The ability to isolate multipotential neuroepithelial precursor cells from the mammalian nervous system provides exciting perspectives for the in vitro analysis of early nervous system development and the generation of donor cells for neural repair. New models are needed to study the properties of these cells in vivo. Neural chimeras have revealed a remarkable degree of plasticity in the developmental potential of neuroepithelial precursor cells. Following transplantation into the cerebral ventricle of embryonic hosts, precursors derived from various brain regions and developmental stages participate in host brain development and undergo region-specific differentiation into neurons and glia. These findings indicate that in the developing nervous system, migration and differentiation of neural precursors cells are regulated to a large extent by extrinsic signals. Neural chimeras composed of genetically modified cells will permit the study of the molecular mechanisms underlying these guidance cues, which may eventually be exploited for cell replacement strategies in the adult brain. Neural chimeras composed of embryonic stem (ES) cell-derived neurons and glia depict ES cells as a versatile and virtually unlimited donor source for neural repair. Generation of interspecies neural chimeras composed of human and rodent cells facilitates the translation of these advances into clinical strategies for human nervous system repair.

“...Countless modifications during evolution have provided living matter with an instrument of unparalleled complexity and remarkable functions: the nervous system, the most highly organized structure in the animal kingdom...”

Santiago Ramón y Cajal (16)

The unparalleled complexity of the mammalian central nervous system makes the study of neural development and repair one of the biggest challenges in contemporary science. Composed of numerous highly specialized and intricately interconnected cell populations, the mammalian brain and spinal cord offer little access to the reductionist approach. However, as with many complex systems, simplicity can be found in the origin.

Origins of plasticity - the neuroepithelial stem cell concept

In 1985, a research team at MIT generated a series of monoclonal antibodies to embryonic rat spinal cord. One of the antibodies they obtained, Rat-401, labeled more than 98% of the cells in the embryonic day (E) 11 neural tube (41, 48). This antibody recognized an intermediate filament strongly expressed in neuroepithelial precursor cells - but not in their differentiated progeny. Accordingly, the group named their newly discovered protein nestin - for neuroepithelial stem cell (67). This discovery had two major implications. On a pragmatic level, it provided a valuable tool for the identification of neural precursors - but not in their differentiated progeny. Accordingly, the group named their newly discovered protein nestin - for neuroepithelial stem cell (67). This discovery had two major implications. On a pragmatic level, it provided a valuable tool for the identification of neuroepithelial precursor cells. On a conceptual level, it supported the notion that neural precursors in different parts of the nervous system may share common features, and that there may be a neural stem cell at the origin of the nervous system.

Today, the neuroepithelial stem cell concept has been put on a solid basis (88, 143). Several studies have shown that individual neural precursors isolated from the developing brain can give rise to neurons and glia...
These cells proliferate in the presence of growth factors such as basic fibroblast growth factor (FGF2) or epidermal growth factor (EGF) and, upon growth factor withdrawal, differentiate into all three major cell types of the nervous system — neurons, astrocytes and oligodendrocytes. Single factors added during growth factor withdrawal are able to promote either of the three differentiation pathways (58). Multipotential neural precursors with similar properties have been derived from the adult mammalian brain and spinal cord (44, 46, 58, 107, 108, 142). The idea that all neuronal and glial cell types originate from a homogeneous population of stem cells has provided a useful basic concept for the study of lineage analysis in the developing nervous system. However, it has also raised new questions. What are the signals that control the development of such a complex and heterogeneous system from a stem cell population? Is an individual precursor cell in a defined area of the neuroepithelium restricted to a local fate? Or are there signals in the surrounding tissue that guide a ‘generic’ precursor to a local fate? These questions are not only relevant for our understanding of brain development but also for the design of cell replacement strategies in the nervous system. A cell-autonomous view would require highly specified donor cell populations tailored to the individual patients’ needs. In contrast, non-cell autonomous concepts would focus on mechanisms that recruit uncommitted cells to the required phenotype (13).

Transplantation is a key tool for exploring commitment and plasticity of neural precursor cells in vivo. Due to the limited access to the developing mammalian brain, most of the initial experiments were done in the embryonic avian or the postnatal rodent brain. Heterotopic and heterochronic displacement of tissue fragments supported the view that the regional identity of neural precursors is acquired early in CNS development (4, 7, 21, 80). However, the question remained whether cells transplanted as tissue fragments are sufficiently exposed to environmental cues in a heterotopic location. This notion was further supported by reports that single cell suspensions grafted into heterotopic regions of the postnatal rodent brain can migrate and differentiate in a manner appropriate for their new location (87, 105, 137). These studies strongly supported a concept of regional guidance of the transplanted cells. They were, however, restricted to the few brain regions exhibiting postnatal neurogenesis. It became clear that a new experimental approach would be needed to comprehensively address the question of regional determination in the developing brain.

**Neural chimeras – Exploring precursor cell plasticity in the mammalian brain**

In 1995, three groups independently reported on an experimental system that permits widespread incorporation of neural precursors cells into the developing rodent brain (12, 17, 36). In this model, the cells were not implanted into the brain tissue but injected through the uterine wall into the cerebral ventricle of embryonic hosts (11) (Figure 1). The transplants were performed between embryonic day (E) 15 and E18, a time of active neurogenesis in many brain regions. Following intrauterine surgery, the transplanted embryos developed normally to term and were born by spontaneous vaginal delivery. Analysis of the host brains at different times after transplantation revealed that the grafted cells had incorporated not only into their region of origin but also into many heterotopic brain regions, where they...
acquired region-specific phenotypes. The study by Fishell et al., for example, showed that fluorescent-labeled striatal precursors incorporate into the host cortex and acquire the morphology and axon projections of cortical neurons. Campbell et al. used a species-specific antibody to trace mouse neural precursors following transplantation into the ventricle of embryonic rat brains. While they noticed a preferential incorporation of precursors from the lateral ganglionic eminence (LGE) into the striatum, precursors derived from both the LGE and the medial ganglionic eminence (MGE) integrated into a variety of fore- and midbrain structures. The preferential incorporation of LGE cells into the striatum was abolished when the donor cells were pre-treated with trypsin, suggesting a potential role of cell adhesion molecules in mediating homotypic association of the grafted cells with the host ventricular zone (98).

Using genetic labeling methods such as transgenic lacZ-expressing donor animals or DNA in situ hybridization, we demonstrated that neural precursors derived from dorsal and ventral mouse forebrain incorporate into telencephalic, diencephalic and mesencephalic regions of the developing rat brain, yielding widespread CNS chimerism (Figure 2). The incorporated cells differentiated into both neurons and glia and acquired morphological and immunochemical features appropriate for their final location (Figure 3). For example, cells incor-
porating into the host hippocampus assumed typical granule and pyramidal cell morphologies and expressed the limbic marker limbic system-associated membrane protein (LAMP; (68)). Transplant-derived hippocampal granule neurons expressed calbindin, a calcium-binding protein typically found in this cell population (5) (Figure 3c). Following integration into the inferior colliculus, neurons derived from the ventral telencephalon assumed morphologies characteristic of tectal neurons in this region. These findings strongly suggested that, during nervous system development, cell migration and differentiation are predominantly regulated by non-cell-autonomous cues. Results of recent studies employing modified intrauterine transplant paradigms support this view. Olsson et al. developed an ultrasound-guided transplant approach to deliver minute volumes of precursors directly into various regions of the E13.5 mouse brain (99). Reciprocal transplantation of precursors from the E13.5 ventral telencephalon and the E10.5 mid-hindbrain region showed that heterotopically incorporated cells express En-1 and Nkx 2.1, i.e., homeotic genes appropriate for a mid-hindbrain and a ventral telencephalic location, respectively. These findings led the authors to speculate that individual neural precursors may be determined with respect to their anteroposterior but not their dorsoventral identity. The apparent lack of congruency of data from different studies should remind us that the precise windows of precursor cell plasticity at different stages of CNS development are still widely unknown.

In the study by Olsson et al., the potential for heterotopic integration of mid-hindbrain precursors decreased when the donor cells were derived after E10.5, suggesting an increased commitment to a local phenotype at later developmental stages. In agreement with this interpretation, cells derived from regions exhibiting continuous neurogenesis appear to retain the capacity for widespread incorporation into the developing brain. One example is the subventricular zone (SVZ) which has been shown to generate olfactory neurons throughout life (1, 73). Lim et al. demonstrated that cells isolated from the postnatal day (P) 5-10 mouse SVZ migrate and differentiate within multiple levels of the developing neuraxis (71). Incorporation of neural precursors grafted into the developing brain also depends on the recipients’ age. The distribution of donor cells following intraventricular transplantation indicates that they do not incorporate randomly but show a strong preference for regions known to undergo neurogenesis at or beyond the time of transplantation (2). Accordingly, intraventricular transplantation at late developmental stages yielded fewer incorporated cells in a more restricted distribution (12, 98). This preferential incorporation into areas of active neurogenesis can be exploited for the targeted introduction of precursor cells into few selected regions.

Intrauterine transplantation provides a unique opportunity to generate brains composed of neurons from different locations and developmental stages. This approach will delineate windows of precursor cell plasticity in different regions of the developing brain and help to explore the relative contribution of cell-autonomous determination and regional guidance at a molecular level. In addition, it permits full exploitation of the steadily growing repertoire of genetically modified mice. Cell-autonomous signals can be investigated using donor cells derived from transgenic mice. Non-cell-autonomous guidance can be studied by grafting wild type or genetically modified donor cells into transgenic recipients. This approach is particularly useful for the study of targeted gene inactivations which result in early embryonic lethality and, therefore, preclude the analysis of a neural phenotype at later developmental stages. Neural precursors isolated from these animals and introduced into a wild type brain can be studied far beyond the natural life span of the respective knockout animal.

Ultimate plasticity: Embryonic stem cell-derived neural precursors

The high degree of plasticity observed after intrauterine transplantation of cells from different host brain regions raised the question whether susceptibility to regional guidance cues is a generic feature of neural precursors. If so, would a precursor cell generated outside the nervous system be able to contribute neurons and glia to the developing brain? To explore this question, we set out to derive neural precursor cells entirely in vitro — from embryonic stem (ES) cells. ES cells are totipotent cells that have been isolated from the inner cell mass of blastocysts from a variety of species, including mouse (33, 79), hamster (28), chicken (101), zebrafish (128), swine (145), cattle (35), and primates (135). Recently, ES cells and ES cell-like stem cells have also been obtained from embryonic human tissue (115, 134). These cells have two unique properties. First, in the presence of leukemia inhibitory factor...
(LIF), they can be proliferated to virtually unlimited numbers without losing their developmental potential (120). Second, they are truly totipotent and can differentiate into all tissues and cell types. Transplanted into another blastocyst, they can contribute to all tissues of the developing embryo, yielding a chimeric organism (9). Today, this approach is most commonly used for the generation of gene deficient (knockout) mice (152).

We were interested in exploiting ES cells for the in vitro generation of somatic precursors for the nervous system. Several groups have demonstrated that induction of ES cell differentiation with retinoic acid yields cells with neuronal and glial properties (6, 34, 38, 125). Upon transplantation into the quinolinic and 6-hydroxydopamine-lesioned striatum, these cells maintain their neuronal identity and differentiate into mature phenotypes expressing various neurotransmitters (26, 27). However, preparations of retinoic acid-induced ES cells generally contain a variety of different cell types precluding the generation of pure proliferative neural precursors. We used a different strategy to obtain neural precursors from ES cells (Figure 4). Following proliferation in the presence of LIF, the cells were aggregated to form embryoid bodies, spheroid aggregates of differentiating ES cells. Four-day-old embryoid bodies were plated in a defined medium that has been shown to promote the survival of neural precursors. Cells generated in this manner can be further proliferated in the presence of basic fibroblast growth factor (FGF2) and, upon growth factor withdrawal, differentiate into neurons and glia (97). To explore whether these entirely in vitro-generated neural precursors are responsive to regional guidance cues, we implanted them into the ventricle of embryonic rats. Similar to precursors derived from the developing brain, ES cell-derived neural precursors left the ventricle and migrated into a variety of host brain regions, including cortex, striatum, septum, thalamus, hypothalamus, cerebellum and tectum (Figure 5). Cells remaining in the ventricle formed conspicuous epithelial structures resembling the developing neural tube and occasional islands of non-neural tissue. Donor cells incorporating into the host brain parenchyma generated all three major cell types of the nervous system — neurons, astrocytes and oligodendrocytes (14) (Figure 6). Morphologically, they were indistinguishable from their endogenous neighbors and only detectable by virtue of their genetic difference. Some of the ES cell-derived cortical neurons displayed polar morphologies with long apical dendrites, pyramidal cell bodies and basal axons projecting into the corpus callosum. Transplanted astrocytes frequently extended processes to adjacent capillary-
transplantation into the developing brain, migration and differentiation of these cells can be analyzed in a functional nervous system. In principle, this approach provides access to the neural phenotype of a targeted gene inactivation without generating a knockout animal.

**ES cell-based neural repair**

The fact that ES cell-derived neural precursors contribute neurons and glia to the developing brain also implies that ES cells have the potential to reconstitute neurons and glia in the diseased nervous system. Offering key advantages such as virtually infinite self-renewal and susceptibility to genetic modification by homologous recombination, ES cells represent a particularly attractive donor source for cell replacement strategies. Combined with recent advances in isolating human ES cells (115, 134) and cloning mammalian embryos from adult tissue (139, 148), this technology can be extended to generate unlimited numbers of donor cells from the same patient. However, the use of ES cells for neural cell replacement also poses several major challenges. These include the generation of highly purified donor cell populations free from tumorigenic undifferentiated ES cells and the establishment of cell culture conditions that permit the targeted differentiation of totipotent stem cells into defined somatic precursors matching the host brain environment and the individual patients’ needs.

**The infinite source.** The possibility to generate virtually unlimited numbers of ES cells, differentiate them to a neural phenotype and use them for cell replacement therapies may, in fact, be the most attractive application of the ES cell technology. So far, neural precursors for central nervous system transplants have been isolated from fetal brain tissue. In the case of Parkinson’s disease, tissue from up to 7 human embryos was used for the treatment of a single patient (60). This illustrates the magnitude of the ethical and logistic problems that would arise from a widespread use of fetal brain tissue transplantation for the treatment of nervous system disorders.

Numerous efforts have been made to bypass the limited availability of embryonic donor tissue. One way of generating large numbers of neural precursors from a limited amount of fetal brain tissue is oncogene-mediated immortalization (20, 66). Several studies suggest that transplanted immortalized neural cells do, indeed, retain the potential for region-specific differentiation. Cerebellar precursor cells transduced with a retroviral vector carrying the v-myc gene and transplanted into the neonatal cerebellum have been shown to generate neurons with granule and basket cell phenotypes (121). Another oncogene frequently used for the immortalization of neural cells is the temperature-sensitive mutant of the SV40 large T antigen (SV40tsA58) which is active at 33°C but instable at 37°C (57). If the cells are switched...
to the higher temperature or transplanted, they cease proliferation and initiate differentiation (40). SV40tsA58-immortalized hippocampal precursors grafted into neonatal hippocampus and cerebellum have been reported to undergo region-specific differentiation (105). Similarly, a SV40tsA58-generated neural cell line from the medullary raphe developed mature regional morphologies following implantation into cortex, hippocampus and striatum (76, 117). At one point, oncogene-immortalized neural precursors were regarded as a major breakthrough in the generation of donor cells for neural replacement. The frequently instable karyotypes of these cell lines (Brüstle and Hayes, unpublished observations), the potential interaction of the introduced vector with the molecular machinery of the cell (74) and the mere fact that the grafted cells carry a potentially tumorigenic gene have dampened the initial excitement. However, their continuous proliferation and susceptibility to genetic manipulation have made immortalized neural cells a preferred tool for gene transfer and cell-mediated gene therapy. Transplantation of the v-myc-immortalized cerebellar cell line C17.2 in mouse models of mucopolysaccharidosis VII and Tay-Sachs disease has been shown to substitute the levels of the missing enzymes β-glucuronidase and β-hexosaminidase to therapeutic levels (63, 123). Similarly, SV40tsA58-immortalized cell lines have been exploited for the delivery of growth factors and neurotrophins in a variety of experimental transplant models (81, 82, 83, 84, 85). Recent observations by Westerman and Leboulch indicate that the CRE-loxP system may be used to remove the immortalizing oncogene prior to transplantation — an interesting step towards a potential clinical use of these cell lines (144).

Another strategy for the generation of large numbers of donor cells is the expansion of undifferentiated neural precursors in growth factor-containing serum-free media. Numerous studies have demonstrated that multipotent neural precursors from various regions of the embryonic and adult CNS proliferate in the presence of FGF2 and/or EGF (19, 25, 45, 46, 58, 59, 103, 104, 106, 107, 108, 138). Recently, ventral mesencephalic precursors expanded in the presence of FGF2 have been shown to survive transplantation into the 6-OHDA-lesioned rat striatum, an animal model for Parkinson’s disease. The grafted cells generated mature dopaminergic neurons and led to a recovery of the abnormal amphetamine-induced rotation behavior (126). However, since only a fraction of the cells is responsive to growth factor-mediated proliferation, it is currently not clear whether this method will permit a significant net increase of cell numbers prior to transplantation.

A key problem of both immortalization and growth factor treatment is the necessity to proliferate cells already committed to a neural phenotype. This critical limitation can be bypassed by generating neural precursors from ES cells. In this case, cell proliferation to any desired number occurs before neural differentiation, and the expanded cells are rapidly converted to a neural phenotype (Figure 7). Thus, in vitro differentiation of ES cells into neural precursors represents, indeed, an infinite source of donor cells for neural repair.

**The essentials: Purity and a matching environment.** The success of ES cell-based neural repair strategies will critically depend on the ability to generate highly
purified precursor cell preparations. It is well known that undifferentiated ES cells transplanted into adult immunocompetent hosts can form teratomas or teratocarcinomas (22). Even a very small number of residual, poorly differentiated cells in the grafted cell suspension could render the transplant tumorigenic or induce the formation of non-neural tissue. Indeed, components of non neural tissue have been found following transplantation of ES cell-derived neural cells into the embryonic and adult rodent brain (14, 26). Thus, a major challenge in the use of ES cells as an alternative donor source will be the generation of homogenous neural precursor cell populations. In principle, there are two strategies to generate lineage-restricted somatic precursors from ES cells. One possibility is the implementation of extrinsic signals which, during normal embryonic development, determine a specific neural phenotype (see below). Another approach is the elimination of non-desired cell populations by lineage-based selection, a method employed in a recent study by Li et al. (70).

Using the knock-in technology, the authors incorporated a neomycin resistance gene into the Sox2 locus, a gene expressed in neuroepithelium, floor plate and early neural crest. The ES cells were then induced to differentiate and subjected to G418 selection. The selection eliminated the Sox2-negative cells, leaving a population of highly purified neural cells. Extended to genes with neural cell-type specific expression, this method may be helpful in generating neuronal and glial subpopulations from ES cells. A critical question is whether cells selected at a timepoint where they already express genes specific for neuronal subpopulations are still immature enough to migrate and incorporate into a foreign brain.

A matching host environment appears to be another important prerequisite for the use of ES cell-derived precursors in cell transplantation. The differentiation of grafted ES cells and ES cell-derived somatic precursors strongly depends on environmental cues. This is best illustrated by the observation that both ES and teratocarcinoma cells can participate in normal development upon injection into mouse blastocysts (9). Exposure to environmental cues appears also to be crucial for the differentiation of ES cell-derived neural precursors grafted into the ventricle of embryonic rats. Whereas donor cells within the host brain tissue developed mature neuronal and glial phenotypes, cells remaining in the ventricle and not sufficiently exposed to the host brain parenchyma embarked on a default program of neural differentiation and formed neural tube-like structures (14). A similar dependence of donor cell differentiation on the host brain environment was noticed in transplant experiments involving the teratocarcinoma cell line NTera2 (89). These findings indicate that both ES cells and their more differentiated progeny require ‘matching’ environments to prevent them from aberrant differentiation.
**ES cell grafts and cloning — a fountain of youth.**

The idea of using ES cell-derived somatic precursors for cell replacement receives tremendous impetus from recent findings showing that mammalian embryos can be generated by transferring nuclei from mature cells into enucleated oocytes (139, 148). This technology will eventually permit the generation of autologous ES cells which can then be used to grow large numbers of somatic precursors for the individual patient (Figure 8). Successful cloning of large mammals such as sheep (18, 148) suggests that this technology can, indeed, be applied to humans. The recent isolation of ES cell-like pluripotent stem cells from embryonic human tissue (115, 134) represents an important step towards a future clinical application of ES cell-based tissue repair. Genetic modification of autologous human ES cells will eventually permit the generation of virtually unlimited numbers of somatic precursors tailored to the individual patients’ disease.

**The challenge: Recapitulating nervous system development.** In vitro generation of neural cells from totipotent ES cells comes with a price: Since these cells have never been exposed to the nervous system, they lack the positional specification found in progenitor cells in vivo. Although the neural chimera experiments have revealed a remarkable degree of precursor cell plasticity, there is fair evidence that precursor cells isolated from a specific part of the developing nervous system and not exposed to heterotopic cues differentiate according to their region of origin. Levitt et al. have called the transition from a generic precursor to a committed progenitor an ‘uncommitted but fated’ stem cell (69). Nakagawa et al. found that both primary and immortalized neural precursors isolated from different brain regions continue to express region-specific transcription factors (92). It is this bias towards a regional differentiation that has allowed the preparation of specific neuronal subpopulations from embryonic brain tissue. For example, neural precursors isolated from the ventral mesencephalon ‘remember’ their origin and differentiate into dopaminergic neurons — even after additional cell division in vitro (126). This does not imply that a ‘fated’ stem cell cannot be recruited to a heterotopic fate. It is, however, not clear, to what extent regional guidance cues are still in place in the adult brain and spinal cord (see below) and whether the transplanted cells would be sufficiently exposed to these cues. Cells grafted into mature nervous system tissue tend to form localized clusters, preventing exposure of individual cells to a new environment. Even after intraventricular transplantation into embryonic hosts, donor cell clusters have been shown to maintain their original identity (78). Thus, it is likely that donor cells used for cell replacement in the adult nervous system will have to be pre-specified with respect to their final phenotype. Neuronal replacement will require different donor cell preparations compared to, for example, remyelination. Similarly, different precursor cells will be needed to reconstitute dopaminergic and GABAergic neurons in Parkinson’s and Huntington’s disease, respectively.

The generation of precursors fated towards but not yet differentiated into a specific neuronal phenotype may be achieved by implementing positional cues that specify regionalization of the developing nervous system in the cell culture protocol. We have, in other words, to recapitulate nervous system development. A first simple step in this direction could be the introduction of Cartesian coordinates. During the last couple of years, numerous factors have been shown to impose dorsoventral or anteroposterior polarity upon the developing neural tube. Sonic hedgehog (SHH), a vertebrate homologue of the *Drosophila* segment polarity gene hedgehog (65, 95), has strong ventralizing activity along the entire neuraxis (31, 53, 109). In the anteroposterior axis, members of the FGF family have been shown to promote a posterior phenotype (75). Using tissue explants, first attempts have been made to combine gradients along both axes. An elegant study by Ye et al. shows that SHH and FGF8 can act in a concerted manner to induce a dopaminergic phenotype at multiple levels of the anterior neural tube. In conjunction with FGF4, the FGF8/SHH intersection defines an inductive center for hindbrain serotonergic neurons (149). These findings suggest the presence of multiple, temporally and spatially overlapping Cartesian grids where the concentration of two molecules defines the neuronal phenotype. Even if a Cartesian system does not suffice to determine all neuronal phenotypes, it may serve as a powerful first step towards the restriction of differentiation pathways to a limited number of choices. As our understanding of early neuronal specification increases, additional strategies will evolve, eventually permitting us to guide the differentiation of ES cells towards a specific neural phenotype.

**From bench to hospital – Exploring human precursor cells in vivo**

Most of our current knowledge about neural development and precursor cell transplantation comes from animal studies. In order to exploit this knowledge for potential clinical applications, findings made in animal
models have to be translated into a human system. This task is complicated by the limited experimental access to the human CNS. Several groups have shown that neural precursors isolated from the fetal human brain divide in the presence of FGF2 and, upon growth factor withdrawal, generate neurons, astrocytes and oligodendrocytes in vitro (15, 90, 112). However, it has not been possible to study the migration and differentiation of human neural precursors within a developing nervous system. Upon transplantation into the mature rodent brain, human neural precursors can grow extensive axonal projections (146, 147) but show little spread into the host tissue (112, 129). Glial scarring and the necessity for immunosuppression further complicate this approach.

Our observations on the widespread incorporation of mouse primary and ES cells into the embryonic rat brain prompted us to explore whether this approach could also be used for the in vivo analysis of human cells. To that end, we transplanted freshly dissociated and growth factor-expanded precursors from 8-10-week-old human embryos into the ventricles of E17-E18 rats (10). Cultured cells were propagated in defined medium containing FGF2 and/or EGF, both as monolayers and sphere cultures. A human-specific DNA probe and a panel of human-specific antibodies were used to trace the cells after transplantation (Figures 9 and 10). Similar to rodent precursors, the human donor cells left the ventricle and migrated in large numbers into multiple regions of the host brain, including the olfactory bulb, cortex, hippocampus, striatum, septum, thalamus, hypothalamus, tectum, cerebellum, and brain stem.

Transplanted human neurospheres got entrapped in periventricular locations and gave rise to numerous precursor cells migrating into the host brain parenchyma. Remarkably, the transplanted cells appeared to follow endogenous migratory routes. For example, cells incorporated into the subventricular zone of the lateral ventricles populated the corpus callosum, cortex and striatum. Human cells originating from the third ventricle migrated into the optic nerve, similar to the migratory...
Human-rat neural chimeras — a tool for the neuropathologist? Interspecies brain chimeras may well develop into a useful tool for the study of neurological disease. Most brain and spinal cord diseases are complex system disorders affecting several cell populations and a multitude of interdependent cellular mechanisms. Not amenable to in vivo experimentation, these disorders are far too complex to be reduced to a cell culture experiment. Here, interspecies brain chimeras can represent a useful intermediate. Cells isolated from patients with nervous system disorders and incorporated into a developing host brain can be studied individually in an environment free of traumatic and reactive changes. These studies may either focus on the affected cells themselves, the pathological phenotype they elicit in the brain tissue or their responsiveness to pharmaceutical and other forms of medical treatment. Since all three neural lineages of donor and host can be reliably distinguished, these studies will help to identify causative and reactive cellular alterations and delineate the primary target population of the disease process.

Neural cell replacement — key problems for the new millennium

Despite the progress in establishing new donor sources and animal models, neural replacement in the adult brain still remains a major challenge. A key problem is the incorporation of cells into an existing functional architecture, a process that would require targeted and in many cases widespread migration, regional differentiation and correct axonal connection of the transplanted neurons. The high rate of cell death following transplantation poses another hurdle in establishing clinical transplant strategies.

How to get there — Cell migration in the adult brain. During development, the migration of newborn neurons from the ventricular zone to their final destination is guided by radial glia (102). Extending from the ventricle wall to the pial surface, these cells permit neuroblasts to migrate through layers of earlier born neurons and reach their final destination. Postnatally, the radial glia scaffold is rapidly dismantled. Thus, other modes of neural migration have to be exploited to move cells from a potential implant site to their target region. There is evidence for at least two forms of migration that do not depend on radial glia. Several studies have shown that during cortical development, some neuroblasts migrate perpendicular to the radial glia network, a phenomenon referred to as tangential migration (96, 131, 140). Neural precursors migrating from the rostral...
into the ventricle of neonatal recipient animals populate osaminidase-deficient mice indicate that cells grafted brain. Studies in this approach can be extended to the postnatal and adult application of intraventricular transplantation is whether sites. One of the key questions concerning the clinical transplantation would require cell delivery to many such as MS affect large and multiple areas of the nervous system, widespread delivery of the transplanted cells to the recipient brain is an important prerequisite for comprehensive cell replacement strategies. Generation of neural chimeras by intrauterine transplantation has shown that neural precursors injected into the ventricle of embryonic hosts circulate throughout the ventricular system and incorporate into all major CNS compartments (12, 17, 36). We have recently demonstrated that this ability for widespread incorporation extends to human neural precursors (10). In these studies, a large proportion of the donor cells assumed oligodendroglial phenotypes and incorporated into gray and white matter regions as remote as the optic nerve and the cerebellum. Such a widespread delivery of oligodendroglial cells to the host brain could be particularly interesting for the treatment of demyelinating diseases such as multiple sclerosis (MS). During the last decade, transplantation of oligodendrocyte precursors has emerged as a promising experimental strategy for the treatment of myelin diseases (30, 39, 61). Transplant experiments in the myelin-deficient (md) rat, an animal model for PMD, have shown that grafted oligodendrocyte precursors efficiently substitute the abnormal host myelin (29, 30). Since many inherited neurological disorders can be diagnosed prenatally, they are, in principle, accessible to perinatal intervention. In contrast, widespread cell replacement in the mature nervous system remains a challenge. A transplant approach for the treatment of diseases such as MS may require additional modifications of both the donor cells and the host tissue to enhance the transependymal incorporation process. However, the periventricular distribution of the demyelinated areas found in many MS patients makes this disease an interesting candidate for intraventricular transplantation.

Efficient cell replacement in adult nervous system disease will not only depend on widespread cell delivery but also on the targeted introduction of donor cells into affected brain and spinal cord regions. For example, treatment of demyelinating diseases by transplantation would be greatly facilitated if the graft cells could be attracted to the demyelinated foci. The pattern of nestin expression following experimental CNS trauma suggests that glial precursor cells may, indeed, migrate towards sites of injury. Using transgenic mice carrying the lacZ gene under control of regulatory elements of the nestin gene, these studies revealed strong nestin induction in subependymal cells close to the lesion site, both in brain (50, 72) and spinal cord (43). With time, the nestin-positive cells appeared to migrate progressively away from the ventricle and towards the lesion site where they assumed the typical morphology of GFAP-expressing reactive astrocytes. These findings could indicate that focal CNS lesions exert a chemotactic stimulus on precursor cells in the subependyma and recruit them to an astrocitic phenotype. It will be particularly interesting to explore whether such a lesion-associated cell recruitment extends to multipotent neural cells or cells already committed to neuronal or oligodendroglial phenotypes.

Searching for environmental cues — Regional differentiation in the mature nervous system. The incor-
poration of additional cells into an existing histoarchitecture and their regional differentiation require a close cross-talk between donor cells and host tissue. Observations on neuronal turnover in the dentate granule cell layer of the adult mammalian hippocampus offer a natural precedent for this process (3). A recently published report on neurogenesis in the adult human hippocampus extends these findings in that it implies that human neural precursor cells may, indeed, incorporate into an existing complex circuitry within the adult human brain (32). Although these observations are restricted to one brain region, they offer an important proof of principle: Neuronal cell replacement in the adult human brain is possible. Experiments in rodents suggest that adult neural precursor cells maintain their ability for regional incorporation even after prolonged propagation in vitro. Gage et al. have cultured cells from the dentate granule cell layer of adult rats up to one year in the presence of FGF2. Upon transplantation into the hippocampus of adult recipient animals, these cells incorporated into the host dentate granule cell layer and acquired morphologies reminiscent of resident granule neurons (44). Remarkably, growth factor-treated adult precursors appear to possess sufficient plasticity to incorporate into heterotopic sites. Hippocampal precursors grown for several months in FGF2-containing medium and grafted into the rostral migratory stream of adult hosts were found to undergo regional differentiation into olfactory neurons which even expressed tyrosine hydroxylase, an enzyme not present in resident hippocampal neurons (127). These observations suggest that brain regions exhibiting postnatal neurogenesis harbor sufficient guidance cues to also recruit cells isolated from heterotopic locations. However, neurogenesis in the adult mammalian brain is restricted to a few regions. Do other regions or lesioned areas show similar guidance cues? Transplant experiments involving the medullary raphe cell line RN33B suggest that this is in fact the case. When these cells were grafted into the adult cortex and hippocampus, they acquired phenotypes indistinguishable from endogenous host neurons in these locations (117).

Only few studies have adequately addressed the question whether precursor cells introduced into lesioned brain regions may find conditions that permit a replacement of lost neurons. This paucity of data most likely results from the complex experimental conditions required for these experiments. Macklis et al. developed an experimental model that uses chromophore-targeted laser photolysis to induce small lesions in the cerebral cortex of postnatal and adult mice. To that end, nanospheres containing the photoactive chromophore chlorin e, are injected into the cortex. The chromophore is retrogradely transported into callosally projecting pyramidal neurons and, upon near-infrared laser radiation, generates singlet oxygen which causes apoptotic cell death of lamina II/III pyramidal neurons (77). When fluorescent-labeled cortical precursor cells were grafted into the lesioned cortex, the labeled cells showed a preferential migration into the depleted laminae and assumed morphologies appropriate for local pyramidal neurons (116). Remarkably, a similar recruitment to a cortical pyramidal phenotype was observed when C17.2 cells, a v-myc-immortalized cell line derived from the external germinal layer of the neonatal mouse cerebellum, were transplanted into these lesions (124). These findings suggest that areas of neuronal apoptosis facilitate the incorporation of grafted neural precursors and promote their recruitment towards a local phenotype.

Functionality of transplanted neurons will critically depend on the establishment of appropriate axonal connections. Several recent findings have turned axonal regeneration in the adult brain into one of the most promising topics in neural repair. Both embryonic and adult neurons have been shown to grow axons upon atraumatic implantation into adult white matter tracts (23, 24). Myelin-associated neurite growth inhibitory proteins present in the adult nervous system can be efficiently neutralized in vivo, yielding long-distance axonal regeneration following transection (8, 114). Remarkably, inhibition of these proteins promotes sprouting and plasticity of both transected and non-transected axons. Following unilateral transection of the corticospinal tract, neutralizing antibody treatment not only increased the target innervation by the transected corticospinal projection neurons but also sprouting of the axons from the non transected corticospinal tract into the denervated hemicord (133). These findings indicate that neutralization of myelin-associated neurite growth inhibitors can restore axonal plasticity in the adult brain to levels similar to those found during CNS development. The innervation of both original target regions and denervated areas implies that the adult nervous system maintains a complex system of axon guidance cues that might be exploited for repair strategies. Several transplant studies have, indeed, shown that fetal neurons transplanted to ectopic sites in the adult brain maintain some degree of axonal target specificity (for an extensive review, see (54)). For example, upon transplantation of ventral mesencephalic cells into the lesioned striatum, the donor-derived dopaminergic axons innervate the striatal gray matter, whereas non-cat-
echolaminergic neurons prefer gray matter regions outside the striatum (55, 56). Together, these findings depict experimental neural transplantation as a versatile tool to elucidate mechanisms involved in regional incorporation and axonal pathfinding of neural precursors grafted into the adult brain.

**Graft survival.** Depending on the transplant paradigm, only a fraction of the grafted cells survives transplantation and differentiates into mature phenotypes. Thus, improvement of graft survival is of paramount importance in clinical neurotransplantation. Recently, several different strategies have been shown to be effective in promoting the survival and function of grafted neural precursor cells, including infusion of growth factors and neurotrophins (86, 110, 111, 118, 150), oxidative stress reduction (93, 94) and inhibition of apoptosis (113). Takayama et al. have shown that survival and function of dopaminergic grafts into the striatum can be significantly improved when the cells are cografted with fibroblasts genetically engineered to express FGF2 (130). These findings indicate that genetically engineered neural cell lines and non neural cells may develop into important tools to support and modulate graft survival and function (122). Strategies that reduce oxidative stress-related neuronal damage include the pretreatment of the donor cell population with lazarooids (93) or overexpression of Cu/Zn superoxide dismutase in the donor cells (94). A recent report by Schierle et al. depicts direct inhibition of the apoptotic pathway as an efficient strategy to improve the survival of fetal nigral transplants. Pretreatment of the ventral mesencephalic donor cell population with a caspase inhibitor resulted in a significant increase of the number of tyrosine hydroxylase-positive cells and improved behavioral recovery of the transplant recipients (113).

**Learning from chimeras**

Although many of the experiments discussed here indicate that grafted neural precursors can successfully incorporate into the adult nervous system, the efficiency of cell migration, regional differentiation and survival does not compare to the tremendous level of plasticity observed in the developing brain. After having established the natural limits of precursor cell recruitment in the adult brain and spinal cord, we are now moving towards an era where active manipulation of both donor cells and host tissue will be required to improve adult neural transplantation. These efforts will be guided by an increased understanding of physiological cell migration and differentiation during CNS development. In analogy to improving axonal regeneration and plasticity by neutralizing neurite growth-inhibitory proteins (133), these approaches will aim at the re-establishment of a developmental host environment and the unmasking of embryonic guidance cues. Chimeric brains generated by intraventricular transplantation provide an exciting experimental model to unveil the molecular mechanisms involved in precursor cell guidance and recruitment. Rodent and human donor cells harboring specific genetic alterations and grafted into wild-type or transgenic recipients will elucidate the role of individual molecules during the integration of individual neural precursors into the CNS tissue. The differential analysis of donor cell migration and differentiation at progressively later developmental stages might reveal crucial changes involved in the conversion of an embryonic environment into an environment less permissive to cellular incorporation. Knowledge gained through these studies will form a valuable basis for the selection of candidate genes that may have to be manipulated to render the adult nervous system more permissive to transplant-based repair.

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