The development of neural stem cells

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The discovery of stem cells that can generate neural tissue has raised new possibilities for repairing the nervous system. A rush of papers proclaiming adult stem cell plasticity has fostered the notion that there is essentially one stem cell type that, with the right impetus, can create whatever progeny our heart, liver or other vital organ desires. But studies aimed at understanding the role of stem cells during development have led to a different view — that stem cells are restricted regionally and temporally, and thus not all stem cells are equivalent. Can these views be reconciled?

The discovery of neural stem cells was rooted in classic studies of haematopoiesis and of invertebrate neural development, which inspired examination of single neural progenitor cells. (In this review, I will use the term 'progenitor cell' to refer to all classes of immature, proliferating cells. Neural stem cells are a subtype of progenitor cells in the nervous system that can self-renew and generate both neurons and glia.) The early studies led to the isolation of stem-like cells from the embryonic mammalian central nervous system (CNS)[1–4] and the peripheral nervous system (PNS)[5]. Since then, stem cells have been isolated from many regions of the embryonic nervous system, indicating their ubiquity (Fig. 1a). After the discovery of neural stem cells in the embryo, the first isolation of stem-like cells from adult brain[6,7] began yet another chapter of neuroscience. Adult neural stem cells have now been found in the two principal adult neurogenic regions, the hippocampus and the subventricular zone (SVZ), and in some non-neurogenic regions, including spinal cord[8–10] (Fig. 1a). These pioneering studies provided a cellular mechanism for adult neurogenesis, which was well-established in birds and becoming accepted in mammals, and raised the possibility that the most intractable of tissues — the CNS — might have regenerative powers.

Markers that define CNS stem cells are only now being developed[11–14]. Hence, they are usually identified retrospectively on the basis of their behaviour after isolation. In adherent cultures, CNS stem cells produce large clones containing neurons, glia and more stem cells; they can also be cultured as floating, multicellular neurospheres[8–10]. PNS neural crest stem cells express the low-affinity neurotrophin receptor p75 (ref. 5), and grow as adherent clones containing peripheral neurons and glia, smooth muscle cells and more stem cells[15].

This review summarizes what we currently know about stem cells in the developing nervous system, and evaluates the idea that embryonic neural stem cells are heterogeneous and restricted. Studies that indicate broad plasticity of adult

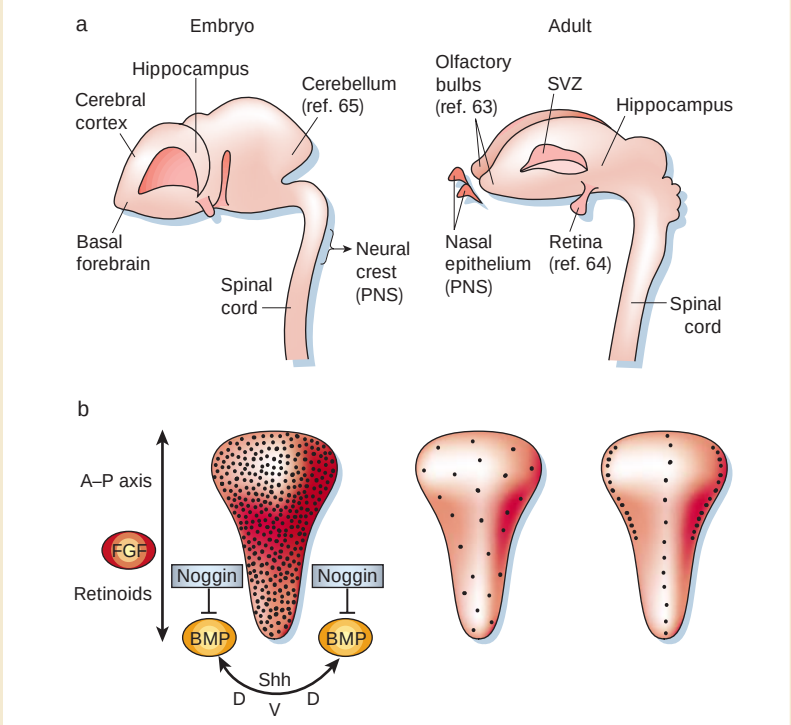

Figure 1 The location of neural stem cells. **a**, The principal regions of the embryonic and adult nervous system from which neural stem cells have been isolated[9,10,63–65]. **b**, Three models describing stem cells in the vertebrate neural plate. All neural plate cells are stem cells (left), or stem cells are a minor population that is evenly distributed (middle) or located in particular regions such as the midline and lateral edges (right). Factors such as bone morphogenetic proteins (BMPs), Noggin, retinoids, Sonic hedgehog and fibroblast growth factors (FGFs), which provide anterior–posterior (A–P) and dorsal–ventral (D–V) patterning information, may regionalize stem cells[24].

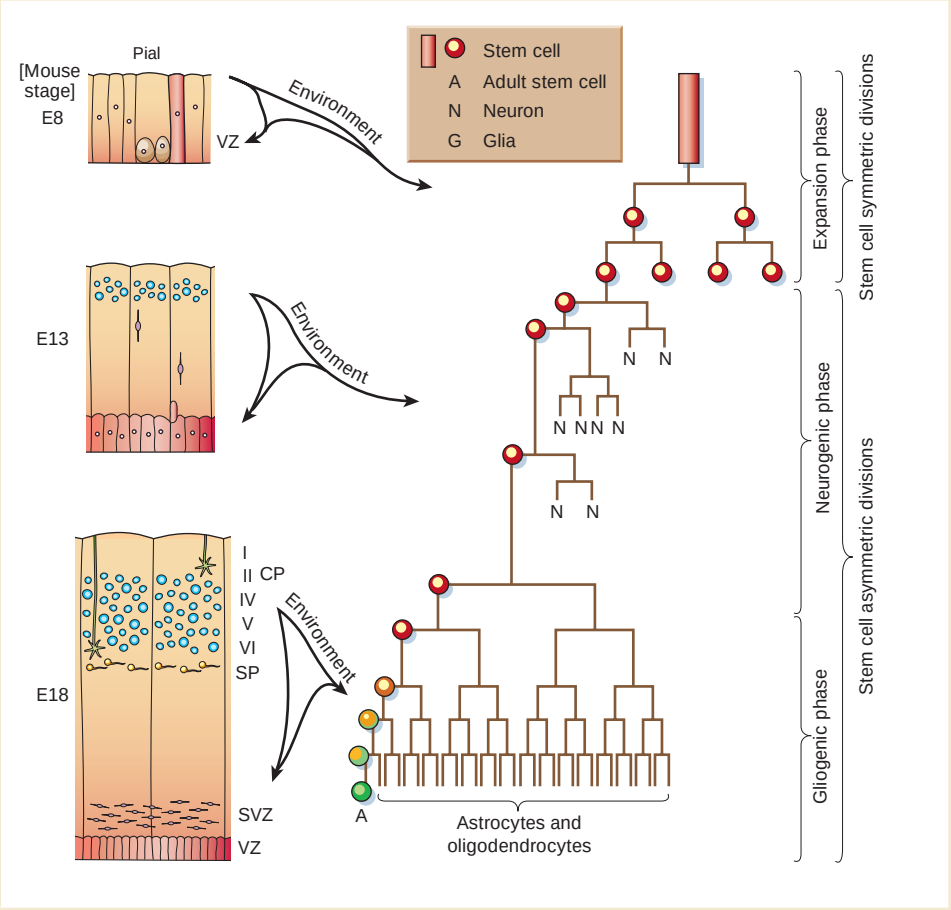

Figure 2 The development of stem cells in the mammalian CNS. Alignment of *in vivo* developmental events with *in vitro* behaviour of stem cells derived from embryonic forebrain has led to the following concept of how neural stem cells change over time. Early neuroepithelial cells are columnar, touching ventricle and pial surfaces during the cell cycle. At mid-gestation, young neurons have migrated above the germinal ventricular zone (VZ), radial glia continue to contact both ventricle and pia, guiding neuronal migration, and a second germinal zone arises, the subventricular zone (SVZ). By postnatal ages, radial glia have transformed into astrocytes and the VZ also disappears, but the SVZ remains into adulthood in some areas. Stem cells present in the early cerebral cortical neuroepithelium divide symmetrically first, and then asymmetrically to generate differentiated progeny. Neurons are produced first, and migrate along radial glia up towards the pial surface where they settle in the subplate (SP) and the cortical plate (CP). After the neurogenic period, the stem cell makes glial progenitor cells that proliferate largely in the SVZ. By birth, the stem cell has developed and has different characteristics, such as responses to growth factors, to those of the original embryonic stem cell. Stem cell development might be driven by a combination of intrinsic temporal programmes and extracellular signals from the changing environment of the developing brain.

stem cells are also discussed. By examining these two different aspects of stem cell research, future directions of exploration are highlighted that might help explain this apparent dichotomy.

Stem cells at the beginning of the nervous system

Many fundamental questions regarding specification of early neural stem cells remain unanswered. When the neural plate first emerges, does it consist solely of stem cells or does it include both stem cells and restricted progenitor types (Fig. 1)? Analysis of adherent clone production suggests stem cells are prevalent at early stages. In spinal neural tube from embryonic day 8 (E8) rat, over 50% of the viable cells at 24 hours are stem cells[16,17]. In telencephalon from E10 mouse, estimates of stem cells range from 5 to 20% (refs 4, 18, 19). Most of the premigratory neural crest consists of stem cells[20].

But the frequency of stem cells declines rapidly, diluted by the production of restricted progenitors and differentiated cells; for example, in spinal cord it drops to 10% at E12 and 1% at postnatal day 1 (P1)[16,17]. Notably, stem cells seem to be much rarer when neurosphere production is used as the assay: only 0.3% of E8.5 mouse anterior neural plate cells make neurospheres[21]. Perhaps neurosphere-generating cells are a subpopulation of early stem cells, or perhaps stem cells are more prevalent in spinal cord than anterior regions at this age.

How are neural stem cells initially specified? The stem cell could be the default state or, alternatively, stem cells might be induced. Pluripotent embryonic stem cells can produce a primitive type of neural stem cell when grown in isolation, but only 0.2% of embryonic stem cells generate neurospheres[22]. Although this study suggests that there is a default pathway for acquiring the stem cell state, the low frequency indicates that it may be normally enhanced by inductive mechanisms.

If stem cells are, or rapidly become, a subset of early neural progenitor cells *in vivo*, how are they distributed (Fig. 1)? Without specific markers, important questions regarding stem cell frequency and location *in vivo* remain open.

The early, widespread presence of stem cells in the embryonic nervous system raises another important issue of their role in development. Given their prolific, multipotent nature *in vitro*, they are likely to be principal progenitors *in vivo*, but this remains to be shown directly. For example, it is possible that early restricted neuroblasts rather than stem cells might generate the preponderance of neurons. Clonal studies suggest that most glia, both astrocytes and oligodendrocytes, originate from stem cells[9,18,19], signifying their importance for gliogenesis. In fact, as described below, there may be an intimate association between glia and the neural stem cell state.

Neural stem cells acquire positional information

Patterning of the body axis occurs through signalling systems that impart positional information. For example, gradients of signalling molecules can regionally specify a population of progenitor cells if the cells respond differently to different concentrations of the signal[23]. In the nervous system, the salient patterning in anterior–posterior and dorsal–ventral axes occurs early, concomitantly with neural induction (Fig. 1)[24].

Do both stem cells and restricted progenitor cells exhibit regionalization, or do stem cells remain unspecified, maintaining their plasticity? In *Drosophila* and grasshopper, each stem cell-like neuroblast has a unique identity based on its position in the neuroectoderm[25]. In vertebrates, this question is just beginning to be explored. Neurospheres generated from different CNS regions express region-appropriate markers, indicating that the original stem cells were indeed regionally specified[26]. Regulatory sequences control region-specific expression of the transcription factor Sox2, so that expression is seen in telencephalic but not spinal cord stem cells[27].

Table 1 Summary of transplantation studies assessing stem and progenitor cell behaviour

Donor	Host site	CNS incorporation	Differentiation	References
Embryonic stem cells				
Mouse ES cells or ES cell-derived neurospheres	Blastocyst	Incorporation into all embryonic tissues	Both glutamate- and GABA-mediated neurons in cortex	50,51
Mouse ES cell-derived neural precursors	E16–18 rat LV	Widespread incorporation	Neurons, oligodendrocytes and astrocytes	52
Embryonic neural cells				
E9.5 and E14.5 forebrain-derived neurospheres	Blastocyst or morula	No aggregation		22
EGF-generated neurosphere cells from E14 mouse fore- and midbrain	E15 rat LV	Fore- and midbrain structures	Astrocytes	53
Fetal human brain-derived neurospheres or primary cells	E17–18 rat or P0 mouse LV	Widespread incorporation in the brain	Neurons (projection), oligodendrocytes and astrocytes	54,55
E10.5 mid/hindbrain	E13.5 MGE	Dispersed into forebrain	Site-specific neuronal differentiation	31
E12–14 mouse forebrain	E15 to P1 rat LV	Into forebrain and midbrain; incorporation and migration reduced as host age increases	Site-specific neuronal differentiation	56,57
E13.5 mid/hindbrain	E13.5 LGE or MGE	No integration into forebrain		31
E36 ferret cortex (making layer 4)	E30 ferret (making layer 6)	Neocortex and hippocampus	Layers 2–5; no transplanted cells in layer 6	29
	P2 (making 2/3)	Neocortex and hippocampus	Layer 2/3 neurons	
Early postnatal				
Postnatal mouse SVZ cells	E15 mouse LV	Septum, thalamus, hypothalamus and inferior colliculus; not cortex or hippocampus	No principal projection neurons	32
Neonatal mouse SVZ neurospheres	Chick embryo	Migrate in isolation	Neural crest derivatives	46
Neonatal mouse SVZ cells	Chick embryo	Chain migration in the neural crest pathway	Progenitors and neurons; not neural crest phenotypes	46
	Neonatal striatum	Migration	Neuronal precursors and olfactory bulb neurons	58
P0–5 mouse SVZ cells	Adult striatum	Some migration	Olfactory bulb interneurons	59
Adult				
Mouse forebrain neurospheres	Blastocyst or morula	No aggregation		22
	Mouse morula or chick embryo	Rare neural chimeras	Cell types not reported	60
Mouse SVZ neurospheres	Chick embryo	Migrate as isolated cells	Phenotypes of neural crest derivatives	46
Mouse SVZ cells	Chick embryo	Cells die		46
	Adult mouse striatum	Minimum migration	Some neurons, mostly astrocytes in striatum	61
	Adult olfactory bulb	Extensive migration	Olfactory bulb neurons	61
Cultured rat hippocampal progenitors	Adult rat hippocampus	Migrate	Neurons in granule cell layer of dentate gyrus	42, 62
	Adult rat rostral migratory stream	Migrate to olfactory bulb	Neuroblasts and olfactory neurons	42
	Adult rat cerebellum	Incorporation	No neurons	42
FGF-responsive rat spinal cord progenitors	Adult hippocampus	Broad dispersion	Hippocampal granule neurons	41
	Adult spinal cord	Broad dispersion	No neurons	41

These studies indicate stage-dependent restrictions in the potential of donor progenitor populations (which include some neural stem cells). They also emphasize the impact of interaction between implanted cells and the host environment. Different treatments of stem and progenitor cells *in vitro* can significantly alter their behaviour after implantation. EGF, epidermal growth factor; ES, embryonic stem; FGF, fibroblast growth factor. LGE/MGE, lateral/medial ganglionic eminence; LV, lateral ventricle.

In addition, stem cells isolated from different neural regions generate region-appropriate progeny. Spinal cord stem cells generate spinal cord progeny[17]. Basal forebrain stem cells cultured at clonal density generate significantly more GABA (γ-amino butyric acid)-containing neurons, which are characteristic of basal regions, than dorsal stem cells cultured under identical conditions[19]. PNS neural crest stem cells, which arise at the lateral edges of the neural plate, express distinct genes and generate progeny distinct from those of CNS stem cells[15,28]. Hence, vertebrate stem cells seem to be positionally specified.

Neural stem cells acquire temporal information

Different neural cell types arise in a precise temporal order that is characteristic for a particular region and species. In general, CNS and PNS neurons arise before glia, and specific types of each cell have specific birthdates. Timing seems to be encoded in progenitor cells, so that besides positional information they have 'temporal information', which is seen as stage-dependent changes in progenitor cells (Table 1). Thus, late-embryonic ferret cortical progenitor cells cannot make cells appropriate for younger stages when transplanted into early cerebral cortex[29]. Rat cortical progenitors become restricted in their ability to generate limbic system-associated membrane protein (LAMP)-positive limbic cortical progeny after E14 (ref. 30). Mid/hindbrain progenitor cells are unable to generate telencephalic phenotypes after E13.5 in mouse[31]. SVZ cells can no longer make projection neurons by birth[32,33], and retinal progenitor cells seem to be similarly restricted temporally[34,35].

Stem cells are a minor component of the progenitor population in these studies, but experiments indicate that they also exhibit stage-dependent changes in potential. Some early neural tube cells produce both CNS and PNS stem cells, suggesting that there is a common progenitor for two separate stem cell lineages with more restricted potentials[28]. Each of these lineages also changes over time. CNS stem cells undergo repeated asymmetric cell divisions, first producing neurons then glia[18], thus reproducing the normal neuron–glia order (Fig. 2). Moreover, they have an active role in this process by altering their intrinsic properties. Thus, stem cells from earlier stage cortices

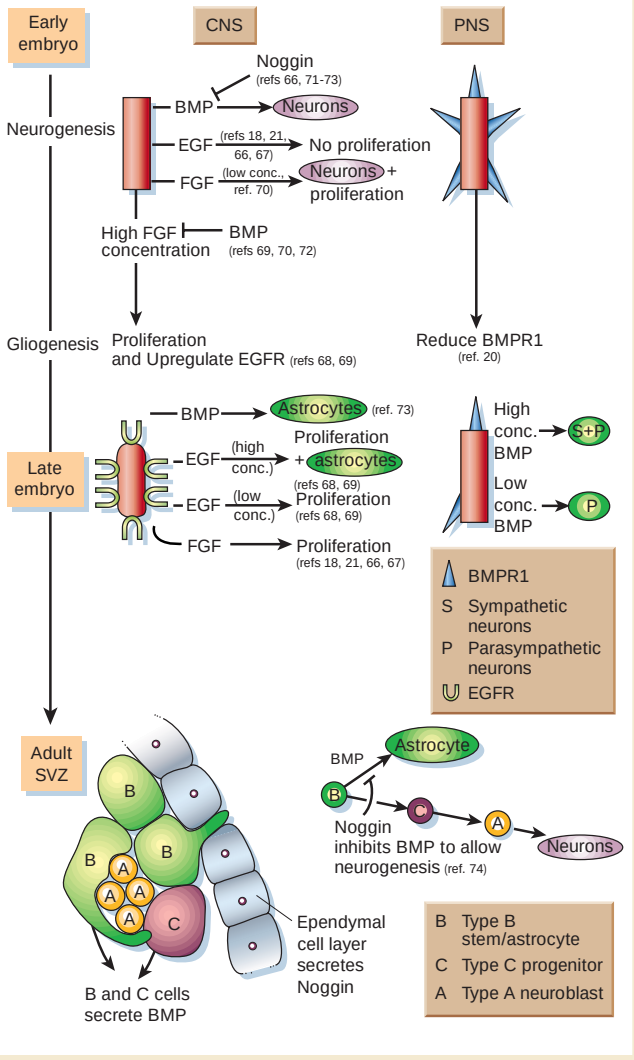

Figure 3 Stem cells alter their responses to growth factors over time. Central and peripheral nervous system (neural crest) stem cells extracted from different ages show differing responses to application of the same growth factor. Changes in levels of growth factor receptors have been observed. In addition, stem cells exhibit different responses to growth factors applied at different concentrations. BMP, bone morphogenetic protein; EGF, epidermal growth factor; EGFR, EGF receptor; FGF, fibroblast growth factor.

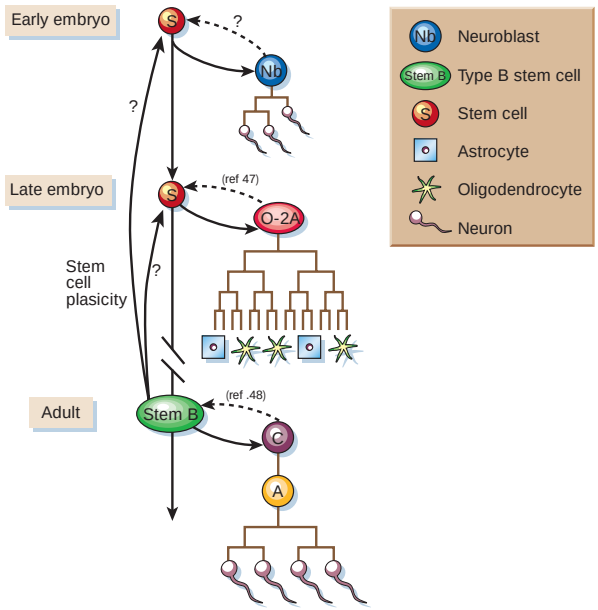

Figure 4 Can the stem cell lineage be reversed? Evidence indicates that neural stem cell progeny can reacquire stem cell properties. An important issue is whether adult stem cells can revert to an earlier embryonic state, with a wider potential.

produce more neurons and have a lower tendency to produce glia than those from later stages.

Temporal specification of stem cell populations also occurs in the PNS. Mouse neural crest stem cells isolated from the rat neural tube have been compared with later stem cells isolated prospectively from E14 sciatic nerve after transplantation into different sites of the chick embryo[20]. Early neural crest stem cells generate significantly more neurons than later stage cells: like CNS stem cells, their neurogenic capacity declines with stage. Furthermore, the range of neurons generated by late neural crest stem cells is more restricted. Early transplants that are highly enriched for neural crest stem cells but contain some restricted sensory progenitor cells can generate dorsal root ganglion (DRG) sensory neurons, and adrenergic and cholinergic autonomic neurons. By contrast, older-stage neural crest stem cells made no DRG neurons, only rare adrenergic sympathetic neurons, but they could generate cholinergic autonomic neurons.

Stem cells also undergo phenotypic changes as germinal zones develop (Fig. 2). It has been suggested recently that some radial glia present at mid-gestation might be stem cells (reviewed in ref. 36). This idea is appealing, given that radial glia are thought to be neurogenic precursors in adult canary brains, and that astrocytes, the lineal descendants of radial glia, have stem cell properties in the adult mammalian SVZ[37].

Developmental changes in stem cells — for example, in their potential and phenotype — are accomplished by, and perhaps driven by, changes in their growth factor responsiveness (Fig. 3; and see accompanying reviews by Spradling and colleagues, pages 98–104, and Weissman and colleagues, pages 105–111). Thus signalling molecules, such as fibroblast growth factors, bone morphogenetic proteins and Noggin, can influence neural stem cells from neural induction through adulthood, but their responses to these factors vary with stage.

The mechanisms underlying temporal changes in neural stem cells are not understood. The lineage trees of mouse CNS stem cells are remarkably similar to those of invertebrates[38]. *Drosophila* CNS neuroblasts express sequentially the transcription factors Hunchback, Krüppel, POU-domain genes 1 and 2, Castor and grainyhead, which specify the production of different neurons at different times[39,40]. The expression sequence may be driven by a cell-intrinsic clock[40]. Perhaps similar intrinsic timing mechanisms, combined with environmental input, temporally specify mammalian CNS stem cells. Growth factor concentrations vary during development and neural stem cells respond differently to different concentrations of the same molecule (Fig. 3). Hence, it is tempting to speculate that, just as positional information can be imparted by spatial gradients of signals[23], temporal information might be imparted by temporal gradients of signalling molecules.

Examining the plasticity of adult stem cells

If stem cells undergo developmental changes, adult stem cells are likely to differ from those in the embryo and to be regionally and temporally restricted.

One way to examine the plasticity of adult neural stem cells is to transplant them directly into the developing embryo and examine

Box 1
Potential therapeutic uses of stem cells to repair the nervous system

Stem cells are under active consideration as a source of donor tissues for neuronal cell therapy[75].

Parkinson's disease. The requirement is to generate cells that synthesize and release dopamine for implantation into the dopamine-depleted striatum. For this therapy to be effective, it is unknown whether these cells must also mature into projection neurons with synaptic host connections — a process that is required for optimal effects of embryonic nigral grafts.

Huntington's disease. If we can control differentiation into mature neuronal phenotypes then many other diseases that involve loss of specific neuronal types, such as the striatal medium spiny projection neurons lost in Huntington's disease, might be suitable for transplantation of neurons expanded from stem cell sources.

Spinal cord injury. Stem cells may be able to repopulate the site of injury, provide a substrate for axon growth across the transection, and block syrinx progression. Each of these effects has been found with primary embryonic cells; however, studies using stem or immortalized precursors are still preliminary.

Stroke. Stem cells and immortalized precursors may be able to migrate through the central nervous system and repopulate sites of ischaemia. In spite of rather limited evidence from animal studies, clinical trials of this strategy are already underway.

Multiple sclerosis. Oligodendrocyte lineages are better characterized than neuron lineages, and oligodendrocyte precursors can differentiate and provide a functional remyelination of axons after focal experimental demyelination. For application in multiple sclerosis, the main problem is how to stimulate the migration of such cells to diverse sites of demyelination that occur sporadically in the human disease. Notwithstanding the potential applications of oligodendrocyte lineages in several diseases, many key technical problems remain to be resolved.

Sources. If we select early embryonic stem cells, then the number of transformations and the complexity of signals required to achieve a specific differentiated phenotype may prove prohibitive; instead, it may be easier to control the phenotypic differentiation of developing neuronal precursors, but that might in turn limit their capacity for expansion. Adult-derived cells may circumvent both ethical and immunological constraints, but their plasticity for expansion and differentiation remains to be established. Cross-lineage transformation offers a new prospect for a more flexible source, in particular to derive autografts from patients themselves. A further advance is that precursor cell are less immunogenic than primary embryonic neurons in xenografts, highlighting a way to overcome one of the main difficulties of transplantation from non-human donors.

Expansion. Neuronal stem cells from species other than mice seem to senesce on repeated passage, with only limited potential for expansion. Conversely, if not fully differentiated at the time of implantation, there is always the possibility of tumour formation — a problem that is still not resolved for either embryonic stem cells or immortalized precursors.

Differentiation. The biggest single problem still to be solved is how to direct and control the differentiation of specific target phenotypes required for replacement and repair in each disease. Selection of appropriate starting cells by embryonic regional dissection and stage of development, as well as diverse parameters of *in vitro* manipulation, are likely to be crucial factors in directing appropriate phenotypic differentiation. Failure can lead not only to a lack of benefit but also to significant side-effects from proliferation of non-neuronal phenotypes.

All these problems can be solved, but it will require more than a single breakthrough to transform the potential attributed to stem cells into a realistic clinical strategy for cellular repair.

what neural cell types they produce; this still remains to be done. Emerging methods for the prospective isolation of adult CNS stem cells[11–14] should facilitate this important experiment. Experiments using culture-expanded adult CNS stem cells indicate that there is some plasticity in adult environments (Table 1). For example, adult spinal-cord-derived stem cells, which do not normally make neurons, can make interneurons if injected into the adult hippocampus[41], and adult hippocampal-derived stem cells can make olfactory interneurons after transplantation to the SVZ[42]. As yet, however, there is no direct evidence that adult-derived stem cells can make the types of projection neuron that are normally generated in the embryo.

Given the vital gaps in our understanding of adult neural stem cells, we cannot yet conclude that they are highly plastic. Evidence that they can generate different somatic cell types is limited, and may be restricted to rare events or rare cells[43–45]. In considering the two different ideas raised at the beginning of this review, there may be in fact no dichotomy. Most neural stem cells might be regionally and temporally specified. There may also be rare stem cells present in the nervous system, perhaps not even of neural origin, that have greater plasticity, at least in terms of producing diverse somatic cell types[43].

Reversing the stem cell lineage

If stem cells are restricted, can these restrictions be reversed (Fig. 4)? For example, can an adult stem cell re-acquire the ability to generate cell types normally made in the embryo? A study has indicated that culturing SVZ stem cells as neurospheres expands their potential and allows them to generate PNS progeny after injection into chick neural crest pathways[46]. Furthermore, descendants of neural stem cells may be able to revert. Optic nerve O2A progenitor cells, which normally produce solely glia, can be converted *in vitro* into multipotent, neurosphere-generating stem cells[47]. In the adult SVZ, both type B stem cells and their progeny, type C progenitor cells, can make neurospheres *in vitro*. Moreover, type C cells are stimulated to convert to stem cells by epidermal growth factor[48]. In the postnatal chick retina after damage, Muller radial glia, which are maintained throughout life, can re-enter the cell cycle, re-express retinal progenitor cell markers and generate new neurons and glia[49].

If environmental factors can enhance the acquisition of neural stem cell fates, or increase the plasticity of stem cells, this may be of enormous benefit therapeutically, as indicated in Box 1.

Future studies

As regions of the embryo are patterned and development unfolds, neural stem cells may be an essential mediator of developmental signals, acquiring a changing repertoire of gene expression, morphology and behaviour. Despite differences in the properties of stem cells isolated from different regions and at different times, they still self-renew. Self-renewal can therefore be considered as the propagation of stem cells, rather than the production of exactly the same type of cell.

It will be important to examine how developmental signals, both spatial and temporal, specify changes in neural stem cells. Markers for neural stem cells will allow their selection from different stages and regions to examine their potential after transplantation into the embryo or adult, and a comparison of their gene expression. Such explorations will help identify essential mediators of stem cell self-renewal, and genes that determine production of different types of progeny. Markers will also help solve the tantalizing issue of which cells *in vivo* are stem cells.

Although research to identify adult sources of highly plastic stem cells for therapeutic use will continue, it seems likely that most neural-generating stem cells might be specified during development. In that case, we must explore this diversity to understand how different neural cells are, and can be, made. □

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Acknowledgements

I thank Q. Shen for her help with Table 1 and for invaluable discussions; K. Kirchofer for preparing the manuscript; and S. Dunnett for summarizing the potential therapeutic applications of stem cells in Box 1.