Cell, Vol. 106, 651–654, September 21, 2001, Copyright ©2001 by Cell Press

Cracking the Transcriptional Code for Cell Specification in the Neural Tube

Minireview

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The bHLH repressor Olig2 participates in the transcriptional code governing cell fate specification in the ventral spinal cord. By temporally selective interactions with other transcription factors, Olig2 first directs motor neuron fate and later switches to promoting oligodendrocyte production.

During development of the CNS, a vast number of distinct types of neurons and glia arise from dividing progenitor cells lining the lumen of the neural tube. Upon exiting the cell cycle, newly born neurons migrate laterally out of the ventricular zone into their final positions in the mantle zone, where they become incorporated into the local neural circuitry. Cell cycle exit and subsequent differentiation is thought to be mediated by the interplay of extracellular signaling molecules and nuclear transcription factors, governing the expression of particular sets of target genes that underlie the acquisition of specific cellular traits.

In general, newly born CNS cells acquire phenotypes that are stereotypic for their site of origin within the ventricular zone. Hence, important steps in establishing the fate of cells appear to occur at the level of the progenitor cells from which they originate. The ventricular zone of the ventral spinal cord was shown to express several homeodomain (HD) transcription factors, which define five discrete domains of progenitor cells (Figure 1; Briscoe et al., 2000). The combinatorial action of these sets of HD transcription factors is thought to control the expression of particular downstream genes encoding cell fate determinants, thereby mediating the production of specific neuronal types from each progenitor domain.

The patterning activity of the secreted signaling molecule Shh has been shown to be essential for the generation of ventral CNS cells, such as motor neurons (Chiang et al., 1996). In the neural tube, the ventral-most cells, forming the floor plate, constitute a prominent source of this molecule, leading to the establishment of a ventral-to-dorsal decreasing concentration gradient of Shh activity. Exposure of neural progenitor cells to different doses of Shh has been shown to either induce or repress the expression of several progenitor cell transcription factors in a graded manner (Figure 1; Ericson et al., 1997), thereby providing a means for generating molecular differences within the ventricular zone. Yet, this still leaves open the question of how a smooth inductive gradient is converted into well-defined progenitor domains.

Based on their responsiveness to Shh, the progenitor cell factors can be grouped into two major categories (Briscoe et al., 2000): class I proteins, which are repressed by various concentrations of Shh, and class II proteins, which are induced by Shh activity (Figure 1). Pairs of class I and class II factors have been shown to negatively regulate each other via mutual transcriptional repression (Figure 1; Muhr et al., 2001). These crossrepressive interactions are thought to result in a delineation of cells either expressing one factor or the other, ultimately leading to a sharpening and stabilization of the boundaries between the initially coarse progenitor domains (Figure 1; Briscoe et al., 2000). A series of loss-of-function and gain-of-function studies elegantly demonstrated that the production of particular cell fates can be predicted by experimentally introduced alterations in this code of HD factors, thereby lending support to this simple combinatorial model for cell fate determination (Ericson et al., 1997; Briscoe et al., 1999; Briscoe et al., 2000).

Until recently, however, this model had a few gaps (A–C in Figure 1,). In particular, it was not well understood how the sharp ventral boundaries of some of the progenitor domains demarcated by the expression of class I factors are established. Another issue that has not been particularly clear is how the specification of neuronal subtypes, as mediated by progenitor factors, is coordinated with the acquisition of panneuronal traits and how these events are necessarily coupled to the steering of progenitor cells out of the cell cycle prior to terminal differentiation. A third issue is how the progenitor code is regulated during the temporal shift toward the production of oligodendrocytes that occurs in the ventral neural tube after the period of neurogenesis (Figure 2B). A number of recent papers, discussed below, have addressed these issues and fill in many of the gaps in our understanding of the transcriptional code that specifies cell identity in the ventral spinal cord.

Transcriptional Repressors in the Specification of Motor Neuron Fate

The bHLH transcription factor Olig2, together with its paralog Olig1, was first identified as a marker for oligodendrocyte progenitor cells (Lue et al., 2000; Zhou et al., 2000). Oligodendrocytes (OCs) are generated in the ventral neural tube well after the peak time of motor neuron (MN) genesis (see below). Olig2, however, displayed a suspicious earlier phase of expression that precisely marks the MN-generating pMN progenitor domain (Figure 2A). Two recent papers have now explored the role of Olig2 in MN fate determination (Novitch et al., 2001; Mizuguchi et al., 2001).

Upon misexpression in the neural tube of chick embryos, Olig2 promotes the generation of ectopic MNs at the expense of more dorsal cell fates, like v2, and v1 interneurons (INs) (see Figure 1). In addition, ectopic MN production by Olig2 was accompanied by the repression of Irx3 from more dorsal progenitor cells. Irx3 is a potent repressor of MN fate (Briscoe et al., 2000), and keeping with the theme of crossrepression, Olig2 was likewise repressed by ectopic Irx3 expression (Figure 2A; Novitch et al., 2001). Novitch and coworkers, as well as Zhou et

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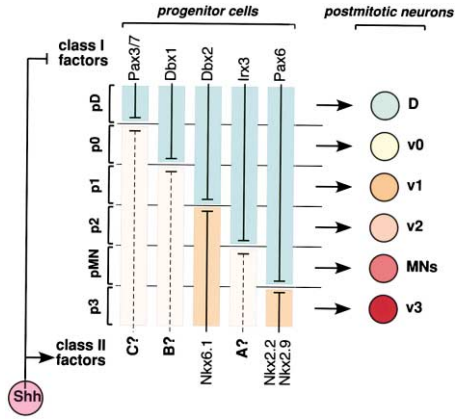

Figure 1. A Combinatorial Code of HD Factors Defines Progenitor Cell Identity in the Ventral Neural Tube

Crossrepressive interactions among class I and class II factors, which are either repressed or activated by Shh, underlie the setting up of defined progenitor domains (pD-p3), each giving rise to a specific class of neurons (D-v3). The identity of three potential class II factors (A–C) counteracting the activity of class I factors remained obscure until recently. "A" has recently been identified as Olig2 and "B" as Nkx6.2 (see text).

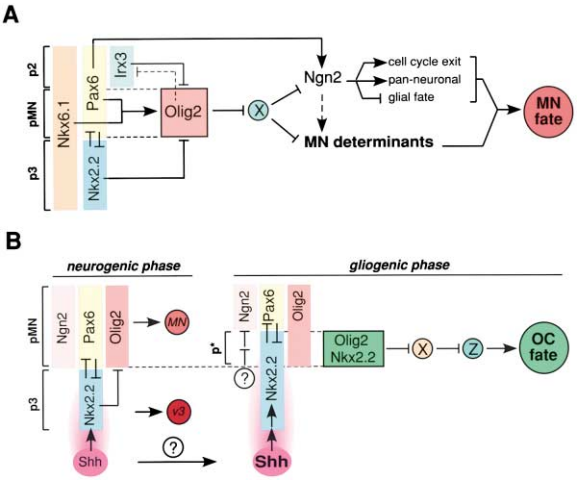

Figure 2. Olig2 First Directs Motor Neuron Fate and Later Switches to Promoting Oligodendrocyte Production by Ventral Progenitor Cells

(A) Olig2 activates MN determinants (such as MNR2, Lhx3, Isl1/2, and HB9) via repression of Irx3 and an unknown repressor, X. Ngn2 becomes derepressed by Olig2 and promotes a neurogenic lineage, as well as cell cycle exit, in addition to possibly activating MN determinants. Olig2 is positively regulated by Nkx6.1 and Pax6 and negatively by Nkx2.2 and Irx3. Dotted interactions refer to (1) Olig2-repressing Irx3 only to a limited extent and (2) Ngn2 being required but not sufficient to induce MN determinants (see text).

(B) A switch from MN to OC fate by ventral progenitor cells, possibly mediated by temporal elevation of Shh activity: coexpression of Olig2 and Nkx2.2 now defines the p* progenitor domain, from which OCs arise. The identity of targets of combined Olig2 and Nkx2.2 activity (X), as well as the derepressed positively acting OC determinants (Z), remains obscure.

al. (2001) (see below), moreover provide evidence that Olig2 exerts its function through its direct actions as a transcriptional repressor.

These results therefore indicate that Olig2 constitutes one of the missing class II factors that participates in the progenitor cell code for the specification of MNs (A in Figure 1). Olig2, however, differs slightly from other constituents of the code: First, it is limited to the pMN domain not only via interactions with the class I factor Irx3 at the p2/pMN boundary, but also ventrally by the class II factor Nkx2.2, which itself is kept in check by indirect repression through Pax6 at the pMN/p3 boundary (Figure 2A; Briscoe et al., 2000). Second, Olig2 is the first example of a bHLH factor to be included into the transcriptional code (see Figure 1). Perhaps this is not a surprise, since recently similar roles for other bHLH proteins have been established in the determination of progenitor cell identity and cell fate in the dorsal neural tube (Gowan et al., 2001).

Olig2 appears to drive progenitor cells toward MN differentiation by utilizing two different strategies: First, it helps to set up the pMN domain by repressing more dorsal identities and, possibly via this activity, allows the derepression of factors subsequently acting as MN determinants (Figure 2A). Second, the repressive activity of Olig2 somehow appears to promote the expression of the neurogenic bHLH transcription factor Neurogenin2 (Ngn2) independently of its repression of Irx3 (Novitch et al., 2001). Neurogenins are thought to promote the acquisition of panneuronal characteristics (Guillemot, 1999; Scardigli et al., 2001; Nieto et al., 2001), although there is evidence in other regions of the CNS that Ngns and other proneural bHLH factors are also involved in more specific aspects of neurogenesis, such as neuronal subtype specification (Guillemot, 1999; Gowan et al., 2001). Ngn2 activity alone, or when induced by Olig2 in the presence of persistent Irx3 activity, promoted the generation of cells displaying neuronal characteristics, but apparently without promoting specific MN or IN fates. Olig2 therefore appears to coordinate the acquisition of panneuronal characteristics, as well as the determination of a specific type of neurons, MNs.

Another important aspect regarding the function of neurogenic bHLH proteins is their capability to drive neural progenitors out of the cell cycle (Farah et al., 2000). Novitch et al. demonstrated that, via the activation of Ngn2, Olig2 appears to restrict the number of cell cycles available to pMN progenitor cells. Ngns are thought to be subject to lateral inhibition by Notch/Delta signaling (Guillemot, 1999), which presumably underlies the mosaic appearance of Ngn2 expression in neural progenitor cells of the neural tube. This control might constitute an essential mechanism to prevent premature depletion of the respective progenitor cell pool by assuring that at any given moment only a subset of progenitors becomes committed to leave the cell cycle.

In another recent paper, the function of the partially redundant HD repressor proteins Nkx6.1 and Nkx6.2 in ventral neural progenitor cells was studied (Vallstedt et al., 2001). Both factors appear to cooperate in the production of MNs in an interesting manner. In the mouse, only Nkx6.1 expression encompasses the pMN domain (Figure 1), while Nkx6.2 becomes confined to the p1 domain, apparently via repressive interactions with the class I factor Dbx1. Nkx6.2 therefore appears

to fill another missing link in the progenitor code (B in Figure 1); again, like Olig2, there are slight deviations from the general scheme, since its ventral boundary seems to be defined by repressive interactions with the class II factor Nkx6.1. In *Nkx6.1* null mutants, Nkx6.2 expression expands ventrally, largely compensating for the loss of Nkx6.1. In *Nkx6.1/Nkx6.2* double null mutants, only residual levels of MNs are produced, which is reflected by the failure to initiate Olig2 expression and accompanied by a shift toward more dorsal IN fates.

One intriguing difference is found when comparing the findings of Novitch et al. and Mizuguchi et al.: the former observed that Irx3 repression and MN production by ectopic Olig2 is constrained to a small region dorsal to the position of endogenous MNs (Novitch et al., 2001). At the same time, the ectopic expression of Ngn2 driven by Olig2 was not restricted to the limited area of Irx3 repression. Why the potent MN inhibitor Irx3 could not be repressed in all regions of the neural tube remains unclear but possibly relates to the absence of essential cofactors in more dorsal regions of the neural tube.

Somewhat in contrast to these findings, Mizuguchi et al. observed the induction of ectopic MNs throughout the dorsoventral extent of the neural tube by utilizing forced coexpression of Olig2 and Ngn2. Therefore, while Olig2 can induce Ngn2 in the dorsal neural tube, the level of Ngn2 activity appears to be limiting for ectopic MN generation, a limitation that might be overcome by exogenous expression of both factors at an equally high level. These results, therefore, indicate that Olig2 and Ngn2 actually collaborate in MN determination, although it remains unclear how this might occur at a mechanistic level. Regarding the findings of Novitch et al., this would suggest the intuitively challenging notion that the combined activity of Olig2, a repressor, and Ngn2, a known transcriptional activator, leads to more effective repression of the MN fate inhibitor Irx3 in the dorsal neural tube.

Together, these studies fill in some prominent gaps in our understanding of the mechanisms coordinating the sequential events leading from mitotic progenitor cell to mature neuron. However, they also clearly show that there is still a way to go for an understanding of how transcriptional repressors (like Olig2 or Nkx6.1) and activators (like Ngn2 or Pax6) expressed within the same cell become functionally intertwined in the control of downstream targets. In particular, do Olig2 and Ngn2 act in strictly parallel pathways, as intuitively suggested by the concomitant function of a repressor and an activator within the same pathway, or do they in some instances interact more directly on the same target promoter? The latter possibility might be suggested by the severely reduced levels in the expression of MN determinants that have been observed in *Ngn2* null mutants (Scardigli et al., 2001).

A Switch from Neuronal to Glial Fate by the Concerted Action of bHLH and Homeodomain Transcription Factors

The ventral neural tube serves as a paradigm of how the prepatterning of the ventricular zone into distinct progenitor domains underlies the specification of a wide range of different cell types (see Figure 1). In other regions of the developing CNS, like the cortex or the neuroretina, the sequential production of different cell types from a common set of progenitor cells appears to constitute the major strategy of cellular diversification (Lillien, 1998). Little is known about the mechanisms underlying such temporal switches from one cell fate to another by neural progenitor cells.

In the spinal cord and hindbrain, oligodendrocytes (OCs), which constitute the myelinating cell type of the CNS, appear to derive from the same set of progenitor cells which earlier generates MNs. In a recent paper, the functional relevance of continued Olig2 expression in these progenitors after the time of MN production and during OC genesis is explored (Zhou et al., 2001). Zhou et al. found that the appearance of emigrating OC progenitors was preceded by the establishment of a progenitor domain coexpressing Nkx2.2 and Olig2 (p*, Figure 2B), an intriguing finding given the fact that Nkx2.2 can act as a potent repressor of Olig2 and that the Olig2 expression domain was shown to expand ventrally in *Nkx2.2* null mutants (Qi et al., 2001).

Moreover, forced coexpression of Olig2 and Nkx2.2 promotes the precocious and ectopic generation of OCs along the dorsoventral extent of the neural tube. Hence, the combined action of Olig2 and Nkx2.2 appears to determine OC fate. The switch from MN to OC production by pMN progenitors therefore appears to be mediated by the shift of Nkx2.2 expression into the formerly $Olig2^{+}Nkx2.2^{-}$ pMN domain (Figure 2B). Interestingly, while forced expression of Nkx2.2 alone results in the repression of Olig2 and the ectopic formation of v3 INs (Briscoe et al., 2000), concomitant expression of both Nkx2.2 and Olig2 on the contrary resulted in the elevation of endogenous Olig2 expression.

In the mouse Nkx2.2 function appears to be dispensable for the initial specification of OC progenitor cells and early aspects of OC differentiation (Qi et al., 2001). Moreover, in Nkx2.2 deficient mice, $Olig2^{+}$ cells can still be seen to emigrate from around the pMN domain in a pattern characteristic of differentiating OCs (Qi et al., 2001). However, the terminal differentiation of the majority of OCs seems to depend on the presence of Nkx2.2 activity. Consistent with these findings, Zhou et al. provide evidence that while being sufficient to promote the specification of OC progenitor cells, Olig2 depends on the concomitant action of Nkx2.2 to guide these cells toward terminal differentiation. These results furthermore suggest a considerable heterogeneity in the population of OCs, already present at the level of OC progenitors. First, Zhou et al. observed a population of $Nkx2.2^{+}Olig2^{-}$ differentiating and mature OCs during normal development. Together with the residual level of mature OCs generated in the absence of Nkx2.2 function, this might indicate that some OCs also derive from $Nkx2.2^{+}Olig2^{-}$ and $Nkx2.2^{-}Olig2^{+}$ progenitor cells in addition to the p* domain expressing both factors (Figure 2B).

How is the temporal shift of Nkx2.2 expression mediated? Recently, it has been shown that OC differentiation can be driven by high levels of Shh activity, which might be mediated via activation of Nkx2.2 (Soula et al., 2001). Zhou et al. suggest that the expansion of Nkx2.2 expression into the MN domain and the concomitant retraction of Pax6 expression to more dorsal levels might be controlled by a temporal increase in the level of Shh activity secreted by the floor plate (Figure 2B). The mechanism by which the repression of Olig2 by Nkx2.2 becomes relieved in the pMN domain still re-

mains to be elucidated. However, a possible means by which this is achieved might involve the temporal regulation of potential corepressors or modulators of Nkx2.2-mediated transcriptional repression.

Another important mechanism of how the temporal switch from neurogenesis to gliogenesis by pMN progenitors is achieved appears to involve the extinction of Ngn expression prior to the onset of OC production (Figure 2B). Ngns are known to be potent antagonists of gliogenesis and appear to be inherently coupled to the acquisition of a neuronal fate (Guillemot, 1999; Nieto et al., 2001). Ngn1 furthermore has been implicated in directly opposing astrocytic differentiation, independently from its neurogenic function, by sequestrating gliogenic transcriptional coactivating complexes (Sun et al., 2001). Consistent with these observations, forced coexpression of Ngn2 together with Nkx2.2 and Olig2 blocked the generation of OCs from neural progenitors (Zhou et al., 2001). Furthermore, the ability of Olig2 to trigger Ngn2 expression was suppressed by coexpression of Nkx2.2, consistent with the idea that downregulation of Ngn2 expression is required for oligodendrogliogenesis. Since Neurogenin expression becomes extinguished in the pMN domain prior to the onset of Nkx2.2 activity, the temporal regulation of Ngn2 in the p* domain, however, remains elusive (Figure 2B).

The study by Zhou et al. begins to provide a clue for how the sequential production of first MNs followed by OCs might be achieved in pMN progenitor cells (Figure 2B). The identity of potential positively acting OC determinants that presumably are the targets of Nkx2.2- and Olig2-mediated derepression (Z in Figure 2B), as well as the direct repressors of these determinants that must in turn be repressed by Olig2 and Nkx2.2 (X in Figure 2B), remains a major open issue. Similarly, since both repressors each act separately in the specification of MNs and v3 INs (Figure 2A), the intriguing question arises of how both factors interact to generate a third cell type, OCs.

Has the Code Now Been Cracked?

The analysis of *Nkx6.1/Nkx6.2* double null mutants lead to the surprising observation that some cell fates can be determined in the absence of the normal progenitor code (Vallstedt et al., 2001). These results are viewed as being consistent with a "derepression model" of cell fate specification: once all repressive constraints are relieved within a progenitor domain, factors can become constitutively activated, directing these cells toward an alternative fate. There are, however, some difficulties with interpreting the results obtained with the *Nkx6.1/Nkx6.2* double null mutants, since other constituents of the code (like Irx3, Pax6, and Nkx2.2) presumably continue to be expressed in these mutants, potentially leading to the formation of partial or mixed codes.

Whether derepression is sufficient to promote the generation of all cell fates of the ventral neural tube, as suggested by these interpretations, is an issue that remains to be addressed. It is conceivable that many progenitor cell factors serving as transcriptional activators remain to be identified within the code.

Finally, the nature and functional consequences of combination within the code of transcription factors remain largely unexplored. A simple "adding up" in the selection of target genes by two or more transcription factors acting in the same progenitor cell could potentially lead to the production of progeny with mixed identities. It therefore remains to be elucidated in which ways factors interact within the combinatorial code and which alternative transcriptional complexes can be formed, ultimately leading to the differential selection of target genes or to contrasting readouts on the same target promoter. In this respect, the differential outcomes obtained by the forced coexpression of Olig2 with either Ngn2 or Nkx2.2 bring up the question of whether such factors always act as either repressors or activators or if they can act bifunctionally in a context-dependent manner.

Many lines of evidence begin to outline the nature of the transcriptional codes governing cell type specification in the developing neural tube. Although the principle features that will help to ultimately decipher the code have been laid out, many aspects of the underlying language remain to be solved.

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