

# Ventral Neurogenesis and the Neuron-Glial Switch

## Minireview

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**In the developing spinal cord, neuroepithelial precursors at different positions along the dorsal-ventral axis generate distinct neuronal and glial subtypes. For example, one group of ventral precursors generates neurons followed by oligodendrocytes. A spate of recent articles, including several in this issue of *Neuron*, are devoted to the mechanisms governing neuronal and glial subtype specification in the ventral cord. We review these studies and discuss the nature of the ventral neuron-oligodendrocyte switch.**

Development of the spinal cord begins with the formation of the neural tube and the establishment of signaling centers both inside and outside the cord. Sonic hedgehog (Shh) from the notochord and floor plate and members of the transforming growth factor  $\beta$  family from the roof plate form opposing gradients of diffusible signaling molecules along the dorsal-ventral axis. Together with analogous gradients along the anterior-posterior axis, they establish a grid of positional information within the cord. These extracellular cues are interpreted by precursor cells and converted into intracellular transcriptional circuits that ultimately determine their differentiated cell fates.

Neurons are generated early during neural tube development; glial cells (oligodendrocytes and astrocytes) are generated later. The progenitors of different species of neurons—motor neurons (MNs) or interneurons, for example—are formed within their own specialized neuroepithelial domains. How are these domains defined? Previous work has shown that ventral domains (of which there are five) are defined by sets of homeodomain (HD) transcription factors that are activated or repressed by different threshold concentrations of Shh (Briscoe et al., 2000, 2001; Briscoe and Ericson, 2001; Jessell, 2000). The particular set of HD factors expressed in each domain regulates downstream genes that determine neuronal identity. An article by Vallstedt et al. (2001) in this issue of *Neuron* reinforces this view and suggests that duplication of HD genes and subsequent modification of their regulatory interactions has been a driving force for generating neuronal diversity in the spinal cord during evolution. Two more papers by Mizuguchi et al. (2001 [this issue of *Neuron*]) and Novitch et al. (2001 [also in this issue of *Neuron*]) show that the recently discovered basic helix-loop-helix (bHLH) protein Olig2 is crucial for

defining the neuroepithelial domain that gives rise to MNs and, in combination with the neurogenic bHLH protein Neurogenin2 (Ngn2), is required to specify MN fate.

Intriguingly, oligodendrocyte progenitors (OLPs) originate from the same region of the ventral neuroepithelium that at earlier times gave rise to MNs and/or V3 interneurons (Soula et al., 2001; Richardson et al., 2000; Sun et al., 1998, Xu et al., 2000). How do these precursors suddenly switch from neuron to OLP production? The studies by Mizuguchi et al. (2001) and Novitch et al. (2001) together with the accompanying article by Zhou et al. (2001 [this issue of *Neuron*]) suggest an elegant mechanism. A temporal shift of gene expression patterns in the ventral cord provides Olig2 with successive regulatory partners, first Ngn1/2 and Pax6, and then Nkx2.2. In collaboration with these factors, Olig2 first specifies MNs, then OLPs.

### *Specification of Neuronal Subtype by Repression of Alternative Fates*

HD proteins in the ventral spinal cord have been classified into two groups on the basis of their expression patterns and mode of regulation by SHH. Class I proteins (e.g., Pax6, Irx3) are repressed by Shh and are therefore expressed at a distance from the floor plate. In contrast, class II proteins (e.g., Nkx2.2, Nkx6.1) are induced by Shh (Figure 1). A three-step model for neuronal specification in the ventral neural tube has been proposed: (1) Shh signaling initiates restrictions in the expression domains of HD proteins, (2) the subsequent sharpening and maintenance of domain boundaries depends on crossregulatory interactions between class I and class II proteins, and (3) the combinatorial activities of class I and class II proteins within each domain specify neuronal subtype (Briscoe and Ericson, 2001; Jessell, 2000).

More recently it has been shown that the class I and class II proteins act directly as transcriptional repressors, via recruitment of Groucho (Gro) corepressors (Muhr et al., 2001). This suggests that neuronal specification is achieved by mutual repression of class I and class II proteins at progenitor domain boundaries, as well as repression of downstream determinants of neuronal cell fate (e.g., the MN-specific HD protein MNR2) (Briscoe and Ericson, 2001; Muhr et al., 2001). Thus, specification of neuronal subtype within a given neuroepithelial domain results from the repression of all other possible fates in that domain. For example, Pax6 represses *Nkx2.2* expression, excluding it from the MN progenitor domain (pMN) and restricting it to the V3 interneuron progenitor domain (p3) (Ericson et al., 1997). In the *Small eye* (*Sey*) mouse mutant, which lacks Pax6 function, *Nkx2.2* is derepressed and expands dorsally into the pMN domain. There, *Nkx2.2* represses MNs and leads to V3 neuron generation. Thus, in normal mice, Pax6 promotes the MN pathway in pMN by repressing a repressor of MN fate—the regulatory equivalent of a grammar built on double-negatives. This has been labeled the *derepression* model of neuronal subtype specification (Briscoe and Ericson, 2001; Muhr et al., 2001).

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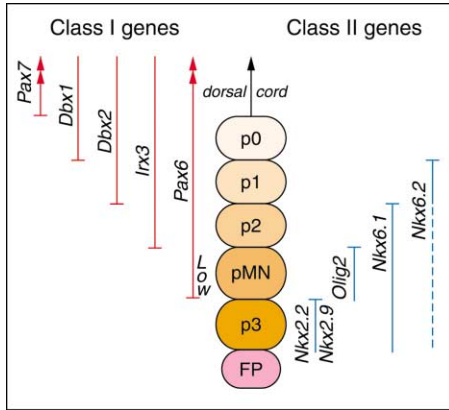


Figure 1. Class I and Class II Repressors in the Ventral Spinal Cord  
All encoded proteins are homeodomain transcription factors except for Olig2, which is a basic helix-loop-helix factor. Nkx6.2 expression is restricted to the p1 domain in mouse (illustrated) but in chick overlaps Nkx6.1 down to the floor plate (dotted line).

### The Search for Predicted Repressor Proteins

The idea that neuroepithelial domain boundaries are defined by crossinhibition between class I and class II proteins predicts that there should be a class I/class II doublet assigned to every boundary. While a full set of class I proteins has been identified, only two of these had known class II counterparts. The known class I/class II pairs were Pax6/Nkx2.2 at the p3/pMN boundary and Nkx6.1/Dbx2 at the p2/p1 boundary. Now, two “missing” class II proteins have been found: Vallstedt et al. (2001) show that Nkx6.2 is a class II partner of Dbx1 at the p1/p0 interface and Novitch et al. (2001) identify Olig2 as a putative class II partner of Irx3 at pMN/p2 (Figure 1).

Nkx6.1 was previously shown to be a class II repressor involved in the specification of V2 interneurons and MNs. With its class I counterpart, Dbx2, it defines the position of the p1/p2 boundary. Mice lacking Nkx6.1 have no V2 interneurons and a reduced number of MNs (Sander et al., 2000). Vallstedt et al. (2001) demonstrate that the reduction—rather than complete loss—of MNs is due to a ventral expansion of Nkx6.2 expression into the p2 and pMN domains, where it compensates for the loss of Nkx6.1. In keeping with this functional redundancy between Nkx6 proteins, ectopic expression of either Nkx6.1 or Nkx6.2 in the chick spinal cord induces ectopic MN production. Note that Nkx6.2 fails to substitute for Nkx6.1 in the p2 domain of the Nkx6.1 knockout. This is because Nkx6.2, being a weaker repressor than Nkx6.1, allows Dbx2 expression to expand ventrally into p2 where it blocks V2 production.

Apart from their redundant functions, Nkx6.1 and Nkx6.2 also have distinct functions. In the normal mouse spinal cord, Nkx6.2 expression is restricted to the p1 domain just dorsal to Nkx6.1. The dorsal boundary of Nkx6.2 expression coincides with the ventral limit of Dbx1, suggesting that these genes might form another class I/class II doublet. Vallstedt et al. (2001) confirm this by demonstrating that in Nkx6.2 null mice, expression of Dbx1 expands ventrally into the p1 domain and induces ectopic V0 neurons. Nkx6.2 therefore fits the bill as a bona fide class II partner of Dbx1.

The bHLH transcription factors Olig1 and Olig2 were recently identified in connection with oligodendrocyte development (hence their names) (Lu et al., 2000; Takebayashi et al., 2000; Zhou et al., 2000). However, Olig2 is expressed in the pMN domain well ahead of the appearance of overt oligodendrocyte progenitors (OLPs), suggesting a role for Olig2 in MN specification. Indeed, Novitch et al. (2001) and Mizuguchi et al. (2001) demonstrate that ectopic expression of Olig2 in the chick spinal cord or hindbrain in ovo represses Irx3 expression and vice versa. Thus, crossrepression between Olig2 and Irx3 fixes the dorsal boundary of the pMN domain, defining them as a class I/class II pair. Olig2 is an unconventional class II protein though because it is a bHLH protein, not an HD protein like all the others.

### Interactions between HD and bHLH Transcription Regulators in Neuronal Subtype Specification

On top of the HD protein pathway of neuronal specification, there appears to be a parallel neurogenic pathway involving bHLH proteins. bHLH genes in *Drosophila* and vertebrates can direct pan-neuronal as well as subtype-specific programs of differentiation (Guillemot, 1999). In the vertebrate spinal cord, several bHLH genes (*neurogenins*, *Mash1*, *Math1*, *Olig1/2*) are expressed in discrete regions of the neuroepithelium. Mizuguchi et al. (2001) and Novitch et al. (2001) demonstrate that the expression pattern of Olig2 in the spinal cord is indeed regulated by HD proteins. In addition to the crossrepressive interactions between Olig2 and Irx3 at the pMN/P2 border, Nkx6.1 appears to induce expression of Olig2 in pMN, and Nkx2.2 sets the ventral limit of Olig2 at the p3/pMN boundary (Novitch et al., 2001). The possibility that HD and bHLH neurogenic pathways intersect was also suggested by Scardigli et al. (2001). By studying mice mutant for the HD protein Pax6 and Ngn2, they found evidence for a two-way genetic interaction. Scardigli et al. (2001) further showed that Ngn2 is necessary for proper MN specification. However Ngn2 is only a permissive component of the MN pathway and is likely to interact with other transcription factors that might have more restricted expression patterns and more specific roles in neuronal subtype specification.

Mizuguchi et al. (2001) and Novitch et al. (2001) now further define the role of Ngn2 in MN generation and demonstrate that it collaborates with Olig2 to direct the expression of both pan-neuronal and MN-specific characteristics. They report that expression of Olig2 in the neural tube precedes that of Ngn2. At the time of MN generation, Ngn2 is expressed strongly in the pMN domain, suggesting that Olig2 may influence its expression. Indeed, ectopic expression of Olig2 in the chick spinal cord and hindbrain showed that Olig2 leads to upregulation of Ngn2 and induction of cells expressing MN differentiation markers. Loss-of-function studies by expression of a putative dominant-negative form of Olig2 (Olig2 bHLH domain fused to the transactivation domain of Herpes Virus Protein VP16) in the pMN domain caused a reduction in the number of cells expressing MNR2/Lim3/HB9 within pMN, indicating that the activity of endogenous Olig2 is indeed essential for MN generation.

In addition, Novitch et al. (2001) ectopically expressed a hybrid protein comprising the bHLH domain of Olig2 fused to the repressor domain of the *Drosophila* En-

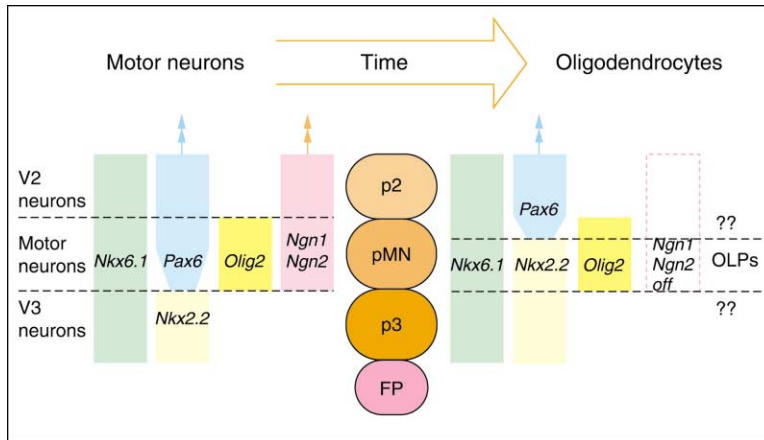


Figure 2. The Neuron-Oligodendrocyte Fate Switch in Chick Spinal Cord

At early times (E3–E5 chick) motor neurons are generated from the pMN domain under the influence of Olig2 in conjunction with Ngns. Later (E6–E7), Ngn1 and Ngn2 are downregulated in this part of the cord. Subsequently, the expression domains of Nkx2.2 and Olig2 shift relative to one another and generate a region of overlap. This triggers a switch in the activity of Olig2 in the overlap, which now generates oligodendrocyte progenitors (OLPs). Note that the situation is different in mouse (see text).

grailed protein (EnR). They found that this hybrid protein was able to mimic the activity of genuine Olig2 in repressing *Ir3* and inducing MN markers, suggesting that Olig2 acts as a direct transcriptional repressor like other class I and class II proteins. In contrast, Mizuguchi et al. (2001) found that an Olig2-EnR hybrid repressor was unable to substitute for wild-type Olig2 in inducing MN differentiation. This discrepancy will be clarified in future by identification of direct targets of Olig2 and Ngn2 as well as true loss-of-function studies in knockout mice. Nevertheless, it seems to be agreed that Ngn2 and Olig2 collaborate in promoting MN generation by inducing pan-neuronal and MN-specific features, respectively.

#### The Switch from MN to OLP Production

At around midgestation, when MN production ceases, OLPs start to be produced from the ventral neuroepithelium instead (Richardson et al., 2000; Miller, 1996). Development of OLPs, like MNs before them, depends on Shh signaling from the ventral midline. How is the switch from neuronal to glial cell production orchestrated? Zhou et al. (2001) have made some key observations that shed light on this question. They report that, shortly after MN production has finished, the proneural bHLH proteins Ngn1 and Ngn2 are downregulated in the ventral neuroepithelium (Figure 2). Subsequently, the domains of Nkx2.2 and Olig2 expression, which previously were mutually exclusive, shift relative to one another to create a region of overlap. Migratory (Olig2, Nkx2.2) double-positive OLPs then appear within the overlap, disperse throughout the spinal cord and differentiate into postmitotic, myelinating oligodendrocytes in white matter. These observations suggest that when the pan-neurogenic program is turned off in the pMN domain, Olig2 acquires a new function in promoting OLP development. By ectopic expression of *Olig2* in the embryonic chick spinal cord in ovo, Zhou et al. (2001) demonstrate that Olig2 by itself is insufficient to induce OLPs unless Ngn1 and Ngn2 are concurrently downregulated by coexpression of either *Nkx2.2* or constitutively active components of the Notch signaling pathway (Notch activation is known to repress Ngns in other developmental contexts).

It is unlikely that Nkx2.2 is responsible for downregulating Ngns during normal development because extinction of Ngn expression in the relevant part of the neuro-

epithelium precedes the dorsal expansion of Nkx2.2 expression in vivo. Nevertheless, it seems an inherently attractive and likely scenario that downregulation of Ngns by whatever mechanism is a key event in the switch from neuronal to glial cell fate specification.

What is the role of Nkx2.2 in OLP development in vivo if not to downregulate Ngns? There is a significant difference between the activities of Nkx2.2 and the Notch signaling pathway in OLP specification. Ectopic *Nkx2.2* in collaboration with *Olig2* drives production not only of OLPs, defined by expression of Sox10 and PDGF receptor- $\alpha$  (PDGFRA), but of seemingly more differentiated stages of the lineage defined by myelin basic protein and myelin proteolipid protein (MBP and PLP). In contrast, Notch signaling together with Olig2 induces OLPs but not MBP or PLP expression. It could be that Nkx2.2 is normally required (in addition to Ngn downregulation) for myelin gene expression, not for OLP specification per se. This would fit with the fact that, in rats and mice, PDGFRA-positive OLPs are generated mainly outside the Nkx2.2 domain (Lu et al., 2000; Sun et al., 1998) and do not express detectable levels of Nkx2.2 as they migrate (N.P. and W.D.R., unpublished data); however, after settling in white matter, differentiating oligodendrocytes in both chicks and rodents express Nkx2.2 strongly (Xu et al., 2000; N.P., N.K., and W.D.R., unpublished data).

#### Remaining Questions

Several intriguing questions remain. Early on, during MN production, Nkx2.2 represses Olig2 and so defines its ventral expression limit. How is it, then, that Nkx2.2 and Olig2 can later be coexpressed in the same cells and collaborate in oligodendrocyte development? Perhaps Nkx2.2 can repress Olig2 only in the presence of Ngns. A major question to address in future is the nature of the direct targets of Olig2, Nkx2.2, and Ngn2. This will be essential to understand how MN and OLP fates are determined and indeed what kinds of molecular events accompany cell fate specification in general.

In chick, both (Nkx2.2, Olig2) double-positive OLPs and Nkx2.2-positive, Olig2-negative cells appear to migrate from neighboring regions of the ventral neuroepithelium. Perhaps these represent different classes of OLPs (Spassky et al., 2000). This is the explanation favored by Zhou et al. (2001). However, they could also

be late-migrating neurons or another class of glial cells—possibly astrocyte progenitors. In mouse, there seem to be separate *Nkx2.2*-positive and *Nkx2.2*-negative OLPs from above and below the p3/pMN boundary (N.P., N.K., and W.D.R., unpublished data); do these give rise to different types of oligodendrocytes? Do any other neuroepithelial domains in the ventral or dorsal spinal cord switch from neuron to glial cell production—for example, from neurons to astrocytes?

What is the role of *Olig1*? In rodents, *Olig1* is coexpressed with *Olig2* in the ventral spinal cord. Gain-of-function experiments in ventral regions of the mouse neural tube show that *Olig1* inhibits production of V3 interneurons but not V2 interneurons or MNs (Sun et al., 2001). Does *Olig1* have a contributory role in neuron-glial switching or in OLP generation? Another recent study shows that when ectopically expressed in the mouse neocortex *in vivo*, *Olig1* generates a mixture of oligodendrocytes and astrocytes and causes neuronal cell death (Lu et al., 2001). This raises additional questions about the relationships among oligodendrocytes, neurons, and astrocytes during development and between the molecular mechanisms of cell diversification in the spinal cord and brain (e.g., Tekki-Kessararis et al., 2001).

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