Chapter 1

Introduction

The central nervous system (CNS) is one of the most important organs of an animal. It is also a most complicated one, comprised of hundreds of cell types and millions of cells in higher vertebrates. Despite popular beliefs, the vast majority of cells in the central nervous system are not neurons, but glial cells, specialized cells that help to construct the nervous system during embryonic development as well as maintaining its daily functions in adult life (Kettenmann 1995). It is estimated that glial cells occupy half of the brain space and outnumber neurons by 10 to 1 (Kettenmann 1995; Zhang 2001).

There are 3 major classes of glial cells residing in the vertebrate CNS. Oligodendrocytes form myelin sheets that wrap around axons, providing insulation as well as enabling saltatory (jumping) conductance for currents traveling down the axons (reviewed in Pfeiffer et al. 1993). The more numerous astrocytes, on the other hand, perform a plethora of tasks including balancing pH and ion concentrations, recycling neurotransmitters, storing energy, regulating vasculature, and forming blood-brain barriers (Kettenmann 1995). Recent findings even indicate that astrocytes communicate with neurons at the synapses (Fields and Stevens-Graham 2002). Microglial cells, the third major class of vertebrate CNS glia, are the immune cells of the brain (Kaur et al. 2001; Kettenmann 1995). Although the origin of microglia is still under debate (Barron 1995; Kaur et al. 2001), oligodendrocytes and astrocytes both derive from the neuroectoderm (Kettenmann 1995).

Traditional studies of glial development focus on isolated glial precursors and their subsequent differentiation steps (Raff 1989; Pfeiffer et al. 1993; Miller 1996; Lee et al. 2000). The earlier fate specification event, i.e., how glial cells are generated from uncommitted neuroepithelial cells, remains largely unexplored. Despite this, it is now clear that glial cells and neurons both derive from multipotent neural stem cells. In addition, neurons are generated before glial cells. These two key observations raised several important questions: What controls the neuron-glia decision? What mechanisms control the temporal switch from producing neurons to producing glial cells? And what factors are involved in neuronal and glial fate specification? I will focus the first part of the introduction on discussing the current understanding of these questions in the context

of neural stem cell biology. In the second part of the introduction, I will review the spatial origins of CNS glial cells and their differentiation steps.

Neural Stem Cells (NSC)

Neural stem cells are generally defined as self-renewing neural precursors capable of generating multiple neural lineages including neuron and glia (Morrison et al. 1997; Gage 2000). To be defined as a NSC, a given neural precursor therefore has to satisfy two criteria: first, to give rise to both neuronal and glial progenies; second, the ability to self-renew. Although the first criterion is relatively easy to assess, the second one is more difficult. It involves either subcloning if the putative NSC can be cultured *in vitro* (Stemple and Anderson 1992; Johe et al. 1996; Reynolds and Weiss 1996; Palmer et al. 1997) or serial transplantation if the putative NSC can not be cultured. The latter approach, however, has never been used in the NSC field. Although prospective isolation of NSCs has been accomplished in a few cases (Morrison et al. 1999; Uchida et al. 2000; Keyoung et al. 2001; Rietze 2001), most NSCs characterized to date are identified from culture.

Classification

Vertebrate NSCs can be broadly classified as either CNS or PNS stem cells, which are responsible for generating all neuronal and glial lineages in the vertebrate CNS and PNS, respectively. Both CNS and PNS stem cells can be further divided as fetal and adult forms. An alternative way of classifying NSCs is by their developmental potential, i.e., the full repertoire of neural cell types that a NSC can give rise to. Given that there are hundreds of different neuronal types and perhaps a dozen glial types, it is very difficult, if not impossible, to determine the full developmental repertoire of a given NSC. Another problem that complicates the determination of true developmental potential of NSCs is the issue of reprogramming (Anderson 2001; Temple 2001). As most NSCs are derived from tissue cultures, where they are exposed to high concentrations of growth factors such as basic FGF and EGF, their developmental potential may be artificially expanded (Kondo and Raff 2000). This problem, however, can be circumvented if freshly isolated

NSCs without any culture are used in transplant experiment to assess the potential of a given NSC (Morrison et al. 1999; White et al. 2001; Bixby et al. 2002).

Adult NSC

The discovery of adult neural stem cells and adult neurogenesis in vertebrate species has come as a surprise given that prior studies suggested that neuronal loss in the vertebrate CNS is irreversible (reviewed by Temple and Alvarez-Buylla 1999; Gage 2000; Anderson 2001; Rakic 2002). It is now generally accepted that there is ongoing neurogenesis in the adult vertebrate brain. In mammals, it seems to be concentrated in the subventricular zone of the forebrain and the subgranular zone of the dentate gyrus of the hippocampus (Temple and Alvarez-Buylla 1999; Gage 2000; Anderson 2001). Some of the adult NSCs in the murine subventricular zone have been identified *in situ* as GFAP+ cells (Doetsch et al. 1999; Laywell et al. 2000). Neurons generated from adult NSCs are electrically excitable and can integrate into existing neural circuits (van Praag et al. 2002). What remains unknown, however, is the functional significance of this continuing neurogenesis in the adult brain.

Neural stem cell heterogeneity

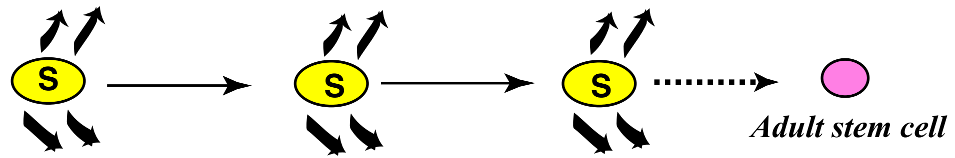
Although we are accustomed to think of neural stem cells as a homogenous population, increasing evidence suggests that in fact they are not. For example, adult PNS stem cells appear to have narrower developmental potential and less self-renewal ability compared to their fetal counterpart (Kruger et al. 2002). In addition, CNS stem cells isolated from late embryonic development produce fewer neurons in clonal cultures than those isolated from earlier stages (Qian et al. 1998; Qian et al. 2000). Even NSCs isolated at the same developmental time point but from different regions of the nervous system exhibit different developmental capacities (Kempermann et al. 1999; Bixby et al. 2002).

How to explain this heterogeneity of NSC? The different populations of NSCs could either have been produced simultaneously from a more primitive stem cell, or alternatively, early NSCs with broader developmental potential may give rise to later stage NSCs with increasingly narrower potentials as time progresses. The second model

would postulate that vertebrate NSCs do not strictly self-renew but rather change over time (Figure 1). Such a model is indeed used by the neuroblasts in the fly central nervous system, which are the fly equivalents of vertebrate CNS stem cells (Pearson et al. 2001; Brody and Odenwald 2002). Fly neuroblasts go through successive stages of development. Each stage is marked by the expression of a unique transcription factor, which endows the neuroblast with the capacity to generate certain cell types (Pearson et al. 2001; Brody and Odenwald 2002). Although there is yet no convincing evidence suggesting that the vertebrate NSCs use a similar mechanism, intuitively, this dynamic change model of NSC development makes sense at least for fetal NSCs. As the construction of the nervous system is a highly ordered process, each neuronal and glial cell type is only produced within a certain time window. There is simply no need for fetal neural stem cells to strictly maintain the full developmental repertoire all the time. Indeed, all fetal NSCs cease to exist once the construction of nervous system is complete. Adult NSCs, on the other hand, may self-renew in a strict manner in order to maintain the stem cell pool that will be required for all adult life, similar to the adult hematopoietic stem cells (Morrison et al. 1994; Weissman 2000; Weissman et al. 2001). If indeed the property of neural stem cells changes quantitatively (in terms of what neural subtypes to generate and how many) but not qualitatively (neuron-glia multipotent) over time, a more fitting definition for neural stem cells is perhaps as the following: neural progenitor cells that are capable of producing at least one multipotent progeny.

Although the dynamic change model can explain the temporal heterogeneity of NSC, i.e., NSCs isolated from the same location but different time are different. Can this model also explain the spatial heterogeneity, i.e., NSCs isolated from the same time but different locations are also different? Indeed, as proposed in the fly model (Pearson et al. 2001; Brody and Odenwald 2002), if NSCs from different regions go through a similar series of transformations but initiate this process at a different time point, the spatial heterogeneity can be explained. According to this model, NSCs in the entire CNS are fundamentally the same at the beginning. Alternatively, there is no one generic NSC, instead, multiple fetal neural stem cell types may coexist before the onset of neurogenesis, for example, fetal spinal cord NSC, fetal forebrain NSC, and fetal PNS

Fetal stem cell



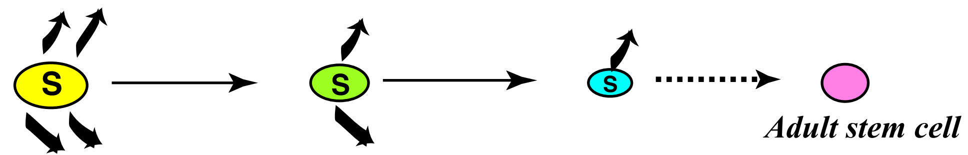


Figure 1. Two models of neural stem cell lineage.
Precise self-renewal vs. Progressive restriction

stem cell. Each fetal NSC type may change with time in a different manner. To distinguish between these possibilities, it is critical to find additional NSC markers and to develop better *in vivo* single cell lineage tracing methods.

Regardless of the theoretical debates, in practice, the property of cultured NSCs varies according to species, strains, the region where they derive from, the developmental stage when they are isolated, and the medium and substrate used to culture them (Gage 2000). As cultured NSCs are now routinely used in different studies, it is important to understand this heterogeneity and efforts should be made to further standardize the culture system.

Control of NSC proliferation and multipotency

What is unique about NSCs, like all other stem cells, is their multipotency. Theoretically speaking, the maintenance of multipotency may need a dedicated set of factors whereas the proliferation of NSCs can be controlled by the same genes that are involved in the mitosis of all other cell types. Alternatively, the proliferation and multipotency of NSCs may be coupled, so that common factors regulate both processes.

Cultured CNS NSCs can only proliferate extensively in the presence of bFGF and/or EGF. Withdrawal of these growth factors results in cell cycle arrest and rapid differentiation of the NSCs (Reynolds et al. 1992; Kilpatrick and Bartlett 1993; Ray et al. 1993; Vescovi et al. 1993; Kilpatrick and Bartlett 1995). In addition, infusion of bFGF or EGF in live animals stimulates proliferation of selective groups of NSCs *in situ* (Craig et al. 1996; Martens et al. 2002). Although bFGF and EGF can clearly promote the proliferation of NSCs, it is not clear whether they also help to maintain the multipotency. Similarly, a nucleolar protein called nucleostemin has been reported to influence the cell-cycle behavior of NSCs (Tsai and McKay 2002). Whether this gene can regulate the multipotent state of NSCs was not examined. The expression of nucleostemin is concentrated in stem cell populations and down-regulated upon differentiation (Tsai and McKay 2002). It remains to be resolved as to why this gene is uniquely required for stem cells.

Contrary to NSC growth factors and neucleostemin, the *pten* tumor suppressor, a lipid phosphatase that negatively regulates the phosphatidylinositol 3-kinase (PI3K) pathway, is a negative regulator of NSC proliferation (Groszer 2001). Compared to wild type animals, more neurospheres (a type of NSC) can be established from *pten* mutants. In addition, the percentage of NSCs per neurosphere is also higher in *pten* mutants (Groszer 2001). These phenotypes have been attributed to shortened cell cycles for *pten* mutant NSCs.

Another approach that has been taken to identify unique factors of stem cells is to compare the transcription profiles of different stem cell populations (Ivanova et al. 2002;Ramalho-Santos et al. 2002). Melton and colleagues compared the mRNA of mouse embryonic, neural and hematopoietic stem cells against differentiated neural and blood cell types with microarrays and found a set of about 200 genes that are only shared by the stem cells (Ramalho-Santos et al. 2002). Similar comparisons by Lemschika and colleagues also revealed a small number of common transcripts (Ivanova et al. 2002). Theoretically, these unique stem cell factors may contain candidate genes that are required to maintain multipotency in all 3 stem cell populations. This conclusion, however, is based on the assumption that there is a common "stemness" program that is shared among different stem cell populations. It is possible that different genes are employed in different stem cell population to define their "stemness." Some examples are the Pou domain transcription factor Oct3/4 in ES cells (Masui et al. 2002), the homeodomain transcription factor HoxB4 in hematopoietic stem cells (Miyazaki et al. 2000; Kyba et al. 2002; Sauvageau et al. 2002), and Wnt/b-catenin pathway in epidermal stem cells (Watt 1998; Watt and Hogan 2000). Recent evidence suggested that Notch might be involved in maintaining the multipotent state of neural stem cells (Hitoshi et al. 2002).

Neuron-glia decision

The most fundamental decision that a neural cell makes is to choose between a neuronal and a glial cell fate. What controls this crucial decision? Accumulating evidence suggests that vertebrate neurogenic bHLH transcription factors promote neuronal fate

while simultaneously repressing glial fate, thus fulfilling the criterion for a molecular switch between neuronal and glial fate.

Most early studies on neurogenic bHLH genes focus on their role in promoting neurogenesis (Bertrand et al. 2002). Recently, however, it was shown that ectopic expression of neurogenin 1 (Ngn1) repressed astroglial fate in cultured neural stem cells (Sun et al. 2001) and over-expression of NeuroD in mouse retina completely blocked the generation of Muller glial cells (Morrow et al. 1999). Conversely, neuronal markers from many brain regions of mash1-/-math3-/- and Mash1-/-Ngn2-/- animals were lost while GFAP and S100b, two astroglia markers, were up-regulated ectopically and precociously (Tomita et al. 2000; Nieto 2001). In NeuroD null mutant retina, the number of Muller glia increased by 3- to 4- fold (Morrow et al. 1999). Similarly, in mash1 and math3 double mutant retina, bipolar neurons were replaced by Muller glial cells (Tomita et al. 2000). These studies are consistent with the idea that neurogenic bHLH factors regulate the neuron-glia decision in vertebrate species. Michael Greenberg and colleagues further demonstrated that promotion of neuronal differentiation by Ngn1 is mediated via a DNA-dependent mechanism whereas repression of astroglial differentiation is DNA independent, and likely involves competition for cofactors with intracellular astroglia-promoting genes (Sun et al. 2001).

Notch is another important molecule that has been implicated in the neuron-glia decision. Activated Notch instructs the generation of glial cells at the expense of neuronal and smooth muscle cell fates in cultured rat neural crest stem cells (Morrison et al. 2000). Ectopic Notch activation promotes Muller glial generation in mouse retina (Furukawa, 2000), and Notch1 and Notch3 instructively commit adult rat CNS stem cells into the astroglial lineage (Tanigaki et al. 2001). Given that Notch activates the Hes family of transcription repressors which in turn inhibit the transcription of neurogenic bHLH factors (reviewed in Kageyama and Nakanishi 1997), and that the glia-promoting activity of Notch can be mimicked by Hes in some settings (Furukawa, 2000), it is highly likely that Notch promotes glial fate at least partially via repressing repressors of glial development, i.e. the neurogenic bHLH factors. What is yet to be resolved, however, is

whether Notch can also activate pro-glial factors. It should be noted that the function of Notch is highly context-dependent (Chambers et al. 2001). In many systems, activation of Notch keeps progenitor cells in an undifferentiated state instead of promoting gliogenesis (reviewed in Artavanis-Tsakonas 1999). In addition, there are examples of Notch promoting neurogenesis (Van Der Bor et al. 2001). It is important to understand the basis of these functional differences elicited by the same Notch pathway.

If neurogenic bHLH factors are the only direct regulators of neuron-glia decision in vertebrate, this mechanism of neuron-glia decision in vertebrate would seem to differ significantly from the one employed by invertebrates (Figure 2). In fly, Gcm is the master glial regulator (reviewed in Jones 2001; Van Der Bor et al. 2002). Expression of Gcm is both necessary and sufficient for the development of most fly glial cells at the expense of neurons (Jones et al. 1995; Hosoya et al. 1995; Vincent et al. 1996). Gcm functions by positively regulating the expression of glial specific genes such as *repo* and *pointed* while simultaneously repressing neuronal differentiation by turning on the transcription repressor *tramtrack* (reviewed in Jones 2001; Van Der Bor et al. 2001).

Vertebrate neurogenic factors and invertebrate Gcm genes may not be the only regulators of the neuron-glia decision. It is possible that there are both neurogenic and gliogenic master regulators in vertebrate as well as invertebrate, and they can cross-repress each other (Figure 2). Whether a neural cell becomes neuron or glia is dependent upon which master regulator's expression dominates in the end. Both the developmental history of the cell (intracellular factors) as well as environments (extracellular factors) may exert influence on this decision. Once a cell fate is chosen, other mechanisms presumably will step in to permanently lock the cell in this fate. This competition model predicts the existence of key glial regulators in vertebrate, which are yet to be found. On the other hand, obvious candidates of invertebrate neurogenic regulators are proneural bHLH factors. To date, however, it has been very difficult to demonstrate that proneural bHLH factors, such as the Achaete-Scute and Atonal genes in fly, also possess "proneuronal" properties because of their early "proneural" function. To distinguish between these two functions, precise temporal control of the proneural gene expression is

required. Additionally, it is also not clear whether fly proneural genes can suppress Gcm transcription or interfere with its function.

Temporal control of Neuron-glia decision

Neurons are generated before glial cells. This conclusion is supported by evidence from many systems. *In vivo*, mature glial cells appear predominantly in postnatal development whereas neurons appear prenatally (Bayer 1991). Although it has been argued that glial precursors may be produced at the same time as neuronal precursors and that only the terminal differentiation of glial cells is delayed, recent studies with early glial precursor markers support the view that glial specification is indeed after that of neurons (Yu et al. 1994; Hall et al. 1996; Zhou et al. 2000; Lu et al. 2000). Additionally, Sally Temple and colleagues have used video microscopy to trace the development of single NSCs in culture and retrospectively reconstructed the family tree of single NSCs (Qian et al. 1998; Qiao et al. 2000). Even in the defined culture conditions, neuroblasts are produced before glioblasts, suggesting that the temporally order of neuron-glia production is largely intrinsic to neural stem cells (Qian et al. 2000). The only violation of this neuron first principle is found in certain grasshopper neural lineages (Condron and Zinn 1994).

Several models have been advanced to explain the temporal control of neuron-glia generation (summarized in Morrison 2000). They can be largely categorized into the intrinsic model, the extrinsic model, and the combination model (Figure 3). The intrinsic model proposes that neural stem cells may contain a cell-intrinsic clock that counts time or cell division and that determines neurons first and glial cells after. The internal timer has been suggested to be telomere length, the cyclin-dependent kinase inhibitor p27, and growth factor receptors for EGF and FGF (Morrison 2000). Although over-expression of EGF receptor and p27, and higher FGF level has been shown to favor the differentiation of glial lineages (Burrows et al. 1997; Ohnuma et al. 1999; Qian et al. 1997), it is not clear whether the expression level of any of these candidate molecules changes over time in neural stem cells. Nor is there any evidence suggesting a link between telomere length and cell fate specification. Methylation of glial gene promoters may be another intrinsic

control mechanism blocking glial generation during early development (Takizawa et al. 2001). Again, what controls the methylation status temporally is unknown.

The extrinsic model proposes that environmental factors present during early embryonic development favor neuronal development whereas those present at later stages favor glial development. That environment plays an important role in neuron versus glial choice is well documented in many systems (Qian et al. 1997; Morrow et al. 2001; Shah et al. 1996; Shah et al. 1994; Cepko 1999; Morrision et al. 1997). In some cases, the identities of these environmental factors are known (Qian et al. 1997; Morrow et al. 2001; Shah et al. 1996; Shah et al. 1994). What is not known, however, is what in turn controls their temporal expression.

Lastly, the sequential production of neuron and glia may be under the influence of both intrinsic and extrinsic cues. This combination model has been proposed for the orderly generation of retinal cell types (Cepko 1999). Again, the exact players and their interactions in retina are not fully understood. By definition, combination models also include the feedback model, which suggests that neural stem cells are programmed to generate neurons first (intrinsic) and that newly produced neurons send a feedback signal to control the subsequent generation of glial cells (extrinsic) from the same stem cells. Although definitive evidence is yet to be found to support this model in the sequential generation of neurons and glia, examples of the feedback model has been discovered in the sequential generation of different neuronal groups from the same precursors (Sockanathan and Jessell 1998). For example, early born motoneurons send a retinoid-based feedback signal to influence the number and subtype of later-born motoneurons in the spinal cord (Sockanathan and Jessell 1998). In addition, it has been reported that in cultured neural crest stem cells, cell-cell interactions mediated by Delta-Notch can gradually increase the ratio of Notch to Numb (a Notch inhibitor) in the stem cells (Kubu et al. 2002). Elevated Notch receptor level increases the responsiveness of these stem cells to the Notch ligand Delta, therefore increases the probability of these cells to generate glial cells (Kubu et al. 2002).

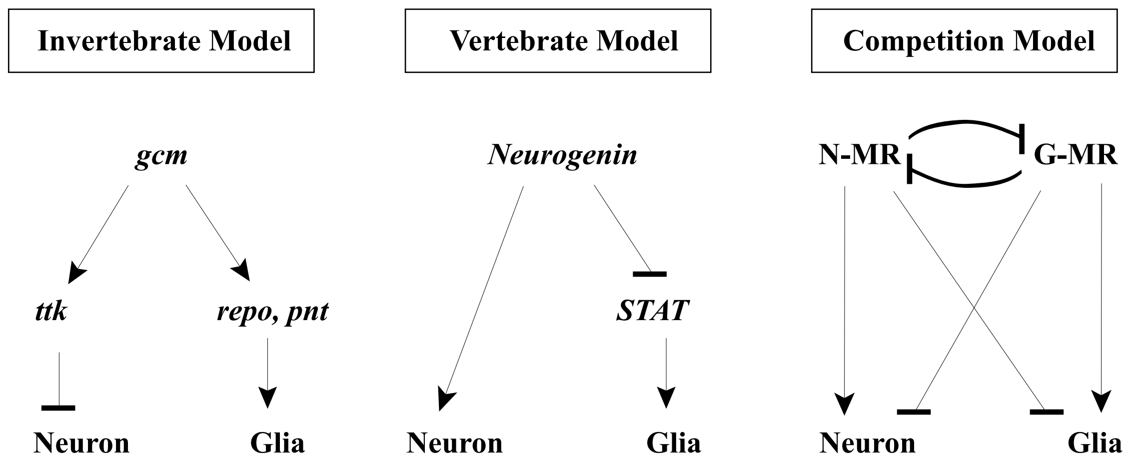


Figure 2. Molecular mechanisms of Neuron-glia decision

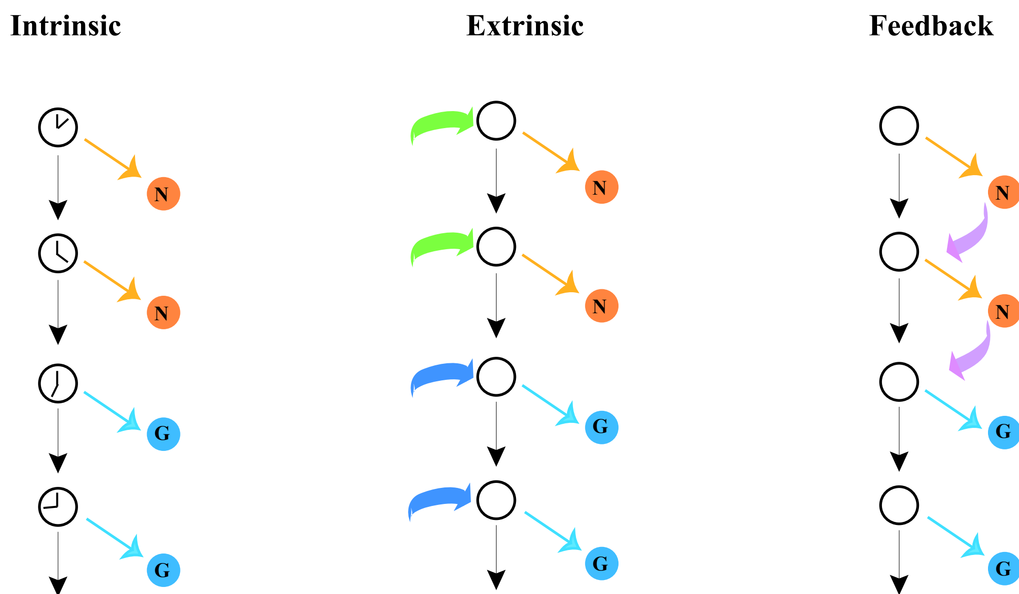


Figure 3. Temporal control of Neuron-glia switch

All the temporal control mechanisms discussed above presumably converge on regulators of neuron-glia fate decision, which includes at least neurogenic bHLH factors in vertebrate and gcm in invertebrate.

Neural subtype specification

Neuronal subtypes

After a neural cell decides to become either a neuron or a glial cell, it has to further choose among hundreds of neuronal and perhaps a dozen glia subtypes. How is this accomplished? In the best studied case of neuronal subtype decision, a combinatorial code of homeodomain transcription factors determines the subtype identity of major spinal neuronal groups, including V0 to V3 interneurons and motoneuron (Briscoe et al. 2000; reviewed in Jessell 2000). The motoneuron group can be further classified into different subgroups according to their axonal trajectory, cell body position and molecular marker differences (reviewed in Jessell 2000). Homeodomain factors, retinoid mediated cell-cell interactions have been implicated in motoneuron subgroup decisions (Sharma et al. 1998; Kania 2000; Socknathan and Jessell 1998). Taken together, these results suggest that the final subtype that a given neuron chooses is decided in a hierarchical, stepwise fashion. At each step, the neuron makes a binary or multiple choice.

Besides homeodomain factors, neurogenic bHLH factors, although primarily known for their proneural functions, can also influence the subtype decisions of the neurons that expressing them (Cubas et al. 1991; Jarman et al. 1993; Lo et al. 1998; reviewed in Bertrand et al. 2002). For example, in fly, the Atonal gene restricts the fate of neurons to that of a chordotonal organ (Jarman et al. 1993) whereas Achaete-Scute specifies external sensory organ fate (Cubas et al. 1991). In addition, expression of the mammalian Achaete-Scute homologue, mash1, has been shown to initiate both a pan-neuronal and a subtype specific program via induction of Phox2a (Lo et al. 1998). Olig2, although itself has no proneural activity, can nevertheless instruct subtype commitment by turning on appropriate homeodomain factors (Mizuguchi et al. 2001; Novitch et al. 2001). These results suggest that it is perhaps a general theme that neurogenic genes regulate neuronal subtype choices via inducing homeodomain factors.

Oligodendrocyte specification

The bHLH transcription factors Olig2 and Olig1, a main focus of my thesis research, are absolutely required for oligodendrocyte fate determination (Lu 2002; Park HC 2002; Takebayashi et al. 2002; Zhou and Anderson 2002). In Olig1,2 double null mutants, no oligodendrocyte precursors are produced from the entire mouse neurotube (Lu et al. 20002; Takebayashi et al. 2002; Zhou and Anderson 2002). Even neurospheres (one type of neural stem cell culture) established from Olig1,2 mutant spinal cord fail to give rise to any oligodendrocytes (Zhou and Anderson 2002). As we discussed before, neurogenic bHLH factors control the neuron-glia decision. Consistently with this, the pro-oligodendrocyte function of Olig genes can only be revealed in the absence of neurogenic factors (Zhou et al. 2001).

Sonic Hedgehog (Shh) is a potent inducer and obligatory for oligodendrocyte generation in both rostral and caudal neural tube (Orentas et al. 1999; Alberta et al. 2001; Nery et al. 2001; Tekki-Kessaris et al. 2001; Park HC 2002). Although some oligodendrocytes still develop in cultures derived from Shh null mutant embryos (Nery et al. 2001), this is likely due to functional compensation from other Hedgehog members such as Indian Hedgehog (Ihh) and/or Desert Hedgehog (Dhh). Shh functions as a morphogen that regulates the transcription of different target genes at different concentrations. At least part of the pro-oligodendrocyte function of Shh can be attributed to its induction of Olig genes (Lu et al. 2000; Nery et al. 2001). Olig genes, however, can not substitute for Shh, as shown in a recent zebrafish study where forced Olig expression largely failed to rescue the oligodendrocyte failure in the absence of Hedgehog signaling (Park HC 2002). Shh therefore must activate directly or indirectly additional genes that cooperate with Olig to promote oligodendroglia fate.

Contrary to Shh, members of the Bone Morphogenetic Proteins (BMP) are potent inhibitors of oligodendrocyte fate (Gross et al. 1996; Mabie et al. 1999; Mehler et al. 2000; Mekki-Dauriac et al. 2002). The inhibitory effect of BMPs can be exerted even in the presence of Shh (Mekki-Dauriac et al. 2002). BMPs, in contrast to Hedgehog, repress Olig gene transcription (Mekki-Dauriac et al. 2002). From a neural patterning point to

view, the antagonistic roles played by BMP and SHH can be attributed to their dorsalizing and ventralized functions as morphogens in ventricular zone patterning (Jessell 2000).

The thyroid hormone T3 has been shown to instructively promote oligodendrocyte fate at the expense of neuronal and astroglial fates in clonal cultures of rate neural stem cells (Johe et al. 1996). It is not clear how exactly T3 promotes oligodendroglial fate, or whether T3 can regulate Olig genes.

In summary, evidence accumulated to date strongly suggests that oligodendroglia fate specification requires at least two conditions. The first is the absence of general glial repressors such as neurogenic bHLH factors. The second is the induction of Olig genes and their partners.

Astrocyte specification

The discovery that oligodendrocytes are respecified as astrocytes in the absence of Olig1 and Olig2 genes revealed a previous unexpected link between oligodendrocyte lineage and the astrocyte lineage (Zhou and Anderson 2002). This result implies that Olig genes are repressors of astroglial fate and that astroglial fate may be the "default" glial fate, i.e., astroglial development occurs in the absence of positive pro-astroglial factors if Olig genes are not expressed. On the other hand, analysis of CNTF/LIF family of cytokines suggests that astroglial fate may have to be actively promoted (Ware et al. 1995; Johe et al. 1996; Bonni et al. 1997; Koblar et al. 1998; Nakashima et al. 1999).

Clonal analysis of multipotent neural stem cells derived from embryonic rat cortices demonstrated that the cytokine ciliary neurotrophic factor (CNTF) can instructively commit these neural stem cells into astroglia lineage at the expense of neuronal and oligodendroglial differentiation (Johe et al. 1996). Leukemia inhibitory factor (LIF), interleukin-6 (IL-6), and members of the bone morphogenetic protein (BMP) family were also shown to promote astroglia fate (Gross et al. 1996; Bonni et al. 1997; Nakashima et al. 1999). As there is yet no clonal analysis on the effect of LIF, IL-

6 and BMPs on neural stem cells, whether they act in an instructive manner or simply by promoting the proliferation or survival of astrocyte precursors needs further examination.

Similar to oligodendrocyte, astrocyte specification is also subject to inhibition by neurogenic factors (Sun et al. 2001). In addition, Olig genes can also repress astroglial fate (Zhou and Anderson 2002). Given that astroglial specification can only occur in the absence of these inhibitors, how could CNTF instruct astroglia generation in neural stem cells at the expense of neuronal and oligodendrocyte fate? It is perhaps because neurogenic factors and Olig genes are not expressed in these cultured neural stem cells, or that CNTF can actively repress such factors. Once CNTF instructs the stem cells into astroglia lineage, certain mechanisms may permanently lock the cell in this state before neurogenic factors or Oligs can be induced and exert their function.

CNTF, LIF and IL-6 all belong to the interleukin-6 (IL-6) family of cytokines which includes IL-6, IL-11, LIF, CNTF, oncostatin-M (OSM), and cardiotrophin-1 (CT-1) (reviewed in Stahl and Yancopoulos 1994; Taga and Kishimoto 1997). All these cytokines use gp130 protein in their receptor complex as a signal transducing component. IL-6 and IL-11 signals trough gp130 homodimer whereas LIF, CNTF, OSM and CT-1 signal through a heterodimer of LIF receptor and gp130. CNTF signaling further requires the formation of a tripartite complex composed of CNTF receptor, LIF receptor and gp130 protein (reviewed in Stahl and Yancopoulos 1994; Taga and Kishimoto 1997).

Consistent with the conclusion that CNTF/LIF is required for astroglial development, few astrocytes were observed in the spinal cord and hindbrain of LIF receptor knockout, which abolishes signaling through both LIF and CNTF (Ware et al. 1995). Cultured neural tissues from LIFR-/- animals also failed to generate significant numbers of astrocytes (Koblar et al. 1998). Similarly, Gp130-/- mice, which die at birth, showed a similarly severe reduction of GFAP+ mature astrocytes *in vivo* at E18.5 (Nakashima et al. 1999).

As LIF receptor and GP130 are widely expressed and multiple phenotypes were observed in many neural and non-neural tissues in LIF and gp130 null animals, whether the observed astroglia defects reflect a cell-autonomous requirement for LIF and gp130 in the astroglial lineage can not be determined. Furthermore, given that the only astrocyte marker used in these studies, GFAP, labels mature astrocytes, loss of GFAP expression in these mutants may well reflect a differentiation phenotype instead of a failure of stem/progenitor cell to commit to astroglial lineage as might be predicted from the *in vitro* culture studies.

Even in gp130 null mutant, in which signaling from all IL-6 family cytokines should be disrupted, some GFAP+ astrocytes were still observed (Nakashima et al. 1999). This might be due to the presence of BMPs (Gross et al 1996). The importance of BMP signaling in astroglia development *in vivo*, however, has not been examined in any knockout studies.

Using *in vitro* cultures of neural stem/progenitor cells, it has been demonstrated that the astroglia promoting activity of CNTF and LIF is mediated by the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway instead of the Ras-Mitogen activated protein kinase (MAP) pathway (Bonni et al. 1997). In addition, LIF and BMP2 can act in synergy to promote astroglial fate, and this synergy is mediated by a STAT3-Smad1 complex bridged by the transcriptional co-activator p300 (Nakashima et al. 1999). Whether any of these results is relevant *in vivo* requires further examination.

Recently, activated Notch pathway was shown to promote astroglial commitment from rat adult hippocampus-derived neural stem cells, independent of the STAT pathway (Tanigaki et al. 2001). This is perhaps due to repression of neurogenic bHLH genes by Notch; and Olig genes, necessary for oligodendrocyte development, are not induced in these cells under the culture conditions used. It is also possible that adult neural stem cells may have a unique propensity to differentiate along the astroglial lineage under the

influence of Notch. Testing the activity of Notch on embryonic stem cells and adult stem cells prepared in different ways may further clear this issue.

Are neuronal subtype and glial subtype decisions coupled?

I discussed neuronal and glial subtype specification above separately. This does not imply, however, that neuronal and glial subtype decisions are unrelated events. New insights from Olig genes suggest that they may be coupled *in vivo* (Zhou and Anderson 2002). This coupling, in the case of sequential generation of motoneurons and oligodendrocytes from the same ventricular domain, is enabled by their common expression of Olig genes, which dictates the subtypes for both neurons and glia (Novitch et al. 2001; Zhou et al. 2001; Zhou and Anderson 2002). Such a mechanism of linking neuronal and glial subtype specification via expressing a common transcriptional regulator may be generally employed by different neural stem cells.

Spatial origin of vertebrate CNS glial cells

Where do different classes of glial cells first arise from the central nervous system? Are they generated simultaneous in different CNS regions? Or do they first appear in certain restricted areas but later migrate and populate the entire CNS? Pinpointing the birth place of glial cells has always been problematic due to a lack of glial precursor markers and the extensive migratory behavior of glial cells. Embryologists have used chimeras to assess the ability of different CNS regions to give rise to different glial cells. For example, chick-quail chimera studies suggest that oligodendrocytes come from ventral but not dorsal spinal cord whereas astrocytes arise from both ventral and dorsal spinal cord (Pringle et al. 1998). Similar experiment in forebrain indicates that oligodendrocytes derive from the ganglionic eminence area (Olivier et al. 2001). Chimeric studies, however, are ultimately limited by the ability to dissect and manipulate increasing smaller pieces of tissues, and hence do not have very good spatial resolutions.

Recently, several glial precursor markers have been discovered, for example, PDGFRa, Sox10, Olig, CNPase and PLP/DM20 label oligodendrocyte precursors (Yu et al. 1994; Ono et al. 1995; Hall et al. 1996; Lu et al. 2000; Takebayashi et al. 2000; Zhou

et al. 2000). In addition, there is increasing understanding about the patterning of the nervous system ventricular zone, in which neural stem cells reside and where different classes of neurons and glial cells first appear. The best studied system of neural patterning is spinal cord (reviewed by Jessell 2000). I will first give an overview of spinal cord patterning and then discuss recent efforts to map spatial origins of glial cells with recently described glial precursor markers.

Spinal cord patterning during Neurogenesis

During the early neurogenesis phase of spinal cord development, a gradient of the morphogen Sonic Hedgehog (SHH), emanating from notochord and floor plate, set up the expression of two classes of patterning molecules (Roelink et al. 1995; Briscoe et al. 2000; reviewed by Jessell 2000). The expression of Class I molecules, including pax6, Dbx1 and Irx3, is repressed by SHH (Ericson et al. 1997; Pierani et al. 1999; Briscoe et al. 2000). In contrast, the expression of Class II patterning molecules, such as Nkx2.2 and Nkx6.1 requires SHH (Ericson et al. 1997; Briscoe et al. 2000). Pairs of Class I and Class II molecules exert cross-repressive interactions, for example, Nkx2.2 and Pax6 cross repress each other (Ericson et al. 1997). This mutual transcriptional repression is hypothesized to sharpen the boundary between each pair (Briscoe et al. 2000). As different Class I and Class II molecules require different concentrations of SHH to repress or induce their expression, distinct boundaries are formed along the dorsal-ventral axis of the spinal cord. These boundaries partition the ventricular zone of the spinal cord into discrete progenitor domains from which different classes of neurons emerge (Jessell 2000). There are 5 progenitor domains described so far in the ventral spinal cord alone. They are, from dorsal to ventral, p0, p1, p2, pMN, p3, which generate V0, V1, V2, motor neuron and V3 interneurons, respectively (Briscoe et al. 2000; Jessell 2000). Intriguingly, all of the patterning molecules, with the possible exception of Olig2 gene (Mizuguchi et al. 2001; Novitch et al. 2001), are homeodomain transcription factors (Jessell 2000), and they likely function as transcriptional repressors (Muhr et al. 2001).

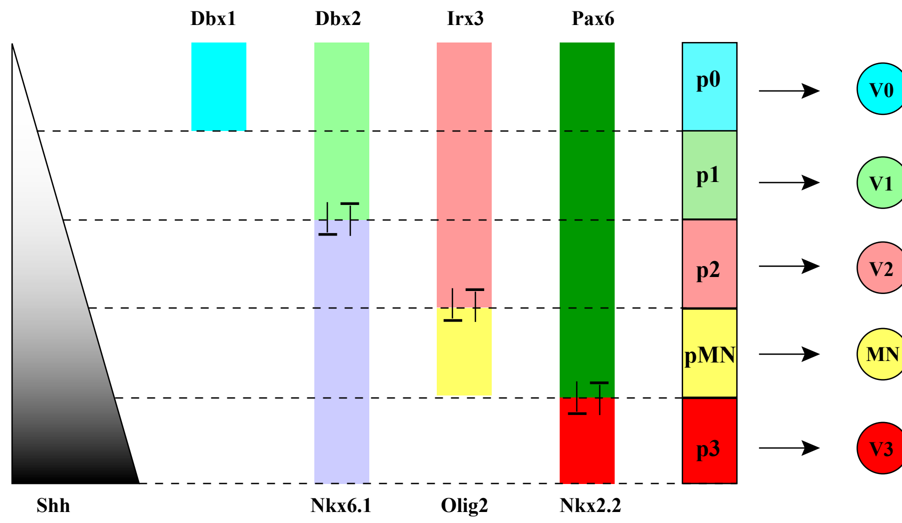


Figure 4. Spinal cord neuronal patterning

Origin of oligodendrocytes

How do glial cells fit into the above picture? For one thing, gliogenesis occurs after neurogenesis. At present, it is not clear whether the same neuronal progenitor domains still exist during gliogenesis, or how the ventricular zone is organized into glial domains. Despite this uncertainty, the simplest model of spinal glial patterning is for each neuronal domain to give rise to a glial domain, and to maintain the old boundaries.

With this line of thinking, William Richardson and colleagues mapped PDGFRa+ oligodendrocyte precursors (OPC) to the boundary between Nkx2.2 and Pax6 at the onset of gliogenesis in the mouse, a location occupied by the erstwhile pMN during neurogenesis (Sun et al. 1998). They interpreted the data as suggesting that oligodendrocyte precursors likely arise from the pMN domain, after motoneuron production (Sun et al. 1998). In contrast, Philip Cochard and colleagues mapped O4+ oligodendrocyte precursors in chick within Nkx2.2+ p3 domain and proposed that OPCs are generated from the p3 domain, not pMN (Soula et al. 2001). What accounts for this wide discrepancy? As we now know, the simplest model of spinal glial patterning that these conclusions are based on is likely incorrect. Recent work with Olig genes in chick demonstrated that upon completion of neurogenesis, the boundary between p3 and pMN no longer exists, and that the pMN patterning molecule Olig2 and the p3 patterning molecule Nkx2.2 come to overlap with each other during gliogenesis (Zhou et al. 2001; Fu et al. 2002). Oligodendrocyte precursors are generated from this domain of overlap. This new domain was named pOlig (Zhou et al. 2001). Are there other spinal glial domains? Do they also not obey the old boundaries established during neurogenesis? These are important issues that need to be resolved.

Compared to the spinal cord, molecular mechanisms governing regional specification of the forebrain ventricular zone during neurogenesis are much less clear (Wilson and Rubenstein 2000). With similar OPC markers, it was determined that forebrain oligodendrocytes originate predominantly from the ganglionic eminences (GM) and later migrate throughout the brain (Tekki-Kessaris et al. 2001; Woodruff et al. 2001). Besides GM, other brain loci of oligodendrocyte production may also exist (Spassky et

al. 1998; Perez Villegas et al. 1999; Spassky et al. 2000; Olivier et al. 2001; Spassky et al. 2001).

Origin of astrocytes

The spatial origin of astroglia has remained enigmatic for a long time due to lack of early astroglial markers. Recently, fibroblast growth factor receptor 3 (FgfR3) was proposed to be an early astrocyte marker in both chick and mouse spinal cord (Pringle et al. 2003). Expression of FgfR3 in the spinal cord is largely confined to the ventral ventricular zone outside the Olig positive domain, potentially demarcating the region where astrocytes are generated (Pringle et al. 2003). Although FgfR3 colocalizes with GFAP+ astrocytes but never with PDGFRa+ oligodendrocytes, it is yet to be established whether all astrocytes in the spinal cord express FgfR3 and whether FgfR3 expression is truly exclusive for astrocytes.

Differentiation of vertebrate CNS glial cells

Oligodendrocyte precursor cell (OPC)

Much of our knowledge about glial cell development comes from studying purified glial precursor cells. In the early 1980s, Martin Raff and colleagues first isolated a glial precursor from the postnatal rat optic nerve (Raff et al. 1983). These cells can give rise to either oligodendrocytes or type-2 astrocytes in culture under different conditions and was subsequently named O-2A cells (Raff et al. 1983; Raff 1989). Later studies, however, have generally failed to confirm the existence of type-2 astrocytes *in vivo*. Most workers in the field now believe that type-2 astrocyte is rather a tissue culture phenomenon. Nonetheless, the bipolar O-2A cells can undergo a series of differentiation steps *in vitro* to give rise to mature, mutipolar oligodendrocytes with myelin sheets (reviewed by Pfeiffer et al. 1993; Miller 1996). In addition, purified and culture expanded O-2A cells can differentiate into mature oligodendrocytes upon transplantation into the brain (Espinosa de los Monteros et al. 1993; Groves et al. 1993). O-2A cells are therefore bona fide oligodendrocyte precursor cells (OPC). An adult O-2A cell has been isolated and may serve as the progenitor pool to replenish oligodendrocytes in adult animals (reviewed by Levine et al. 2001). It is often assumed that adult O-2A cells

derive lineally from embryonic O-2A cells, but this may not be the case, as they could equally derive from adult neural stem cells.

A number of extracellular factors have been found to influence the proliferation of OPC. For example, OPC proliferates extensively in culture before differentiation in the presence of platelet derived growth factor (PDGF). Without PDGF, OPC undergoes terminal differentiation immediately (Noble et al. 1988; Raff et al. 1988). The combined action of PDGF and bFGF can further keep OPC in a proliferative state almost indefinitely (Bogler et al. 1990). There are two PDGF ligand isoforms, A and B, which form homodimers AA, BB or heterodimer AB (reviewed by Heldin and Westermark 1989). PDGF-AA and -AB signal through receptor a (PDGFRa) whereas PDGF-BB and AB signal through receptor b (PDGFRb) (Heldin and Westermark 1989). PDGFRa, but not PDGFRb is predominantly expressed in OPC (Hart et al. 1989). Over-expression of the ligand (PDGF-A) in transgenic mice resulted in over-proliferation of OPC (Calver et al. 1998) whereas both the ligand (PDGF-A) and receptor (PDGFRa) knockout showed a severe loss of oligodendrocytes (Fruttiger et al. 1999; Klinghoffer et al. 2002). These experiments firmly established PDGF-PDGFRa ligand-receptor pair as one major regulator of OPC proliferation. Neuregulin is another growth factor for OPC (Canoll et al. 1996; Vartanian et al. 1999). Neuregulin signals through erbB family receptor tyrosine kinase (reviewed by Carraway and Burden 1995). All three erbB receptors are expressed in OPC (Canoll et al. 1996).

Terminal differentiation of oligodendrocyte is a carefully orchestrated event *in vivo* to ensure timely myelination of the axons. Available evidence strongly suggests that Notch pathway plays a critical role in this process (reviewed by Wang and Barres 2000). In the rat optic nerve system, Notch1 is expressed in OPCs while Jagged, a Notch ligand, is present on the optic nerves (Wang et al. 1998). Activation of Notch1 by Jagged can powerfully inhibit differentiation of OPC in culture (Wang et al. 1998). Moreover, the expression of Jagged on optic nerves is controlled developmentally *in vivo* such that Jagged levels decrease while embryonic development progresses, thus allowing myelination to occur in a controlled manner (Wang et al. 1998). More recently, Notch1

was shown to inhibit premature differentiation of spinal cord OPC as well (Genoud et al. 2002).

Notch activation generally leads to the expression of Hes family inhibitory basic helix-loop-helix (bHLH) transcription factors (reviewed by Kageyama and Nakanishi 1997). The expression of one Hes family member, Hes5, was found in optic nerve OPCs and ectopic Hes5 expression blocked OPC differentiation *in vitro* (Kondo and Raff 2000). These results suggest that Notch1 acts through Hes5 to inhibit terminal differentiation of optic nerve OPC. Id2, another inhibitory bHLH factor, was also found to negatively regulate OPC differentiation (Wang et al. 2001). As both Hes and Id family bHLH factors are known to antagonize the functions of tissue specific bHLH transcription factors (Kageyama and Nakanishi 1997), it was hypothesized that unknown E-box bHLH factors might positively promote the terminal differentiation of OPCs (Kondo and Raff 2000; Wang et al. 2001). Whether these might be the Olig family of bHLH genes identified recently (see Chapters 2, 3, 4) is yet to be determined. It should be cautioned that the series of studies on Notch, Hes5 and Id2 were mostly undertaken in culture or using optic nerve as the *in vivo* model system. Large numbers of different Notch receptors and ligands, Hes and Id family members are expressed in complex overlapping patterns in the spinal cord and brain (Kageyama and Nakanishi 1997; Artavanis-Tsakonas et al. 1999; Andres-Barquin et al. 2000). None is specific to the oligodendrocyte lineage. Whether some or all of them participate in controlling the timing of myelination in different brain regions is yet to be elucidated.

Two transcription factors necessary for the terminal differentiation of oligodendrocyte have recently been discovered. One is Sox10, a SRY related, High-Mobility-Group (HMG box) transcription regulator (Zhou et al. 2000; Stolt et al. 2002). Another is Nkx2.2, a homeodomain transcription factor (Xu et al. 2000). Loss of either Sox10 or Nkx2.2 results in hypomyelination and loss of mature oligodendrocyte markers such as myelin basic protein (MBP) and proteiolipid protein (PLP) without affecting the development of oligodendrocyte precursors (Qi et al. 2001; Stolt et al. 2002). While these mouse knockout studies suggest that Sox10 and Nkx2.2 are necessary for

oligodendrocyte terminal differentiation, a reciprocal gain-of-function study has not been done to test their sufficiency in this process. Moreover, it is not clear what the epistatic relationship between Nkx2.2 and Sox10 is, and whether Notch signaling can regulate their expression.

Recently, it was reported that the neurotransmitter adenosine released during action potential firings could potently inhibit OPC proliferation while promoting their terminal differentiation and myelination (Stevens et al. 2002). Adenosine thus acts as a positive whereas Notch a negative signal for OPC differentiation *in vivo*. The thyroid hormone T3 and retinoic acid (RA) can also promote terminal differentiation of OPCs in culture and has been argued to be part of a timer that regulates the timing of OPC differentiation (Durand et al. 1997). It is yet to be understood how exactly adenosine, T3 and RA exert their functions.

Astrocyte precursor cells (APC)

Astrocytes are classically divided into protoplasmic and fibrous types, the former found in gray matter, the latter in the white matter (Kettenmann 1995). Additional morphological, biochemical and functional criteria can be used to further divide astrocyte into more subtypes (Wilkin et al. 1990; Kettenmann 1995). In addition, at least five different types of astrocytes have been identified from spinal cord cultures (Miller and Szigeti 1991). These studies suggest that either multiple classes of astrocyte precursors exist, or alternatively, one class of astrocyte precursor is capable of generating all subtypes of mature astrocytes under varying environmental influences. There is currently no evidence to support either hypothesis.

A putative astrocyte precursor has been purified from rat optic nerve and shown to give rise to mature astrocytes *in vitro* (Mi and Barres 1999). A more definitive test of the putative APC's developmental potential *in vivo*, i.e., transplantation into the embryos, however was not undertaken. Cells with antigenic profiles of the putative APC, i.e., pax2+ A2B5+ GFAP+ S100b+, are only found in optic nerves (Mi and Barres 1999). This may imply that other classes of APCs give rise to astrocytes elsewhere in the brain.

However, if like the oligodendrocyte precursors, astrocyte precursors also go through a series of differentiation steps before terminal differentiation, the putative APC may then represent an intermediate and not the earliest precursor. Interestingly, zebrafish mutants of Noi, which encodes a fish pax2 homologue, exhibit a differentiation defect of astroglia in the optic nerve as well as errors in optic nerve path-finding (Macdonald et al. 1997). Given that the fish optic nerve path-finding error is likely caused by the astroglia defect (Macdonald et al. 1997), and that a similar optic nerve trajectory phenotype was observed in the mouse Pax2 mutant (Torres et al. 1996), it is worth re-examining the original pax2 mutant for optic nerve astroglia defect.

Glia restricted precursor (GRP)

A glia restricted precursor (GRP) has been isolated from early embryonic spinal cord (Rao et al. 1998). *In vitro*, GRP can give rise to oligodendrocyte, type-1 astrocyte, type-2 astrocyte but not neurons (Rao et al. 1998). GRP has been cited as evidence that oligodendrocytes and astrocytes are derived from common glioblasts *in vivo* (Rao et al. 1998; Lee et al. 2000). Lineage tracing experiments, however, suggest that oligodendrocyte does not share a common precursor with astrocyte (Lu 2002) so there could be no glioblast *in vivo*, at least not in the mouse spinal cord. Either GRP is a culture anomaly, or alternatively, bi-potential, gliogenic GRP does exist *in vivo* but they give rise to either oligodendrocyte or astrocyte, but not both (reviewed in Rowitch et al. 2002). Identifying unique makers for GRP so that the fate of their descendants can be traced *in vivo* should help to resolve this issue.

Astrocytes as neural stem cells?

It was reported recently that rodent subventricular zone neural stem cells are GFAP+ and the authors labeled them “astrocytes” (Doetsch et al. 1999). This raised several interesting questions: are all neural stem cells GFAP positive? Can any GFAP+ cell in the brain be called astrocyte? While it remains unknown whether adult stem cells from other brain region, e.g., dentate gyrus is GFAP positive, embryonic CNS stem cells are clearly GFAP-. High GFAP expression may be unique to adult stem cells from the subventricular zone.

Can any GFAP+ cell in the brain be called astrocyte? With few unique markers for astrocytes available, unambiguous identification of astrocytes *in vivo* is problematic. Although GFAP is the most widely used marker to identify mature astroglia, some astrocytes do not express GFAP (Wilkin et al. 1990; Zhang 2001). Other markers, such as glutathione transferase, glia specific glutamate transporter (GLAST) and the calcium binding protein S100b, are more or less non-specific to the astroglia lineage and only label sub-populations of astroglia (Wilkin et al. 1990; Zhang 2001). Whenever possible, a combination of these markers plus the morphology of the cells should be applied to identify astrocytes. Relying only on GFAP poses certain dangers as the GFAP promoter could be activated in non-astroglial cells under certain conditions (Michalowsky and Jones 1989).

Development of other glial types

In addition to CNS glial cells such as oligodendrocyte and astrocyte, other classes of glial cells exist throughout the vertebrate nervous system. For example, Schwann cell of the peripheral nervous system (PNS), enteric glia of the enteric nervous system, Muller glia of the eye, olfactory ensheathing glia of the olfactory system, etc. I will hereby discuss the development of a few important glial types.

radial glia

Radial glial cells are morphologically distinct bipolar cells present in the developing vertebrate brain (reviewed in Parnavelas and Nadarajah 2001; Campbell and Gotz 2002). One process of radial glia extends to the pial, the other to the ventricular zone. Radial glial cells possess certain characteristics of astrocytes such as glycogen granules and GFAP (unique to primates). Rodent radial glial cells further express astrocyte specific glutamate transporter (GLAST) and brain lipid binding protein (BLBP), both are found in mature astrocytes. For these reasons, radial glia have been considered to be a type of glial cells whose function is to provide a scaffold for the radial migration of neurons (Parnavelas and Nadarajah 2001; Campbell and Gotz 2002). Recently, however, results from a series of papers suggest that radial glia are a rather heterogeneous group of

stem/progenitor cells, not glial cells (Malatesta et al. 2000; Hartfuss et al. 2001; Miyata et al. 2001; Noctor et al. 2001).

Gotz and colleagues analyzed the developmental potential of isolated radial glial cells in culture and found them to be either neuronal progenitors, glial progenitors, or multi-potential stem cells (Malatesta et al. 2000). Lineage tracing studies in living brain slices further demonstrated that radial glial cells can generate neurons *in situ* (Miyata et al. 2001; Noctor et al. 2001).

Activated Notch1 promotes radial glia development when introduced into mouse forebrain before the onset of neurogenesis (Gaiano et al. 2000). It is not clear, however, whether Notch actively promotes radial glia fate commitment or is simply involved in the maintenance of radial glia fate. The second interpretation is consistent with the demonstrated ability of Notch to keep cells in an undifferentiated precursor stage (Artavanis-Tsakonas et al. 1999).

Despite recent advances in our understanding of radial glia development, many important questions remain unanswered: how many different types of radial glial cells are there? What mechanisms control the general morphological similarity of radial glial cells? Are radial glial cells lineally related to adult stem cells? To answer these questions, it is essential to find additional unique molecular markers for radial glia.

Muller glia

The vertebrate retina, which contains 6 neuronal types and one glial type (Muller glia), has served as an important model system for cell type specification (reviewed in Cepko 1999). Lineage analysis revealed that different cell types of the retina are born in a temporal order of ganglion cell first, followed by horizontal cell, cone photoreceptor, amacrine cell, rod photoreceptor, bipolar cell, and Muller glia (Young 1985; Cepko 1999). This temporal order, however, is not absolute, as multiple progenitors exist at any given time. Lineage analysis further suggests that there is no glioblast; neurons and glia are derived from a common progenitor in the retina (Turner and Cepko 1987).

As I have discussed before, neurogenic bHLH transcription factors are negative regulators of Muller glia development (Morrow et al. 1999; Tomita et al. 2000). In addition, several genes have been shown to positively regulate the development of Muller glial cells including Rax (a homeodomain protein), Notch1, Hes1 and Hes5 (Tomita et al. 1996; Furukawa et al. 2000; Hojo et al. 2000). The expression of these genes first comes on broadly in many early retinal progenitors, and later restricts to Muller glia. Forced expression of Rax, Notch1, Hes1 and Hes5 each promotes Muller glia development (Furukawa et al. 2000; Hojo et al. 2000). Conversely, there is 30-40% decrease of Muller glia number in Hes5 mutant retina (Hojo et al. 2000). The effect of loss of Rax and Hes1 in Muller glia development can not be assessed due to early retinal phenotypes (Tomita et al. 1996; Tucker et al. 2001). Inducible knockouts of Rax, Notch1, and Hes1 during late retinal development should circumvent this problem. While Hes1 and Hes5 are clearly downstream of Notch1, the epistatic relationship between Rax and Notch1 is less clear, although there are indications that Rax may induce Notch1 expression.

In Xenopus frog, over-expression of p27xic1, an inhibitor of cyclin-dependent kinase (Cdk), has been shown to cause early cell cycle arrest and promote Muller glia development (Ohnuma et al. 1999). p27xic1 induced Muller cell differentiation seems to operate within the context of the Notch pathway: coexpression of p27xic1 with a dominant-negative Delta1, which blocks Notch-Delta signaling, completely abolished the Muller glia inducing activity of p27xic1, whereas co-expression of p27xic1 with Notch significantly potentiated this activity (Ohnuma et al. 1999). Despite these experiments, the mechanisms of pro-Muller function of p27xic1 are largely unknown. Analysis of p27kip1, the mouse homologue of p27xic1, revealed no Muller glia defect (Levine et al. 2000), suggesting that either p27kip1 is not involved in mouse Muller glia development, or that other Cdk inhibitors are redundant for this function.

Invertebrate glial development

Invertebrate glial development is best studied in the genetic model organism, *Drosophila melanogaster* (reviewed in Jones 2001). The main function of fly glial cells is to ensheath both central and peripheral axons and form a blood-nerve barrier (Jones 2001).

Unlike the vertebrates, fly hemolymph is not confined in a vasculature. Failure of glial development often results in breach of the blood-nerve barrier, exposing nerves to the high K+ environment of the hemolymph, and causing paralysis (Auld et al. 1995; Baumgartner et al. 1996). In this respect, the function of fly glial cells is perhaps akin to the astrocytes that form the vertebrate blood-brain barrier.

The glial cell missing (*gcm*) gene is a master regulator of most fly glial cells except the midline glia (Hosoya et al. 1995; Jones et al. 1995; Vincent et al. 1996). Two vertebrate homologues of gcm, gcm1 and gcm2, have been discovered, but neither is involved in vertebrate glial development (Akiyama et al. 1996; Altshuller et al. 1996; Kim et al. 1998). While gcm1 is essential for branching morphogenesis of the placenta (Schreiber et al. 2000), Gcm2 is a master regulator of parathyroid development (Gunther et al. 2000).

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