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An 'oligarchy' rules neural development

David H. Rowitch, Q. Richard Lu, Nicoletta Kessar and William D. Richardson

Recent reports show that *Olig* genes, which encode the basic helix–loop–helix Olig transcription factors, are essential for development of oligodendrocytes. Surprisingly, Olig function is also required for formation of somatic motor neurons. These findings alter our views of how the oligodendrocyte lineage is generated and raise further questions about the underlying developmental relationships between neurons and glia.

Oligodendrocytes engage in complex interactions with nerve cell bodies and axons in the CNS, notably in the formation of myelin sheaths [1]. Myelin is an elaborately structured proteolipid that serves to insulate axons and facilitate rapid, saltatory (jumping) nerve conduction, which is crucial for nervous system function. When oligodendrocytes die and myelin sheaths break down, as in diseases such as multiple sclerosis, severe debilitation results. The recent identification of transcription factors that mark or determine key stages of oligodendrocyte development has provided new genetic tools with

which to further dissect demyelinating disease and other disorders of oligodendroglial cells.

Transcription factors as arbiters of oligodendroglial cell fate

The roles of transcription factors in neuronal cell fate specification in the CNS have been intensively studied over the past decade (reviewed in Refs [2–4]) but cell-intrinsic determinants of glial cell fate remain poorly understood. Genes such as *glial cells missing* (*gcm* or *glide*) and *pointed* encode transcription factors that regulate formation of glia in *Drosophila*, but so far there is no indication that their orthologues are involved in development of oligodendrocytes or other glia in vertebrates. Loss-of-function studies have demonstrated roles for *Sox10* [5] and *Nkx2.2* [6] in the maturation of oligodendrocyte precursors (OLPs) in transgenic mice, but have left open the question of how OLPs are initially specified.

Box 1. Let's do the locomotion: the motor neuron–oligodendrocyte lineage as a primitive functional unit

Myelinating oligodendrocytes are found all over the CNS – abundantly in white matter tracts, but also in grey matter. It was therefore surprising to discover that oligodendrocyte progenitors (OLPs) are generated in a restricted part of the embryonic neural tube. In mice it seems that the majority of OLPs are generated in the pMN domain of the neuroepithelium – the same region that generates somatic motor neurons. This apparent link between OLPs and motor neurons is curious. Present day oligodendrocytes, after all, do not myelinate the axons of motor neurons in preference to those of other neurons. However, the developmental connection between motor neurons and oligodendrocytes might be an evolutionary relic of a previous age, when there could have been a special functional relationship between these cells. We have proposed that there might originally have been adaptive pressure to myelinate motor circuits and thereby accelerate locomotion, allowing escape from predators [a,b]. Once invented, oligodendrocytes would quickly have been pressed into service throughout the CNS because of their overwhelming general advantages.

Neuroglial lineages – form and function

The colocalization of motor neuron precursors and OLPs in the ventral neuroepithelium is suggestive of a lineage connection. Clonal analysis *in vivo* and *in vitro* has previously hinted at a common motor neuron–oligodendrocyte progenitor but failed to demonstrate the exclusivity of this relationship [c–e]. The striking demonstration that loss of *Olig2* function eliminates both spinal motor neurons and OLPs, apparently without affecting astrocytes or other classes of neurons [f,g], reinforces the notion of a common precursor (Fig. 1a).

However, the form of the lineage remains elusive. It is possible that the motor neuron and oligodendrocyte lineages separate early and that the pMN contains a salt-and-pepper mixture of committed 'neuroblasts' that generate only motor neurons and 'glioblasts' that generate OLPs (Fig. 1b). In this model, both neuroblasts and glioblasts express *Olig2*. In addition, the glioblasts must lie dormant in the neuroepithelium for several days because migratory OLPs do not appear until motor neuron production is over. Alternatively, the pMN might contain a homogeneous set of *Olig*-positive neuroglial precursors that undergo asymmetric, self-renewing divisions to create motor neuron progenitors in one cycle and OLPs in the next (Fig. 1a), analogous to the way certain *Drosophila* neuroblasts bud off successive ganglion mother cells with different fates [h]. Time-lapse microscopy will be required to distinguish between these and other possibilities.

Zhou and Anderson [f] have shown that in *Olig1;Olig2* double knockout mice, precursors in the pMN undergo a homeotic transformation. Instead of generating motor neurons and OLPs, they generate V2 interneurons and astrocyte progenitors, implying that this is the usual fate of neuroepithelial precursors in the p2 domain (Fig. 1). It could even be that for every neuronal subclass there is a corresponding glial partner. Since there are several different kinds of spinal neurons, might there be an equivalent number of glial subtypes? A variety of astrocytes with intrinsically different morphologies and/or antigenic phenotypes have been described in spinal cord cultures [i]. Perhaps these astrocytes originate from distinct regions of the neuroepithelium *in vivo* in response to the same organizing signals that are responsible

for generating diverse neuronal subtypes along the dorsal–ventral axis [j].

Neuron–glial precursors versus glial-restricted precursors

If platelet-derived growth factor receptor α (PDGFR α)-positive OLPs from rat optic nerve are cultured in the presence of 10% fetal calf serum, or ciliary neurotrophic factor plus extracellular matrix, they generate type-2 astrocytes rather than oligodendrocytes [k]. For that reason, OLPs were originally called oligodendrocyte–type-2 astrocyte (O–2A) progenitors [l]. However, type-2 astrocytes have proved elusive *in vivo*, suggesting that OLPs/O–2A progenitors do not normally encounter conditions that promote astrocyte differentiation [m].

A different kind of oligodendrocyte–astrocyte precursor has been identified in cultures of dissociated spinal cord cells [n,o]. *In vitro*, these precursors generate oligodendrocytes, type-1 astrocytes or type-2 astrocytes (depending on the culture conditions) but not neurons, leading Rao *et al.* [o] to call them glial-restricted precursors (GRPs). The relationship between GRPs and OLPs, or between GRPs and the presumed motor neuron–OLP precursors described above, is not clear because there is, as yet, no positive marker for GRPs *in vivo*. Perhaps GRPs correspond to the 'glioblasts' (G cells) depicted in Fig. 1b? If so, they might be formed at all levels of the neural tube but, depending on positional cues in their environment, would then convert either to OLPs (in pMN) or astrocyte progenitors (ASPs) (in p2 and elsewhere). Note that this interpretation does not necessarily conflict with the observation that oligodendrocytes and astrocytes come from separate precursors *in vivo* [g], because the actual *fate* (as opposed to the *potential*) of any given GRP would depend on its precise location in the neuroepithelium.

Olig genes

Several laboratories recently identified a novel family of '*Olig*' genes, encoding basic helix–loop–helix (bHLH) transcription factors [7–9]. During development, *Olig* genes are expressed in discrete sub-populations of neural progenitors. Strikingly, *Olig* expression persists beyond the embryonic period and is detected in oligodendrocytes of the adult rodent CNS, as well as in other cells that might represent immature progenitors. Although *Olig* genes were named for this distinctive pattern of expression in developing and mature oligodendrocytes, it is clear that the roles of *Olig* proteins are diverse and include other fundamental processes, such as neural pattern formation and specification of motor neurons. Indeed, it now appears that an 'oligarchy' governs multiple aspects of progenitor cell function in the ventral neural tube.

In humans and mice, *Olig1* and *Olig2* genes lie ~40 kb from each other, on chromosomes 21 and 16 in the two species, respectively. Thus, a recent evolutionary duplication might account for the

generation of *Olig1* and *Olig2*. Although *Olig2* homologues have been found in chick and zebrafish [10–12], *Olig1* has been identified only in mammals, suggesting that *Olig2* is the more ancient of the two genes. This review focuses on *Olig1* and *Olig2*, although a third family member, *Olig3*, is known to be expressed in a non-overlapping pattern [8], and reports of additional homologues are emerging.

Olig function: necessary and sufficient for oligodendrocyte development

Expression analysis of *Olig1* and *Olig2* suggested possible roles for these genes in the pMN-precursor region of the ventral neural tube, which is known to give rise both to motor neurons and oligodendrocytes (referred to hereafter as the pMN–OL domain) (Box 1, Fig. 1). The pMN–OL and other distinct precursor domains in the ventral neural tube have been defined by limits of the expression of genes encoding transcription factors, including *Olig* proteins, that are activated at different threshold concentrations of

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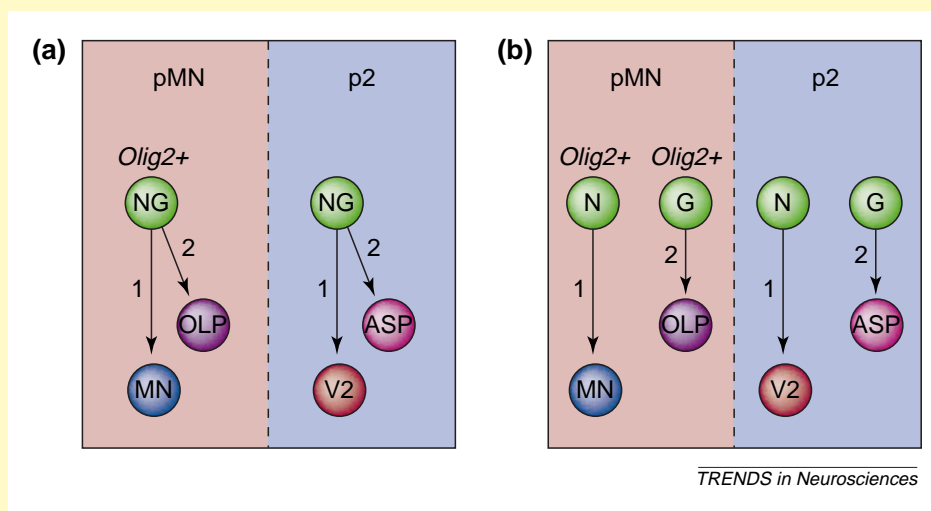


Fig. 1. Models for oligodendroglial lineage development. Several lines of evidence suggest that motor neurons and oligodendrocytes share common precursors, separate from astrocyte precursors. The form of the lineage linking motor neurons and oligodendrocytes is not known. Two possible lineages are depicted; in both cases, numbers indicate the temporal order in which cells are generated. (a) 'Switching' model: common *Olig*-positive 'neuroglialblasts' (NG) in the pMN domain generate first motor neuron (MN) progenitors and subsequently oligodendrocyte precursors (OLP). This is similar to the way in which certain *Drosophila* neuroblasts generate different species of ganglion mother cells (committed neuronal and glial progenitors) by successive asymmetric divisions. In the p2 domain, the NG cells generate V2 interneurons followed by astrocyte progenitors (ASP). (b) 'Segregating' model: separate 'neuroblasts' (N) and 'glioblasts' (G) co-exist side-by-side in the neuroepithelium. In the pMN, the neuroblasts generate motor neurons and the glioblasts generate OLPs, whereas in p2 the neuroblasts generate V2 interneurons and the glioblasts generate ASPs. The main difference between these two models is the time at which neuronal and glial lineages separate – before or during neural tube patterning in (b) and after patterning in (a). Note that G cells in (b) might correspond to the glial-restricted precursors (GRPs) studied by Rao *et al.* [c]. If so, we can postulate that, although GRPs might have the potential to generate both OLPs and ASPs *in vitro*, they are constrained by positional cues *in vivo* to generate only OLPs (in pMN) or ASPs (p2 and elsewhere).

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floor-plate-derived Sonic hedgehog (Shh) [2]. Regulation of *Olig* genes by Shh [7] is possibly indirect, via homeodomain proteins Nkx6.1 and Nkx6.2 [13].

An initial clue to *Olig* function was the finding that virus-mediated expression of *Olig1* in rat neuroepithelial cells was sufficient to promote development of OLPs but not differentiated oligodendrocytes *in vitro* [7]. Subsequently, retroviral infection *in vivo* confirmed that *Olig1* can drive the production of differentiated oligodendrocytes in the mouse neocortex [14]. In the spinal cord, however, forced expression of either *Olig1* or *Olig2* failed to generate OLPs [9,15,16]. When *Olig2* was coexpressed with *Nkx2.2*, it was possible to form *Sox10*⁺ OLPs and differentiated oligodendrocytes [15,16]. Thus, *Olig* proteins evidently require context-dependent protein interactions to specify oligodendrocytes [17,18]. These experiments indicate roles for class B bHLH–homeodomain interactions in neural tube development but leave open the question of

whether such interactions are direct or indirect. Furthermore, because the overlap between *Olig* and *Nkx2.2* is only partial in the mouse spinal cord at embryonic day (E) 12.5 [15], it is likely that factors other than *Nkx2.2* collaborate with *Olig* proteins to generate OLPs outside the region of overlap.

Recent studies from several laboratories provide compelling evidence that *Olig* proteins are essential for development of all oligodendrocytes in the CNS. Lu *et al.* [19] performed individual knockouts of *Olig1* and *Olig2*, while Zhou and Anderson [20] targeted *Olig1* and *Olig2* simultaneously. (The single gene mutations cannot be recombined by breeding because the *Olig* genes lie close together on the same chromosome.) Comparison of the three knockout phenotypes indicates a spectrum of severity. *Olig1*-null animals are viable, but show a delay in oligodendrocyte maturation. *Olig2*-null animals entirely lack OLPs in the spinal cord. They do, however, generate small pockets of OLPs in the forebrain and near-normal numbers in the midbrain

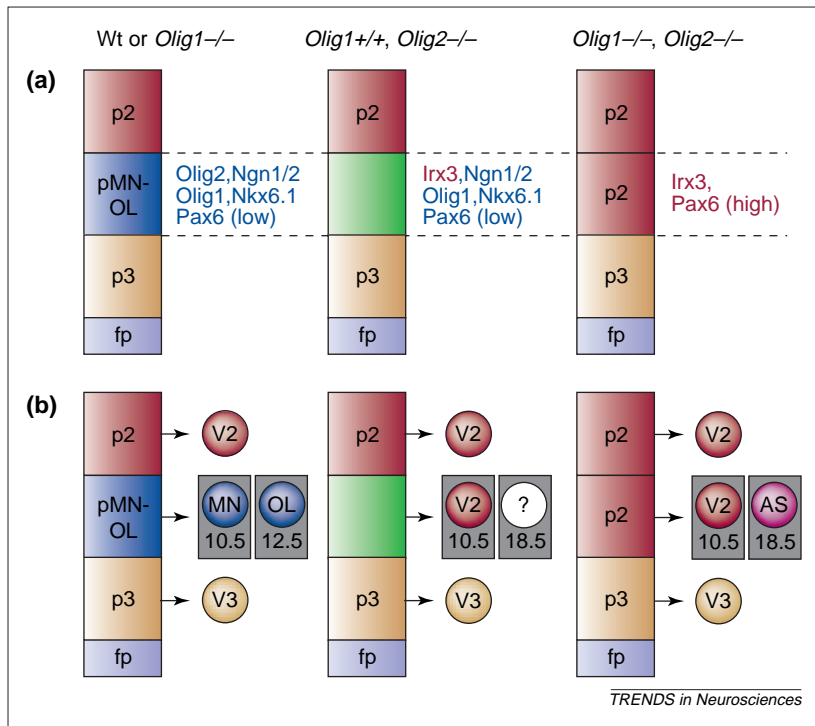


Fig. 1. Olig function is essential for neural pattern formation and cell fate acquisition in the ventral neural tube. (a) Relative differences in pattern of pMN-OL progenitors of the spinal cord in wild-type (WT) and *Olig* mutant animals. Note that many pMN markers are preserved in *Olig2* mutants. By contrast, there is a pMN-to-p2 conversion in compound *Olig1;Olig2* mutants. (b) pMN-OL progenitors normally give rise to motor neurons and oligodendrocytes between embryonic day (E)9 and E13 in the mouse spinal cord. However, these cell types fail to develop in *Olig2* mutants, and instead appear to be partially converted to V2 interneurons and a second type of *Olig1*⁺ cell, the lineage of which is undetermined. Dramatically, Zhou and Anderson found that a complete pMN-to-p2 conversion takes place in compound *Olig1;Olig2* mutants, resulting in production first of V2 interneurons and then astrocytes [20]. Could it be that *Olig* expression is antagonistic to astrocyte development? It seems not, because ectopic expression of *Olig1* in the developing mouse brain indicates that *Olig1* does not block astrocyte development [14].

production of motor neurons in dorsal regions of the neural tube [10]. Thus, it appears that Olig proteins act in concert with other transcription factors to promote a motor neuron fate. The details of the transcriptional complexes with which Olig proteins normally interact remain to be identified, as do their downstream targets during motor neuron development. Data indicating that Olig proteins act as transcriptional repressors raise the possibility that the transcriptional targets of Olig proteins are themselves antagonists of motor neuron development – so that Olig proteins effectively promote the motor neuron fate by repressing the repressors of that fate [10,11].

The *Olig1* knockout mouse is viable, whereas *Olig2* null mice die at birth from failure of spontaneous breathing. Further analysis of *Olig2*^{-/-} animals revealed a total absence of somatic motor neurons in the hindbrain and both somatic and visceral motor neurons in the thoraco-lumbar spinal cord. As previously mentioned, *Olig1* is able to compensate partially for *Olig2* function, at least in the development of OLPs in the rostral CNS. Conversely, even though *Olig1* is expressed within precursors of motor neurons and *Olig1* expression is upregulated in the pMN-OL domain of *Olig2* mutant animals, *Olig1* appears to be insufficient for production of motor neurons anywhere along the neuraxis. Might these results reflect differences in early patterning functions of *Olig1* and *Olig2* proteins? In the absence of *Olig2* function, neural pattern is relatively unaffected, showing unaltered expression of some pMN markers, including *Olig1*, *Nkx6.1*, *Ngn2* and others (Fig. 1). However, *Irx3* expression expands ventrally into the pMN from p2 [19]. Dramatically, in *Olig1;Olig2* compound mutants, all known markers of the pMN are obliterated and the pMN undergoes a homeotic transformation into p2 (Fig. 1). Thus, the failure of motor neuron development in *Olig2* mutant mice could result from the limited patterning capability of *Olig1*, especially the inability of the latter to repress *Irx3* within the pMN [21]. Alternatively, *Olig2* could have unique functional properties that are necessary for motor neuron production.

and hindbrain. All surviving OLPs in *Olig2*-null animals appear to express *Olig1*, suggesting functional redundancy between the two genes in certain region of the CNS. Strikingly, no OLPs were observed anywhere in the CNS of compound *Olig1;Olig2* mutants [20]. Combined, these results indicate overlapping functions for Olig proteins in oligodendrocyte specification, although the activities of *Olig1* and *Olig2* appear to be highly context dependent. Finally, loss-of-function studies in zebrafish indicate that the fish homologue *Olig2* has a conserved role in formation of both oligodendrocytes and motor neurons [12].

***Olig* function: required for the development of all somatic motor neurons**

Olig proteins are expressed in motor neuron precursors in pMN-OL but are downregulated in differentiated motor neurons – a pattern associated classically with proneural genes [4]. Gain-of-function studies show that *Olig* genes can in fact promote motor neuron development [11]. Ectopic expression of *Olig2* along the entire dorso-ventral axis of the chick spinal cord resulted in a dorsal expansion of the pMN-OL domain and led to increased numbers of motor neurons. The expansion was only modest, however, being limited to the neuroepithelium immediately dorsal to pMN-OL. These experiments indicate intrinsic functional limitations of the *Olig* genes. By themselves (i.e. at ectopic locations) they are evidently insufficient to promote motor neuron specification and presumably must work in concert with other factors. In keeping with this, Mizuguchi *et al.* reported that coexpression of the gene encoding Neurogenin 2 (*Ngn2*) with *Olig2* resulted in ectopic

Table 1. Olig function in the developing CNS^a

Age	Role	Species	Factors implicated	Refs
E8.0–E8.5	Necessary for pMN patterning	Mouse	Olig2	[19,20]
	Sufficient for aspects of pMN patterning	Chick	Olig1, Olig2	[10,11,19]
E8.5–E10.5	Necessary for MN specification	Mouse	Olig2	[19,20,38]
		Zebrafish	Olig2	[12]
E12.5	Sufficient for MN specification	Chick	Olig2 (with Ngn2)	[10,11]
	Necessary for OL development	Mouse	Olig1, Olig2	[19,20,38]
		Zebrafish	Olig2	[12]
	Sufficient for OL development and maturation	Rodent	Olig1	[7,14]
		Chick, mouse	Olig2 (with Nkx2.2)	[15,16,24]

^aSummary of Olig functions, based on recent reports in the literature. Note that Olig functions are separable into early phases of neural patterning (~E8–E8.5), specification of motor neurons (E8.5–E10.5) and specification of oligodendrocytes (>E12.5). Abbreviations: E, embryonic day; MN, motor neuron; OL, oligodendrocyte; pMN, motor neuron precursor.

Regulation of Olig function during specification of neurons and glia

Shh is required during a prolonged period of spinal cord development for specification of motor neurons and OLPs [22]. This presumably reflects a prolonged requirement for Shh-inducible factors, including the Olig proteins. What accounts for the time-dependent change in the activities of Olig proteins during the switch from motor neuron to OLP production? One possibility is that interactions between Olig proteins and cofactors that are contemporaneously expressed in the pMN at early stages (E9–E10.5) might drive production of neuronal subtypes [10,11]. Subsequently, at E12.5, the pMN–OL might express genes encoding a different set of Olig-interacting proteins that lead to OLP production, one candidate being the homeodomain factor Nkx2.2. However, things are probably that simple. *Olig2* is expressed widely throughout the ventral neural tube at E9.5, so that there is clear colocalization of *Olig2* and *Nkx2.2* in ventral precursors well before the emergence of OLPs [15]. Moreover, platelet-derived growth factor receptor α (PDGFR α)-positive OLPs in rodents appear mainly outside the Nkx2.2 domain [7,23,24] – in sharp contrast to chick, where they are restricted to the Olig–Nkx2.2 overlap region [25,26]. Although ectopic coexpression of *Olig2* and *Nkx2.2* in the E4 chick neural tube results in premature activation of the OLP marker *Sox10*, establishment of bona fide oligodendrocytes requires ongoing expression until at least at E6 [15,16]. Thus, complex cell-intrinsic and/or environmental factors evidently modulate Olig functions. It is possible that inhibitory factors are involved in regulating the timing of initial OLP specification in the pMN–OL. For example, it has been proposed that downregulation of Ngn2 is a necessary pre-requisite for OLP specification [16,20]. Negative HLH factors of the Notch pathway (e.g. Id, HES), which are thought to regulate the timing of oligodendrocyte differentiation [27,28], might well have additional roles in the regulation of OLP specification. Further insights into this process will no doubt require a detailed understanding of

Olig protein interactions based on genetic and biochemical studies.

Olig genes in the brain

What is the nature of oligodendroglial precursors in the rostral CNS? It is known that a common signal, Shh, is required for oligodendrocyte development in both the spinal cord and brain [22,29–32]. Moreover, in the brain a precursor for both oligodendrocytes and GABAergic neurons has been proposed [33], consistent with fate-mapping analysis of *Olig-cre* animals [19]. An intriguing possibility is that the oligodendrocyte lineage is universally coupled with production of neurons – motor neurons in the spinal cord and brainstem, and as yet unidentified neurons in more anterior regions. The finding that *Olig* function is required for production of oligodendrocytes from neurospheres suggests that Olig activity might also be essential for regulation of neural stem cell fate in the adult brain [20].

Olig genes and human disease

There are ~25 000 new cases of glial brain tumors diagnosed per year in America, yet the biology of these tumors is poorly understood. Recent findings that *Olig* genes are strongly expressed in human oligodendroglial tumors suggests that they could serve as useful diagnostic reagents and help to unravel the complex composition of these malignancies. For instance, oligodendrogliomas do not express markers of mature oligodendrocytes, but do express *Olig1*, *Olig2* [34,35] and the chondroitin sulfate proteoglycan NG2 [36], suggesting similarities to OLPs.

Multiple sclerosis (MS) is a major cause of disability in adults, yet the causes for this disorder are controversial and the reasons for failure of remyelination obscure. It will be interesting to determine whether Olig markers are expressed by OLPs at lesion sites, and whether such early lineage markers will be helpful in the characterization and understanding of MS lesions [37].

Finally, since the *Olig* genes in humans are located on chromosome 21 and are potentially upregulated in

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Down syndrome, a role for *Olig* genes in the neurological aspects of this common genetic disorder cannot be ruled out. In sum, investigation of *Olig* genes has provided substantial new insights into the development of neuronal and glial lineages and, further, raises the prospect of new insights into human diseases.

Conclusions

The past two years have seen remarkable advances in our understanding of transcription factors that determine early glial lineage, and prominent roles for *Olig* genes as 'master regulators' in this process now seem clear (Table 1). *Olig* genes have fundamental roles in neural patterning and are essential mediators of ventralizing signals such as Shh. *Olig* proteins have further roles in the specification of

motor neurons and oligodendrocyte progenitor cells at early stages of neural tube development. The persistent expression of *Olig* genes throughout the life of the oligodendrocyte suggests later roles in oligodendrocyte maturation and/or survival. The fact that oligodendrocyte maturation is delayed in the *Olig1* knockout mouse supports this notion. The biological capabilities of *Olig1* and *Olig2* are distinct, raising the question of which mechanisms account for their distinct biochemical properties. A major area of research in the future will be to determine the transcriptional partners of *Olig* genes, which might reveal the basis for their diverse functional roles. Finally, it is anticipated that identifying the downstream transcriptional targets of *Olig* proteins will yield further important insights into specification of both motor neurons and oligodendrocytes.

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