Transplantation as a Tool to Study Progenitors within the Vertebrate Nervous System

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ABSTRACT: In recent years, many studies have focused on the fate and potential of neural progenitors in vertebrates. While much progress has been made, many questions remain about the mechanisms which lead to neural diversity, in terms of both the regionalization of the nervous system and specification of cell fates within those regions. Studies aimed at addressing these questions have fallen into three main categories: *in vivo* lineage tracings, *in vitro* differentiation analyses, and *in vivo* cell transplantation studies. This body of work has pointed to the existence of both pluripotent and unipotent neural progenitors, and has suggested that both cell intrinsic and extrinsic cues play a role in the determination of neural cell fate. In addition, the existence of neural ''stem cells'' maintained into adulthood has been suggested. This review will focus on transplantation studies in mammals, and will emphasize how this method has been useful as a means of determining the changing potential of neural precursors and their environments within the developing nervous system. © 1998 John Wiley & Sons, Inc. J Neurobiol 36: 152–161, 1998

From very early in neural development, it is apparent that different parts of the neural tube are destined for different fates. While the caudal-most region, which will give rise to the spinal cord, remains a relatively simple structure, the rostral end undergoes a combination of evaginations and constrictions which produce the subdivisions of the presumptive brain (Neal, 1919; Martin and Jessell, 1991). As neural development proceeds, the nervous system grows increasingly complex in terms of regional architecture and cellular diversity. Concomitant with this process, neural progenitors, which will give rise to all of the mature cells of both the central nervous system (CNS) and peripheral nervous system (PNS), are required to make increasingly refined fate decisions regarding both their regional identity and cellular phenotype.

It is now well recognized that cues from both mesoderm and ectoderm act to establish regional pattern within the nervous system (Tanabe and Jessell, 1996; Sasai and De Robertis, 1997). Signaling molecules such as sonic hedgehog (shh), bone morphogenetic proteins (Bmps), and Wnts have been shown to play a role in the establishment of both the anterior-posterior and dorsal-ventral identity of the neural tube (Ericson et al., 1995; Pöpperl et al., 1997; Dale et al., 1997; Chang and Hemmati-Brivanlou, 1998). Less clear is the manner in which these extrinsic positional cues are translated into intrinsic regional and cell type restrictions within neural progenitor populations (for overview, see Fishell, 1997). Consequently, numerous fundamental questions remain to be explored regarding the cellular and molecular mechanisms which give rise to the mature vertebrate nervous system. These questions fall into three primary categories: (a) To what degree are there either intra- or interregional differences in the developmental potential of progenitor populations? (b) To what extent does the potential of these populations change as development proceeds? (c) What molecular cues are responsible for generating cellular diversity within and between different regions? With these issues in mind, we will first discuss what is currently understood about neural progenitors and the experimental

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approaches which have led to that understanding. Then we will consider the utility of cell transplantation as an experimental approach for studying both regional identity and cell fate specification during mammalian neural development.

STUDYING THE FATE AND POTENTIAL OF NEURAL PROGENITORS

The fate of a cell is defined as the phenotype that cell will acquire during the process of unperturbed development. However, a given cell's fate may not reflect the full range of what that cell has the potential to become. Some cells may be ''pluripotent,'' or capable of giving rise to numerous phenotypes, depending upon the available cues. Others may be ''unipotent'' and restricted or ''committed'' to a single fate regardless of extrinsic cues. Critical to gaining an understanding of neural development in particular is discerning what neural progenitors are fated to become versus what phenotypes they have the potential to achieve if given appropriate cues. Underlying this issue is the question of what molecular mechanisms control these processes.

Numerous *in vivo* studies addressing the fate of neural progenitors at a variety of developmental stages have been performed in mammals and birds using vital dyes and retroviral vectors as lineage tracers (Fraser et al., 1990; Figdor and Stern, 1992; Walsh and Cepko, 1992; Fishell et al., 1993; Szele and Cepko, 1997). Some of these studies have addressed the regionalization of the neural tube, and have revealed that regional borders in both the forebrain and hindbrain restrict the dispersion of neural cells by acting as boundaries to progenitor movement (Fraser et al., 1990; Figdor and Stern, 1992; Fishell et al., 1993; Neyt et al., 1997). Other *in vivo* lineage studies have considered the phenotypic fate of clonally related cells (Luskin et al., 1988; Walsh and Cepko, 1992; Golden and Cepko, 1996). Such studies in the rodent neocortex, for example, have shown that during early to midneurogenesis (E12–14 in the mouse; E15–17 in the rat), at least some progenitors are pluripotent, giving rise to both neurons and glia, while others may be unipotent giving rise only to a single cell type. While work of this sort has characterized the *in vivo* lineal relationships of neural cells, it is not particularly informative regarding the extent to which these cells are intrinsically committed to their observed phenotypes.

A widely used approach to study cell plasticity has been to culture neural progenitors *in vitro.* Such studies have examined what mammalian neural progenitors from numerous stages and locations can differentiate into in culture (Vescovi et al., 1993; Davis and Temple, 1994; Ghosh and Greenberg, 1995; Qian et al., 1997). Some pioneering work of this sort found that glial progenitors present in the neonatal rat optic nerve could be induced to develop into two types of glia, oligodendrocytes and what have been termed type II astrocytes (Raff et al., 1983). More recent *in vitro* studies have examined the potential of neural crest cells (Stemple and Anderson, 1992; Shah et al., 1994), neocortical and striatal progenitors (Cattaneo and McKay, 1990; Reynolds et al., 1992; Davis and Temple, 1994), and cells from the subependymal layer of the adult brain (Reynolds and Weiss, 1992). This work has found that while the majority of progenitors appear to be restricted to neuronal or glial fates, a small fraction of progenitors can differentiate into both neurons and glia in culture (see Shen et al., this issue). Furthermore, some of these studies have shown that the addition of exogenous factors such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), the neurotrophins (i.e., NT-3), and glial growth factor can influence the fate of these cells (see Cameron et al., Gage et al., and Weiss and van der Kooy, this issue).

Both the *in vivo* and *in vitro* studies referred to suggest that some neural progenitors are specified and possibly committed to a single fate, while others are pluripotent. Of particular interest has been the suggestion by several groups that at least some pluripotent progenitors may be ''stem cells'' capable not only of generating progeny with numerous cells types but also of self-renewal (Davis and Temple, 1994; Morshead et al., 1994; Reynolds et al., 1996). The existence of such a cell type would have major implications for both the mechanisms that generate neural diversity during embryonic development and the possibility of using such cells for therapeutic reintroduction of neurons and glia into the brain (for reviews, see McKay, 1997; Martinez-Serrano and Björklund, 1997). Currently, however, there is little evidence to support or refute the existence of neural progenitors which actually carry out a stem cell–like program *in vivo.*

CELL TRANSPLANTATION AS A METHOD TO STUDY NEURAL DEVELOPMENT

While *in vitro* work has demonstrated plasticity among neural progenitors, it has been limited in that the methodologies used only examine the behavior of progenitors in artificial culture conditions. In particular, it has been difficult to characterize putative

environmental signals which may specify neural cells within a developing structure as distinct from those in neighboring territories. Several *in vitro* studies have attempted to identify if progenitors are restricted to certain cellular phenotypes (Kilpatrick and Bartlett, 1993; Davis and Temple, 1994; Williams and Price, 1995), although none have yet determined if regional identity can be changed *in vitro* at the single-cell level. Regardless of whether such *in vitro* manipulations are possible, transplantation offers an attractive means of addressing this question *in vivo.*

Numerous studies have been done over the past two decades in which embryonic precursors have been grafted into the adult CNS as a means of exploring the potential of cell replacement therapies (see Martinez-Serrano and Björklund, 1997). Increasingly, it has been recognized that transplantation can also be used as a means of investigating cell commitment within the developing CNS. Grafting studies have challenged a multitude of different progenitor populations and have revealed much about the potential of neural progenitors. Studies in which intact pieces of tissue are transplanted between chick and quail have been used to fate map much of the avian CNS, including the neural crest, hindbrain, and forebrain (Couly and Le Dourain, 1985, 1987; Bronner-Fraser and Fraser, 1988; Lumsden et al., 1994). In addition, grafts in which pieces of the neural tube have been shifted in terms of their dorsal/ventral or anterior/posterior position have been informative regarding how regional induction within the spinal chord and hindbrain occurs (Simon et al., 1995; Itasaki et al., 1996).

In mammals, although the limited accessibility of the developing nervous system makes solid grafts difficult, numerous studies have examined when different territories within the cerebral cortex are established. This has been done by heterotopically transplanting rat cortical grafts from various embryonic periods (E12–17) into the neonatal cerebrum (O'Leary and Stanfield, 1989; Schlaggar and O'Leary, 1991; Barbe and Levitt, 1991, 1995). These experiments have shown that at E17, when grafts between territories of the neocortex are plastic, transplants between limbic cortex and neocortex are not. That the decision to become limbic cortex versus neocortex is established earlier in development than the determination of areal specificity within neocortex suggests that progressive restrictions may occur during cortical regionalization. Marked regional differences reflecting cytoarchitecture and function, such as those that distinguish neocortex from limbic cortex, are established first, and more restricted regional character is established later.

In addition to the transplantation of solid grafts in mammals, studies using dissociated cells have been performed into the brains of neonatal and adult animals (McConnell, 1988; Dunnett et al., 1991; Espinosa de Los Monteros et al., 1993; Suhonen et al., 1997). Such transplants are accomplished by injecting cells directly into the tissue of specific brain regions, or ''intraparenchymally.'' While cells injected in this way often remain clumped, significant numbers of individual cells do migrate away from the injection site (McConnell, 1988). This migration makes it possible to evaluate transplanted cells that are surrounded by the host environment and its regional cues. Perinatal intraparenchymal injections within the forebrain have shown that grafted cells often show stereotypic patterns of migration and differentiation typical of the host region (Olsson et al., 1997a). This is not true, however, when embryonic grafts are transplanted into adults (Graybiel et al., 1989; Wictorin et al., 1991).

Recently, embryonic transplantation of dissociated cells has also been shown to be possible in both rats and mice simply by injecting the donor cells into the ventricular cavities of E14–18 rat embryonic brains (Fishell, 1995; Brustle et al., 1995; Campbell et al., 1995). Perhaps surprisingly, cells injected in this manner can efficiently integrate into and disperse throughout the host tissue. This method, in contrast to intraparenchymal transplantation, minimizes damage to the host and permits the widespread distribution of donor cells. Another advantage of embryonic transplantations of this sort is that they permit introduction of donor cells into hosts at earlier stages of development than previously possible. Taken to the extreme, the recent development of a real-time ultrasound imaging system (Olsson et al., 1997b) has permitted the transplantation of cells into murine hosts prior to the onset of neurogenesis, making it possible, at least in principle, to transplant cells throughout the full range of the neurogenic period.

WHAT HAVE WE LEARNED FROM TRANSPLANTATION STUDIES SO FAR?

There are two primary transplantation approaches which have been used to study the mechanisms controlling the regional and cellular identity of neural progenitors: heterochronic and heterotopic (Fig. 1). Heterochronic transplantation is an excellent way to evaluate the extent to which progenitors and/or their environments change during development,

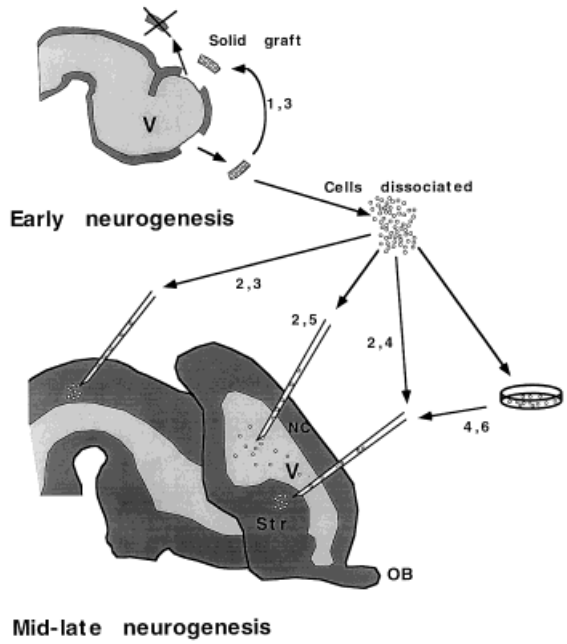

Figure 1 Schematic representation of the various types of cell tranplantation methods discussed. 1 = solid tissue graft; 2 = heterochronic transplantation; 3 = heterotopic transplantation; 4 = intraparenchymal injection; 5 = intraventricular injection; 6 = cultured cells as donors. OB = olfactory bulb; NC = neocortex; Str = striatum; V = ventricle.

while heterotopic transplantation allows the regional commitment of progenitors, as well as the availability of environmental cues within different regions to be evaluated.

An excellent example of the utility of heterochronic transplantation has been its application in studying laminar fate determination in the ferret neocortex (McConnell, 1988; Frantz and McConnell, 1996). During neocortical development neurons are generated in an inside-out pattern with the deep-layer neurons being born first and each more superficial layer of neurons being born sequentially as development proceeds. This pattern of neurogenesis prompts the question: Are the earlier progenitors committed to a deep layer fate and the later progenitors a superficial one, or are signals from the intermediate zone, cortical plate, and marginal zone specifying cortical cell type?

Using heterochronic transplantation, it was found that nearly half of the progenitors isolated from early in neurogenesis could give rise to neurons in more superficial layers when placed in an older environment (McConnell, 1988). While this work suggested that some early progenitors could change their fate, it also suggested that many progenitors at that time were committed to a deep-layer fate. This result indicated that neocortical histogenesis is controlled by a combination of both cell-intrinsic and cell-extrinsic cues, and that early progenitors are heterogeneous in their potential. The same group also performed the converse experiment of transplanting late progenitors into an early environment (Frantz and McConnell, 1996). They found that donor cells normally fated to give rise to superficial layer neurons did so even when placed in a host environment generating deep-layer neurons, suggesting that late progenitors are intrinsically restricted to an upper-layer fate.

Heterochronic transplantation has also been used to examine the behavior of postnatal subventricular zone (SVZ) progenitor cells in the mouse (Lim et al., 1997). SVZ cells from P5–10 were dissociated and transplanted into E15 host brains by intraventricular injection. The donor cells were isolated from a time when they are producing olfactory bulb interneurons. By transplanting these cells into hosts undergoing widespread neurogenesis, it was possible to ask the question: Can postnatal SVZ cells which normally have the potential to generate olfactory bulb neurons also generate neurons in other parts of the neuraxis if present during neurogenesis in those regions?

Transgenic markers and dye labeling were used to track the fate of the transplanted cells, and it was found that postnatal SVZ cells underwent widespread integration (although not into the neocortex or hippocampus), and gave rise to neurons in numerous heterotopic locations including the septum, hypothalamus, and inferior colliculus, in addition to the olfactory bulb. This result demonstrates that the neurogenic potential of SVZ progenitors is not limited to olfactory neurons, and suggests that spatial and temporal position influences the fate of these cells. However, this work also suggests that these cells are specified to some extent, since they cannot contribute to the neocortex or hippocampus, but do give rise to olfactory neurons as they would normally. Furthermore, although SVZ progenitors from postnatal mice could integrate into a range of host areas, they appeared to give rise only to interneuronal phenotypes, albeit with local characteristics.

To address questions of regional commitment, heterotopic transplants have been used. Such transplants can be performed either isochronically or heterochronically, opening the way to simultaneously testing the extent to which local inductive cues within specific CNS regions change during development. Several groups have examined the behavior of mouse neural progenitors during embryogenesis when transplanted to heterotopic locations (Fishell, 1995; Brustle et al., 1995; Campbell et al., 1995).

These experiment were done using progenitors from both neocortical and ventral telencephalic proliferative zones, including the regions which give rise to the globus pallidus and the striatum. Intraventricular injection of dissociated cells was used to perform the transplants. Host animals were examined after transplantation to evaluate the extent to which progenitors from the dorsal and ventral telencephalon could integrate into different regions of developing brain and whether the integrated donor cells could take on phenotypes characteristic of their new environments. Despite these grafts being done at a variety of times from early to late neurogenesis, all groups observed that ventral telencephalic cells are able to integrate widely and differentiate appropriately within a number of different telencephalic host regions. This was judged by their morphology, their expression of host region–specific markers, and their ability to make host-specific axonal projections. Specifically, it was shown that striatal progenitors which integrated into the cortex took on a pyramidal morphology and made commissural projections to the contralateral cortex (Fishell, 1995), whereas those that integrated into the septum were induced to express the low-affinity nerve growth factor (NGF) receptor (Campbell et al., 1995).

It may be tempting to conclude that if progenitors are able to integrate and persist within heterotopic regions, then they are likely to have adopted a fate appropriate to that host region. Such suggestions should be treated with caution. Recently, our group has shown that even though ventral telencephalic cells can integrate throughout the diencephalon and mesencephalon, they continue to express telencephalic-specific markers through to adulthood (Na et al., submitted). BF-1 is a winged-helix protein whose expression is restricted solely to the telencephalon, and within this structure it is present ubiquitiously and at high levels throughout life. Interestingly, the *lacZ* gene directed by the endogenous BF-1 locus remains strongly expressed in telencephalic progenitors which have integrated into either the diencephalon or mesencephalon, even after long-term survivals. Hence, while striatal precursors can trans-fate into other telencephalic phenotypes, they appear to be unable to acquire normal diencephalic or mesencephalic identities.

Numerous lines of evidence suggest that all progenitor populations are not equivalent. For instance, ventral telencephalic precursors (i.e., striatal or globus pallidal precursors) appear to be more promiscuous in terms of where they can integrate compared with either their dorsal telencephalic counterparts (i.e., cortical cells) (Olsson et al., 1998; Frantz and McConnell, 1996) or diencephalic cells (Na et al., submitted). Interestingly, it also appears that even identical progenitor populations behave quite differently depending on the means used to isolate them. In particular, the methods used to dissociate cells for transplantation can significantly effect whether they integrate heterotopically or homotopically. In studies which observed preferential homotopic integration of grafted progenitors, the donor cells were not treated with proteases to remove cell surface molecules (Campbell et al., 1995). Indeed, a recent study comparing the integration of trypsinized versus nontrypsinized telencephalic precursors noted that the removal of surface adhesion molecules greatly increases heterotopic integration (Olsson et al., 1998). This is consistent with *in vitro* work which has demonstrated that striatal and cortical progenitors can sort out from one another based on cell-surface adhesion molecules (Götz et al., 1996). Together, these studies suggest that part of how regional territories are maintained during development is through homotypic adhesion mechanisms. Interestingly, homotypic adhesion appears to be more involved in maintaining regional coherence than regional identity: when regionally specified adhesive properties are removed enzymatically, many cells can integrate heterotopically and differentiate according to cues in the host environment.

While the work described above has clearly demonstrated the utility of intraventricular injections to transplant progenitors, it has also highlighted a potential limitation of this method: The transplantation of cells in this way relies upon their ability to integrate of their own accord. Although at least in the case of ventral telencephalic precursors protease treatment seems to relieve the problem of selective integration, it remains possible that only a subpopulation of the transplanted cells are successfully integrating. This possibility suggests that embryonic intraparenchymal injections would provide a valuable complement to intraventricular injections. Until recently, however, precisely directed intraparenchymal injections were not feasible during the majority of embryogenesis owing to an inability to visualize the injection site. The use of real-time ultrasound imaging to guide injections into mouse embryos has made a wide variety of highly precise intraparenchymal injections now possible. Experiments by Olsson et al. (1997b) have demonstrated the feasibility of such an approach. Progenitor cells isolated from early embryonic telencephalon and the midhindbrain region were transplanted by injection directly into either the ventral telencephalon or developing midhindbrain targets. These studies have found that different precursor populations become more restricted in terms of the phenotypes they can

adopt as development progresses, and that different positions within the neuraxis become restricted to their regional phenotypes at different times. For example, while at E13.5 telencephalic striatal cells can still adopt a cerebellar phenotype upon heterotopic transplantation, at this time in development, midhindbrain progenitors cannot adopt a globus pallidus fate. Notably, however, when midhindbrain progenitors from earlier times such as E10.5 are transplanted to the E13.5 ventral telencephalon they can trans-fate. This general approach should continue to prove a powerful system for testing the potential of progenitors.

TRANSPLANTATION OF PRIMARY VERSUS CULTURED PROGENITORS

The majority of the transplantation studies described above have used primary cells as donors. Currently, there are several limitations to this sort of approach. First, the extent to which primary donor cells are a heterogeneous population is difficult to assess. For instance, it seems likely that progenitors within any given proliferative zone are not equipotent. Numerous lines of evidence, including the purported existence of cell type–restricted progenitors in the E12–14 mouse forebrain (Luskin et al., 1988) and the salt-and-pepper expression of genes such as *Delta* (Bettenhausen et al., 1995), *Mash-1* and *dlx2* (Porteus et al., 1994) within the proliferative zones, support this notion. Such heterogeneity obscures whether the phenotypes generated in grafting experiments result from trans-fating of specified progenitors or the selective differentiation of a subpopulation of plastic progenitors contained among the grafted pool. A second limitation of working with primary donor populations is that they are difficult to genetically modify. Such modifications would permit the analysis of the effect of certain genes on the behavior of progenitors in the context of different regional cues. While it is possible to use retroviral vectors to transduce genes into progenitors *in vivo* (e.g., Ishibashi et al., 1994; Burrows et al., 1997), such studies are limited because it is difficult to account for the preexisting differences between infected progenitors within and between different regions. The standardized comparison of both regional cues and intrinsic cues requires the use of a donor population which is homogeneous, or at least well-characterized with respect to any existing heterogeneity.

In the past, as an alternative to the use of primary cell donors, immortalized cell lines were used for transplantation studies (Renfranz et al., 1991; Snyder et al., 1992; Gao and Hatten, 1994). These studies showed that neurally derived cell lines immortalized with oncogenes such as v-*myc*, and the SV40 large T antigen, can be transplanted back into either the cerebellum or hippocampus and take on both neuronal and glial phenotypes appropriate for the host region. For example, one study demonstrated that immortalized cerebellar granule cell progenitors can give rise to stellate neurons, Golgi neurons, Bergmann glia, and astrocytes, in addition to granule cells, upon transplantation back into the cerebellum. In contrast, primary granule cell progenitors transplanted in the same manner are limited to a granule cell fate (Gao and Hatten, 1994). This work shows that these cerebellar progenitors can be genetically modified to expand their potential. Furthermore, this use of an apparently homogeneous donor population suggests that the postnatal cerebellar environment may possesses cues capable of instructing the development of a wide variety of cell types.

Other prospects for cultured donors include progenitors which have been propagated in culture but remain pluripotent as judged by *in vitro* differentiation assays (Reynolds et al., 1992; Reynolds and Weiss, 1992; Kilpatrick and Bartlett, 1993; Davis and Temple, 1994). Considering the degree of interest in the possible existence of neural ''stem cells,'' it is surprising that more studies have not considered the behavior of these cells *in vivo.* This is particularly true in light of the fact that while O2A cells generated a great deal of excitement because they are bipotential *in vitro,* when these cells were transplanted back into hosts *in vivo,* they give rise only to a single cell type, oligodendrocytes (Espinosa de Los Monteros et al., 1993). This result is a sobering reminder that while *in vitro* studies may be informative, ideally they should be coupled with *in vivo* studies where possible, to more fully understand the process being examined.

Along these lines, a very recent study has addressed the behavior of EGF-responsive neural progenitors after transplantation *in vivo.* Based upon *in vitro* work which has demonstrated that such cells possess stem cell–like properties and can differentiate into neurons and glia in culture (Reynolds and Weiss, 1996), it might have been predicted that *in vivo* they would do the same. Instead, EGF-responsive progenitors taken from both the forebrain and midbrain differentiated predominantly into glia *in vivo* (Winkler et al., 1998). Considered together with the O2A work and the embryonic transplantation studies described earlier, it is increasingly clear that *in vivo* environments can influence the behavior

of transplanted cells in ways that are not predictable from *in vitro* studies.

Interestingly, the fact that EGF-responsive progenitors differentiate into glia *in vivo* fits in nicely with several other recent reports suggesting that EGF signaling may promote glial differentiation. One such study found that overexpression of the EGF receptor in the developing neocortex led to the premature expression of glial markers (Burrows et al., 1997). Other studies which have continuously perfused EGF into the adult rodent brain have found that gliogenesis is strongly stimulated, apparently at the expense of neuronal production (Craig et al., 1996; Kuhn et al., 1997). This is suggested by the fact that in animals treated in this manner, the generation of olfactory neurons appears to be inhibited. A picture is emerging from this work which suggests that although most glia are normally generated during late embryogenesis and early postnatal ages, what limits progenitors from acquiring a glial fate early may be a cell-intrinsic property (e.g., the inability to respond sufficiently to an EGF-like ligand), and not the absence of environmental cues.

FUTURE DIRECTIONS FOR TRANSPLANTATION

What, then, can be garnered from the recent spate of heterochronic and heterotopic transplantation experiments? Clearly, long after regional gene expression and differentiation is initiated, some precursors remain competent to adopt novel fates when challenged through transplantation. In addition, local inductive cues appear to persist within regional territories throughout much of development. On the other hand, the repertoire of fates that specific progenitor populations can adopt becomes increasingly limited as development proceeds. While ongoing transplantation studies continue to define the potential of groups of neural progenitors isolated from different regions, the next challenge will be to determine the role of heterogeneity within regional progenitor pools in generating cellular diversity. Ultimately, it will be interesting to directly test the extent to which specific patterns of gene expression are markers of cell potential.

It will also be of interest to define the molecular basis of regional inductive cues. While recent studies of early regional patterning have shown that relatively long-range signaling by molecules act to establish general territories, it seems likely that later inductions such as those seen in heterotopic or heterochronic grafts rely on shorter-range or cell–cell-mediated signaling (reviewed in Fishell, 1997). Numerous ectopic and overexpression studies have considered the role of molecules which may regulate short-range signals determining neural fate in vertebrates including genes in the Notch-Delta signaling pathway (see Nye and Kopan, 1995) and the EGF receptor (Burrows et al., 1997). While such studies will continue to provide indications of how short-range signals direct cell fate within the CNS, the possibility of combining gain of function gene expression studies with heterochronic or heterotopic transplantation studies presents an attractive method for testing the role of specific molecules in development.

What can we expect from the next generation of transplantation studies? At its heart, grafting remains an essential test of progenitor potential. While many details remain to be elucidated concerning the plasticity of neural progenitors, it seems likely that future studies will shy away from gross methods where regional populations are grafted en masse. Ongoing work throughout many labs will no doubt gradually bring to light the cell-intrinsic molecules which act to generate progenitor heterogeneity. The identification of such molecules should permit a more refined selection of progenitor pools. This prospect, coupled with the genetic manipulation of environmental signals using transgenic and mutant mouse strains, promises to create a powerful system for understanding the interplay between cell-intrinsic and -extrinsic cues during progenitor maturation. Then the real work of teasing apart the molecular mechanisms which regulate vertebrate neural development will begin.

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