REVIEW

Why Stem Cells?

Derek van der Kooy[1] and Samuel Weiss[2]

Stem cells are viewed from the perspectives of their function, evolution, development, and cause. Counterintuitively, most stem cells may arise late in development, to act principally in tissue renewal, thus ensuring an organism's long-term survival. Surprisingly, recent reports suggest that tissue-specific adult stem cells have the potential to contribute to replenishment of multiple adult tissues.

Expanding on an earlier assertion by J. S. Huxley, the Nobel laureate ethologist Tinbergen suggested that there are four separate ways to answer any "why" question in biology: how does a biological entity function currently, how did it evolve, how does it develop, and what are the proximate causes that regulate its behavior (*1*). These questions are known colloquially as the four "whys," and are addressed below with respect to stem cells.

Function

The definition of stem cells must be on a functional basis. Even as the identification of structural attributes of stem cells at the morphological or molecular levels becomes possible [current candidates include high levels of expression of the multidrug resistance gene (*2, 3*) and certain combinations of integrin expression (*4*)], it will always be the seductive function of stem cells that will be their defining feature.

Functionally, stem cells are the multipotential, self-renewing cells that sit at the top of the lineage hierarchy and proliferate to make differentiated cell types of a given tissue in vivo. It is important to restrict this definition to single cells that, once developed, self-renew for the lifetime of the organism in order to distinguish stem cells from the many types of more transient progenitor cells (with limited self-renewal lifespans) that are present, especially in complex organisms. This may be more than semantic classification, because stem and progenitor cells defined by self-renewal ability may constitute different classes of cells under different molecular regulation across tissue types. In vivo in adult organisms, stem cells can divide repeatedly to replenish a tissue or may be more quiescent, as in the mammalian brain (*5*). Rather than considering stem cells as undifferentiated cells, it may be more productive to think of them as appropriately differentiated for their specific tissue niches (*6*), with perhaps the ability to display more potential phenotypes in alternate niches. Stem cells can divide symmetrically during development to expand their numbers and asymmetrically to self-renew and give rise to a more differentiated progeny (*7*). Indeed, as suggested for mammalian hematopoietic stem cells, the differentiation of specific blood progenitors from the asymmetric division of stem cells may be stochastic (*8*), with only the rate of proliferation of the stem cells under specific regulation.

Evolution

Should the first cell to evolve (a unicellular organism) be considered a stem cell? In a trivial sense, this suggestion reduces organism reproduction to stem cell behavior. The cell that self-renews itself is essentially reproducing itself. In a unicellular organism, that cell must have both the ability to self-renew and to carry out differentiated functions. A similar argument has been made in simple multicellular organisms with relatively few cell types, such as the hydra, where its head and foot can be regenerated in adult from a piece of body column representing only 2% of the tissue mass. In hydra, single epithelial cells appear to carry out several steady-state physiological functions as well as serving as stem cells (*9*). The recent suggestion that the neural stem cells in the adult mammalian forebrain have at least some properties of differentiated astrocytes can be seen as another example of stem cells perhaps also carrying out differentiated adult tissue functions even in complex organisms (*10*).

Although most evidence suggests that multicellularism evolved separately in plants and animals, the homology between the *piwi* gene in *Drosophila*, which controls germ line stem cells, and the *ZWILLE* gene in *Arabidopsis*, which controls the stem cells of the shoot meristem, has led to the suggestion that "stemness" evolved in a single-celled ancestor, or at least that plants and animals shared a multicellular ancestor (*11*). However, as with all evolutionary theorizing based on single gene homologies, other interpretations are possible, such as cross-kingdom gene transfer or, more plausibly, convergent evolution through separate co-opting of similar biochemical machinery in plants and animals (*12*). At any rate, plants may have been overlooked as a resource for stem cell research. Single cells from adult plants such as carrot and tobacco have the ability to make complete new adult plants (*13*).

By the strict definition that stem cells, once developed, must self-renew over the entire lifetime of the organism, the supposed stem cells of the *Drosophila* sensory neuron lineage perhaps are better viewed as progenitor cells with more limited self-renewing abilities. The same may be said of the cells of the *Drosophila* imaginal discs and the so-called set-aside cells of metamorphizing amphibians [cells that are set aside in the larva to later make the tissues of the adult organism (*14*)]. Rather than being true stem cells, these cells may represent transient progenitor populations with functions limited to specific developmental stages. Perhaps adult meristem cells that replenish plant leaves and flowers (under homeostatic control) are better analogies to mammalian tissue stem cells in blood, brain, gut, and skin. Whether they have homologous features remains to be seen.

Development

In mammalian development, most would consider embryonic stem (ES) cells, the cell culture derivative of the blastocyst inner cell mass (*15*), to be primitive. Although the multipotency of such cultured ES cells has been firmly established and is indeed the basis for making transgenic and knockout mice, there is no evidence that the primary blastocyst cells can self-renew in vivo. Moreover, blastocyst cells clearly do not function throughout the lifetime of the organism. In fact, it may be argued that cells of the germ line carry out the in vivo function of totipotent self-renewal of the organism. Are germ line cells the true developmental descendants of primary blastocyst cells? In most animals, embryonic primordial germ cells eventually give rise to germ line stem cells (oocyte- and/or sperm-producing). These germ line stem cells first appear at the onset of preadult gonadogenesis (*16*). Mammals are distinguished from most other animals in that only male spermatogonial stem cells are

[1]Department of Anatomy and Cell Biology, University of Toronto Faculty of Medicine, Toronto, Ontario, M5S 1A8 Canada. [2]Genes & Development Research Group, Department of Cell Biology and Anatomy, University of Calgary Faculty of Medicine, Calgary, Alberta, T2N 4N1 Canada.

E-mail: derek.van.der.kooy@utoronto.ca; weiss@ucalgary.ca

present throughout lifetime—a finite number of oocytes is established shortly after birth. The embryonic primordial germ cells themselves are not considered stem cells, because they do not self-renew nor are they present throughout the lifetime of the organism. Surprisingly, although primordial germ cells injected into blastocysts do not contribute to either the germ line or the soma, cultured embryonic germ cell lines derived from primary germ cells behave remarkably similar to ES cells in their ability to contribute widely to tissue development in chimeras (*17*). The significance of this in vitro, culture-induced transformation/dedifferentiation will be revisited below under cause, in terms of the role of cell culture studies in understanding stem cell plasticity and tissue lineages.

If the definitive stem cells of the germ line appear relatively late in development, what do we know about the developmental appearance of stem cells in somatic tissues? Perhaps the best-studied stem cells are the hematopoietic stem cells that give rise to all the blood and immune cells. The precise origin of hematopoietic stem cells is somewhat controversial (*18*). In mice, the first cells of hematopoietic origin are found in the blood islands of the yolk sac (extra-embryonic mesoderm) at embryonic day 7 (E7). A separate population of intra-embryonic hematopoietic precursors appears in the paraaortic splanchnopleura/aorta-gonad-mesonephros region between E8 and E10. The relationship between the two remains uncertain; however, it is generally agreed that the site for definitive hematopoiesis shifts to the fetal liver at about E10 or E11, finally moving to the spleen and bone marrow after E15. Although aorta-gonad-mesonephros and fetal liver hematopoietic precursors show similar, if not identical, repopulating potential when injected into lethally irradiated hosts, the phenotype of cells produced and pattern of gene expression of adult bone marrow–derived hematopoietic stem cells is thought to be distinct (*19*). The differences, in particular in erythroid lineages that show smaller cell size and adult-specific globin gene expression, are thought to be a reflection of the unique homeostatic requirements of postnatal to adult life. Although this in no way challenges the contention that these adult hematopoietic stem cells arose from early embryonic counterparts, the intriguing question is whether adult hematopoietic stem cells have been programmed to function differently and whether this program is irreversible.

There appears to be a clear increase in numbers of hematopoietic stem cells capable of repopulating a lethally irradiated host, when advancing from aorta-gonad-mesonephros to fetal liver to adult bone marrow (*18*). Similarly, a huge increase is seen in the numbers of forebrain neural stem cells during late embryogenesis in mice (*7*). Therefore, the accumulating evidence points to an increasing appearance both of germ line stem cells and those of somatic tissues in the perinatal to adult phase of the mammalian life cycle, as part of the organism's capability for repopulation and renewal (Fig. 1). This leads to the somewhat counterintuitive (and controversial) conclusion that stem cells may not be the first cells that are present embryonically in a specific tissue to create that tissue, but rather appear later in development where they can replenish adult tissue populations.

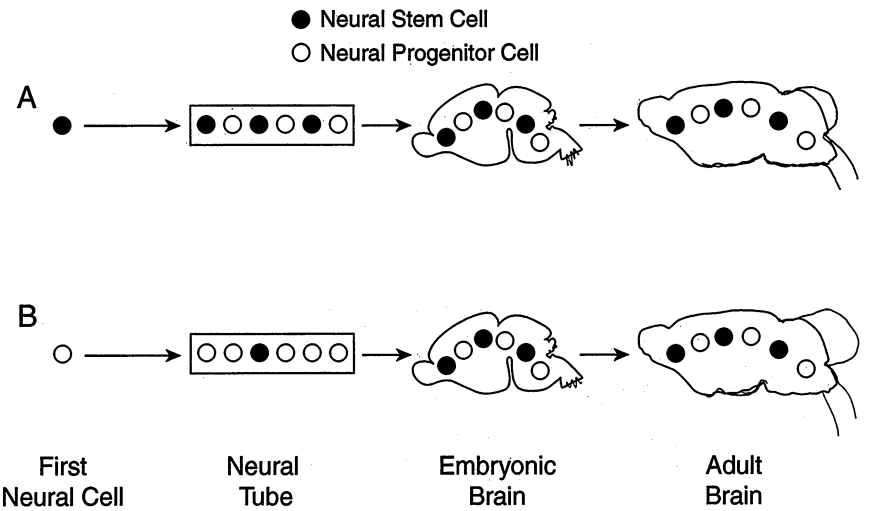

Fig. 1. Two possible models for the developmental emergence of (neural) stem cells. Although the models are schematized for neural tissue, they may apply to all tissues with stem cells. (**A**) The neural stem cell (having the longest self-renewal capability) is the first cell produced in the developing nervous system, and it makes all the neural progenitor cells (with less self-renewal capability) and their progeny—the differentiated neurons, astrocytes, and oligodendrocytes. (**B**) A neural progenitor cell (having less self-renewal capacity) is the first cell that arises in the developing nervous system, with neural stem cells emerging later in development to make larger numbers of later neural cells and to replenish adult neural structures.

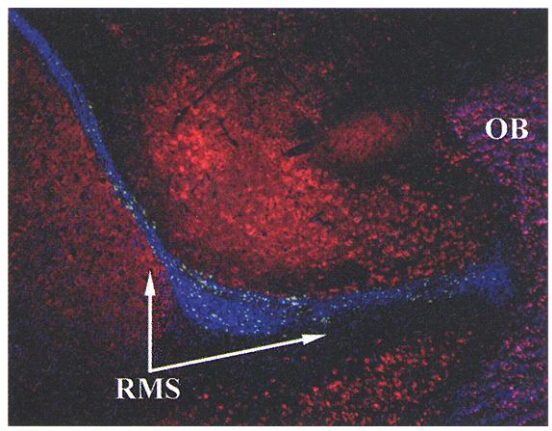

Fig. 2. Adult forebrain neurogenesis. A sagittal section of the adult mouse forebrain [with mature neurons labeled for NeuN (a neuronal antigen) in red] shows neuronal precursors labeled with bromodeoxyuridine (green) migrating along the rostral migratory stream (RMS; blue Hoechst stain) toward their final destination in the olfactory bulb (OB).

Cause

The shift from a large number of more restricted progenitors capable of tissue formation to a later-emerging population of multipotent, lifetime self-renewing stem cells participating in repopulation suggests that these stem cells may be differentiated for a specific adult task necessary for the organism's survival. Recently, compelling support has been generated for such a phenomenon in the forebrain of adult mammals. The discovery of adult forebrain neural stem cells (*20*) in the adult remnant of the embryonic brain germinal zone surrounding the lateral ventricle was followed by evidence for their participation in repopulating the adult lateral ventricular subependyma following irradiation (*21*). Subsequently, the adult subependyma has been shown to be the source of new neurons, which migrate along a glial pathway to the olfactory bulb of rodents (Fig. 2) (*22*) and

putatively along an unknown pathway to the association cortex of nonhuman primates (*23*). If new adult neurons are contributing to repopulating regions of olfaction in rodents and memory retention in primates, then this would support the notion of stem cell participation in renewal, probably acting for the organism's survival. On the other hand, a recent study found that adult forebrain neural stem cells injected into the circulation of irradiated adult hosts could contribute to hematopoietic lineages (*24*). Do these results challenge the notion of specification, in particular for adult neural stem cells?

An intriguing series of recent reports on adult bone marrow stem cells suggests that these cells have a relatively unrestricted developmental potential. At the same time, these studies may help shed light on some of the recent reports of surprising plasticity of adult neural stem cells. Adult bone marrow stem cells include both hematopoietic stem cells and stromal stem cells, the latter of which give rise to cells of the mesenchymal lineages such as bone and cartilage. When injected into the circulation of irradiated adult mouse hosts, mixed bone marrow stem cells were shown to contribute new microglia and astroglia in various regions of the brain (*25*), new skeletal muscle cells in tibialis anteriors that had been induced to degenerate (*26*), and new hepatic oval cells [precursors to differentiated liver cells (*27*)]. More recently, differential purification showed that stromal stem cells injected directly into the neonatal lateral ventricles could produce the differentiated astroglia (*28*), whereas hematopoietic stem cells contributed cells to new muscle fibers, and postnatal muscle stem cells could also make blood (*3, 29*).

How does contributing to new brain, muscle, and liver cell in adults alter our understanding of the lineage commitment of adult hematopoietic stem cells? The adult microenvironment, in particular with the stress of irradiation or muscle degeneration, may be especially critical in permitting adult hematopoietic stem cell phenotypic plasticity. In contrast, when adult hematopoietic stem cells are transplanted into blastocysts, their contributions into chimeras is almost completely faithful to their phenotype, with little or no evidence for donor cells in other adult somatic tissues (*30*). These very different observations of phenotype potential of adult hematopoietic stem cells underscore the need to consider the in vivo microenvironment, distinguishing between physiologically ongoing versus injury-derived situations, before drawing any conclusions about adult lineage commitment. Indeed, injury-induced modifications of microenvironments may be necessary to induce some of the more marked phenotypic changes in cells.

In fact, such caution may be particularly important when considering the issues of lineage specificity of adult neural stem and progenitor cells. The ability of bone marrow stem cells to contribute astrocytes, that astrocyte-like cells in the subependyma are neural stem cells, and that adult neural stem cell can contribute to hematopoietic lineages, raise intriguing possibilities about long-term relationships between cells of the circulation and brain in adult mammals. This may have relevance to the origin of brain neoplasms, although no data speak directly to this point. However, whether adult neural stem cells in situ ever contribute to tissues other than those they are originally specified for remains less certain. While bone marrow cells appear to directly (isolated with little or no in vitro culture nor absolutely requiring host irradiation) contribute to other tissues, protracted expansion and long-term culture of the adult neural stem and progenitor cells may be necessary for their shift in lineage commitment (*24, 31*). Long-term proliferation of previously specified stem or progenitor cells in cell culture may permit their dedifferentiation (loss of identity) and respecification. These distinctions have been emphasized previously by Slack, who pointed out the difference between the specification of cell phenotype (in the normal or a neutral environment) and whether that cell phenotype also is determined—that is, irreversibly committed to that phenotype in a range of environments (*32*).

Conclusions

The "whys" of stem cells are inextricably linked with issues of cell lineage. In light of the recent reports suggesting that stem cells from one tissue type can produce cells of other tissues (in plants to mammals), one may ask whether the study of cell lineage remains relevant today. If we can find the transcription factors that turn one differentiated cell into another differentiated cell, do we lose all the predictive capability that cell lineage studies (from stem cells to tissue differentiated cells) give us? Differentiation becomes essentially any change in a cell, such that there are no firm cell lineages and no progressive differentiation. Although this may be true in some cases, the evidence is not yet convincing. Such a program would make definitions on a cellular level useless: differentiation would be reduced to a listing of the combinations of genes expressed in a cell at one time. It is worth noting that a similarly narrow approach to development emerged from the one-dimensional application of molecular biological principles (*33*). In both cases, what is missing is the appreciation of cell lineage—that cells have lineage histories in vivo, and that their further differentiation often depends on those histories. The comparison of the in vivo lineages of stem cells and the changes of those lineages after exposure to new environments is "why" stem cells are so intriguing.

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