

*Invited Review*

## Understanding Glial Differentiation in Vertebrate Nervous System Development

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WAKAMATSU, Y. *Understanding Glial Differentiation in Vertebrate Nervous System Development*. Tohoku J. Exp. Med., 2004, **203** (4), 233-240 — Recent progresses in molecular and developmental biology provide us a good idea how differentiation of glial cells in the vertebrate nervous system is regulated. Combinations of positional cues such as secreted proteins and cell-intrinsic mechanisms such as transcription factors are essential for the regulation of astrocyte and oligodendrocyte differentiation from the neural epithelium. In contrast, regulatory mechanisms of glial differentiation from neural crest-derived cells in the peripheral nervous system are less understood. However, recent studies suggest that, at least in part, the peripheral gliogenesis is regulated by mechanisms, such as Notch signaling, that are also important for the gliogenesis in the developing central nervous system. ——— glia; oligodendrocyte; astrocyte; Schwann cell; satellite glia; differentiation

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### *Regulation of glial differentiation*

During development of the vertebrate nervous system, many types of neurons and glial cells differentiate. Recent progresses in molecular and developmental biology have uncovered the mechanisms that regulate how distinct neurons and glial cells are generated from neuroepithelium (NE) or neural crest-derived tissues including undifferentiated stem cells. In particular, we now have a good idea how neurogenesis and subtype determination of different classes of neurons are regulated. Recently, the regulation of glial cell differentiation in the central nervous

system (CNS) has been well studied, and major components of the regulatory system have been identified. In contrast, the regulation of glial differentiation in the peripheral nervous system (PNS) remains largely unknown. In this review, I summarize the present knowledge in the regulation of glial differentiation, and clarify problems in understanding fate determination of PNS glia.

### *Glial differentiation in CNS*

There are two major classes of CNS glia, astrocytes and oligodendrocytes. The most important role of astrocytes is to nurture neighboring

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neurons, while oligodendrocytes make myelins. It has long been believed that astrocytes and oligodendrocytes are derived from a common glial precursor cell (O-2A progenitor), suggested by in vitro culture experiments (see text books, such as Jacobson 1991). Recent studies, however, have revealed that these glial cells differentiate from distinct parts of NE, and that the NE cells generating oligodendrocytes also generate motor neurons, suggesting that astrocytes and oligodendrocytes derive from distinct precursors (Fig. 1, see also below). Regardless of their origin, however, there is one common feature: These glial cells differentiate from NE only after neurons differentiate (Sauvageot and Stiles 2002, for a review).

NE tissue contains neural stem cells, and the neural stem cells are believed to generate both neurons and glial cells, while undergoing self-renewal. It is therefore important for neuron-generating NE to maintain the pool of stem cells, otherwise there would be no source of glial cell precursors. The most important maintenance mechanism appears to be lateral inhibition mediated by Notch signaling (Lewis 1998; Artavanis-Tsakonas et al. 1999; Frisen and Lendarhl 2001). *Notch* receptor genes are expressed in the NE cells, and their ligands, such as *Delta* and *Serrate/Jagged* genes, are transiently expressed in new-born neurons, which are leaving the NE layer (ventricular zone) after the last cell division. Therefore, these ligand-expressing young neurons activate the Notch signal of neighboring NE cells, resulting in the inhibition of their neuronal differentiation. Thus, when Notch signal activation is interrupted, the pool of undifferentiated NE cells cannot be maintained, and neurons differentiate prematurely (e.g. Chitnis et al. 1995). This indirectly leads to the loss of glial cells, due to the absence of stem-cells.

Recent studies indicate that Notch signal is more directly involved in a glial differentiation (Gaiano and Fishell 2002). For example, at the spinal cord level, the pMN domain of NE (a ventral part of the developing neural tube) initially generates motor neurons, and subsequently,

the same domain generates oligodendrocytes (Richardson et al. 2000; Zhou and Anderson 2002). In this case, while premature neurogenesis caused by the inhibition of Notch signal results in the depletion of NE cells, forced activation of Notch signal increases oligodendrocyte precursor cells (Park and Appel 2003, Fig. 1). Furthermore, in other parts of the CNS, forced activation of Notch signal promotes expression of *Glial Fibrillary Acidic Protein (GFAP)*, an astrocyte marker (Tanigaki et al. 2001; Ge et al. 2002). These reports indicate that Notch signal is involved both in the early inhibition of neuronal differentiation and the later promotion of glial differentiation (Fig. 1).

How, then, do NE cells respond differently to the Notch activation in different time of the development? One possibility is that the micro-environment may be changed during the course of the neural development, and that such changes may alter the response of NE cells to the Notch activation. Another possibility is that the NE cells themselves have an internal clock, which alters the response to the signal. Whichever the case, there will be change(s) in NE cells over time, and N-CoR, a transcriptional co-repressor, may be involved in this process (Hermanson et al. 2002). Thus, it was suggested that the level of N-CoR expression in the NE is decreased as development proceeds. N-CoR knockout mice, furthermore, showed an elevated expression of *GFAP* in the NE. The relationship between N-CoR and Notch signal, however, remains to be examined.

As discussed above, astrocytes and oligodendrocytes differentiate from different parts of the developing CNS (Fig. 1). To induce different kinds of glial cells in the different parts of the CNS, similar mechanisms to those that determine the neuronal subtypes appear to be used. Thus, dorsal neural tube cells are affected by Bone Morphogenetic Protein (BMP) signaling mechanisms, and ventral cells are exposed to Shh (Sonic Hedgehog) signal (Jessell 2000; Fig. 1). For example, *Olig2* gene, which encodes a bHLH transcription factor and is involved in

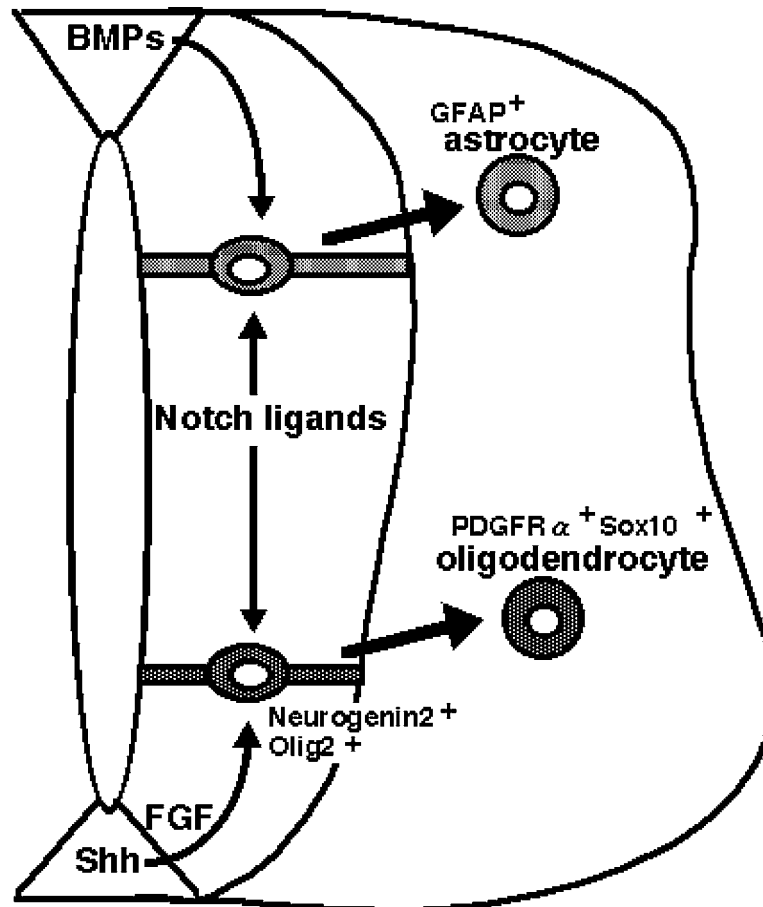


Fig. 1. Locally provided signaling molecules determine astrocyte and oligodendrocyte fates. The activation of Notch signal by ligands expressed in young neurons inhibits neuronal differentiation of NE cells. Under the influence of dorsalizing factors, such as BMP proteins, NE cells differentiate into GFAP-positive astrocyte. In the ventral neural tube, Shh and/or FGF signals promote Sox10 and PDGFR $\alpha$ -positive oligodendrocyte differentiation from NE cells by inducing expression of neurogenin2 and Oligo2 transcription factors.

BMP, Bone Morphogenetic Protein; FGF, Fibroblast Growth Factor; GFAP, Glial Fibrillary Acidic Protein; PDGFR $\alpha$ , Platelet-Derived Growth Factor Receptor  $\alpha$ ; Shh, Sonic Hedgehog.

oligodendrocyte differentiation as well as motor neuron differentiation (Lu et al. 2000; Zhou et al. 2000), has been identified as a gene induced by Shh signal in the pMN domain (Lu et al. 2000). *Olig2* expression, followed by the expression of a related gene, *Olig1*, as well as the HMG-type transcription factor *Sox10*, appears to promote oligodendrocyte differentiation. Recent studies, however, indicate that Fibroblast Growth Factor (FGF) signaling promotes oligodendrocyte differentiation in vitro (Chandran et al. 2003; Kessar et al. 2004; Fig. 1). It is possible, therefore, that

FGF signal relays the Shh signal to promote oligodendrocyte differentiation. On the other hand, relatively little is known about the spatial regulation of astrocyte differentiation. Since BMP signal promotes astrocyte differentiation in vitro (Yanagisawa et al. 2001), and since over-expression of BMP4 promotes astrocyte differentiation, while inhibiting oligodendrocyte differentiation in vivo (Mekki-Dauriac et al. 2002; Gomes et al. 2003), the inhibitory effect of the dorsal neural tube for oligodendrocyte differentiation (Wada et al. 2000) can be explained by the strong expres-

sion of BMP-related genes in this region (Fig. 1).

### *Peripheral glial subtypes*

The PNS is mostly generated by neural crest-derived cells, except for some neurons in the cranial ganglia that are derived from placodes. Neural crest cells also give rise to melanocytes, some endocrine cells, and may also contribute to smooth muscle and connective tissues in the head. Crest-derived cells in the PNS include satellite glia of peripheral ganglia and the enteric nervous system, as well as myelin-forming Schwann cells along the spinal nerves.

### *Neuregulin signal*

Neuregulin1 (also called as, ARIA, Glial Growth Factor, and Neu differentiation factor) has been shown to be involved in the glial differentiation from neural crest-derived cells (Jessen and Mirsky 2002, for a review). Isoforms of Neuregulin, such as a diffusible form and a membrane-bound form, are generated by alternative splicing, but all the isoforms share a common EGF-like domain, which binds to ErbB family receptors.

Previous reports have revealed that Neuregulin1 instructively induces glial differentiation (e.g. Shah et al. 1994; Leimeroth et al. 2002). Consistently, knockout mouse embryos that lack *Neuregulin1* or the genes for its receptor showed a severe reduction of Schwann cell precursors along the spinal nerve (Jessen and Mirsky 2002). While Neuregulin1 has also been suggested to regulate migration and survival of Schwann cells (Jessen and Mirsky 2002), these results suggest an important role for *Neuregulin1* in glial development. It is important to note, however, that we still do not know (1) the timing of Neuregulin1 signal in Schwann cell differentiation, (2) the source of Neuregulin1 in Schwann cell differentiation, and (3) if the *Neuregulin1* gene is involved in satellite glia differentiation.

We have previously identified a novel gene encoding a secreted molecule with EGF-like repeats, designated *Seraf* which regulates distri-

bution of Schwann cells in vivo (Wakamatsu et al. 2004b). The expression of *Seraf* can be first detected in a subset of avian crest-derived cells migrating on the medial pathway between the neural tube and the somite. Subsequently the *Seraf* expression is detected only in cells associated with the ventral nerve, suggesting that *Seraf*-positive cells are early Schwann cell precursors. Thus, we have suggested that *Seraf* expression is the earliest indicator yet found for the Schwann cell lineage. As development proceeds, *Seraf* expression gradually diminishes from the proximal portion of the nerve cord, and this down-regulation of *Seraf* expression coincides with the up-regulation of *P0*, a PNS glial marker. When cultured neural crest cells are exposed to the EGF-fragment of Neuregulin1, a robust expression of *Seraf* is induced, further supporting the idea that Neuregulin1 promotes Schwann cell differentiation. Neuregulin1 provided through axons of motor neurons and sensory neurons may account for the *Seraf* expression in Schwann cell precursors along the nerve cord (Fig. 2). Alternatively, it is possible that endogenous expression of *Neuregulin1*, provides an autocrine trigger for the initial induction of endogenous *Seraf* expression, but such an autocrine pathway has yet to be identified.

Although the involvement of *Neuregulin1* in Schwann cell differentiation is most likely, it is still unclear if *Neuregulin1* is also involved in satellite glia differentiation. As mentioned above, *Neuregulin1* expressed by sensory neurons in the dorsal root ganglia may be able to promote satellite glia differentiation (see also below), but due to a lack of definitive marker(s) for satellite glia, it is difficult to assay the effect in vitro. There is no clear description for the satellite glia phenotype in *Neuregulin1/ErbB* receptor knockouts, partly because some researchers do not distinguish satellite glia and Schwann cells.

### *Satellite glia differentiation*

Based on a few observations in culture, it has been suggested that Schwann cells and satellite

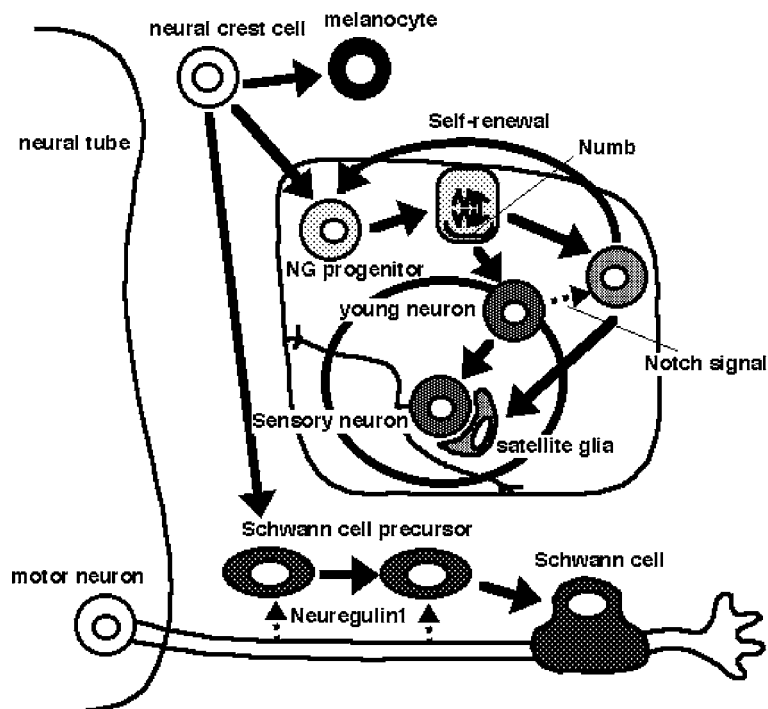


Fig. 2. Differentiation of PNS glial cells. Neural crest-derived NG progenitors in the periphery of nascent ganglia undergo asymmetric cell divisions initially to generate neurons, and subsequently to generate satellite glial cells. Sensory neurons and satellite glia later intermingle to form mature ganglia. A subset of crest-derived cells differentiate into Schwann cell precursors under the influence of Neuregulin1, likely to be provided by motor neurons. Maturing Schwann cells subsequently rap the axons to form myelins.

glia segregate from the common glial precursors. However, since these glial cells differentiate in different locations and at different times, like astrocytes and oligodendrocytes, they are more likely to segregate independently from neural crest-derived cells (Fig. 2). If this be the case, when and how are these distinct cell lineages be established in vivo?

Previous studies have shown that neuroglial (NG) precursors are present in early avian crest-derived populations in vitro (Henion and Weston 1997), and it is most likely that satellite glial cells originate from these cells. The earliest neuronal differentiation from neural crest can be detected during the migration on the medial pathway (Wakamatsu and Weston 1997; Wakamatsu et al. 2000). Subsequently, in nascent dorsal root ganglia (DRG), differentiating neurons form a core, surrounded by a layer of undifferentiated,

proliferative cells (Wakamatsu et al. 2000; Fig. 2). Our BrdU-labeling experiments suggested that the peripheral undifferentiated cells initially generate additional sensory neurons, and later give rise to satellite glia that subsequently intermingle with neurons (Wakamatsu et al. 2000; Fig. 2). The behavior of peripheral DRG cells is similar to the CNS NE cells, so it is tempting to speculate that these cells possess stem cell properties, undergoing self-renewal while generating both neurons and glia. There are also other similarities in regulating the differentiation of neurons in the CNS and the PNS. For example, young neurons in the developing PNS express *Delta1*, and undifferentiated peripheral cells in the nascent ganglia express *Notch1* and *Sox2* (Wakamatsu et al. 2000, 2004a; Fig. 2). Notch activation inhibits neuronal differentiation, and *Sox2* is involved in this process (Wakamatsu et al. 2000, 2004a). Furthermore,

when undifferentiated crest-derived cells divide, Numb protein, a Notch antagonist, localizes asymmetrically, and is segregated unevenly. Thus, asymmetrical segregation of Numb appears to modulate Notch signaling so that daughter cells assume different fates (Wakamatsu et al. 1999, 2000; Fig. 2).

Is Notch signaling directly involved in satellite glia differentiation? We have previously reported that the forced activation of Notch signal by a transfection of a constitutively-active form of Notch1 did not promote glial differentiation from cultured crest cells, based on the expression of *P0* (Wakamatsu et al. 2000). In contrast, there is a report showing that a transient treatment of cultured crest-derived cells with a soluble form of Delta1 instructively promotes differentiation of *GFAP*-positive cells (Morrison et al. 2000). It is not clear how this difference occurred, but the lack of definitive satellite glia marker makes the interpretation of data difficult. Nevertheless, as is the case in the CNS, Notch signal alone seems to be insufficient to explain the timing difference of neuronal and satellite glial differentiation. Although, as discussed above, there are similarities between CNS and PNS neurogenesis and gliogenesis, the analogy is not fully applicable, since *Oligo1/2* genes are not expressed in the developing PNS. One possibility is, as we previously suggested (Wakamatsu et al. 2000), that *Neuregulin1* expressed in newborn neurons cooperates with Notch signaling to promote glial differentiation of neighboring cells.

As mentioned above, oligodendrocyte precursors express *Sox10*, a member of group E Sox genes. Previous reports indicated that *Sox10* is important for the PNS glial development (e.g. Britsch et al. 2001; Jessen and Mirsky 2002), suggesting an analogy in glial differentiation of the CNS and the PNS. However, *Sox10* is expressed not only in the glial cells, but also in undifferentiated crest-derived cells. Furthermore, *Sox10* function is also important for the other crest-derived sublineages (e.g. Dutton et al. 2001). Alternatively, another group has suggested that

*Sox10* preserves crest-derived cells as stem cells (Kim et al. 2003). Although these workers considered *Sox10*-positive peripheral cells in nascent ganglia to be satellite cells, there is no basis for this suggestion (see Fig. 2). In any case, even if *Sox10* function were required for glial differentiation by crest-derived cells, *Sox10* could not be the master gene for gliogenesis. Since Sox family proteins are well known to require partner proteins for target activation (Kamachi et al. 2000), it is more likely that spatial and temporal differences of expression of such partners account for multiple roles of *Sox10* in neural crest-derived cell lineages.

### Summary

Since our understanding of neurogenesis is now well advanced, researchers have turned their attention in recent years to the regulation of gliogenesis. Yet, many questions still remain unanswered, including (1) the basis for temporal distinctions between glial and neuronal development, (2) the migration of nascent glial cells and their association with neurons, (3) the regulation of myelin formation, and (4) the function of satellite cells. Although these issues could not be addressed in this review, they will surely be the basis for additional exciting and informative work in the future.

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