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Review

Receiving mixed signals: uncoupling oligodendrocyte differentiation and myelination

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Abstract. The development and maturation of an oligodendroglial cell is comprised of three intimately related processes that include proliferation, differentiation, and myelination. Here we review how proliferation and differentiation are controlled by distinct molecular mechanisms and discuss whether differentiation is merely a default of inhibited proliferation. We then address whether differentiation and myelination can be uncoupled in a similar manner. This task is particularly challenging because an oligodendrocyte cannot myelinate without first differentiating, and these processes are therefore not mutually exclusive. Is it solely the presence of the axon that distinguishes a differentiated oligodendrocyte from a myelinating one? Uncoupling these two processes requires identifying specific signals that regulate myelination without affecting the differentiation process. We will review current understanding of the relationship between differentiation and myelination and discuss whether these two processes can truly be uncoupled.

Keywords. Oligodendrocyte, myelination, differentiation, proliferation.

Introduction

Saltatory conduction, the process by which action potentials are rapidly and efficiently transmitted along axons, is dependent on the formation of the myelin sheath. In the central nervous system (CNS), myelination takes place following the maturation of oligodendroglial cells [1]. During development, oligodendrocyte precursor cells (OPCs) arise from specific loci in the ventricular zone [2]. These actively proliferating cells migrate throughout the CNS populating axons in the brain and the spinal cord. After reaching their final destination, OPCs cease to proliferate and differentiate into oligodendrocytes. Mature oligodendrocytes then myelinate surrounding axons (Fig. 1). What are the molecular mechanisms that drive proliferation, differentiation, and myelination? Determining whether these processes are regulated by distinct mechanisms could enhance our understanding of oligodendroglial development and broaden our outlook on treating various demyelinating diseases. Studies of OPCs suggest that proliferation and differentiation are controlled independently, and differentiation is not merely a default of halted proliferation. In this review, we will discuss some of the key mechanisms that regulate the transition of an oligodendroglial cell from a proliferating OPC to a terminally differentiated oligodendrocyte.

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Oligodendrocytes are multipolar cells, and each cell is capable of myelinating multiple axons. This is in direct contrast to Schwann cells, the myelin-forming cells of the peripheral nervous system (PNS). Each Schwann cell ensheaths and myelinates only one axon. While the functional role of myelin is the same in both the CNS and the PNS, the composition of the myelin membrane, the signals regulating myelination, and the timing of myelination differ somewhat between the two systems. In the PNS, myelination begins immediately after birth, while the onset of myelination in the CNS varies, beginning anywhere from 2 days until approximately 2 weeks postnatally, or even later, depending on the specific brain region [3]. What factors are responsible for the asynchronous appearance of myelin in the CNS?

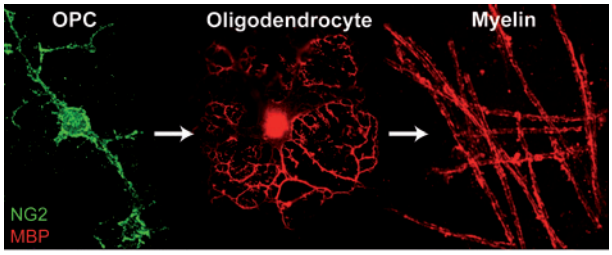

Figure 1. Stages of oligodendroglial development. Oligodendrocyte precursor cells (OPCs) are bipolar progenitor cells that actively proliferate as they migrate throughout the CNS. After reaching their final destination, OPCs stop proliferating and differentiate into multipolar oligodendrocytes. Mature oligodendrocytes then form myelin around surrounding axons. The OPC (green) is stained with an antibody to the chondroitin sulfate proteoglycan, NG2. The oligodendrocyte and myelin segments (red) are stained with an antibody to myelin basic protein (MBP).

At present there are two leading theories that could potentially explain the regional variability in the timing of CNS myelination. The first is that OPCs represent a heterogeneous population of cells capable of forming myelin at different times. The second hypothesis is that region-specific environmental cues are responsible for initiating myelination at the appropriate time. In this review we will discuss data supporting both of these mechanisms, as well as the possible contribution from both models. Results from *in vivo* studies suggest the existence of extrinsic signals that regulate CNS myelination. Examination of myelinated axons in P7 and P9 rat pups using electron microscopy reveals that myelination of the optic nerve takes place in a graded fashion, with axons near the eye myelinated earlier than axons at the optic chiasm [4]. Surprisingly, the onset of differentiation in the optic nerve occurs in a gradient inverse to that of myelination, with differentiation initiated near the chiasm prior to differentiation of OPCs near the eye (Fig. 2A). These intriguing results suggest that myelination and differentiation are distinct processes and can be independently regulated. This study seems to support the existence of a graded myelination signal, which ensures that oligodendrocytes near the eye form myelin sooner, despite the fact that they differentiate later than their counterparts near the chiasm. Is it possible that signals from the axons themselves are responsible for these temporal differences in myelination? The temporal variability of CNS myelination may result from regional differences not only in the regulation of myelination, but also in the generation of oligodendrocytes. Extensive work by Raff and colleagues suggests that oligodendrocyte differentiation is orchestrated by an intrinsic program, capable of functioning in the absence of environmental influence [5]. It is therefore possible that the graded differentiation seen in the optic nerve is not dependent on extrinsic cues, but results instead from the heterogeneity of OPCs along the optic nerve. If differentiation and myelination are regulated by distinct mechanisms, then it is plausible that differentiation may be controlled by an intrinsic program, whereas myelination depends on extrinsic signals.

In order to truly understand oligodendroglial development, it is essential to determine the relationship between proliferation, differentiation, and myelination (Fig. 1). This includes investigating whether these processes are independently regulated by distinct mechanisms. Therefore, in this review we will examine studies that have helped uncouple proliferation and differentiation. We will then discuss whether it might also be possible to uncouple differentiation and myelination. This task is particularly challenging because an oligodendrocyte must differentiate before it can form myelin. Therefore these processes are not mutually exclusive. Is it solely the presence of the axon that distinguishes a differentiated oligodendrocyte from a myelinating one? Uncoupling these two processes requires identifying specific signals that regulate myelination without affecting the differentiation process. In this review, we will discuss the current understanding of the relationship between differentiation and myelination and examine whether these two processes can truly be uncoupled.

An intrinsic program regulates OPC development

Differentiation and myelination represent intimately related steps in the maturation of an oligodendrocyte. As described previously, studies of myelination in the optic nerve suggest that differentiation and myelination can be differentially controlled [4]. Uncoupling these two processes depends upon the identification of

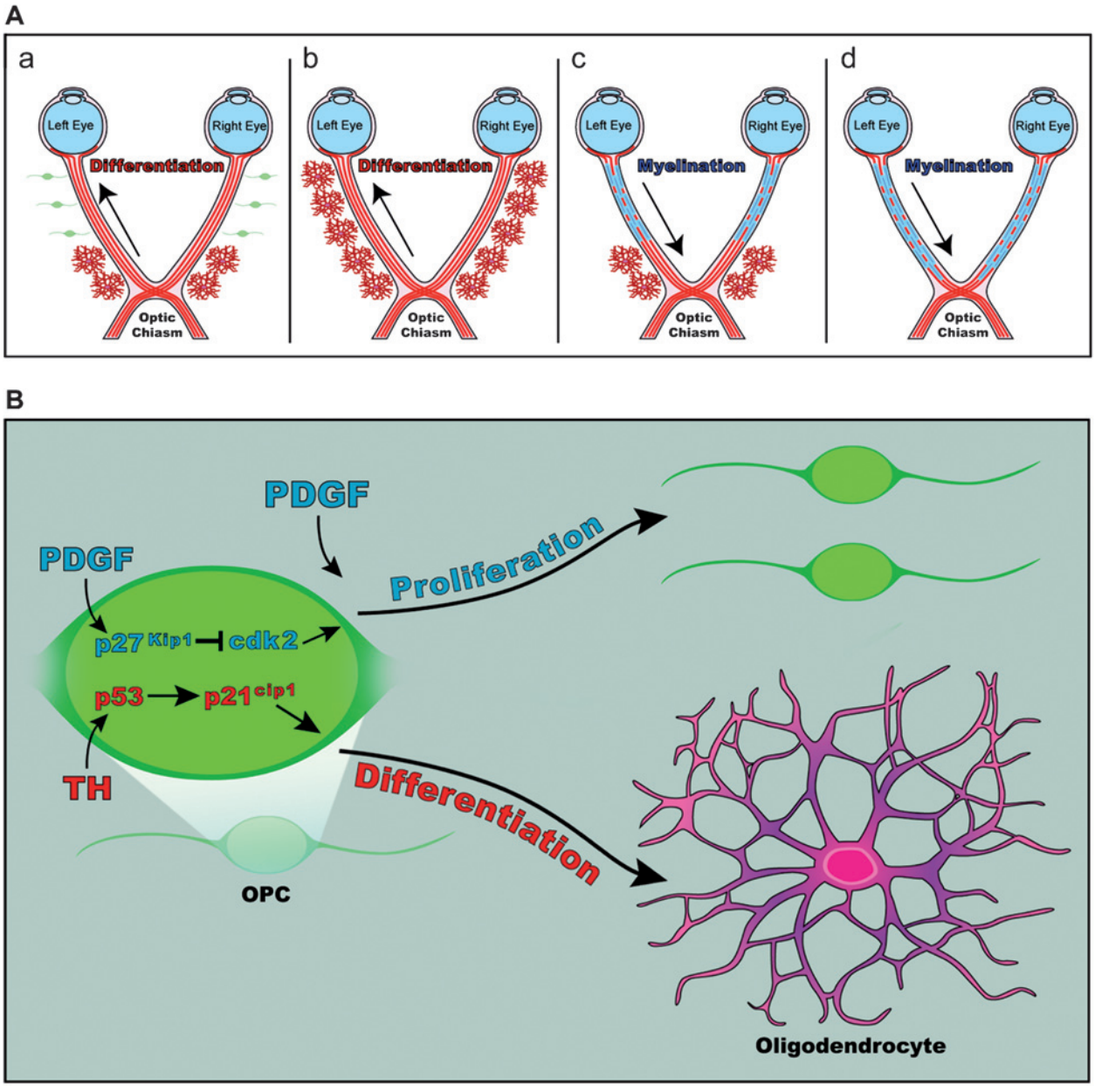

Figure 2. (*A*) Uncoupling differentiation and myelination in the developing Optic nerve. As observed by Collelo et al. [4], there is a regional gradient in the differentiation of OPCs (green) into oligodendrocytes (red) in the optic nerve. Differentiation begins near the optic chiasm (a) and continues along the nerve in a graded fashion toward the eye (b). Myelin (blue) formation around the axons of the optic nerve occurs in a reverse gradient that begins at the eye (c) and ends at the optic chiasm (d). These findings suggest that myelination and differentiation are distinct processes and can be independently regulated. (*B*) Proliferation and differentiation are regulated by distinct mechanisms. An OPC must decide whether to continue to proliferate and divide into two daughter cells or to stop dividing and differentiate into an oligodendrocyte. Intrinsic factors are responsible for both differentiation and proliferation. Interactions between cyclin E and the cyclin-dependent kinase, cdk2, promote cell division. The cell cycle inhibitor $p27^{Kip1}$ inhibits proliferation by inhibiting the formation of the cyclin E-cdk2 complex [18]. Platelet-derived growth factor (PDGF) is an extrinsic signal that promotes proliferation, but also promotes an increase in the expression of $p27^{Kip1}$. The $p27^{Kip1}$ pathway may represent one element of the intrinsic timer that helps halt proliferation at the appropriate time [13, 19, 22]. Differentiation is controlled by a $p27^{Kip1}$-independent pathway. One component of the intrinsic program that regulates differentiation is $p21^{cip1}$. $p21^{cip1}$ promotes the differentiation of OPCs into oligodendrocytes. Thyroid hormone (TH) is an extrinsic signal that promotes OPC differentiation under certain conditions. Tokumoto et al. [24] suggest that TH may regulate a p53 pathway that initiates differentiation by increasing transcription of $p21^{cip1}$.

distinct mechanisms that can independently regulate one process without affecting the other. This is particularly difficult in the case of differentiation and myelination since it is impossible for myelination to precede differentiation. However, this type of distinction has recently been established in studies of OPC proliferation and differentiation. It appears that proliferation and differentiation are clearly separate

processes, each independently regulated by distinct factors. Here we examine some of the studies that have helped to establish the differential regulation of proliferation and differentiation. Hopefully these studies will provide insight into understanding whether similar distinctions can be established between differentiation and myelination.

Extensive efforts have been made to examine the relationship between proliferation and differentiation of OPCs. As mentioned previously, the differentiation of OPCs into oligodendrocytes is preceded by a period of active proliferation. The ability of OPCs to proliferate appears to be dependent on extrinsic cues. *In vitro* studies demonstrate that one of the main promoters of OPC proliferation is platelet-derived growth factor (PDGF), which is secreted by astrocytes and neurons [6–10]. In addition, transgenic mice with neurons overexpressing PDGF exhibit a significant increase in the number of OPCs [11]. OPCs cultured in the presence of PDGF continue to divide indefinitely, unless factors regulating cell cycle arrest are also present [12, 13]. For example, the addition of growth factors such as thyroid hormone (TH) and retinoic acid (RA) limits the proliferative capacity of cultured OPCs [12, 14]. In fact, in the presence of both PDGF and TH, OPCs experience a fixed period of cell division, followed by differentiation. Under these conditions, the transition from OPC to oligodendrocyte appears to be regulated by an intrinsic program [5]. Studies concerning this intrinsic program suggest that it may be responsible for monitoring the amount of time an OPC spends proliferating, and for initiating cell cycle arrest at the appropriate time [15].

The existence of an intracellular mechanism regulating OPC division was first suggested by experiments in which single OPCs, isolated from P1 and P7 rat optic nerves, were individually plated onto monolayers of purified astrocytes [16]. In these experiments, the majority of OPCs within an individual clone divided a set number of times before undergoing simultaneous differentiation. These results suggest that the progeny of a single dividing OPC possess some intrinsic determinant that regulates the timing at which proliferation ends and differentiation begins. Although the number of divisions varied between clones, the majority of OPCs exhibited a cell cycle time of 1–2 days, and no cells experienced more than eight total divisions [16]. These findings provide compelling evidence that variability can exist within a given population of purified OPCs. Because all of the cells were cultured under similar conditions, it is likely that intrinsic determinants are responsible for variations in the timing of differentiation between clones. These results are relevant to the studies performed in the optic nerve in which differentiation occurred in a graded fashion [4]. Is it possible that heterogeneity among OPCs is responsible for the temporal differences observed in the appearance of differentiated cells? This scenario could support the uncoupling of differentiation and myelination if an intrinsic program is responsible for regulating differentiation, and myelination is controlled by extrinsic signals.

Additional support for the existence of an intrinsic regulatory mechanism is provided by supplementary experiments in which two progeny from a single clone were individually replated on separate astrocyte monolayers [16]. Amazingly, although the two sister OPCs were replated in isolated environments, the time at which their successive progeny ceased to divide and began to differentiate differed by less than 1.5 cell divisions. These *in vitro* studies further suggest the existence of an intrinsic mechanism that ensures the synchronized differentiation of related OPCs. Subsequent work has provided added insight into the role of this intrinsic regulatory mechanism in OPC differentiation. The intracellular timing mechanism functions when OPCs are actively dividing, as in the presence of PDGF. However, in the absence of PDGF and TH, over 98% of cultured OPCs fail to divide extensively and instead will differentiate into oligodendrocytes within 48 h [12, 17, 18]. The ability of OPCs to differentiate in the absence of PDGF and TH suggests that neither the timing mechanism nor TH is necessary or sufficient to induce differentiation in the absence of mitogens. Instead, it appears that the timing mechanism is responsible for determining when an appropriate number of OPCs has been generated and proliferation is no longer necessary. It is at this juncture that TH contributes to the cessation of proliferation, allowing differentiation to take place [12]. However, in the absence of mitogens, proliferation does not occur, and there is no need to count cell divisions or to halt the cell cycle [12, 17, 18]. The differentiation of OPCs in the absence of mitogens demonstrates that differentiation is not dependent on proliferation. However, it does not answer the question whether the termination of proliferation and the initiation of differentiation are controlled by a common mechanism. In order to truly uncouple the regulators of proliferation and differentiation, it is necessary to examine a model system in which both processes are occurring actively. The studies described next employ a series of elegant experimental approaches to accomplish this task.

Uncoupling proliferation and differentiation

In order to uncouple proliferation and differentiation, it is necessary to demonstrate that the factors regulat-

ing the cessation of proliferation are distinct from those that control the initiation of differentiation. We begin by describing a signaling pathway that is capable of halting proliferation, but is unable to promote the induction of differentiation. This pathway, which is regulated by the cell cycle inhibitor, $p27^{Kip1}$, provides a mechanism by which the intrinsic timer can determine when sufficient proliferation has occurred and when it is appropriate to induce cell cycle arrest. Normal progression through the cell cycle is modulated by interactions between cyclins and cyclin-dependent kinases (cdks). The formation of cyclin-cdk complexes, such as the cyclin E-cdk2 complex, activates signaling cascades that promote cell division. Formation of the cyclin E-cdk2 complex is regulated by the cell cycle inhibitor protein, $p27^{Kip1}$ [19]. Overexpression of $p27^{Kip1}$ in OPCs *in vitro* arrests the cell cycle by inhibiting cdk2 activity [20]. The control of cell division by $p27^{Kip1}$ has been shown to play a regulatory role in the proliferation of OPCs. Interestingly, in the presence of PDGF, actively proliferating OPCs exhibit a gradual increase in $p27^{Kip1}$ expression [21]. In the absence of PDGF, when OPCs differentiate within 48 h, the rate at which $p27^{Kip1}$ expression increases is upregulated dramatically. This increase in $p27^{Kip1}$ expression correlates with withdrawal from the cell cycle [13, 22, 23]. These studies suggest that increased expression of cell cycle regulators such as $p27^{Kip1}$ may represent one element of the intrinsic timer that helps halt proliferation at the appropriate time (Fig. 2B). Surprisingly, the rate at which $p27^{Kip1}$ expression levels increase can be modulated not only by the presence of mitogens, but also by the temperature at which cells are cultured. The level of expression of $p27^{Kip1}$ increases twice as rapidly in OPCs cultured at 33 °C compared to cells cultured at 37 °C [15]. Interestingly, OPCs cultured at 33 °C divide more slowly but differentiate after fewer divisions than OPCs cultured at 37 °C. These findings suggest that neither the rate of increase of $p27^{Kip1}$ expression nor the timing of OPC differentiation is dependent on the number of times that an OPC divides. Instead, it appears that the intrinsic timer uses some measurement other than the number of cell divisions to determine when it is appropriate to halt proliferation.

Are the factors responsible for down-regulating proliferation also responsible for initiating differentiation? This question is addressed by examining the role of $p27^{Kip1}$ in initiating OPC differentiation. OPCs overexpressing $p27^{Kip1}$ experience a premature induction of cell cycle arrest. However, instead of differentiating immediately, these cells remain in an extended period of quiescence and eventually differentiate at the same time as control OPCs [20]. These findings suggest that the inhibition of proliferation, as a result of cell cycle arrest, is not sufficient to induce differentiation of OPCs. Corresponding *in vivo* experiments examine how the loss of $p27^{Kip1}$ affects differentiation. In $p27^{Kip1-/-}$ mice, the number of proliferating OPCs increases [24]. However, the timing of differentiation is similar between knockout and wild-type mice, despite the increased proliferative capacity of $p27^{Kip1-/-}$ OPCs. Together, these studies support the idea that proliferation and differentiation are regulated through distinct mechanisms. Similar results were obtained after transfection of purified OPCs with a dominant-negative cdk2 (dn-cdk2), which inhibits the interaction between endogenous cdk2 and cyclin E [19]. Because an upregulation of $p27^{Kip1}$ also inhibits the formation of the cyclin E-cdk2 complex, one would expect the dn-cdk2 vector to inhibit proliferation. As expected, transfection with dn-cdk2 significantly decreased the proliferation of OPCs. However, the dn-cdk2 did not affect OPC differentiation when compared to controls [19]. These results, combined with the $p27^{Kip1}$ experiments, suggest that the $p27^{Kip1}$ pathway represents one mechanism by which OPCs control cell cycle arrest. These results also suggest that while the $p27^{Kip1}$ pathway regulates the inhibition of proliferation, the initiation of differentiation appears to be controlled by a $p27^{Kip1}$-independent pathway. These findings indicate that proliferation and differentiation can be uncoupled and are regulated by distinct mechanisms (Fig. 2B).

Factors regulating differentiation

Uncoupling proliferation and differentiation could potentially offer great insight into general development and even into the treatment of demyelinating diseases. Therefore, it is valuable to understand that the factors that inhibit proliferation are not sufficient to induce differentiation. It is also important to understand the mechanisms responsible for initiating differentiation. As described earlier, TH is not required for oligodendrocyte differentiation, as OPCs cultured in the absence of either mitogens or TH actively differentiate within 1–2 days [12, 17, 18]. This ability of OPCs to spontaneously differentiate suggests that oligodendrocyte differentiation is largely mediated by an internal program. However, in the presence of PDGF, TH is required for differentiation to occur [12]. What is the role of TH in the initiation of differentiation? As mentioned previously, the $p27^{Kip1}$ pathway represents an intrinsic mechanism that inhibits proliferation by inducing cell cycle arrest. Interestingly, $p27^{Kip1}$ expression can differ depending on the temperature at which cells are cultured [15],

and on whether or not PDGF is present [21]. This finding suggests that an intrinsic mechanism like the $p27^{Kip1}$ pathway can be modulated by extrinsic signals. It appears that TH may also serve as an extrinsic modulator of an intrinsic program. Recent work demonstrates that TH may mediate the expression of the cell cycle inhibitor protein $p21^{cip1}$ [25], which is required for the differentiation of OPCs [26]. The role of $p21^{cip1}$ is specific to the differentiation process, as cells isolated from $p21^{cip1-/-}$ mice exit the cell cycle in a timely manner. This is in direct contrast to cells purified from $p27^{Kip1-/-}$ mice, which proliferate longer than wild-type and $p21^{cip1-/-}$ cells [26]. This finding demonstrates that while $p27^{Kip1}$ is necessary for cell cycle arrest, $p21^{cip1}$ is not. However, myelination in the cerebellum of $p21^{cip1-/-}$ mice is decreased compared to wild-type mice [26]. This result suggests that $p21^{cip1}$ may be important for the proper differentiation of OPCs. These experiments further suggest that proliferation and differentiation can be uncoupled by demonstrating that the $p27^{Kip1}$ pathway is responsible for inhibiting proliferation, whereas a mechanism mediated by $p21^{cip1}$ plays a role in initiating differentiation. How is the regulation of differentiation by $p21^{cip1}$ affected by the presence of TH? Interestingly, treatment of OPCs with TH was shown to promote an increase in the expression of $p21^{cip1}$ [25]. Additional experiments suggest that the effects of TH on $p21^{cip1}$ expression are mediated through the tumor suppressor protein, p53, which has been shown to activate transcription of $p21^{cip1}$ [27, 28]. The infection of purified OPCs with a dominant-negative form of p53 substantially inhibits TH-induced differentiation [25]. Based on this finding, the authors of this study suggest that TH may regulate a p53 pathway that initiates differentiation by increasing transcription of $p21^{cip1}$. Together, these results provide a potential mechanism for the role of TH in promoting differentiation (Fig. 2B).

TH represents an extracellular signal that promotes OPC differentiation under certain conditions. In contrast, Jagged1 and Delta1 represent extracellular signals that can inhibit the differentiation of OPCs through activation of the Notch signaling pathway [29]. OPCs from the optic nerve express the Notch1 receptor both *in vivo* and *in vitro*. In cultures of purified OPCs, addition of the soluble Notch ligand Delta1 significantly inhibits the appearance of oligodendrocytes. Purified OPCs cultured on top of cells expressing Jagged1, another Notch receptor ligand, also fail to differentiate. Importantly, the expression of Jagged1 does not impair the ability of OPCs to divide, thereby demonstrating that activation of the Notch pathway specifically inhibits differentiation without affecting the proliferation process. These findings correlate with *in vivo* studies examining oligodendrocyte development in Notch1 conditional knock-out mice. These mice exhibit premature oligodendrocyte differentiation in multiple regions of the CNS [30]. Additionally, the gene inactivation of Notch1 results in the ectopic appearance of oligodendrocytes in the gray matter of the spinal cord. Together, these findings suggest that activation of the Notch pathway by extracellular signals can specifically inhibit OPC differentiation. We have reviewed the pathways described here because they specifically regulate differentiation, and are distinct and separate from the factors that control proliferation. It is important to note that the studies discussed here are by no means the only examples of signals that have been shown to regulate differentiation without affecting proliferation. For example, recent findings have highlighted the specific effects of transcription factors such as Nkx2.2, Sox10, and Olig2 on the differentiation of OPCs [31–33]. However, a thorough review of these and other studies is beyond the scope of this review. Clearly, there now exists a significant body of evidence which suggests the possibility of uncoupling proliferation and differentiation. This uncoupling could allow therapeutic targeting of factors that specifically affect a single process, which may improve the treatment of demyelinating conditions.

Axonal regulators of myelination

As demonstrated by the work discussed previously, proliferation and differentiation are distinct and independently regulated processes. Here we investigate whether differentiation and myelination are also separate events that are controlled by different mechanisms. This is particularly challenging because differentiated oligodendrocytes express the proteins and lipids that comprise the myelin membrane. Thus, it is primarily the wrapping of myelin around an axon that distinguishes a mature oligodendrocyte from a myelinating one. What are the potential biological benefits of separating these two processes? One explanation is that oligodendrocytes play multiple roles in the CNS in addition to their primary purpose of myelinating axons. This idea is supported by studies demonstrating that prior to myelination, oligodendrocytes play a role in clustering proteins at nodal domains [34]. Oligodendrocytes are also responsible for synthesizing *de novo* cholesterol found in the CNS, a role that has implicated oligodendrocyte death as a factor in plasticity deficits observed in Alzheimer's disease [35]. In addition, studies of mice lacking CNPase expression suggest that oligodendrocytes play a role in maintaining axonal integrity that is

independent of myelin formation [36]. In light of these and other findings regarding the function of oligodendrocytes, it is important to distinguish signals regulating myelination from those regulating differentiation. Identifying the signals responsible for initiating myelination could provide new targets for promoting remyelination. Here we will focus specifically on axonal signals that regulate oligodendrocyte myelination. We will discuss whether these signals specifically affect myelination, thus allowing the uncoupling of differentiation and myelination.

Based on the fact that some axons are myelinated while others are not, it seems plausible that signals from the axons themselves could help regulate myelination by oligodendrocytes. Indeed, coculturing oligodendrocytes with neurons promotes an increase in the clustering of membrane lipid rafts mediated by myelin basic protein (MBP) as compared to oligodendrocytes cultured alone [37]. However, the presence of axons cannot be the sole factor responsible for inducing myelination, because not all axons are myelinated. What are the specific axonal characteristics that determine which axons are myelinated and which are not? One study suggests that axon diameter may represent a crucial regulator of myelination [38]. In these studies, an increase in the size of an axonal target was shown to promote a corresponding increase in axon diameter. Results from this work demonstrate that an increase in axon diameter can promote the myelination of previously unmyelinated axons. Although these experiments were performed on peripheral axons, changes in axon diameter were also regarded as a likely regulator of CNS myelination. More recent studies suggest that an increase in axon diameter is not the only axonal factor responsible for myelination by oligodendrocytes. This was demonstrated by experiments in which the addition of nerve growth factor (NGF) to neuron-OPC cocultures inhibited myelination [39]. Importantly, the effect of NGF was mediated through modulation of an axonal signal and not through a direct effect on oligodendrocytes. This was demonstrated by experiments examining the effects of NGF on myelination of TrkA-expressing neurons compared to TrkB-expressing neurons. NGF failed to inhibit myelination of TrkB-expressing neurons, suggesting that the effect of NGF was mediated through a specific interaction with neuronal TrkA. These results therefore imply that activation of TrkA by NGF modulates an axonal signal that controls oligodendrocyte myelination. Interestingly, the presence of NGF, similar to an increase in the size of an axonal target, induces an increase in axon diameter. How does NGF simultaneously promote an increase in axon diameter and an inhibition of oligodendrocyte myelination? Is it possible that NGF activates an inhibitor of oligodendrocyte myelination, which overrides the pro-myelinating effect of increased axon diameter? In any event, the axonal control of myelination is not solely due to an increase in axon diameter. Identification of the axonal signal(s) regulated by NGF will help resolve this question.

Additional experiments showed that adding NGF to cocultures of TrkA-expressing dorsal root ganglion (DRG) neurons and mature oligodendrocytes significantly inhibited myelin formation [39]. Because these experiments were performed on cells that had already differentiated, it is possible to conclude that NGF has a specific effect on myelination. However, this does not rule out the possibility that NGF can also affect the differentiation process. In fact, adding NGF to DRG-OPC cocultures greatly reduces not only the appearance of myelin, but also the generation of oligodendrocytes. This result demonstrates that NGF may also inhibit the differentiation process. While NGF clearly regulates oligodendrocyte myelination, these studies are not sufficient to uncouple differentiation and myelination. Separating these two processes requires the identification of two distinct mechanisms that regulate each process independently. Is it possible that NGF regulates two different axonal signals, one controlling differentiation and the other controlling myelination? Answering this question requires identification of the signals downstream of NGF. LRR and Ig domain-containing, Nogo receptor-interacting protein (LINGO-1) was recently identified as an inhibitor of oligodendrocyte myelination that is expressed on both oligodendrocytes and axons [40, 41]. NGF signaling through TrkA promotes an increase in the axonal expression of LINGO-1 [40]. Inhibition of LINGO-1 using either a DN-LINGO-1 lentivirus or LINGO-1-Fc greatly enhances oligodendrocyte differentiation and myelination. These studies suggest that LINGO-1 is capable of inhibiting both differentiation and myelination. However, because the myelination studies were performed on OPC-DRG cocultures, it is possible that the effects of LINGO-1 are specific to differentiation, and that the observed reduction in myelination is simply a result of decreased numbers of oligodendrocytes. If signals such as LINGO-1 are to be used as targets for treating demyelinating diseases, it will be important to determine the specific developmental process that is impacted by manipulation of these factors.

Another possible candidate for an axonal signal that regulates myelination is the cell adhesion molecule, polysialic acid-neural cell adhesion molecule (PSA-NCAM). This is suggested by experiments in which dissociated cultures of cerebral hemispheres were treated with an anti-PSA-NCAM antibody. Addition

of the antibody resulted in an increase in the number of myelinated axons [42]. This effect appears to be specific to the process of myelination, as the addition of the anti-PSA-NCAM antibody had no effect on the number of MBP-expressing oligodendrocytes. These results suggest that PSA-NCAM represents an axonal inhibitor of oligodendrocyte myelination. If future studies can establish that the effects of PSA-NCAM are restricted to the process of myelination and do not impact differentiation, then these results may help uncouple differentiation and myelination.

Discussion

Myelin is responsible for the proper transmission of signals along an axon. How does the developing nervous system ensure that a sufficient number of oligodendrocytes are generated to myelinate all of the axons? The secretion of axonal factors that control OPC proliferation represents a clever method by which the number of precursor cells can be appropriately matched to the number of axons requiring myelination. Yet, it is not the axons but the OPCs themselves that appear to be primarily responsible for down-regulating proliferation and initiating differentiation. This represents an alternative and yet reasonable mechanism when considering that it may be difficult to fine-tune the levels of mitogens once they are secreted. The studies discussed here suggest that differentiation is not merely a default of inhibited cell division. Instead, proliferation and differentiation can be independently regulated. Is there a reason that these processes are controlled by two separate mechanisms? If OPCs represent a heterogeneous population of cells, then the differential regulation of these two processes would allow some OPCs to continue to proliferate at the same time that others begin to differentiate. The extrinsic modulation of proliferation and differentiation suggests that the location of an OPC within the CNS may help dictate whether it chooses to continue dividing or to differentiate. In this way, the nervous system ensures the presence of adult OPCs, which might be generated specifically to replace adult oligodendrocytes that are damaged as a result of injury or disease. However, the ability of adult OPCs to differentiate following demyelination is an issue that is currently being studied extensively. If these cells are to be used as a therapeutic target, it is important to identify the factors that both promote and inhibit the differentiation of OPCs. It is also important to understand whether differentiation and myelination are separate processes regulated by different mechanisms. If these two processes are distinct from one another, then treatment of demyelinating conditions may require the manipulation of both processes. Inducing the differentation of adult OPCs may not promote recovery unless the surrounding environment is also conducive to remyelination. Therefore, it is necessary to identify the specific factors controlling both differentiation and myelination. Whether these two processes can be differentially regulated is not yet clear. Is there a biological advantage gained by uncoupling differentiation and myelination? Perhaps the separation of these two processes allows oligodendrocytes to communicate with each other regarding which cell is responsible for myelinating a certain axon. After all, if differentiation and myelination are regulated by a single mechanism, what factors ensure that two oligodendrocytes do not try to myelinate the same axonal segment? Perhaps intercellular communication between oligodendrocytes, prior to the initiation of myelination, could guarantee the appropriate placement of oligodendrocyte processes, preventing overlap between separate myelin internodes. This theory is supported by studies suggesting that interactions between the processes of neighboring OPCs help ensure that cells are evenly spaced along an axon [43]. In addition, the localized ablation of developing OPCs *in vivo* is compensated for by increased proliferation of surrounding OPCs [44]. These findings suggest that generation of the appropriate number of OPCs is regulated in part by competition between cells for available space. Therefore, it is possible that communication between neighboring oligodendrocytes could also play a role in both differentiation and myelination. It is our hope that future studies will help decode the mixed signals regulating oligodendroglial development, thus allowing for the uncoupling of differentiation and myelination. Uncoupling these two processes could answer many questions regarding the behavior of oligodendrocytes both during development and after demyelination.

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