Neuron
Previews

Oligodendrocytes and the "Micro Brake" of Progenitor Cell Proliferation

Klaus-Armin Nave[1,*]
[1]Max-Planck-Institute of Experimental Medicine, Department of Neurogenetics, D-37075 Goettingen, Germany
*Correspondence: nave@em.mpg.de
DOI 10.1016/j.neuron.2010.02.026

A large fraction of mammalian genes is regulated posttranscriptionally by microRNAs. In the developing nervous system, a small subset of these short RNA molecules is important to orchestrate the rapid switch from OPCs to myelin-forming oligodendrocytes. But putting the miRNA brake on oligodendrocyte gene expression is required throughout life.

Oligodendrocytes are the last cell type to differentiate in the developing brain. In rodents, most oligodendrocytes are generated in the first 2–3 postnatal weeks. One function of this highly specialized subtype of glia, the wrapping of axons with a multilayered myelin sheath, is well known. The unique membrane architecture of myelin reduces axonal energy expenditure and provides the physical basis for rapid impulse propagation (Lazzarini et al., 2004). There is also growing evidence that oligodendrocytes maintain the functional integrity of myelinated axons, i.e., motor-driven transport processes and the long-term survival of axons, a support function that is shared by myelin-forming Schwann cells in the periphery. With this crucial role of glia in neuronal function, there is an obvious interest in better understanding the molecular regulation of oligodendrocyte differentiation in a number of human myelin diseases, in which myelin either fails to form or is secondarily lost, such as in multiple sclerosis.

Myelination in the rodent brain and spinal cord involves two phases of unusually high developmental dynamics: first, the number of oligodendrocyte precursor cells (OPCs) expands rapidly, beginning before birth in the ventral and dorsal embryonic spinal cord, followed by the ganglionic eminence and the cortex of the developing forebrain (Richardson et al., 2006). These proliferating OPCs migrate and populate all future white matter tracts and are initially produced in excess. This necessitates that oligodendrocytes are matched to the available number of myelination-competent axons, which is achieved by a combination of rate-limiting growth and survival factors and the electrical activity of axons on one hand and programmed glial cell death on the other (Barres and Raff, 1994). Among the many factors that stimulate the growth of OPC in culture, PDGF has been shown most convincingly to be a crucial driving force of proliferation also in vivo (Calver et al., 1998). Later and prior to myelination, a careful orchestration of cell cycle withdrawal and differentiation is essential. Recent investigations have revealed that this involves the coordinated repression of several transcriptional inhibitors, such as Hes5, Id4, and Tcf4 (He et al., 2007). Immediately after cell cycle exit, myelin lipid and protein synthesis increases dramatically for the deposition of large amounts of myelin membrane, a process completed within a few days (Pfeiffer et al., 1993). This enormous cell growth requires unusual synthesis rates of myelin structural proteins, many of which are encoded by cell-specific mRNAs that are present at high copy numbers.

The switch from proliferating OPCs that are migratory to postmitotic oligodendrocytes that synthesize myelin occurs rapidly and exactly at the time when axon and oligodendrocyte numbers appear to match. How is it possible to put a sudden "brake" onto the pool of progenitor cells that still express multiple transcriptional inhibitors and live in the continued presence of growth factors, such as PDGF? In this issue of *Neuron*, two papers from Ben Barres' and Richard Lu's lab provide evidence that this switch is aided by the expression of a set of microRNAs (miRNAs) that are induced in maturating OPCs (Dugas et al., 2010; Zhao et al., 2010). Their work suggests a model in which posttranscriptional attenuation of exactly those genes that normally maintain the proliferating OPC phenotype becomes a "brake" in the system that promotes the rapid transition of OPCs to mature oligodendrocytes.

The discovery that miRNAs provide a new layer of gene expression control in apparently all multicellular organisms is one of the major breakthroughs of the last decade (Bartel, 2004; Filipowicz et al., 2008). It is thought that human cells harbor about one thousand different miRNAs, a superfamily of single-stranded RNA molecules 20–24 nucleotides in length. These are derived from larger hairpin-folded precursors that are often noncoding transcripts ("pri-miRNAs"). Processing of pri-miRNAs to mature miRNAs involves at least two steps. In the nucleus, the endonuclease Drosha generates a pre-miRNA that is exported. In the cytosol, the RNase Dicer continues the processing to a mature single-stranded miRNA, which incorporates into a functional ribonucleoprotein, the "miRNA induced silencing complex" (miRISC).

In the 3′ untranslated region (UTR) of mRNAs, often more than one binding site for known miRNAs can be predicted using database mining tools and assuming perfect base pairing of nucleotides 2–8 (the "seed"). Binding of the miRISC to the 3′ UTR inhibits translational initiation and can also destabilize mRNAs, resulting in rapid degradation (which mechanism prevails is not well understood). Either way, it is estimated that 30% or more of our genes are posttranscriptionally regulated by miRNAs.

Not surprisingly, the complexity of miRNA expression is highest in the brain, and the miRNA profile of oligodendrocyte lineage cells has recently been established (Lau et al., 2008). To address functional importance, Dugas et al. and Zhao et al. started out by generating conditional mouse mutants, in which the critical *Dicer* nuclease gene was cell-specifically inactivated (using mice expressing Cre recombinase under control of the *Olig1*, *Olig2*, or *Cnp1* promoter at earliest stages of oligodendrocyte development).

In general, the consequences of *Dicer* ablation are similar in the two studies. Lack of visible myelin sheaths in brain and spinal cord is associated with ataxia, tremors, and premature death, reminiscent of other myelin mutants, with differences in detail that depend on the Cre driver line. Those that also target Schwann cells (i.e., *Cnp1-Cre*) die postnatally with a severe developmental defect of the PNS. In all mutants, CNS dysmyelination is caused by the virtual lack of mature oligodendrocytes, as assessed by late marker gene expression, and a concomitant increase of proliferating OPCs that continue to express PDGF receptors. The differentiation arrest of OPCs is cell autonomous (i.e., not caused by axonal signaling problems), as evidenced by similar defects of purified OPCs, maintained under conditions that should promote their differentiation. Interestingly, in older mice, CNS myelination appears not to be blocked, but only severely delayed. This, however, is likely due to the expansion of OPCs that have simply escaped Cre-mediated recombination. Thus, the current analysis has been largely restricted to early stages of development.

Dicer ablation may be considered a crude and unspecific insult to cells, prompting the question of which miRNAs are so important for the differentiation of OPC? Out of several hundreds, few candidate genes were narrowed down by focusing onto miRNAs that showed a regulated expression in oligodendrocyte lineage cells (Barres lab) or were abundant in developing white matter tracts (Lu lab) and which could be confirmed by northern blotting and in situ hybridization experiments. These differences at the outset might explain why an overlapping but not identical set of top candidates emerged in these studies. Importantly, both groups confirmed the presence of miRNAs that were identified in independent studies, notably miR-219 (Lau et al., 2008; Shin et al., 2009), but also went an important step further by demonstrating functional significance for differentiation.

The role of specific miRNA can be tested by either adding "mimetics" to cultured cells (i.e., chemically modifed oligonucleotides that efficiently mimic a specific miRNA species) or corresponding "inhibitors" (i.e., blocking anti-sense oligonucleotides that inhibit endogenous miRNAs). In these in vitro experiments, OPCs were either induced with PDGF to continue proliferating or were forced to exit the cell cycle and to differentiate (–PDGF/serum removal; also thyroxin was used to stimulate differentiation). Under most conditions, although not in all, miR-219 emerged as a dominant player that enhanced the expression of all myelin protein genes analyzed. Interestingly, when *Dicer* mutant OPCs were tested by complementation, miR-219 alone appeared to "rescue" a significant fraction of cells from a developmental arrest, at least when judged by the expression of MBP, a late myelin protein marker.

What are the critical target genes of developmentally expressed miRNAs in oligodendrocytes, such as miR-219 (which may also have a neuronal function; see Kocerha et al., 2009)? By scanning the 3′ UTR of mRNAs that were previously found in oligodendrocyte lineage cells for the presence of conserved miRNA binding sites, several promising candidate genes were picked. Importantly, this included among others the gene for PDGFR alpha, the very receptor tyrosine kinase whose activation keeps OPCs proliferating and undifferentiated in vitro and in vivo. Indeed, miR-219 very efficiently attenuates the expression of PDGFR alpha at the protein level, and this effect is clearly dependent on the identified miR-219 binding site in the 3′ UTR. Similarly, the transcriptional inhibitors of differentiation Hes5 and Sox6 are downregulated by miR-219. Thus, while the true regulatory network of miRNA-controlled genes in oligodendrocytes is undoubtedly much more complex, already a simple working model (Figure 1) can be sketched with these observations. Upon serum or PDGF withdrawal from cultured OPCs (the in vivo correlate is not so clear), the expression of miR-219 is induced, which then "inhibits the inhibitors," e.g., it rapidly downregulates the responsiveness of OPCs to PDGF stimulation. Repressing PDGFR alpha and Hes5 expression further enhances oligodendrocyte differentiation and creates a positive feedback loop (Figure 1). This model may also explain the irreversible nature of differentiation, an issue perhaps relevant to the role of miRNAs in cancer. It also fits the overall concept that microRNAs have evolved to control the timing of cell division and differentiation in organ development (Pasquinelli and Ruvkun, 2002).

The late differentiation of oligodendrocytes in the mammalian brain has the remarkable consequence that even severe developmental defects cause dys- and demyelinating disease (in children with leukodystrophies) rather than embryonic lethality. One common feature of some leukodystrophies is the apparent inability of myelinating glia to handle elevated copy numbers of genes for abundant membrane proteins. For example, duplications of *PLP1* are the most frequent cause of demyelination and axon loss in Pelizaeus-Merzbacher disease. It is plausible that cells, already geared for high-level membrane synthesis, cannot tolerate protein overexpression due to the associated ER stress. Also in the adult brain, the steady-state level of many myelin protein mRNAs appears higher than predicted for myelin membrane turnover. This raises the possibility that mature oligodendrocytes also require posttranscriptional mechanisms to control the expression of myelin-associated genes.

Support for this idea comes from a study of Louis Ptacek's and Ying-Hui Fu's lab, reported in the first of the three recently published papers on *Dicer* and miRNA function in oligodendrocytes (Shin et al., 2009). In this study, *Dicer* expression was disrupted in mature oligodendrocytes of adult mice, using tamoxifen-inducible Cre recombination under control of the *Plp1* promoter. Thus, bypassing the critical early stages of development, these mutant mice

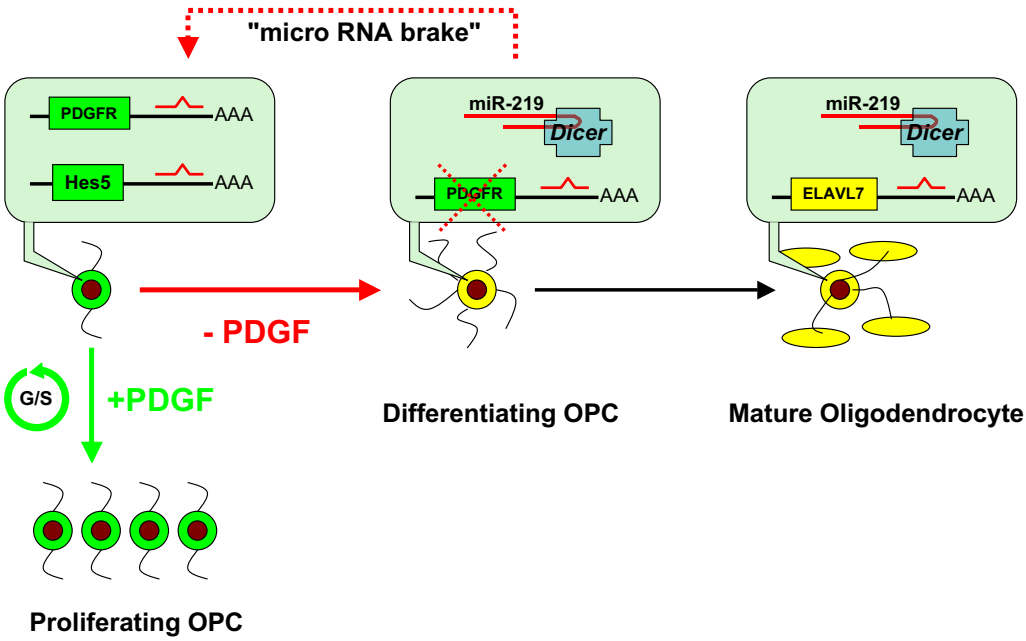

Figure 1. MicroRNA-Mediated Loss of Progenitor Properties Causing Rapid Oligodendrocyte Differentiation
In this model, the proliferation of OPC (left) is stimulated by gowth factors (+PDGF), and the differentiation into oligodendrocytes (right) is prevented by transcriptional inhibitors (e.g., Hes5 and Sox6). Following serum removal (–PDGF), the induction miR-219 and other miRNAs, processed by *Dicer*, is a "brake" on the expression of transcriptional inhibitors and PDGF receptor (middle, other target genes not shown). This lack of PDGF responsiveness and transcriptional inhibition constitutes a positive feed back loop that promotes a more rapid differentiation. In mature oligodendrocytes, the same miRNA regulates genes for other proteins, such as fatty acid elongase ELAVL7.

exhibited a severe neurodegenerative phenotype later in life, which included demyelination, inflammatory changes, and axon loss. Using very similar techniques as used in this year's papers, the authors identified miR-219 as the most interesting candidate miRNA, and determined its binding site in a regulated target gene. This gene, however, encodes an enzyme (ELAVL7) for the synthesis of very long chain fatty acids, which marks the "far end" of oligodendrocyte differentiation. Again, also the inducible *Dicer* mutant phenotype is complex, and the cause of demyelination is not understood yet, but miRNAs (such as miR-219) appear to be critical for regulating gene expression also in mature oligodendrocytes.

Collectively, these papers have identified the expression of miRNAs as a post-transcriptional "brake" of gene expression that remains essential throughout the lifespan of oligodendrocytes.

Given the sensitivity of all myelinating glia to the overexpression of myelin membrane proteins and the intriguing finding that a clinically relevant myelin protein, PMP22, is regulated by miR-29A (Verrier et al., 2009), one wonders how soon miRNAs themselves will be associated with a human myelin disease.

REFERENCES

Barres, B.A., and Raff, M.C. (1994). Neuron *12*, 935–942.

Bartel, D.P. (2004). Cell *116*, 281–297.

Calver, A.R., Hall, A.C., Yu, W.P., Walsh, F.S., Heath, J.K., Betsholtz, C., and Richardson, W.D. (1998). Neuron *20*, 869–882.

Dugas, J.C., Cuellar, T.L., Scholze, A., Ason, B., Ibrahim, A., Emery, B., Zamanian, J.L., Foo, L.C., McManus, M.T., and Barres, B.A. (2010). Neuron *65*, this issue, 597–611.

Filipowicz, W., Bhattacharyya, S.N., and Sonenberg, N. (2008). Nat. Rev. Genet. *9*, 102–114.

He, Y., Dupree, J., Wang, J., Sandoval, J., Li, J., Liu, H., Shi, Y., Nave, K.-A., and Casaccia-Bonnefil, P. (2007). Neuron *55*, 217–230.

Kocerha, J., Faghihi, M.A., Lopez-Toledano, M.A., Huang, J., Ramsey, A.J., Caron, M.G., Sales, N., Willoughby, D., Elmen, J., Hansen, H.F., et al. (2009). Proc. Natl. Acad. Sci. USA *106*, 3507–3512.

Lau, P., Verrier, J.D., Nielsen, J.A., Johnson, K.R., Notterpek, L., and Hudson, L.D. (2008). J. Neurosci. *28*, 11720–11730.

Lazzarini, R.A., Griffin, J., Lassmann, H., Nave, K.-A., Miller, R., and Trapp, B. (2004). Myelin Biology and Disorders (San Diego, CA: Elsevier - Academic Press).

Pasquinelli, A.E., and Ruvkun, G. (2002). Annu. Rev. Cell Dev. Biol. *18*, 495–513.

Pfeiffer, S.E., Warrington, A.E., and Bansal, R. (1993). Trends Cell Biol. *3*, 191–197.

Richardson, W.D., Kessaris, N., and Pringle, N. (2006). Nat. Rev. Neurosci. *7*, 11–18.

Shin, D., Shin, J.Y., McManus, M.T., Ptácek, L.J., and Fu, Y.H. (2009). Ann. Neurol. *66*, 843–857.

Verrier, J.D., Lau, P., Hudson, L., Murashov, A.K., Renne, R., and Notterpek, L. (2009). Glia *57*, 1265–1279.

Zhao, X., He, X., Han, X., Yu, Y., Ye, F., Chen, Y., Hoang, T., Xu, X., Mi, Q.S., Xin, M., et al. (2010). Neuron *65*, this issue, 612–626.