muscle cells is unknown.

There are some related forms of cell plasticity, however, that start with differentiated cells or precursor cells rather than with stem cells and are unlikely to be confounded by cell fusion. These examples of plasticity have also challenged some cherished beliefs of developmental biologists, although they have received much less attention than the adult stem cell plasticity experiments. They may also have implications for cell therapy and other forms of regenerative medicine, as I now discuss.

DEDIFFERENTIATION AND CONVERSION OF PRECURSOR CELLS TO STEM CELLS

The steps of mammalian cell development normally proceed in one direction only. Stem cells, for example, produce precursor cells, which, in turn, produce differentiated cells. The developing cells become progressively more restricted with each step and normally do not go backward. A classic example of progressive restriction in development occurs during gastrulation, when the three germ layers (ectoderm, endoderm, and mesoderm) form. Each germ layer then gives rise to a characteristic set of cell types, tissues, and organs: Endoderm, for instance, forms the gut, liver, pancreas, and lungs; mesoderm forms muscle, blood, bone, and fat;

and ectoderm forms skin and the nervous system. Cells produced by one germ layer usually do not produce cells derived from another germ layer, although there are exceptions: Cranial neural crest cells, for example, derive from neuroectoderm but give rise to muscle, cartilage, and bone cells in the head (Le Douarin et al. 1997).

There is increasing evidence that the rules of irreversibility and germ layer restriction can be broken, especially following injury and in cell culture. In these cases, extracellular cues seem to reprogram some precursor or differentiated cells so that the cells acquire characteristics of either a less mature state or a new differentiated state. When a differentiated cell converts to a precursor cell or stem cell, the process is called dedifferentiation. When the conversion is to a different type of differentiated cell, it is called transdifferentiation. It is uncertain whether transdifferentiation always involves dedifferentiation, although this seems likely. The first evidence for dedifferentiation in vertebrate cells came from studies of urodeles and avians.

Dedifferentiation in Urodeles and Avians

Non-mammalian vertebrates provide many examples of dedifferentiation, either during injury and regeneration or in culture (Eguchi & Kodama 1993, Slack & Tosh 2001). Following limb amputation in urodeles, for example, chondrocytes and skeletal muscle cells dedifferentiate, proliferate as blastemal cells, which then redifferentiate into the specialized mesoderm cell types of the regenerated limb (Brockes 1997, Brockes & Kumar 2002). Similarly, removal of the lens in a salamander causes pigmented epithelial cells in the dorsal iris to dedifferentiate and form a new lens (Eguchi & Kodama 1993, Stone 1967), whereas removal of the neural retina causes pigmented cells of the retina to dedifferentiate and form a new neural retina (Stroeva & Mitashov 1983). In these cases, the dedifferentiated cells adopt the character of local precursor cells and still obey the rules of germ-layer restriction (Brockes & Kumar 2002). Recently, however, it was shown that, during the process of tail regeneration following amputation in axolotyls, individually marked radial glial cells of the spinal cord can dedifferentiate, proliferate, and, surprisingly, produce some skeletal muscle cells and chondrocytes of the regenerating mesodermal tissues, in addition to the expected glial cells and neurons within the regenerating spinal cord (Echeverri & Tanaka 2002). This is the first definitive demonstration of naturally occurring germ layer switching in an adult vertebrate.

Similar dedifferentiation processes occur in avians. As in urodeles, chick pigmented retinal epithelial cells can dedifferentiate and form either lens or neural retina, both in culture and in vivo (Eguchi & Kodama 1993). More recently, the cytokine endothelin 3 was shown to induce quail skin melanocytes in culture to dedifferentiate into glial-melanocyte precursor cells, which normally give rise to both Schwann cells and melanocytes (Dupin et al. 2000). Similarly, treatment of Schwann cells with endothelin 3 induces them to dedifferentiate into glial-melanocyte precursor cells (Dupin et al. 2003). Although it is unclear if similar reversions occur in vivo, the use of cultures derived from single cells eliminates any ambiguity in the interpretation of the in vitro findings.

Dedifferentiation in Mammals

Some of the examples of dedifferentiation in mammals are less dramatic but no less convincing than those in non-mammalian vertebrates. When an adult rodent peripheral nerve is cut, for example, the Schwann cells dedifferentiate into Schwann cell precursors, which proliferate and then redifferentiate, much as occurs in limb and organ regeneration in urodeles (Brockes & Kumar 2002). After the axons regenerate, the precursors redifferentiate into either myelinating or non-myelinating Schwann cells, depending on the type of axon, rather than on whether the original cell had been a myelinating or non-myelinating Schwann cell before the nerve was cut (Aguayo et al. 1976, Weinberg & Spencer 1976). An example of mammalian transdifferentiation occurs during the normal development of the mouse esophagus, where smooth muscle cells in the esophageal wall transdifferentiate into skeletal muscle cells around the time of birth (Kablar et al. 2000, Patapoutian et al. 1995).

There are also a number of examples of mammalian dedifferentiation in cell culture. Cultured chondrocytes, for instance, can revert to fibroblast-like precursor cells and back again, in response to changes in extracellular matrix (Benya & Shaffer 1982). Remarkably, even multinucleated, postmitotic, skeletal muscle cells can be induced by treatment with the purine derivative myoseverin to break up into mononuclear cells that reenter the cell cycle (Rosania et al. 2000). This effect of myoseverin partly mimics the effect of ectopic expression of the transcriptional repressor msx1, which, in addition, induces the transfected mononuclear cells to dedifferentiate into precursor cells that can produce adipocytes, chondrocytes, and skeletal muscle cells (Odelberg et al. 2000).

Conversion of Mammalian Precursor Cells to Stem Cells

A change in extracellular environment can also induce some mammalian precursor cells to convert to stem cells. An important example is the conversion of mouse (Matsui et al. 1992, Resnick et al. 1992) and human (Shamblott et al. 1998) primordial germ cells (PGCs) to ES-like EG cells when cultured in appropriate growth factors. Whereas PGCs normally give rise only to germ cells, mouse EG cells can form any type of cell in a mouse (Donovan & Gearhart 2001). More recently, it has been possible to induce purified rat oligodendrocyte precursor cells (OPCs) in culture to acquire some characteristics of NSCs so that they can form neurospheres and produce neurons and astrocytes, as well as oligodendrocytes (Kondo & Raff 2000). Interestingly, for both PGCs and OPCs, the conversion depends on FGF-2.

Precursor-to-stem-cell conversion has also been reported to occur in vivo. As mentioned above, there is evidence that precursor cells in intestinal crypts (Marshman et al. 2002) and in adult brain (Doetsch et al. 2002) may be induced in vivo to acquire the properties of stem cells. In one remarkable example, a region of adult rabbit corneal epithelium known to be devoid of stem cells was associated with mouse dermis and transplanted into an immunodeficient nude mouse. The genetically marked corneal precursor cells apparently converted to skin stem cells under the influence of the dermis and produced epidermis, hair, and sweat glands (Ferraris et al. 2000).

These various cell conversion experiments suggest that many precursor cells and differentiated cells are not as irreversibly committed to a particular pathway of development as originally believed. Thus it would be better to use the term specified rather than determined or committed when referring to precursor cells, especially as it is not possible to test all environmental conditions to establish that a particular cell type cannot convert to another or that a particular developmental stage cannot be converted to an earlier one. The ability of precursor cells to acquire stem cell characteristics also makes it increasingly difficult to define a stem cell and distinguish it from a precursor cell. It also raises the possibility that some of the stem cells identified in culture may have developed in vitro by the conversion of precursor cells, or even of differentiated cells. Adult NSCs, for example, can be identified in cultures prepared from many regions of the CNS (Gage 2000), but it is possible that in some cases they may have developed in culture by the conversion of OPCs (Kondo & Raff 2000). Such conversion might be exploitable for cell therapy: As precursor cells in adult tissues are generally more abundant and easier to purify and expand than stem cells, if they can be isolated, expanded, and then converted to stem cells, they may provide a convenient source of stem cells for therapy.

The cell conversion results also raise a number of fundamental questions, answers to which could have important implications for both developmental biology and cell therapy. When precursor cells are induced to acquire stem cell characteristics, do they retrace the original steps by which they developed, or do they follow alternative routes? In either case, do such converted cells retain a memory of their unusual history, in their chromatin or gene expression pattern, for example, that could distinguish them from nonconverted cells of the same type and state? If the converted cells always retrace their original steps, then their position in a lineage hierarchy might determine how easy it is and how long it takes to induce them to acquire characteristics of the stem cells located at the top of the hierarchy. The neuroepithelial cells that give rise to NSCs, for instance, arise early in development from the primitive ectoderm. Thus some NSCs may be only a few steps removed from ES cells, which may be why they more closely resembled ES cells than HSCs in a recent transcriptional profiling study (Ramalho-Santos et al. 2002).

There is surprisingly little evidence for environment-induced dedifferentiation or precursor-to-stem-cell conversion in the hematopoietic system. Is this because it has not been rigorously looked for, or is there something special about the hematopoietic cell lineage? Many hematopoietic cells migrate to a variety of organs and thus may need to have an unusually robust ability to maintain their specification in different environments.

CONCLUSIONS

Despite the problems with many of the adult stem cell plasticity experiments, the intense recent interest in these cells has greatly advanced our understanding of them. Even the discovery that cells produced by adult HSCs can fuse spontaneously with other cell types in vivo (Vassilopoulos et al. 2003, Wang et al. 2003) is an important advance, with potential implications for gene therapy. Although some

adult stem cells in new environments may be able to produce cell types other than those they normally produce, this probably occurs at too low a frequency to be clinically useful at present. On the other hand, the ability to obtain rodent and human MAPCs with some ES-cell-like properties in cultures prepared from readily accessible adult tissues such as BM and muscle holds great promise (Jiang et al. 2002a,b). It will be important to see how robust these findings are and whether they reflect the preexistence of rare MAPCs in mammalian organs or the rare conversion of MSCs in culture.

As Anderson has pointed out (Anderson 2001), there is a big difference between what cells normally do and what they can do, if put in culture, for example, or if transplanted to a new location. From the perspective of cell therapy, however, it is what cells can do that may matter most. Although the approaches and perspectives of those who wish to use stem cells for therapy and those who study stem cells to understand normal development and tissue homeostasis can be very different (Anderson 2001), it is likely that the two approaches will continue to inform each other. In both cases, there is a pressing need for better ways to identify and purify adult mammalian stem cells prospectively (Anderson et al. 2001), especially as most of these cells still cannot be unambiguously identified, let alone purified. Gene profiling studies should prove invaluable in this regard (Ivanova et al. 2002, Ramalho-Santos et al. 2002). It would also be helpful to be able to follow individual adult stem cells and their progeny continuously, both in culture and in vivo, so that their proliferation, migration, differentiation, and death can be accurately tracked.

Perhaps the greatest challenge in stem cell biology is to uncover the extracellular and intracellular mechanisms that determine whether a daughter cell of a stem cell division self-renews or commits to a particular pathway of differentiation. Cracking this problem for the adult mammalian stem cells of interest will be a crucial step for both developmental biology and cell therapy.

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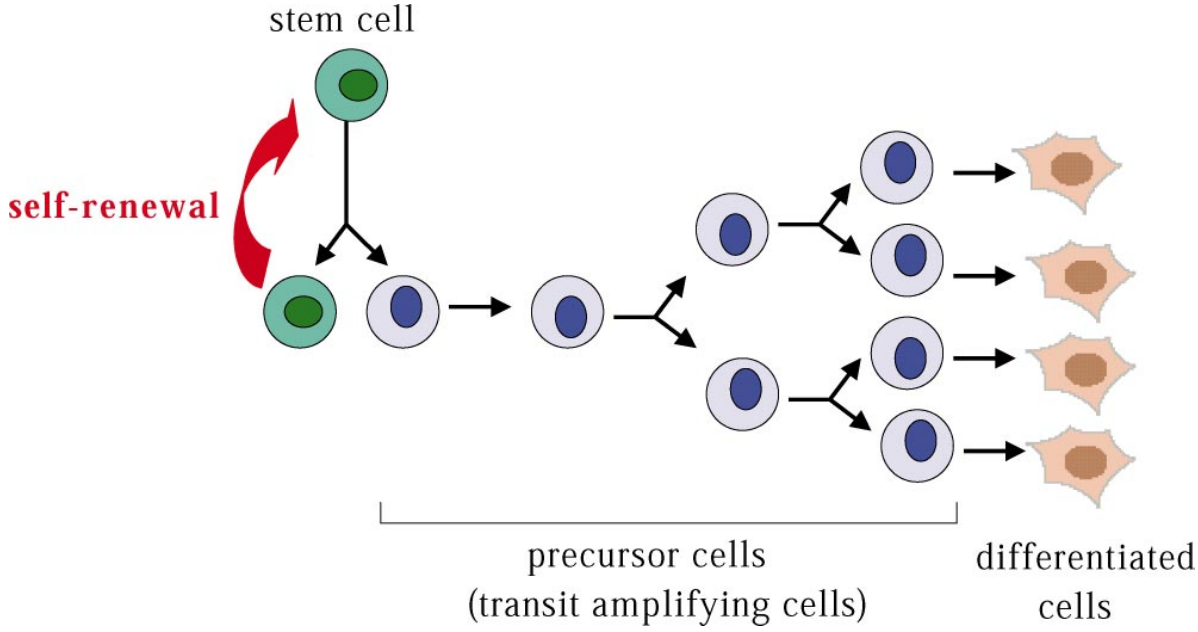

Figure 1 The possible choices for a daughter cell of a stem cell division. It can either self-renew (that is, remain a stem cell) or commit to a pathway leading to differentiation. In many cases where it commits to differentiation, it first becomes a precursor cell, which proliferates before differentiating. (Redrawn from Alberts et al. 2002, with permission.)

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