Chapter 5

Summary and Future directions

Much of our knowledge about glial development in the vertebrate CNS comes from studies of purified oligodendrocyte precursor cells (Raff 1989; Pfeiffer et al. 1993; Miller 1996; Lee et al. 2000). Very little is known, however, as to how the different glial cell fate is specified from uncommitted neuroepithelial cells. The discovery and series of studies on *Olig* genes provided crucial insight on this important issue (reviewed by Rowitch et al. 2002). Of the three *Olig* genes found to date, *Olig2* is best studied. The expression of *Olig2* comes on sequentially first in motoneuron precursors and later in cells of the oligodendrocyte lineage (Lu et al. 2000; Takebayashi et al. 2000; Zhou et al. 2000). Loss-of-function studies suggest that *Olig2* is absolutely necessary for oligodendrocyte development (Lu 2002; Park HC 2002; Takebayashi et al. 2002; Zhou and Anderson 2002), whereas gain-of-function studies suggest that *Olig2*, together with co-factors, is sufficient to induce ectopic oligodendrocyte formation (Zhou et al. 2001; Sun et al. 2002). The sequence and function of *Olig2* is highly conserved among different vertebrate species including fish, bird and mammals (Lu et al. 2000; Takebayashi et al. 2000; Zhou et al. 2000; Zhou et al. 2001; Lu 2002; Park HC 2002; Takebayashi et al. 2002; Zhou and Anderson 2002).

In contrast to *Olig2*, the *Olig1* gene is only found in mammalian species. Although *Olig1* is co-expressed with *Olig2* in oligodendrocyte (Lu et al. 2000; Zhou et al. 2000), its function is dispensable for oligodendrocyte precursor formation; instead, *Olig1* is required for the terminal differentiation of oligodendrocytes (Lu 2002). The third member of the *Olig* family, *Olig3*, does not co-express with *Olig2* and may function in the development of certain types of interneurons (Takebayashi et al. 2000; Takebayashi et al. 2002).

The unexpected discovery that motoneurons and oligodendrocytes are respecified as interneurons and astrocytes in the absence of *Olig1* and *Olig2* suggests that *Olig* genes control the subtype identities of both neurons and glial cells (Zhou and Anderson 2002). This result, together with the fact that neurogenic factors control neuron-glia decision, leads us to propose that a simple combinatorial model of *Olig* genes and neurogenic factors may underlie the generation of all three major cell types in the vertebrate CNS,

i.e., oligodendrocyte, astrocyte, and neuron (Zhou and Anderson 2002). In addition, given that motoneurons and oligodendrocytes likely derive from common neural stem cells (Richardson et al. 2000; Lu 2002), the spatial confinement of *Olig* gene expression *in vivo* suggests that different neural stem cells may exist *in vivo* (Zhou and Anderson 2002). Study of *Olig* genes thus also shed important light in neural stem cell biology.

Despite these exciting discoveries with *Olig* genes, many important questions remain unanswered. I will discuss below several outstanding issues and potential experimental approaches to address them.

What are the target genes of Olig?

Olig genes belong to the basic helix-loop-helix transcription factors. Like other tissue-specific bHLH factors, they presumably bind ubiquitous E-proteins to form dimmers, which then bind E-box sequences in DNA and regulate the transcription of target genes (reviewed by Bertrand et al. 2002). Unlike most bHLH factors, however, Olig2 was shown to act as a transcriptional repressor in promoting the ectopic generation of both motoneurons and oligodendrocytes in chick embryos (Novitch et al. 2001; Zhou et al. 2001). This observation has led to the proposal that Olig2 functions by repressing a repressor of oligodendrocyte development (Zhou et al. 2001). Despite this, the possibility that *Olig* genes may also function as activators in promoting oligodendrocyte fate can not be entirely ruled out. Additionally, the transformation of oligodendrocytes to astrocytes in the *Olig1,2-/-* double mutant spinal cord further indicates that *Olig1,2* negatively regulate astroglial development as well (Zhou and Anderson 2002). These data are summarized into a simple model (Figure 5).

According to this model, specific classes of *Olig* target genes will be either up-regulated or down-regulated in the absence of Olig function. For example, oligodendrocyte specific genes and putative oligodendrocyte activators (A) will be down-regulated whereas the putative repressors of oligodendrocyte fate (R) as well as astrocyte specific genes will be up-regulated. Uncovering these *Olig* target genes will not only help us to understand exactly how the function of *Olig* genes is executed, more

importantly, the potential discovery of astrocyte specific genes and regulators of astroglia fate will provide badly needed tools and crucial insight on the development of the astroglia.

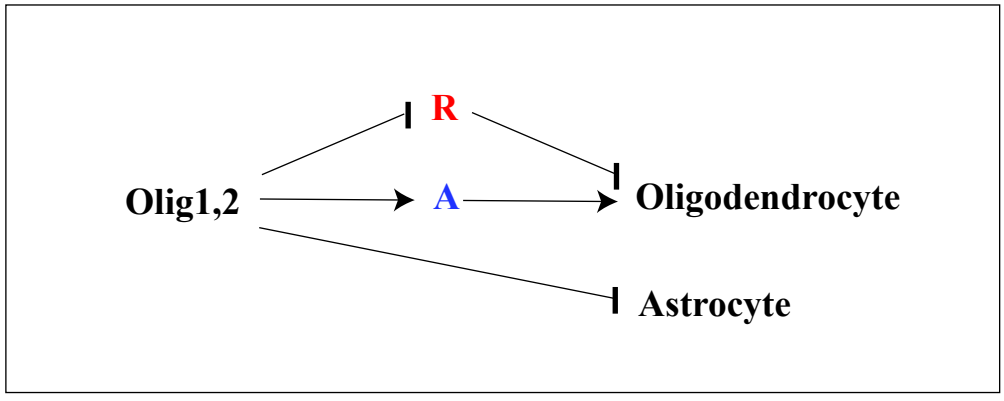
Taking advantage of the fact that a histone-EGFP fusion gene is knocked into the mouse *Olig2* locus (Zhou and Anderson 2002), GFP+ precursor cells can be isolated via fluorescence activated cell sorting (FACS) from embryonic mouse spinal cord of both *Olig1,2+/-* heterozygous and *Olig1,2-/-* homozygous mutants and their mRNA profiles compared with Affymetrix gene chips. Genes regulated by *Olig* can thus be identified and their functions further tested in different assays. Details of this gene chip comparison and preliminary results are presented in Appendix I.

One potential drawback of this approach is that although no oligodendrocyte precursor defect has been observed in *Olig1,2* heterozygous animals (Zhou et al. 2002; Lu et al. 2002; Takebayashi et al. 2002), it remains possible that the expression of some precursor genes may be impaired. This possibility should cause concern especially in light of the differentiation defect of oligodendrocytes in *Olig1,2+/-* spinal cord (Zhou and Anderson 2002). It would therefore be best to FACS sort oligodendrocyte precursors from gene conserving knockin animals such as an *Olig2-IRES-GFP* line.

Although the comparison discussed above is carried out with glial precursor cells, similar gene chip comparisons may also be conducted with cells derived from the neurogenesis period to study the target genes of *Olig* in motoneuron fate specification.

Are there multiple oligodendrocyte lineages?

The fact that development of all oligodendrocytes in mouse requires *Olig* genes does not necessarily mean that there is one unitary oligodendrocyte lineage. As *Oligs* are only necessary but not sufficient for oligodendrocyte specification (Zhou et al. 2001; Park HC 2002), there must be other genes that cooperate with Olig in this process. Should there be more than one Olig partner and they act only in subsets of oligodendrocytes, then multiple oligodendrocyte lineages can be defined. At present, it is not clear what the Olig



Olig1,2 -/- mutant

Up-regulate	***Down-regulate***
Astrocyte genes	**Oligodendrocyte genes**
Repressor of oligo (R)	**Activator of oligo (A)**

Figure 5. Olig target genes

partners could be and how they can be identified easily without resorting to biochemical purification. Nevertheless, given the many molecular differences in the development of forebrain versus spinal cord (Jessell 2000; Wilson and Rubenstein 2000), a gene chip comparison between oligodendrocyte precursors isolated from forebrain and spinal cord of the mouse may yield important clues.

Is there an Olig2+ stem cell in vivo?

It is often assumed that in the spinal cord, motoneurons and oligodendrocytes are sequentially generated from common Olig2+ stem cells *in vivo*. The fact that both cell types sequentially appear from roughly the same ventricular region is certainly no proof, as they could have developed from separate neuronal and glial precursors that co-exist in a salt-and-pepper fashion. Even lineage tracing experiments with *Olig1* or *Olig2* genes can not distinguish between these two possibilities (Lu 2002). The most definitive proof has to come from tracing the lineage of single Olig2+ cells *in vivo*, similar to the one conducted for neural crest cells (Bronner-Fraser and Fraser 1989; Fraser and Bronner-Fraser 1991). Access to Olig2+ cells in a live embryo, however, is extremely difficult as the Olig2+ ventricular area is embedded deep inside. Alternatively, an "open-book" spinal organ culture may prove more useful (Wada et al. 2000). By using embryos derived from either *Histone-GFP* or *Olig2-IRES-GFP* knockin mouse lines, Olig2+ expressing cells can be visualized and single GFP+ or GFP- cell injected with a tracer dye, e.g., rhodamine dextran. After a certain culture period, the progenies derived from a single injected cell can be analyzed by their morphology and molecular marker expression. The behavior of labeled cells may also be traced with live video microscopy.

Unlike invertebrate such as the fly and the worm, cell lineages in the CNS of vertebrates are almost unknown due to the vast number of cells and the inability to locate the same cell from different individuals. Retroviral lineage tracing, although informative, becomes ambiguous if the labeled cells migrate extensively, as in the case of glial cells. The lineage tracing experiment proposed above, although far from ideal, is perhaps the best system available right now, and much about neural stem cells and their *in vivo* behavior may be learned from it.

Spinal cord patterning during gliogenesis

A beautiful model of spinal cord patterning during neurogenesis has been proposed in which different neuronal groups emerge from discrete dorsal-ventral domains (Briscoe et al. 2000; Jessell 2000). Is there a similar map during gliogenesis? Given that oligodendrocytes arise from a distinct domain (Zhou et al. 2001), this is certainly possible. It would be extremely interesting if subtypes of astrocytes are produced from separate domains and, similar to the motoneuron-oligodendrocyte pair, the generation of astroglial subtypes is also coupled to different interneurons. To test these hypotheses, the key is to find early markers for different astroglia subtypes and potential patterning molecules during gliogenesis. It is possible that some of the same molecules used in neuronal patterning such as Nkx2.2, Pax6, Nkx6.1, may also be employed in glial patterning, as in the case of Olig2. One approach of identifying glial patterning molecules is therefore to systematically test all the neuronal patterning molecules for their expression and function in gliogenesis.

Hierarchical control of neural cell type specification

Specification of neuronal fate requires the function of neurogenic bHLH factors (Bertrand et al. 2002). Specification of oligodendrocyte fate requires *Olig* genes (Lu 2002; Park HC 2002; Takebayashi et al. 2002; Zhou and Anderson 2002). Astroglia development, however, occurs in the absence of both neurogenic bHLH factors and *Olig* genes (Zhou and Anderson 2002). Does that mean the "default" fate of a CNS stem cell is astroglia? It is possible that a neural stem cell is intrinsically programmed to be an astrocyte. Alternatively, the astroglial fate has to be actively promoted by pro-astroglia factors such as CNTF and BMP (Gross et al. 1996; Johe et al. 1996). According to the second model, the effect of neurogenic factors dominants over glia-promoting factors, and *Olig* genes dominate over pro-astroglial factors, thereby setting up a hierarchical control for the specification of all neural cell types. This model is essentially the same as the combinatorial model proposed in Chapter 4 except that a third axis of pro-astroglia factors has to be introduced.

Based on the model above, the following equations can be drawn: (1) astrocyte + (*Olig* genes + partners) oligodendrocyte; (2) (astrocyte or oligodendrocyte) + Neurogenic factors neurons. Although perhaps too simplistic, these hypotheses are nevertheless testable. For example, if this model is correct, then ectopic expression of neurogenic bHLH factors in astrocytes may transform some of the astrocytes into neurons. Interestingly, it has been reported that pax6 expression in astrocytes can induce neuronal differentiation (Heins et al. 2002). Given that Pax6 cross-regulate with Neurogenin2 (Scardigli et al. 2001), perhaps Pax6 induced the expression of neurogenin2, which in turn transformed astrocytes to neurons. One potential problem of studying cell type transformation by ectopically expressing genes in differentiated cells is that the cell fate of a differentiated neural cell may be permanently "locked", for example, by methylation. It is therefore best to use purified progenitor cells instead of mature cells.

Olig genes in adult

Most studies on *Olig* genes focus on their role in embryonic development. My unpublished results suggest that *Olig1* and *Olig2* genes are likely co-expressed in adult oligodendrocyte precursors as well. Although further work is needed to confirm this, if it is true, we will have a unique tool in hand to study these adult glial progenitors.

Although it has always been thought that adult OPCs are involved in replenishing the adult nervous system with oligodendrocytes (Levine et al. 2001), it has never been directly tested whether the adult OPCs are really indispensable for adult CNS integrity and function. Nor do we know whether they give rise exclusively to oligodendrocytes in the adult animals. To trace the progenies of adult OPCs, a transgenic mouse line can be constructed in which an inducible CRE recombinase-estrogen receptor (CRE-ER) fusion gene behind an IRES element is inserted into the 3UTR of the endogenous *Olig2* or *Olig1* locus (*Olig-IRES-CRE-ER*). Permanent lineage tracing can be conducted in the adult by crossing this line with any of the reporter strains, e.g., *ROSA-stop-lacZ-stop*, and administer Tamoxifen in adult animals. To eliminate adult OPCs, an *Olig-IRES-rtTA*

mouse line can be made and crossed to *TetO-DTA* line. Upon administration of Doxycycline in adult, *Olig* expressing adult OPCs can be eliminated.

References

Bertrand, N., D. S. Castro and F. Guillemot (2002). "Proneural genes and the specification of neural cell types." Nature Reviews Neuroscience 3(7): 517-30.

Briscoe, J., A. Pierani, T. M. Jessell and J. Ericson (2000). "A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube." Cell 101: 435-445.

Bronner-Fraser, M. and S. Fraser (1989). "Developmental potential of avian trunk neural crest cells in situ." Neuron 3: 755-766.

Fraser, S. E. and M. E. Bronner-Fraser (1991). "Migrating neural crest cells in the trunk of the avian embryo are multipotent." Development 112: 913-920.

Gross, R. E., M. F. Mehler, et al. (1996). "Bone morphogenetic proteins promote astroglial lineage commitment by mammalian subventricular zone progenitor cells." Neuron 17: 595-606.

Heins, N., P. Malatesta, et al. (2002). "Glial cells generate neurons: the role of the transcription factor Pax6 [erratum appears in Nat. Neurosci. May 2002; 5(5): 500]." Nature Neuroscience 5(4): 308-15.

Jessell, T. M. (2000). "Neuronal specification in the spinal cord: inductive signals and transcriptional codes." Nature Reviews Genetics 1: 20-29.

Johe, K. K., T. G. Hazel, T. Muller, M. M. Dugich-Djordjevic and R. D. G. McKay (1996). "Single factors direct the differentiation of stem cells from the fetal and adult central nervous system." Genes & Dev. 10: 3129-3140.

Lee, J. C., M. Mayer-Proschel and M. S. Rao (2000). "Gliogenesis in the central nervous system." GLIA 30(2): 105-21.

Levine, J. M., R. Reynolds and J. W. Fawcett (2001). "The oligodendrocyte precursor cell in health and disease." Trends in Neurosciences 24(1): 39-47.

Lu, Q., Sun, T., Zhu, Z., Ma, N.m Garcia, M., Stiles, CD., and Rowitch DH (2002). "Common developmental requirement for Olig function indicates a motor neuron/oligodendrocye connection." Cell 109(1): 75-86.

Lu, Q. R., D. Yuk, et al. (2000). "Sonic hedgehog-regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system." Neuron 25(2): 317-29.

Miller, R. (1996). "Oligodendrocyte origins." Trends Neurosci. 19: 92-96.

Novitch, B. G., A. I. Chen and T. M. Jessell (2001). "Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2." Neuron 31(5): 773-89.

Park HC, M. A., Richardson JS, Appel B. (2002). "Olig2 is required for zebrafish primary motor neuron and oligodendrocyte development." Dev Biol 248(2): 356-68.

Pfeiffer, S. E., A. E. Warrington and B. R. (1993). "The oligodendrocyte and its many cellular processes." Trends Cell Bio. 3: 191-197.

Raff, M. C. (1989). "Glial cell diversification in the rat optic nerve." Science 243: 1450-1455.

Richardson, W. D., N. P. Pringle, W.-P. Yu and A. C. Hall (1997). "Origins of spinal cord oligodendrocytes: possible developmental and evolutionary relationships with motor neurons." Dev. Neurosci. 19: 58-68.

Richardson, W. D., H. K. Smith, et al. (2000). "Oligodendrocyte lineage and the motor neuron connection." Glia 29: 136-142.

Rowitch, D. H., Q. R. Lu, N. Kessaris and W. D. Richardson (2002). "An 'oligarchy' rules neural development." Trends in Neurosciences 25(8): 417-22.

Scardigli, R., C. Schuurmans, G. Gradwohl and F. Guillemot (2001). "Crossregulation between *Neurogenin2* and pathways specifying neuronal identity in the spinal cord." Neuron 31: 203-217.

Sun, T., N. Pringle, A. P. Hardy, W. D. Richardson and H. K. Smith (1998). "Pax6 influences the time and site of origin of glial precursors in the ventral neural tube." Mol. Cell Neurosci. 12: 228-239.

Sun, T., Z. Zhu, et al. (2002). "Olig bHLH proteins interact with homeodomain proteins to regulate cell fate acquisition in progenitors of the ventral neural tube." Cell 109(1): 75-86.

Takebayashi, H., Y. Nabeshima, S. Yoshida, O. Chisaka and K. Ikenaka (2002). "The basic helix-loop-helix factor olig2 is essential for the development of motoneuron and oligodendrocyte lineages." Current Biology 12(13): 1157-63.

Takebayashi, H., T. Ohtsuki, et al. (2002). "Non-overlapping expression of Olig3 and Olig2 in the embryonic neural tube." Mechanisms of Development 113(2): 169-74.

Takebayashi, H., S. Yoshida, et al. (2000). "Dynamic expression of basic helix-loop-helix Olig family members: implication of Olig2 in neuron and oligodendrocyte differentiation and identification of a new member, Olig3." Mechanisms of Development 99(1-2): 143-8.

Wada, T., T. Kagawa, et al. (2000). "Dorsal spinal cord inhibits oligodendrocyte development." Developmental Biology 227(1): 42-55.

Wilson, S. W. and J. L. Rubenstein (2000). "Induction and dorsoventral patterning of the telencephalon." Neuron 28: 641-651.

Zhou, Q. and D. J. Anderson (2002). "The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification." Cell 109(1): 61-73.

Zhou, Q., G. Choi and D. J. Anderson (2001). "The bHLH transcription factor Olig2 promotes oligodendrocyte differentiation in collaboration with Nkx2.2." Neuron 31: 791-807.

Zhou, Q., S. Wang and D. J. Anderson (2000). "Identification of a novel family of oligodendrocyte lineage-specific basic helix-loop-helix transcription factors." Neuron 25: 331-343.