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Dorsally Derived Oligodendrocytes Come of Age

While oligodendrocyte precursors have previously been localized to the ventral midline, two papers in this issue of *Neuron* (Cai et al. and Vallstedt et al.) provide compelling evidence for a second dorsal origin of oligodendrocyte precursors in the spinal cord and hindbrain. These cells share expression of the PDGFα receptor and *Olig2* with their ventral counterparts but differ in the requirement for *Nkx6* and hedgehog signaling.

Oligodendrocytes, the myelinating cells of the vertebrate CNS, are ubiquitously distributed throughout the adult CNS. In white matter, the majority of these cells produce myelin sheaths that enwrap axons and facilitate rapid axonal conduction, while in gray matter they may provide trophic support to adjacent neurons. Given the widespread distribution of oligodendrocytes, it was somewhat surprising that the founder cells of the oligodendrocyte lineage were highly localized in the developing neural tube. For example, in caudal regions of the CNS, such as the spinal cord, the founder cells of the oligodendrocyte lineage first arose in a restricted domain of the ventral ventricular zone of the neural tube. Myelination of lateral and dorsal regions of the cord was thus dependent on long-distance migration of oligodendrocyte precursors (Miller, 2002). The restricted localization of spinal cord oligodendrocyte precursors led to an analysis of the molecular regulation of their induction and their cellular ancestry.

In keeping with other ventrally derived neural cells, the appearance of spinal cord oligodendrocyte precursors was shown to be largely dependent on induction through local expression of the patterning molecule Sonic Hedgehog (Shh) by the notochord and floor plate (Orentas and Miller, 1996; Pringle et al., 1996). Further, the inductive influence of Shh was balanced by inhibitory cues such as members of the bone morphogenetic protein family (BMPs) from the dorsal regions of the spinal cord that inhibited oligodendrocyte precursor appearance (Mekki-Dauriac et al., 2002; Miller et al., 2004). The localized appearance combined with insights into the inductive signals has allowed for a dissection of the cellular ancestry and transcriptional control of oligodendrocytes. In one current popular model, oligodendrocyte precursors are derived from a precursor cell that initially generates motor neurons and subsequently, under the influence of the transcription factors *Olig1* and *Olig2*, generates oligodendrocyte precursors (Lu et al., 2002; Richardson et al., 2000; Zhou and Anderson, 2002). This ventral induction of oligodendrocyte precursors is dependent on the additional transcription factors *Nkx6.1* and *Nkx6.2*. While there is considerable evidence to support this hypothesis, there are also a number of observations that do not fit easily into this simple ventral motor neuron/oligodendrocyte model.

The potential of the dorsal neural tube to generate oligodendrocytes has been suggested for some time. Reporter genes driven by "myelin-specific" promoters revealed occasional dorsal ventricular expression. Dorsal-ventral transplants in chick quail chimeras demonstrated either ventral or dorsal and ventral origins depending on the precise approach (Cameron-Curry and Le Douarin, 1995; Pringle et al., 1998). Further, isolated explants of dorsal spinal cord gave rise to oligodendrocytes in culture, and these cells also developed in neurospheres derived from animals lacking hedgehog signaling (Chandran et al., 2003). Whether these data really indicated a "dorsal origin" of oligodendrocytes was unclear. One complication is the realization that in some cases the conditions under which the cultures were grown had the capacity to switch the identity of cells from dorsal to ventral (Gabay et al., 2003). Thus, the generation of oligodendrocytes in dorsally derived cultures may simply reflect a loss of dorsal identity as a result of the culture conditions.

In this issue of *Neuron*, two independent papers from Cai et al. (2005) and Vallstedt et al. (2005) provide convincing evidence for a dorsal origin of oligodendrocytes at multiple levels of the mouse neural tube. A breakthrough utilized by both groups was to take advantage of the critical requirement for the *Nkx6.1* and *Nkx6.2* transcription factors in the regulation of *Olig2* expression and thus the generation of oligodendrocyte precursors from the motor neuron domain in the ventral spinal cord. What Cai and Vallstedt have done is to examine oligodendrocyte development in mutants that lack *Nkx6* signaling and thus *Olig2* and ventral oligodendrocytes. What both groups demonstrate is that oligodendrocyte precursors that are defined by expression of Olig transcription factors and a number of other markers including PDGFαR arise in the dorsal spinal cord. The studies by Cai follow the development of these cells and show that in *Nkx6*$^{-/-}$ animals dorsally derived oligodendrocyte precursors arose slightly later in development and migrated laterally and ventrally.

One potential complication in these studies is that in the absence of *Nkx6* some dorsal cells might have assumed a more ventral fate. To address this issue, Cai et al. demonstrated the colocalization of the known dorsal markers *Pax7* and *Mash1* in dorsally derived *Olig2*+ cells in vitro and the capacity of isolated dorsal wild-type spinal cord explants to produce *pax7*+ oligodendrocytes. These studies effectively blunt the "fate switch" explanation and combined with the ventricular localization of *Olig2* cells between *Dbx2* and *Dbx1* domains provide strong evidence for a dorsal source of oligodendrocytes. Using somewhat overlapping approaches, Vallstedt et al. also provide compelling evidence for oligodendrogenesis from spinal cord cells that retain a dorsal identity.

The identification of a dorsal source of oligodendrocytes in the spinal cord raises important questions related to

specification of these cells. Here, the two manuscripts provide complementary insights. Ventrally derived OPCs require Shh signaling that is mediated by the Smoothened pathway, and the data presented by Cai et al. provide several independent lines of evidence that the appearance of the later-arising, dorsally derived oligodendrocyte precursors is independent of either Shh or Smoothened signaling. These observations suggest differences in the molecular control of dorsal and ventral oligodendrogenesis. Differences between the dorsal and ventral *Olig2*+ cells are further explored by Vallstedt and colleagues, who show the lack of *Nkx2.2* expression in dorsal *Olig2*+ cells. Some characteristics are shared between ventrally and dorsally derived cells, however. Clearly, both are *Olig2*+, and cells from both sources express PDGFαR. Importantly, Vallstedt and colleagues demonstrate that dorsally derived oligodendrogenesis is inhibited by BMP signaling as has previously been described for ventral regions, and addition of inhibitors of BMP to explants of dorsal spinal cord resulted in significantly increased number of oligodendrocytes. These findings then raise the issue of how oligodendrocytes emerge from dorsal regions of the intact spinal cord adjacent to a source of BMPs. Vallstedt neatly argues that the critical issue is timing. Dorsal oligodendrocytes do not arise until later in development than their ventral counterparts, and their appearance correlates with a reduction in the functional signaling of BMPs. Thus, timing, not patterning per se, is the critical role of BMPs in dorsal oligodendrogenesis.

The dorsal origin of oligodendrocytes is not restricted to caudal regions of the neural tube. In hindbrain, Vallstedt and colleagues show dorsally located *Olig2*+ cells, and in vitro explants of dorsal but not intermediate hindbrain generated *Olig2*+ cells without losing the expression of dorsal markers.

The incontrovertible demonstration of a dorsal origin of oligodendrocyte precursors along the neural axis by the two papers raises a number of important questions. First, do these cells actually generate oligodendrocytes that contribute to myelin formation in the adult nervous system? It seems likely that the dorsally derived oligodendrocyte precursors are capable of generating myelinating cells (see Figure 4 in Cai et al.) and possibly in sufficient numbers to make a substantial contribution to myelination. Second, are dorsally and ventrally derived cells functionally equivalent? It is an attractive hypothesis that dorsally derived cells differ functionally from their ventral counterparts. Perhaps dorsal oligodendrocytes are destined to gray matter, become "adult progenitors," and serve as a reservoir for repair, which might explain their later emergence. Alternatively, different pools of oligodendrocytes may be targeted to myelinate different populations of axons. Since dorsal regions (alar) are largely sensory, while ventral (basal) are largely motor, perhaps the modality of the axon is "matched" to the type or source of oligodendrocytes. Different morphological types of oligodendrocytes have been reported, but functional studies, other than gross transplantation, have yet to be performed. Third, it becomes critical to identify the molecular cues responsible for the localized appearance of dorsal oligodendrocyte precursors. Previous studies have implicated bFGF signaling, and perhaps this pathway controls oligodendrocyte development independently of dorsal/ventral fate. Fourth, if ventrally derived OPCs arise from the pMN domain from ancestral cells that first generate motor neurons, then what is the cellular ancestor of the dorsal oligodendrocytes? It seems likely that the precursor to dorsal oligodendrocyte precursors may also generate neurons, since in rostral CNS regions a common ancestor for cortical interneurons and oligodendrocytes has been reported. Alternatively, these dorsal oligodendrocytes could be generated from the "glial restricted precursor cells" (Noble et al., 2004) identified by in vitro assays, in which case the ancestor or sibling of these cells would be an astrocyte. Additional studies involving cell tracing from this dorsal domain are required to resolve this issue. Finally, it remains to be determined if the phenomena of multiple origins of oligodendrocytes is restricted to caudal regions of the CNS. Again, this seems unlikely. While the development of oligodendrocytes in the cortex is less clearly resolved, it is easy to infer from existing data that there are multiple different sources of cells. Whether they differ in the mechanisms of molecular specification, generate distinct populations of cells, are specialized to development, or repair or myelinate distinct pools of axons are questions for the future.

The importance of defining the cellular and molecular control of oligodendrocyte origins is not simply restricted to developmental epochs. A full understanding of the early phases of oligodendrogenesis has enormous implications for the development of therapies for myelin repair in demyelinating diseases such as multiple sclerosis as well as developmental disorders such as the leukodystrophies. If oligodendrocytes can be generated in multiple ways, then the potential for harnessing that capacity to promote recovery in the adult CNS is significantly enhanced.

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Hear, Hear for the Zebrafish

Our senses of hearing and balance rely on the function of specialized receptor neurons called "hair cells." In this issue of *Neuron*, Grant et al. report a series of elegant zebrafish experiments that reveal a previously unappreciated role for glia in the regulation of hair cell proliferation and differentiation. This work is a beautiful example of how zebrafish are particularly useful model systems for studying hair cell development and function.

Hair cells are essential for our ability to detect both sound and motion. In the cochlea, hair cells detect a wide range of sound frequencies and intensities, while those in the semicircular canals and otolith organs pick up changes in gravity, linear accleration, and angular accleration, information that keeps us properly oriented with the three dimensions of the external world. Historically, these amazing cells have been difficult to study because they are housed within the inner ear, which itself is buried deep in the temporal bone of the skull, a region that is considerably less accessible than the eye or nose. Hair cell biology has entered a new era of exciting discoveries with the advent of zebrafish genetics. The experiments reported by Grant et al. (2005 [this issue of *Neuron*]) use zebrafish to show that glia are responsible for an inhibitory cue that determines when and where quiescent precursors go on to differentiate as hair cells.

Hair cells detect vanishingly small motions of fluids in the inner ear in response to sound or motion. The part of the cell responsible for this feat is the stereociliary bundle, a characteristic array of actin-rich microvilli on the apical surface of every hair cell. A bundle contains 20 to 300 stereocilia which vary in height but are always precisely arranged from smallest to tallest, like a staircase. Neighboring stereocilia are connected to each other by a helical filament called a "tip-link." When the bundle is deflected, tension on the tip-link opens a mechanically gated ion channel and depolarizes the cell. Movement in the opposite direction inhibits activation, while movement of the bundle from side to side has no effect. All hair cells, in all vertebrates and in both auditory and vestibular organs, share this essential mechanotransduction machinery.

Like other vertebrates, zebrafish use hair cells to detect sound and motion. In addition, fish have a lateral line system of hair cells whose stereocilia are directly exposed to the water and sense its movement along the surface of the head and body. Information from the lateral line is used for a variety of behaviors, including schooling and avoidance of predators. The sensory organs of the lateral line are called "neuromasts" and are distributed at precise locations (Figure 1). Each neuromast contains a central cluster of hair cells that is surrounded by supporting cells and is innervated by sensory ganglion neurons. It is the neuromasts of the lateral line that make zebrafish a particularly attractive system because, unlike the hair cells of the inner ear, neuromasts are present on the surface of the animal and are readily accessible to analysis. For example, hair cells can be visualized by simply letting fish swim around in dyes such as DASPEI or FM1-43, both of which pass through the transduction channel and serve as reliable indicators of hair cell function. Despite their unusual location, the hair cells of the lateral line are placodally derived like other hair cells and undergo similar stereotyped patterns of development and differentiation. The lateral line is therefore an excellent and exceedingly simple system in which to study hair cells (Ghysen and Dambly-Chaudiere, 2004).

Making the lateral line is a tall order for development, since controlled numbers of neuromasts need to differentiate at specific locations all along the length of the animal, even as the animal continues to grow. A solution to this problem is to establish a basic lateral line early in development, when the embryo is small, and elaborate this system as the animal matures. Consistent with this idea, the first wave of neuromasts comes from the posterior division of the lateral line placode, primI, which migrates from the head to the tail, periodically dropping off groups of precursors that will eventually give rise to the hair cells and support cells of each neuromast. The anterior half of the placode generates a sensory ganglion, pLL, which projects axons along the length of the body to form the lateral line nerve. This nerve is ensheathed by glia, which are produced by the neural crest and then migrate along the surface of the extending axons of pLL. By the end of 3 days of development, small clusters of primary neuromasts have been deposited along the body and are innervated by pLL ganglion neurons whose axons are surrounded by glia. Over the next month, a second wave of neuromast development occurs, with secondary neuromasts eventually added to every somite of the animal. Some neuromasts may come from a second placode, primII, which follows primI later in development. However, it has been unclear exactly where these new hair cells come from or how they integrate into the initial lateral line system.

To understand what regulates the development of secondary neuromasts, Grant, Raible, and Piotrowski exploited the many experimental advantages of the zebrafish. The work begins with the identification of a previously undescribed population of cells, the interneuromast cells. These cells sit in a thin line between the primary neuromasts, along the horizontal myoseptum. Using a combination of keen observations and sophisticated dye labeling and imaging, the authors show that interneuromast cells migrate ventrally away from the lateral line nerve to form new clusters of secondary neuromasts on the surface of the trunk and tail. Because these cells differentiate only after they have moved away from the nerve, the authors postulate that the nerve itself inhibits proliferation and/or differentiation of secondary