Neural Stem Cells
An Overview
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Abstract—This review focuses on the nature and functional properties of stem cells of the adult mammalian central nervous system (CNS). It has recently been shown that cell turnover, including neurons, does occur in the mature CNS, thanks to the persistence of precursor cells that possess the functional characteristics of bona-fide neural stem cells (NSCs) within restricted brain areas. We discuss how the subventricular zone of the forebrain (SVZ) is the most active neurogenetic area and the richest source of NSCs. These NSCs ensure a life-long contribution of new neurons to the olfactory bulb and, when placed in culture, can be grown and extensively expanded for months, allowing the generation of stem cell lines, which maintain stable and constant functional properties. A survey of the differentiation potential of these NSCs, both in vitro and in vivo, outlines their extreme plasticity that seems to outstretch the brain boundaries, so that these neuroectodermal stem cells may give rise to cells that derive from developmentally distinct tissues. A critical discussion of the latest, controversial findings regarding this surprising phenomenon is provided. (Circ Res. 2003;92:598-608.)

Key Words: stem cells ▪ transdifferentiation ▪ neurogenesis

The nervous tissue, particularly that of the central nervous system (CNS), is endowed with several peculiar characteristics that make it unique among the other bodily tissues. Ultimately, these properties relate to the CNS heterogeneous cell composition that underlies the elaboration of an enormous amount of information into a complex output. Historically, this complexity has been seen inextricably linked to the lack of any cell turnover in the adult brain.

The dogmatic view of an ever-immutable neural tissue in mammals is now been replaced by the notion that cell replacement occurs within specific brain regions throughout adulthood. This continuous neurogenetic process is sustained by the life-long persistence of neural stem cells (NSCs) within restricted CNS areas.

In the adult mammalian brain, the genesis of new neurons has been consistently documented in the subgranular layer of the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles. From the SVZ, newly generated neurons reach their final destination in the olfactory bulb after long-distance migration through a well-defined path called the rostral migratory stream (RMS) (Figure 1). The SVZ is the adult brain region with the highest neurogenetic rate, from which NSCs have been firstly isolated and characterized for their ability to give rise to non-
of the chains of migrating neuroblasts within the astroglial tubes (in black). Tubes are not present in the distal tip of the OB (gray shadowed area), within which the neuroblasts depart from the terminal end of the tubes, leave the chains by detaching from each other, and migrate outwardly (radially) as isolated cells (n) through the OB parenchyma. T indicates tangential chain migration; R, radial migration; N, differentiated neurons in the olfactory bulb; SC, area in which the stem cells are found; and MP, area containing migrating precursors but not NSCs (modified from Peretto P, Merighi A, Fasolo A, Bonfanti L. The subependymal layer in rodents: a site of structural plasticity and cell migration in the adult mammalian brain. *Brain Res Bull.* 1999;49:221–243).

Figure 1. A, Global view of the adult rodent brain that depicts (in gray) the regions within which continuous neurogenesis occurs throughout adulthood, namely the olfactory bulb (OB) and the hippocampus (H). Areas that are known to contain NSCs are outlined in black: the subventricular zone (SVZ) with its rostral extension reaching the OB and the dentate gyrus (DG) of the hippocampus. B, Schematic sagittal section of the adult rodent brain through the SVZ-RE system; the dotted line outlines the contour of the lateral ventricle (LV). The SVZ is composed of a posterior part (dark gray) lining the lateral ventricle (SVZ-LV) and an anterior part (light gray) forming the rostral extension (SVZ-RE). CC indicates corpus callosum; CX, cerebral cortex; and OB, olfactory bulb. C, Transverse sections of the SVZ at the levels 1 and 2 as indicated in A and B; 1, SVZ-RE; 2, SVZ of the lateral ventricle (LV); see also Figure 2A). Gial tubes are in black; CH, chains of neuronal precursors; CC, corpus callosum; e, ependymal monolayer. D, Schematic representation of the resident proliferating cell population occurs every 12 to 28 days in the SVZ,13 with about 30,000 new neuronal precursors (neuroblasts) being produced every day and migrating to the OB1 (Figure 1).

Two main cell types are found in the SVZ: migratory, proliferating neuroblasts and astrocytes (Figure 1). The latter form a meshwork throughout the whole SVZ and are organized into channels that are oriented along the anteroposterior axis and are called glial tubes.14–16 Within these glial tubes, NSC-generated neuroblasts undergo rostralward, tangential migration as cells grouped into tightly packed chains.14,17 Eventually, neuroblasts reach the distal tip of the OB, separate from each other and leave the tubes, shifting their migration pattern from tangential to radial. In this way, they reach the more superficial OB layers and terminally differentiate into granule and periglomerular neurons. Importantly, tangential migration toward the OB has also been documented in the adult brain of primates18,19 and even in the infant human brain.20 Also, the possible migration of cells from the SVZ to the neocortex has been proposed in mouse21,22 as well as in adult macaque monkeys,23–25 although this view has recently been questioned.18

Glial tubes are composed of a special type of astroglia that expresses the marker of mature CNS astrocytes, namely glial fibrillary acidic protein (GFAP), but also contain the cytoskeletal proteins vimentin and nestin.16,17 The presence in glial tubes of such molecules, known to abound in those immature and radial glial cells that guide young neurons during CNS development, suggested a role for glial tubes in influencing the migration/guidance of neuroblasts in the SVZ. However, the actual role of astroglial cells in this process remains unclear.26 They may simply act as a barrier to prevent...
neuroblasts from escaping the proper migratory route before they reach the OBs. Although the presence of astrocytes is not essential for chain migration, they do secrete factors that facilitate this process. Notably, astroglial tubes and NSCs do not coexist solely within the periventricular aspect of the SVZ but also within the rostral migratory stream that extends into the OB, with the former perhaps contributing to create an appropriate stem cell “niche” for the maintenance of NSCs all along the pathway.

In an appealing functional/structural parallelism with the hemopoietic system, it has been proposed to use terms like “neuropoiesis” to portray the persistence of neurogenesis in adulthood, and “brain marrow” to describe the anatomical regions that contain the NSCs supporting neurogenesis. Thus, whereas hematopoiesis takes place within the boundaries of the marrow in the deep adult bone, adult neurogenesis is a spatially confined process, constrained within the boundaries of the brain-deep SVZ. In both “marrows”, we can find (1) proliferating stem/progenitor cells, (2) cell types, which can carry out stromal function(s), and (3) extracellular matrix that can modulate cell proliferation, adhesion, and migration. Stromal cells of the bone marrow are known to act as an interface between peripheral blood and the stem/progenitor pool, thus modulating the fate of hematopoietic stem cells (HSCs). In a similar fashion, astrocytes and ependymal cells of the SVZ may act as “stromal” elements of the CNS by producing molecules as bone morphogenetic proteins that affect the neuronal versus glial fate of the stem/progenitor cells. Furthermore, the extracellular matrix of both the SVZ and bone marrow contains tenasin and proteoglycans, molecules that are important in the formation of developmental compartments and in the control of cell adhesion, migration and differentiation. Finally, similarly to the bone marrow, the proximity of the SVZ with the cerebrospinal fluid, the enlarged intercellular spaces, the reduced cell-cell contacts, and the presence of molecules linked to water cotransport contribute to create in the SVZ a cytoarchitectural/biochemical niche, which is very different from the environment of the mature CNS parenchyma.

The Nature and Origin of the Adult Neural Stem Cell In Vivo

A series of observations indicate that a specific subtype of SVZ astroglial cells is the actual NSC (see review). In fact, after the killing of all the proliferating SVZ neuroblasts by administration of subdural, cytotoxic doses of antimitotic drugs, only astroglial cells were left, which surprisingly, were shown to be able to regenerate the entire SVZ cell system. According to this view, some of the SVZ astrocytes (named type B cells) are thought to represent relatively quiescent stem cells that normally proliferate at a low rate and generate the neuronal precursors (type A cells), through the generation of a third, intermediate cell type: the C cell (or D cell in the developing cerebral cortex can act as a bipotent stem cell, it is most likely that NSCs originate from type B cells that retain radial glia-like features within specific regions of the adult brain (see review).

It should be emphasized, however, that NSCs can also be isolated and grown in vitro from nonneurogenic periventricular regions, in which the mature parenchyma is directly in contact with the ependymal monolayer, such as the fourth ventricle or the spinal cord. These findings may indicate that cells endowed with some extent of stem-like potential may exist throughout the whole adult CNS tissue. However, only those NSCs that occur within adult brain areas that retain a brain marrow-like environment, as the SVZ, take up an actual stem-cell behavior, self-renew, and generate a mature progeny in vivo, whereas those residing within nonneurogenic regions may remain dormant.

Ex Vivo Behavior of NSCs

To carry out the vital function of maintaining cell homeostasis and integrity throughout life, stem cells are endowed with peculiar functional characteristics that provide suitable criteria to identify and classify them. In fact, stem cells are highly undifferentiated cells possessing a significant proliferation potential, a broad developmental fate, and most and foremost, an intrinsic capacity for self-perpetuation—better defined as self-maintenance or self-renewal—throughout adulthood.

Historically, hemopoietic stem cells (HSCs) have been the first to provide a useful prototypic functional model in order to devise conceptual and practical strategies to tackle investigations on other stem cell types. When comparing HSCs and NSCs with regard to their functions, the two systems appear to sit at opposite sides of the functional spectrum. Because enormous amounts of new blood cells are generated every day from stem cells of the hemopoietic tissue(s), it has been possible to develop an in vivo functional assay by which bona fide stem cells (even a single candidate cell) have been identified for their capacity to reconstitute the hemopoietic stem compartment after its deadly experimental ablation. Moreover, a reliable antigenic fingerprint based on the expression of multiple, lineage-specific markers is now available to distinguish various hemopoietic precursors. However, one of the most significant hurdles in the field of HSCs is their resilience to undergo extensive proliferation in culture.

Fascinatingly, a mirror-like situation has emerged in the field of NSCs. Given the relative overall quiescence and structural/cytoarchitectural “rigidity” of the brain parenchyma, the classical hemopoietic experimental paradigms of reconstitution of ablated stem cell compartments are rarely used to investigate and characterize NSCs. Thus, the study of NSCs received much of its initial impulse from in vitro findings that firstly demonstrated and exploited the amazing proliferation capacity that NSCs display in culture. Under appropriate conditions and in the presence of mitogens (epidermal growth factor (EGF) and/or fibroblast growth factor 2 [FGF2]), it is possible to induce the proliferation of rapidly dividing precursors from the SVZ. At least in vitro, these precursors fulfill most of the criteria of bona fide stem cells. Lately, these cultured NSCs have been suggested to derive from the C type cells of the SVZ. Since in vivo type C cells represent transit-amplifying progenitors rather than bona fide stem cells, the concept has been put
forth for the possibility that tissue culturing and/or mitogens can re-awake a latent stem cell program in what can be considered “potential stem cells.” On removal of the mitogens, the progeny of NSCs promptly differentiate into the three main cell types of the CNS (astrocytes, oligodendrocytes, and neurons) (Figure 2).

It should be noted that similar results could be obtained with adult hippocampal cells. The most important concept regarding this method is that it represents a selective system by which, in a heterogeneous primary culture, the more committed progenitors and/or differentiated mature cells rapidly die and thus are eliminated, whereas the undifferentiated NSCs are positively selected and forced to access a state of active proliferation. NSCs start proliferating initially as adherent cells and attach to each other, eventually giving rise to spherical clusters that float in suspension and form the so-called “neurospheres.” In giving rise to neurospheres, NSCs undergo multiple symmetric cell divisions by which two new NSCs are generated at each cycle (Figure 3). It should be noted that not all the NSC progeny found in a neurosphere are stem cells. Indeed, only 10% to 50% of these progeny retain stem cell features, whereas the other 50% to 90% of the neurosphere cells are differentiated cells.

Figure 2. Neural stem cells of the adult rodent brain. A, Transverse section through the periventricular SVZ is shown. This section corresponds to the plane section 2 as shown in Figure 1. V indicates ventricle; SVZ, subventricular zone. Arrows point to some of the proliferating cells in the SVZ that were labeled after intraperitoneal injection of the thymidine analogue, 5-bromodeoxyuridine. SVZ contains fast and slow proliferating precursor populations with a cell cycle time comprised between 12 hours and 28 days. B through D, Stem cells isolated from the SVZ are shown in serum-free cultures in the presence of both EGF and FGF2. Under these conditions, stem cells undergo proliferation, eventually giving rise to neurospheres. One day (B), 4 days (C), 7 days (D) after plating. E, On removal of growth factors and plating onto a laminin-coated substrate, cells in the sphere differentiate into mature brain cells that express the typical markers of neurons (red, β-tubulin), astrocytes (green, glial fibrillary acidic protein), and oligodendrocytes (blue, O4). Bars: A=100 μm; B and C=20 μm (bar in B); E=30 μm.

Figure 3. Schematic outline of how stem cell expansion is achieved in cultured neural stem cells. A, Under serum-free conditions and in the sustained presence of mitogens such as EGF and/or FGF2, NSCs (red, S) are thought to undergo symmetric divisions in which either (i) two more differentiated cells (likely transient amplifying precursors, green, T) (symmetric differentiative divisions) or (ii) two NSCs are generated (symmetric proliferative divisions); (iii) asymmetric cycles giving rise to one NSC and to one more differentiated (not shown) or apoptotic cell (yellow) can also take place at the same time. If within a NSC population the frequency of proliferative cycles equals that of differentiative divisions, the overall number of NSCs within the pool is maintained at a steady level; (iv) under the conditions used for NSCs expansion, the number of proliferative divisions normally exceeds that of differentiative cycles, so that an increasing number of NSCs is generated within the stem cell pool at each cell generation. B, Starting from a single NSC (red) all the types of cell division described in A concur to the formation a clonal cluster of cells called neurosphere. Therefore, each neurosphere contains a mixture of many NSCs, as well as transient amplifying precursors (green) and dead/dying cells (yellow). When neurospheres are dissociated, all but the stem cells die (light blue shadowed area), due to the stringent culture conditions, whereas NSCs continue to proliferate, giving rise to more, secondary neurospheres. This procedure can be repeated sequentially over many months, resulting in an exponential increase in the overall number of NSCs and in the establishment of NSC stable cell lines.
remains are cells that undergo spontaneous differentiation. Consequently, a neurosphere is a mixture of NSCs, differentiating progenitors, and even differentiated neurons and glia, depending on the neurosphere size and time in culture. This is the reason why neurospheres are subcultured by harvesting, followed by mechanical dissociation and by re-plating under the same growth conditions. As in the primary culture, differentiating/differentiated cells rapidly die while the NSCs continue to proliferate, giving rise to many secondary spheres and exponential growth in vitro. In this way, stable NSCs cell lines can be obtained.12

Due to these properties, it has been possible to establish continuous mouse transgenic/genetically modified66 or human NSC lines57,58 that expand merely by growth factor stimulation and under completely chemically defined conditions. In particular, human NSC lines represent a renewable source of normal nervous cells that might facilitate basic studies on human neurogenesis and drug discovery and, remarkably, may virtually eliminate the need of fetal human tissue for therapeutic neural transplantation. It is well known that some CNS disorders may require grafting of specific neuronal subtypes. Notably, human NSC, which are available in huge amount as compared with fetal tissues, can be easily induced to differentiate into specific cell types and/or neurotransmitter phenotypes by simply modifying the culture medium. For example, NSC-derived GABAergic neurons could be used to replace GABAergic spiny neurons lost in Huntington’s disease. Similarly, we have been able to induce a significant fraction of the progeny of various human NSC lines to express catecholaminergic neuronal features59 that may prove useful for cell replacement therapy in Parkinson’s disease. Very recently, human NSCs have been shown to efficiently integrate in the adult rat brain where they differentially acquiring a cholinergic phenotype.60 Thus, in addition to markedly reducing the use of human fetal brain tissue, stem cell lines may eventually eliminate the need for tissue exclusively from selected embryonic regions.

The issues discussed so far prompt some final remarks as to the classification of NSCs with respect to their ex vivo behavior. Recently, the identification of putative NSC markers as AC133, PNAwm/HSAwm, and LeX/sea-18–10 has provided researchers with a valuable tool for identifying and enriching bona fide NSCs. This antigenic characterization will eventually help to resolve the state of confusion in the NSC area in which classification based on functional approaches presents severe limitations due to the different techniques, the variety of the brain regions investigated, and the variable ages of the donors yielding the isolation of different precursor cell types that all promiscuously go by the name of NSCs.

Once again, it is tempting to borrow from the hematopoietic field, in which a lineage of stem cells endowed with progressively narrowing self-renewal capacities has clearly been defined.61 Although limited to the in vitro studies and mainly for practical purposes, we speculate that a similar criterion of classification may be initially assumed for NSCs, thus identifying them as long-term or short-term self-renewing NSCs based on the extent of their self-maintenance ability in culture. At this point, we ought to remember that (1) challenging a cell in vitro only unveils its developmental potential and not its actual in vivo fate, and (2) the extent by which a cell can self-renew in vitro may depend on the culture conditions adopted. Nevertheless, it has also emerged that some NSCs from distinct regions display clearly distinct self-renewal capacities under identical culture conditions, thus lending to the attempt to classify cultured NSCs based on the self-renewal capacity that they display in vitro.62,63

**Plasticity of NSCs Within the CNS**

Although in vivo NSCs appear to generate almost exclusively neuronal cells, their actual developmental potential as observed ex vivo is much broader than expected. In fact, cultured NSCs do give rise to neurons, astrocytes, and oligodendrocytes and are therefore normally classified as being multipotent in nature. The concept of NSC plasticity and of their dependence on environmental cues is strengthened by transplantation and manipulation/recruitment studies in vivo. For example, intrahippocampus transplantation of hippocampal stem cells results in the generation of the neuronal types normally found within this region.55 However, if the same hippocampal stem cells are transplanted heterotopically, that is into the SVZ, they produce cell types that are normally found in the latter, rather than hippocampal ones.55 Further, when transplanted in adult brain regions in which no neurogenesis takes place, NSCs produce exclusively glial cells. After intracerebral administration of growth factors, namely EGF,64 FGF2,65 or transforming growth factor-α (TGF-α),66 the proliferation of cells in the SVZ is dramatically increased and the fate of the progeny can change depending on the type of factor(s) used. In particular, EGF infusion results in an increased production of cells in the SVZ, in a diversion of their migration pattern from tangential to radial,65 and in the eventual generation of cells of the glial lineage rather than neurons,64,65 These findings altogether re-emphasize the concept expressed earlier in this review that NSCs appear to possess a rather broad developmental potential and functional repertoire whose expression is strongly influenced by extracellular cues.

**Plasticity of NSCs Outside the CNS**

Some important considerations have emerged so far. First, the nervous tissue contains bona fide stem cells that support neuronal cell turnover throughout life. Second, despite their origin from one of the most quiescent tissues in the body, NSCs can undergo effective long-term culturing, proliferation and expansion while retaining stable functional characteristics. Third, when properly challenged, the overall developmental potential appears to be broader than that observed under physiological conditions in vivo.

This section reviews recent observations suggesting that this plasticity may perhaps outstretch the brain boundaries, so that NSCs (neuroectodermal in origin) can give rise to cells that normally derive from germ layers other than the neuroectoderm. This is discussed later, in the more general perspective that many somatic stem cells may give rise to cells of an embryonic origin different from their own. One of the most intriguing cases is the virtual pluripotency of bone marrow–derived cells67; however, multiple examples of SCs...
Somatic Stem Cells Give Rise to Mature Progeny That May Belong to Other Tissues and Organs, Also of a Distinct Embryological Origin

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<td>Kidney (meso)</td>
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Meso indicates mesoderm; ecto, ectoderm; and endo, endoderm.

giving rise to cells normally found in other tissues have become available. In some cases, both the original SCs and the cells to which they give rise derive from the same embryonic germ layer (intragerm layer conversion). For instance, intramesoderm conversion has been documented by showing the genesis of skeletal or cardiac muscle cells from bone marrow cells. Similarly, muscle precursors can give rise to hematopoietic cells, although it has now been shown that the original muscle population undergoing conversion are Sca-1 and CD45-positive cells, which are hematopoietic in origin. Finally, muscle satellite cells retain an osteogenic and adipogenic differentiation potential that is normally retrieved in mesoderm-derived stromal cells.

More striking examples of transgerm layer conversion—hereby also defined as transdifferentiation—in which SCs and their progeny belong to developmentally unrelated cell lineages have been reported (Table). Thus, mesoderm-derived mesenchymal stem cells appear to generate astrocytes and neurons, both in vivo and in vitro, and bone marrow SCs can give rise to endoderm-derived liver cells. Of note, Wagers et al have recently demonstrated that, in the absence of a tissue-specific lesion, there is not appreciable contribution of single repopulating hematopoietic stem cell to nonhematopoietic tissues, suggesting that the production of nonhematopoietic cell types is not a physiological function of HSCs. Nonetheless, in the presence of sustained damage as caused by lethal irradiation, adult bone marrow cells were found to integrate and differentiate within the brain tissue after systemic injection. In these mice, 0.2% to 0.3% of the total neurons in the olfactory bulbs appeared to derive from the bone marrow cells. In a similar study, bone marrow cells from wild-type male mice were injected intraperitoneally in PU.1-mutant, immunodeficient females. In these animals, 0.2% to 2.3% of the male cells found within the brain parenchyma expressed neuronal markers. Nonetheless, a recent report by Castro et al has questioned the “blood-into-brain” conversion. These authors failed in detecting side population or unfractionated donor bone marrow cells integration in the brain of lethally irradiated mice even after injury. Importantly, in the same experimental model, blood-into-muscle conversion appeared to take place. At present, this issue remains open for discussion, although it is perhaps possible to infer that we are dealing with a rather rare phenomenon that takes place only under specific conditions with a low frequency, whose detection may be elusive under certain circumstances, but which seems to be effective also in humans.

A similar situation has arisen in a neighboring area that is the field of investigation in which the opposite phenomenon, ie, brain-to-blood conversion, is studied. The initial discovery that transgerm layer differentiation can occur in adult mammals came from cells that were derived from the adult brain. In 1999, we reported that clonally derived adult NSCs could give rise to hematopoietic cells in vivo. In these experiments, the progeny of genetically tagged (ie, constitutively expressing β-galactosidase, the product of the bacterial LacZ gene), single NSCs were injected into sublethally irradiated mice. NSCs integrate into many of the host hematopoietic tissues, including spleen, thymus, and bone marrow and gave rise to various types of blood progenitors. These progenitors did eventually differentiate into a wide range of blood cells, including megakaryocytes, granulocytes, macrophages, and B- and T-lymphocytes; however, erythroid cells were not observed. Furthermore, although not described in the initial study (R. Rietze, C.R. Bjornson, A.L. Vescovi, unpublished observations, 2002), NSCs increased viability in recipient animals, suggesting that they may partially reconstitute and, in fact, rescue lethally irradiated animals. It is worth noting that neurohemopoietic conversion has now been documented also by using human NSCs. These cells were transplanted into SCID-Hu mice, which carry transplanted human fetal bone and thymic fragments, thus providing physiologically relevant human bone marrow and thymic microenvironments. Under these conditions, human NSCs were able to establish long-term hemopoiesis, as shown by serial reconstitution experiments. In further support of brain into blood conversion, a recent work has provided evidence that hematopoietic epigenetic factors can induce human fetal neural tissues to give rise to blood cells in vitro and that this wider lineage potential is characteristic of the AC133-positive fraction.

Rather peculiar circumstances appear to be necessary for the expression of the latent, broad developmental potential of
NSCs that underlies neuromesodermal transdifferentiation. First, it appears that only bona fide stem cells can undergo transdifferentiation. As described in Galli et al., only SVZ cells, but not cells from regions that do not appear to contain actual stem cells, such as the stratum and the cerebral cortex, undergo neuromyogenic conversion. This was confirmed by Rietze et al. who enriched their cultures for NSCs by cell surface markers (PNA−/HSA+) and showed how this resulted in an increased neuromyogenic conversion rate—from 2.5% to 57% of the total cells. Furthermore, when the differentiated progeny of NSCs were exposed to the same cues that induce myogenesis in enriched NSC cultures, negligible conversion to skeletal muscle, if any, was observed.

The fact that only NSCs, but not their differentiated neuronal or glial progeny, can give rise to nonneural cells highlights an important difference between this phenomenon and transdifferentiation as conceived in a classical view. In fact, transdifferentiation implies the ability of differentiated cells to acquire a new identity by turning off one set of lineage-specific genes and activating genes of another differentiated cell type. The two sets of antigens sometimes colocalize in cells undergoing transdifferentiation. Conversely, transgerm layer differentiation seems to reflect the de novo expression of a broader developmental potential of NSCs that may become evident only under peculiar conditions. Accordingly, no molecular and biochemical overlapping of specific neural and nonneural markers were ever observed in either NSCs or their nonneural progeny. Thus, markers of hematopoietic precursors were not detected in NSCs before they were injected into injured animals, and expression of muscle determination factors or other muscle differentiation markers such as MyoD, Myf5, myogenin, and myosin was never observed in NSCs before their exposure to myogenic environments. Similarly, lack of expression of mesodermal and endodermal antigens, such as TROMA-1, was detected in NSCs before injection into the developing blastula. Yet these cells can give rise to derivatives from both these germ layers.

It can be speculated that, in order to undergo transgerm layer differentiation, NSCs ought to be exposed to microenvironments that contain rather peculiar, as yet unidentified cues. CNS stem cells have been shown to repopulate the injured hemopoietic system and muscles. Similarly, bone marrow cells regenerate the muscle and liver only in the presence of an injury. Hence, it can be argued that specific instructive cues may become available during the early and regenerative phases that follow an injury in a given adult tissue, which may resemble those found in embryonic development. Importantly, recent data suggest that transgerm layer differentiation might be an integral part of tissue regeneration when happening in a physiological context, like the regenerating tail in amphibians. In this system, neuroectodermal radial glial cells, which normally regenerate the spinal cord after tail amputation, also contribute to mesoderm lineages such as cartilage and, more intriguingly, muscle. This study suggests that the existence of a blastema (ie, undifferentiated proliferative cells) in a regenerative milieu is most likely accountable for this phenomenon, which resembles or can be considered neuromesodermal transdifferentiation. One significant hurdle in the identification of the cues involved in neuromesodermal conversion depends on the fact that most of the models used rely on in vivo assays. Yet, in vitro models of neuromesodermic conversion are now available that allow for some tentative hypotheses to be drawn. Using these systems, it has emerged that neuromesodermal conversion could only be observed when NSCs were cocultured with C2C12 myogenic cells or with primary myoblasts—and never with other cell types. Furthermore, when NSCs underwent myogenic induction as clustered cells into neurospheres, the proportion of cells that converted to skeletal muscle dropped by almost 80% as compared with dissociated cells. Thus, when neural cells are grouped together, neural-to-neural signals override the myoblast-derived myogenic cues, blocking neuromyogenic conversion. Hence, some type of “neuralizing” signal(s), occurring between neighboring neural cells, counteract the induction of the muscle fate on NSCs. Thus, this phenomenon might be interpreted as a classical “community effect.” In vivo, these signals can be found in the extracellular microenvironment that has been perturbed by an injury and may be elicited through a direct cell-to-cell interaction between the host and the donor cells.

Notably, conversion required direct cell-to-cell contact and did not take place when neural and myogenic cells were physically separated by a porous membrane, nor when NSCs were exposed to muscle cell-conditioned medium. This requirement might suggest that spontaneous cell fusion between inducing and induced cells might account for neuromyogenic conversion in this system. Although specific experiments based on cell selection with transgenic markers need to be undertaken to completely rule out this possibility, the presence of a significant number of mononucleated NSC-derived myocytes in this culture (authors’ unpublished observations, 2003) clearly suggests that cell fusion alone may not justify brain-into-muscle conversion in this system. In this context, it is possible that, in the case of polynucleated NSC-derived myotubes—in which fusion of NSCs with C2C12 myoblasts does occur—cell fusion might be responsible for the activation of the muscle-specific reporter gene in NSCs, without the latter undergoing an effective cell fate shift. Yet, we ought to emphasize that the expression of many other muscle-specific markers, such as MyoD, myosin heavy chain (MHC), together with ultrastructural analysis, have been associated with the neural-to-muscle transition in this coculture model. Based on these findings, we can infer that, at least in the specific case of neuromesodermal conversion, cell-to-cell contact is required, underlying the necessity of a direct exchange of information between the “inducing” and the “induced” cells. This may imply the interaction of cell surface receptors and ligands, the formation of gap junction structures, as well as the involvement of short range–acting molecules.

A fascinating extension of the these findings came from the observation that NSCs could integrate into many different tissues derived from the three main germ layers on injection into the mouse blastocyst. Thus behaving as embryonic stem (ES) cells. Intriguingly, the contribution of NSCs to some major mesodermal lineages, such as blood and the skeletal muscle, was never observed in this work. Yet, it was soon
showed that the cells that they used were different from any culture functional characteristics described by Morshead et al finding on neurohemopoietic conversion. As it turns out, 91 the detected in a recent study.90 This situation exemplifies the debated issue. Particularly, despite the ability of NSCs to give variable results.

It was evident that less than 1% of the cells in Morshead’s system were NSCs, whereas Bjornson et al’s data agreed with the literature’s data pointing to a minimal standard value of 20%.56,58,92,93 Bjornson et al contended that the combination of low NSC number and significant transformation found in the Morshead et al cultures would lead to the transplantation of a negligible number of NSCs and to the negative outcome of these experiments.

Perhaps, the most knowledgeable conclusion to draw—also given the constant state of flux of this novel field of investigation—is that transdifferentiation is an uncommon phenomenon that takes place only under very peculiar circumstances. Because the capacity to undergo transdifferentiation is a characteristic of specific cell types, namely somatic stem cells, and because the latter normally represent a minor fraction of the overall cell population in vivo or a pool whose relative size fluctuates in vitro depending on culture conditions, detection of transdifferentiation is rather difficult, and requires appropriately refined and sensitive methodologies concerning both cell culturing and fate assessment.

Recently, explanations that are alternative to transdifferentiation have been invoked to explain, at least in part, the findings discussed so far. Two distinct studies94,95 reported that adult stem cells from the CNS and the bone marrow can give rise to cell types as a consequence of cell fusion with ES cells, rather than by transdifferentiation. The first consideration to make in this context is that these results are limited to cell fusion between adult and embryonic stem cells and do not impinge on the interpretation of data in which adult somatic cells undergo transdifferentiation in vivo in adult animals, or in vitro in adult cells.

Yet, it is possible that part of the transdifferentiation events reported so far could be explained by cell fusion. Nonetheless, due to the very low frequency of hybrid formation (an average frequency of $10^{-5}$ in the two reports), owing to the fact that ES cells might be more susceptible to cell fusion than somatic cells and to the observation that the eventual polyploid fusion products are highly unstable, a main role of the sole cell fusion in the conversion of somatic stem cell into different cell types is unlikely.

In fact, as shown in these two studies and as argued by others,96 at least 30% of the bone marrow–derived cells injected in vivo transdifferentiated46 and, similarly, 10% of NSCs cocultured with inducing myoblasts gave rise to muscle cells.6 These frequencies are orders of magnitude higher than the fusion efficiency between ES and somatic stem cells. Particularly, concerning brain into muscle conversion, the

![Figure 4](http://circres.ahajournals.org/)

**Figure 4.** An example of the transdifferentiation capacity of NSCs. Generation of skeletal muscle from NSCs. A, NSCs that were derived from a strain of transgenic mice in which the promoter of the myosin light chain gene drives the expression of β-galactosidase in the nucleus of muscle cells activate the transgene when cocultured with C2C12 myocytes. NSC-derived polynucleated myotubes are identified by the nuclear expression of β-galactosidase (X-gal reaction, blue). Bar=40 μm. B, Clonally derived NSCs from ROSA 26 β-galactosidase–expressing transgenic mice cocultured with C2C12 cells differentiate into myotubes. NSCs expressing β-galactosidase immunoreactivity are shown in red (concave arrowhead). C2C12 cells labeled with the antisarcomeric myosin heavy chain (MHC) antibody are shown in green (arrowhead). NSCs-derived polynucleated myotubes (blue, DAPI nuclear staining) appear as yellow cells due to the overlapping MHC and β-galactosidase staining (arrow). Bar=20 μm.

The discrepancy between the various transdifferentiation examples described above and the initial study of NSCs injection into blastocyst87 most likely lies in the fact that a number of inductive factors competitively act on NSCs cells in this system. The prevalence of the effect of a given set of instructive cues that acts on NSCs will ultimately depend on the initial, very variable site of deposition/integration of the cells on their injection into the blastocyst, thus yielding rather variable results.

Transdifferentiation of somatic stem cells is a hot, highly debated issue. Particularly, despite the ability of NSCs to give rise to blood cells has been reported in both human and mouse cells by 4 independent groups, this phenomenon could not be detected in a recent study.90 This situation exemplifies the state of confusion that plagues the field of neural stem cells. Particularly, it emphasizes the need for standardization of the terminology adopted when it comes to defining NSCs or, at least, of the tissue culture systems used to grow and expand them. In fact, Morshead et al90 stated that they failed in reproducing brain-into-blood conversion when using the very same cells and system used by Bjornson et al5 in their initial finding on neurohemopoietic conversion. As it turns out,91 the culture functional characteristics described by Morshead et al showed that the cells that they used were different from any known NSCs, particularly those used in previous transdifferentiation experiments.5,6 In fact, Morshead’s cultures consistently underwent transformation. This is not a property that was ever observed in studies from many groups. In fact, neither human nor mouse NSCs undergo consistent transformation on subculturing. Over extensive time in culture, these cells exhibit strict growth factor dependency, unchanged growth kinetics and prompt differentiation on removal of mitogens.56,58,92,93 Furthermore, a clear difference emerged when comparing the content of NSCs (ie, clonogenic cells in culture) in Morshead et al cultures with that of other groups.

It was evident that less than 1% of the cells in Morshead’s system were NSCs, whereas Bjornson et al’s data agreed with the literature’s data pointing to a minimal standard value of 20%.56,58,92,93 Bjornson et al contended that the combination of low NSC number and significant transformation found in the Morshead et al cultures would lead to the transplantation of a negligible number of NSCs and to the negative outcome of these experiments.
possibility that cell fusion might be responsible for transdiffer-
nentiation seems more remote, due to the following facts:
(1) proliferating C2 myoblasts are strongly resistant even to
Sendai virus–mediated cell fusion.27 (2) myoblasts fuse with
other myoblasts only when both are committed to differenti-
ation and not when they are still proliferating as in our
experimental paradigm;6 (3) myoblasts do not normally fuse
with nonmyogenic cells8 and, on this rare event, the fusion of
a myocyte with a nonmyogenic cells generally results in the
extinction of muscle-specific properties in the immediate
fusion product,29 and (4) in our in vitro model, we observed
high frequency of mononucleated NSC-derived myocytes.5
Thus, the chances that cell fusion between NSCs and induc-
ning myoblasts might occur spontaneously are quite low.

Whichever interpretation one may adopt, it is cogently
clear that the field of transdifferentiation is in its infancy and
that this phenomenon is far from being a widely accepted and
well-established one. Many questions remain unanswered.
The topic is open to heated discussion(s).

Conclusions
The field of neural stem cells is in a state of rapid growth.
Every month, new pieces are added to the puzzle of the
neurogenetic process in adult mammals. As expected for such
a young field, stimulating new findings go hand in hand with
the raising of new outstanding issues that heat the debate
around these cells. This area is, therefore, in a state of rapid
flux and will clearly benefit from the isolation of specific
antigenic markers that allow for the univocal identification of
the NSC and its derivatives.8,9,10 A somewhat more conser-
ervative use of the term stem cell as applied to different types
of brain precursors would also be beneficial. In this view,
studies that aim at unraveling the relationship between
candidate stem cells as they are identified in vitro and in vivo
will certainly provide more rigorous criteria for the identifi-
cation of NSCs.

Nevertheless, these cells represent one of the most aston-
ishing discoveries of the last decade. It is intriguing that cells
isolated from what has long been viewed as the most
quiescent among the bodily tissues can display such an
amazing degree of plasticity and, equally important, such a
remarkable growth capacity. The latter properties will cer-
tainly provide the necessary basis to unravel the most
intimate mechanisms underlining the genesis of neural cells
and the process of development, degeneration, and repair in
the CNS.

Finally, the availability of a renewable source of human
neural cells as provided by the establishment of NSC lines
promises to open new, and as of yet, unpredictable therapeu-
tic venues for the treatment of neurological disorders.

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