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The Role of Neurotrophins in the Regulation of Myelin Development

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Abstract
Neurotrophins comprise a family of growth factors that are expressed in a variety of cell types, and which exert influences on a large range of cellular activities that are important for development and the maintenance of the nervous system, as well as in neurodegenerative and psychiatric disorders. More recently, neurotrophins have been implicated in influencing the dynamic and complex signals that occur between neurons and glial cells, including Schwann cells in the peripheral nervous system and oligodendrocytes in the central nervous system that regulate myelination. Here we review the recent studies that identify neurotrophins as important regulators of both peripheral and central myelination, highlight some of the many questions that remain to be answered, and identify possibilities for further research.

Introduction

Acquisition of the myelin sheath is part of an evolutionary process that has been of critical importance to the development of vertebrates. Myelinating glial cells, oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS), are present in invertebrate species and ensheath axons; however, in all but a few exceptions they do not generate myelin. In contrast, myelin is present in all but the most primitive vertebrates [1]. Thus, vertebrate myelination did not require the evolution of new cells per se, but rather evolution of their phenotype [2]. Myelinated nerves, regardless of their central or peripheral origin, have a helical or concentrically wrapped multi-lamellar sheet of insulating plasma membrane comprising specific proteins and lipids. This myelin sheath plays two crucial roles: first, it is necessary for survival of the axons which it ensheaths, and second, it also permits saltatory conduction of action potentials. Substantial progress has been made in the identification of the nature of the signals, as well as the signalling pathways activated that regulate myelination [3]. Here we review the literature implicating one particular growth factor family, the neurotrophins, and the roles they play in regulating both peripheral and central myelination. Coincident with the evolution of myelin, evolutionary expansion of the neurotrophin family occurred early in the vertebrate lineage [4], and recent data have begun to identify and further our understanding of the complex roles these factors play in regulating myelination. In this review, we focus on the neurotrophins and their receptors, and the evidence garnered from both in vitro and in vivo studies of myelin development.

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We also outline some of the many questions that remain unanswered, and highlight possibilities for further research.

Neurotrophins and Their Receptors

The neurotrophins represent a family of growth factors comprising nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). They are initially synthesised as precursor proteins, known as proneurotrophins, and are subsequently cleaved to release the mature neurotrophin proteins. Recent evidence indicates that the proneurotrophins are biologically active, and signal through a receptor complex consisting of the p75 neurotrophin receptor (p75NTR) and sortilin. Once cleaved, the mature neurotrophins are no longer able to interact with this complex, but rather interact with two distinct classes of transmembrane receptors: the receptor tyrosine kinase tropomyosin-related kinase (Trk), and the structurally unrelated p75NTR, a member of the tumour necrosis factor α receptor superfamily. Neurotrophins exhibit some selectivity in their interaction with Trk receptors, such that NGF binds selectively to TrkA, BDNF and NT-4/5 to TrkB, and NT-3 to TrkC. In addition, all the neurotrophins can bind non-selectively to p75NTR. Recent reviews have addressed these ligand-receptor interactions and resulting signalling events [5–8], so will not be detailed here.

Neurotrophins and Their Receptors in Myelination

It is well established that both peripheral and central neurons, as well as Schwann cells and oligodendrocytes express and secrete neurotrophins. In addition, a variety of neurotrophin receptors can also be found on these cells. Thus, although this family of growth factors is both temporally and spatially well placed to influence the interactions between neurons and glial cells that regulate myelination, identification of the cell type, receptor and signalling mechanisms the neurotrophins utilise to regulate myelination has proven difficult. However, the recent adoption of reductionist approaches to the study of myelination in vitro has provided new insights into the roles the neurotrophins are playing in this context. We will review recent progress in identifying the roles that the neurotrophins and their receptors play in regulating myelination firstly by Schwann cells, then by oligodendrocytes, and finally speculate on whether proneurotrophin signalling could also play a role in this process.

Neurotrophins Regulate Axonal Signals to Influence Myelination by Schwann Cells

The difficulty in identifying the precise cellular and molecular basis of how neurotrophins regulate peripheral myelination is compounded by the fact that both neurotrophin and neurotrophin receptor knockout mice display severe peripheral neuronal loss, with approximately 80% of dorsal root ganglion (DRG) neurons dependent on NGF for survival [9], ~30% dependent on BDNF [10] and ~70% on NT-3 [11, 12]. As a result, analysis of the effects these molecules have on peripheral myelination in vivo, in the absence of substantial populations of DRG neurons, has been problematic.

The in vitro myelination assay, encompassing the coculture of defined populations of DRG neurons with Schwann cells, replicates aspects of the fundamental processes that occur during myelination in vivo, and has been utilised as models for both PNS and CNS myelination [13, 14]. Recently, important insights into the roles that the neurotrophins play in regulating myelination have been obtained using this assay, and by (i) culturing distinct DRG neuronal sub-populations for myelination, and (ii) segregating purely neuronal from mixed axonal-Schwann cell aspects of the culture in Campenot chambers [15], a much clearer understanding of the influence that the neurotrophins exert on myelination is emerging.

NGF Exerts a Promyelinating Influence

It is now apparent that NGF exerts a positive influence on Schwann cell myelination, but interestingly only on certain subpopulations of DRG neurons. In a seminal paper that re-ignited interest in the neurotrophins as regulators of myelination, Chan et al. [14] utilised co-cultures of NGF-dependent (TrkA+) DRG neurons and Schwann cells, and found that exogenous NGF enhances Schwann cell myelination. As NGF retained the ability to promote myelination in explants from p75NTR–/– mice, this demonstrated that NGF enhanced peripheral myelination via activation of neuronally expressed TrkA receptors [14]. To further support this, Chan et al. [14] showed that NGF had no effect on myelination of BDNF-dependent (TrkB+) DRG neurons, and as these DRG neurons do not express TrkA, the parsimonious explanation was that NGF was exerting its role in this context upon neuronally expressed TrkA receptors, which signalled to upregulate axonal permissiveness to myelination. While these analyses provided unequivocal insight into the influence that NGF

could exert on myelination in vitro, what of its role in vivo? Approximately 80% of the DRG neuron population is dependent on NGF for survival; thus, analysis of NGF–/– mice are of little benefit in this context, as the NGF-dependent neurons are lost during development [9]. A similar loss of DRG neurons is observed in TrkA–/– mice [16]. Interestingly, studies analysing DRG neuron phenotypes have shown that, in general, NGF-dependent (TrkA+) DRG neurons are nociceptive, small, and unmyelinated [17]. This presents a conundrum, as the in vitro data support a promyelinating influence on these neurons, whereas in vivo these neurons appear largely unmyelinated. Perhaps the answer lies in the developmental profile of TrkA receptor expression. Analysis of NGF–/– and TrkA–/– mice indicate that ~80% of DRG neurons must express TrkA at some stage during development, whereas, in contrast, at postnatal day (P) 14, only approximately 40% of DRG neurons express this receptor [18, 19]. It is now apparent that 30–40% of DRG neurons downregulate TrkA expression in the early postnatal period [18, 20], which is coincident with the commencement of the peripheral myelinating phase [21]. It is possible that this population of DRG neurons, that ultimately downregulate TrkA in the postnatal period, comprise a significant component of the total myelinated pool of axons in the adult, providing a promyelinating role for NGF in this context. This of course raises the issue of how NGF could discriminate between those cells that are destined to remain TrkA+ and those that only transiently express TrkA in the postnatal period, for which there is no readily apparent reason. It could be that relative levels of TrkA expression are important, on the other hand, the ratio of TrkA:p75NTR expression can alter the dynamics of NGF ligand binding [22] and TrkA signalling [23], so perhaps this could account for the differences. Unfortunately, the p75NTR–/– mouse also exhibits substantial (>50%) DRG neuronal loss in development [24], it will therefore be challenging to dissect the molecular basis of the influence that NGF exerts on peripheral myelination in vivo.

NT-3: A Positive or Negative Regulator of Myelination?

The effect NT-3 exerts on Schwann cell myelination is less clear, with evidence to support either a potentiating or inhibitory effect. In NGF-dependent DRG neuron-Schwann cell co-cultures, endogenous expression of NT-3 is high during the premyelinating phase and downregulated before myelination begins, and the addition of exogenous NT-3 to these same co-cultures significantly inhibits myelin formation [25, 26]. Injection of NT-3 adjacent to the sciatic nerves of myelinating neonatal mouse pups also results in reduced myelin protein formation [26], supporting an inhibitory role for NT-3 upon peripheral myelination; however, whether this was a uniform response across all sub-populations of peripheral neurons is unclear. The fact that exogenous NT-3 exerted a similar inhibitory influence upon the myelination of cultures in vitro and of the sciatic nerve in vivo from p75NTR–/– mice led to the conclusion that this effect is mediated through TrkC [26], although whether this activity is mediated via neuronal or Schwann cell signals remains unclear. To address this issue, in vitro analyses have found that NT-3, signalling via Schwann cell-expressed TrkC receptors, regulates Schwann cell migration. This is achieved through two distinct but parallel pathways; one in which TrkC directly phosphorylates the guanine-nucleotide exchange factor Dbs to regulate Cdc42 activity, and the other by Ras-dependent activation of Tiam1 to regulate Rac1 activity. Both these pathways converge via c-Jun N-terminal kinase (JNK) to promote Schwann cell migration [27–29]. These important insights indicate that NT-3 has the capacity to act through Schwann cells in the premyelinating phase to promote their migration, which is a necessary preamble to myelination. The ultimate test of these in vitro findings is their verification in vivo. However, analyses of peripheral myelination in NT-3 mutant mice suggest it exerts a promyelinating influence. It is important to note that NT3–/– mice lose ~70% of their DRG neurons during embryonic development, and the majority of mice die in the early postnatal period [11, 12], rendering analysis and interpretation of peripheral myelination difficult. Nevertheless, analysis of brachial plexus nerves from P0 NT-3–/– mice shows that peripheral nerve ensheathment and the earliest stages of myelination in these mice are similar to that of controls. On the other hand, analyses of P21 mice that are heterozygous for NT-3, which have relatively normal DRG neuron numbers [30], demonstrate a reduction in peripheral myelin mRNA and protein levels, suggesting that NT-3 exerts a positive influence on myelination. In addition, an increase in the number of caspase3+ Schwann cells has been detected in P0 NT-3–/– mice, suggesting that the aforementioned promyelinating influence could, at least in part, be supported by promoting survival of maturing Schwann cells [31]. The nexus identified between these studies is difficult to reconcile; however, as NT-3–/– mice display substantial (~70%) DRG neuronal loss early in development, how representative is the phenotype observed in this reduced DRG population to the study of myelination in normal

mice? Analyses of the influence that NT-3 exerts on Schwann cell myelination have yet to employ the more reductionist approaches of Campenot chambers and utilisation of different subsets of DRG neurons as substrates for myelination in vitro, so it is difficult to definitively identify precisely which cell type NT-3 is acting through or to ascertain whether its influence is uniform across all DRG neuron subtypes. Based on the in vitro data, perhaps another cogent approach to adopt in order to identify the role NT-3 is playing in this context would be to target deletion of TrkC specifically in Schwann cells. This would most likely overcome the problems associated with substantial DRG neuronal loss and early postnatal death, and at least unambiguously address the role that NT-3 signalling in Schwann cells plays in myelination. Clearly, further studies are required to definitively identify the basis of the influence that NT-3 exerts in this context.

BDNF Can Both Promote and Inhibit Myelination

In contrast to both NGF and NT-3, we have recently found that BDNF can either promote or inhibit Schwann cell myelination, depending on the phenotype of the DRG neuron being myelinated [32]. In vitro analysis of NGF-dependent (TrkA+) DRG neurons previously demonstrated that exogenous BDNF significantly enhanced myelin production, identifying a promyelinating influence for BDNF in this subpopulation of DRG neurons [25, 26]. To identify the cell type that BDNF was acting through to promote myelination, we utilised compartmentalised Campenot chambers and, surprisingly, found that it was sufficient for BDNF to act solely on the DRG neurons to promote myelination [32]. This was surprising as Chan et al. [25] previously demonstrated that NGF promotes myelination via activation of neuronally expressed TrkA receptors, and as these NGF-dependent (TrkA+) DRG neurons do not express the full-length TrkB receptor, it became immediately clear that the promyelinating influences of NGF and BDNF are mediated by distinct mechanisms.

To find how BDNF promotes myelination, we utilised 'xenocultures', co-culturing NGF-dependent DRG neurons isolated from p75NTR–/– mice with Schwann cells isolated from neonatal rats, and demonstrated that exogenous BDNF was unable to promote the myelination of these neurons. Through this analysis, we found that BDNF was acting through neuronally expressed p75NTR receptors to promote myelination, and therefore showed a fundamental difference in how NGF and BDNF promote Schwann cell myelination: NGF promotes myelination of NGF-dependent neurons via activation of neuronally expressed TrkA receptors [14], whereas BDNF promotes myelination via activation of neuronal p75NTR receptors [32]. Interestingly, while p75NTR has previously been shown to be critical for BDNF-induced myelination [26], identification of the cell type mediating this effect remains elusive. In vitro studies investigating the effect of BDNF on Schwann cells found that BDNF was able to inhibit Schwann cell migration in a p75NTR-dependent manner, through Src kinase-dependent activation of the guanine-nucleotide exchange factor Vav2 which ultimately influences RhoA activation [33]. In separate experiments, BDNF was also implicated in regulating Schwann cell polarity, an important process in the initiation of myelination, by promoting the association of p75NTR with Par-3 [34]. Collectively, these data suggest that BDNF could be acting directly via p75NTR on Schwann cells to promote myelination. However, our data are supported by additional experiments using an siRNA approach to knockdown p75NTR expression in Schwann cells, whereby we demonstrated that Schwann cells with knocked-down expression of p75NTR retain the same ability to myelinate DRG neurons in vitro as control cells, and also increase myelin formation in response to BDNF [32]. This further verified the critical role that neuronally expressed p75NTR receptors play in this context.

Perhaps the most surprising effect that BDNF exerts upon peripheral myelination was seen when we analysed its effect upon a population of DRG neurons distinct to those that are NGF-dependent. Through culture of BDNF-dependent (TrkB+) DRG neurons, we found that exogenous BDNF exerts an inhibitory effect upon Schwann cell myelination. Campenot chamber experiments further supported these data, and additionally showed that BDNF inhibits myelination of BDNF-dependent neurons by acting via neuronally expressed receptors. We were subsequently able to show that the Trk tyrosine kinase inhibitor K252a could block the inhibitory effect of BDNF upon the myelination of BDNF-dependent DRG neurons. As Schwann cells do not express full-length TrkB [26, 32], these data suggest that the inhibitory influence of BDNF on myelination is mediated by activation of neuronally expressed full-length TrkB receptors [32].

The BDNF data, while compelling, raised a number of issues that challenged its veracity. Firstly, given that NGF signalling through neuronal TrkA promotes Schwann cell myelination [14], how is it that BDNF signalling through neuronal TrkB inhibits it [32]? Given the structural and signalling similarities between TrkA and TrkB, it is difficult to reconcile how these closely related receptors can mediate opposing actions. However, TrkA+ and TrkB+ DRG neurons represent phenotypically distinct

neurons within the DRG, and the receptors are rarely co-expressed in the same neuron [35], so it could be that the cell context specificity of Trk signalling accounts for these contrasting effects. Clearly, further analyses investigating the molecular changes following BDNF signalling in these distinct neuronal populations are warranted to identify the basis of this dichotomy of BDNF-mediated activity. Secondly, Chan et al. [25] demonstrated that BDNF injection adjacent to the myelinating sciatic nerve in rat pups not only enhances sciatic nerve myelin protein expression but, on average, also increases the number of myelinated axons and thickness of the myelin sheath [25]. This was further supported in a mouse model where BDNF was transgenically overexpressed over a prolonged period, under the control of the β-actin promoter. In this model, myelin formation is accelerated during development, and while it did not alter the number of myelinated axons, the overexpression of BDNF generated thicker myelin sheaths and increased axonal diameter [36]. So how is it possible that BDNF can inhibit myelination? As TrkB is only expressed in a relatively small proportion of DRG neurons (estimates vary from 10 to 25% [35, 37, 38]), whereas 70% of these neurons express TrkA in the myelinating phase, it is perhaps not surprising that the inhibitory effect that BDNF exerts upon the minority TrkB+ subpopulation would be difficult to identify in vivo. Nevertheless, it is clearly important to establish whether BDNF exerts selective inhibitory effects upon TrkB+ DRG neurons in vivo. This ultimately may only be definitively achieved by genetically pre-labelling TrkB-FL+ DRG neurons, and injecting BDNF into the developing sciatic nerve to establish the specific influence upon myelination of these pre-labelled neurons. Finally, it appears counterintuitive that, as identified above for NGF, BDNF promotes the in vitro myelination of NGF-dependent (TrkA+) DRG neurons, a population that is unmyelinated in the adult in vivo, yet it inhibits the myelination of BDNF-dependent (TrkB+) DRG neurons in vitro, which becomes myelinated in vivo. Ultimately, the answer to this conundrum could come down to the limitations of studying such a complex biological process as myelination in vitro. The likelihood of an individual axon becoming myelinated in vivo is almost certainly dependent on the orchestration of any number of independent factors, rather than simply being due to Trk- or p75NTR-mediated signalling, in isolation. The influences that the neurotrophins exert on peripheral myelination in vitro should therefore be taken in context, where promyelinating effects demonstrated in vitro are interpreted as important cues that could influence rather than, in isolation, control the initiation or activation of different stages of myelination in vivo. In this context, effects that are demonstrated to be inhibitory in vitro could act to delay myelination during Schwann cell migration or at critical stages of development when synaptogenesis and neuronal pruning are being effected in vivo, rather than definitely block the process. Clarification and resolution of these issues clearly requires detailed in vivo analysis, but this will be challenging given that neurotrophin and neurotrophin receptor knockout mice all display severe deficits in DRG neuron survival, an important confounder. In this regard, the application of inducible transgenic mice that over-express neurotrophins and their receptors could help to overcome some of the limitations.

Taken together, neurotrophin-mediated influences upon the capacity of individual peripheral axons to become myelinated are clearly determined by the complement of cell-specific expression profiles of neurotrophin receptors at critical times in development. At least for NGF and BDNF, the available evidence strongly argues that these responses are directed via neuronally derived signals to enable neurons to subsequently direct Schwann cell myelination. Recent reviews have covered the candidate molecules that the neurotrophins could regulate in this regard [39], as well as the prominent role that axonally derived signals play in regulating myelination [40, 41], so will not be detailed here. It is interesting to note, however, that neuregulin-1 (NRG1), a key axonally derived signal, is known to drive not only Schwann cell growth and differentiation but also myelination [40, 42]. It is known that the soluble NRG1 type I and II are upregulated in response to both NGF and BDNF in NGF-dependent DRG neurons in vitro [43], However, it remains unknown whether the expression of membrane-bound NRG1 type III can also be regulated by neurotrophins: this is important to examine as it could provide the molecular explanation for the promyelinating influences of the neurotrophins. In this scenario, Schwann cells would secrete neurotrophins which would, in turn, stimulate adjacent axons to secrete NRG1 type III which would then induce the Schwann cell to myelinate. In this model, dynamic developmental changes in the expression and location of the neurotrophins and their receptors in both Schwann cells and axons could be critical for co-ordinated peripheral nerve myelination.

These data also identify that the neurotrophins must induce a range of signals to exert such distinct effects upon peripheral nerve myelination. Identification of the signals that regulate peripheral myelin development is important for our progression into designing rationally

based therapies that promote remyelination in the context of disease and injury. The neurotrophins may also be instructive in this regard, as the fact they exert positive as well as negative influences on myelination highlights another challenge: the importance of a thorough examination of the effect that putative remyelinative therapies have upon all DRG neuronal subpopulations, rather than assuming a unified response.

Neurotrophin Signalling in Schwann Cells

The propensity for the neurotrophins to exert their influence upon myelination via neuronally derived signalling invites speculation on the role that Schwann cell-expressed neurotrophin receptors may play in this context. Schwann cells express p75NTR, TrkC and truncated TrkB receptor 1 (TrkB-T1) receptors [26, 32]. Of these receptors, it is the function of p75NTR that has been the focus of the most attention. Phenotypic analyses of p75NTR–/– mice reveal that axons within the sciatic nerve have less than normal myelin thickness at P5, and a greater than 50% reduction in the number of myelinated axons in the adult [26], suggesting that p75NTR is necessary for proper myelination. In support of this, it has been demonstrated that Schwann cells from the p75NTR–/– mouse migrate less on peripheral nerve sections in vitro and in vivo [44]; however, it must be noted that studies utilising different models of migration have observed disparate results [27, 33, 45]. The finding that remyelination is impaired in p75NTR–/– mice following sciatic nerve injury [46] further supports a role for Schwann cell p75NTR in myelination; however, it is important to note here that p75NTR is also expressed in DRG neurons, and the fact that more than 50% of the DRG neurons are lost in the p75NTR–/– mouse [24] complicates the analysis of Schwann cell migration and myelination in these mice. This was overcome to a degree in a study where either p75NTR–/– or wild-type mouse-derived nerve grafts were transplanted into nude mice following sciatic nerve injury, supplying a source of Schwann cells for remyelination. Functional and histological analysis of the injured nerve of these mice up to 10 weeks following grafting indicated that those receiving p75NTR–/– grafts had poorer motor performance, reduced myelin sheath thickness, decreased axonal diameter, and reduced myelin protein expression [47]. Although the study was performed in the context of remyelination rather than development, these data also suggest that Schwann cell p75NTR plays an important role in the myelinating process.

The possibility that p75NTR could be important in regulating myelination has also arisen from a number of studies analysing Schwann cell signalling. One of the first identified p75NTR-signalling targets in Schwann cells was activation of the transcription factor NF-κB [48], and it was subsequently identified that the adapter proteins Traf-6 [49] and RIP2 [50] were important mediators of this response. The fact that activation of NF-κB in Schwann cells is required for peripheral myelin formation [51, 52] suggests that p75NTR signalling could play a pivotal role in this process; however, this has yet to be demonstrated conclusively. Additionally, the recent finding that Par-3, a component of the Par polarity complex, asymmetrically co-localises with p75NTR to the inner Schwann cell membrane adjacent to the axon during ensheathment suggests an additional role for p75NTR in Schwann cell myelination. As Schwann cells begin to myelinate, they must become highly polarised to permit topographically targeted insertion of specific myelin lipids into the spirally ensheathing membrane. Analyses indicate that Par-3 and p75NTR co-localise at the axo-glial junction and transiently associate during the period of active myelination. This association is enhanced by BDNF, suggesting an important role for p75NTR in inducing Schwann cell polarity and myelination [34]. However, we have recently shown that following lentiviral-mediated knockdown of p75NTR in Schwann cells, myelination in vitro is not impaired, and BDNF maintained its capacity to enhance myelination [32], suggesting Schwann cell p75NTR plays a limited role in myelination under these in vitro circumstances. This must be interpreted cautiously, as the possibility that the isolation and culturing of Schwann cells from animals at different ages or under different in vitro conditions could precondition them to respond differentially to the same promyelinating stimuli.

Together, analyses of both p75NTR–/– mice and p75NTR signalling in Schwann cells have provided enticing data suggesting that the neurotrophins can influence myelination by directly acting on Schwann cells. Despite this, the precise roles that p75NTR signalling in Schwann cells play in regulating peripheral myelination remain obscure. Ultimately however, even in the face of a substantive breakthrough from an in vitro perspective, it will not be until a conditionally targeted approach is taken to delete p75NTR specifically from Schwann cells that its role in these cells will be revealed.

A Role for the Neurotrophins in Regulating CNS Myelination?

While substantive effort and progress have been made in identifying the key roles that the neurotrophins play in regulating PNS myelination, in contrast our understand-

ing of the precise roles played by these molecules in CNS myelination remains relatively unclear. This could, in part, be explained by the simple fact that a method for the isolation and purification of primary Schwann cell cultures pre-dated that of cells of the oligodendroglial lineage. Nevertheless, the recent description of a method to isolate bulk quantities of myelin-competent oligodendrocyte progenitor cells has opened up this area for in vitro investigation. Adaptation of the in vitro myelination assay, utilising oligodendrocyte progenitor cells (OPCs) isolated from the neonatal rat as the myelinating glial cell instead of primary Schwann cells, results in oligodendrocyte ensheathment of DRG axons [14], retinal ganglion cell axons [53] and spinal motor neuron axons [54], generating compact myelin and the expression of myelin-specific proteins [14]. Among these in vitro myelination models, the DRG-OPCs co-cultures are the most broadly used [13, 14, 55, 56]. Using this model, Chan et al. [14] demonstrated that, in contrast to what was observed in Schwann cell myelination, exogenous NGF inhibited oligodendrocyte myelination of NGF-dependent DRG neurons. Utilising xenocultures (in this case the co-culture of rat DRG neurons with OPCs derived from the p75NTR–/– mouse), Chan et al. [14] found that this effect was also dependent on activation of neuronally expressed TrkA receptors, which was later found to regulate axonal expression of LINGO-1 [57]. These observations are important from a biological perspective, showing that Schwann cells and oligodendrocytes respond in contrasting ways to the same NGF-regulated axonal signal, and that, therefore, axonal control over CNS and PNS myelination will likely be mediated via unique and mutually exclusive mechanisms. These observations are also important from a technical perspective, and effectively enable a range of reductionist techniques for the analysis of oligodendrocyte-induced myelination. There is reason to think that further analysis of the roles that the neurotrophins play in regulating CNS myelination could well be productive, as analyses of both BDNF and NT-3 mutant mice suggest they play important roles in this context.

BDNF – A Promyelinating Regulator of CNS Myelination?

The majority of BDNF–/– mice die within a few days of birth, although a fraction are observed to survive for up to ~3 weeks [10]. Homozygote mice exhibit deficits in cardiac development, and display respiratory dysfunction with aging, resulting in early postnatal death [58]. From a nervous system perspective, while these mice exhibit marked peripheral and cranial sensory neuron losses, CNS development appears to be relatively normal with no gross structural or cytoarchitectural abnormalities. However, those knockout mice that survive up to P15 have significantly reduced brain size [59], as well as alterations in the expression profile of some peptides within CNS neurons, suggesting some functional abnormalities [10]. Important to our consideration of myelination, in P13 BDNF–/– mice, while the number of retinal ganglion cells and the size and thickness of the retina is normal, the proportion of myelinated axons in the optic nerve is reduced by approximately 50% compared to littermate controls. This reduction was also present at P21, with an accompanying significant reduction in optic nerve axon diameter, indicating that the BDNF–/– mouse develops with a greater number of small unmyelinated axons. In addition, analysis of the hippocampus and cortex showed significant reductions in myelin basic protein (MBP) and proteolipid protein (PLP) mRNA levels [60]. However, as insufficient numbers of these mice survive past P21, it remains unclear whether this was a true deficit in myelination or rather a developmental delay in normal myelination. These data suggest a role for BDNF in the control of CNS myelination; however, the precise mechanism underlying this phenotype remains unclear. Insights from the analysis of PNS myelination have highlighted the importance that neuronally expressed receptors can play in regulating myelination; thus, it remains unclear whether these effects are mediated by BDNF acting directly on oligodendrocytes or via neuronally expressed receptors. Indeed, the effect could also be indirect, as the cardiorespiratory deficits these mice exhibit could predispose them to early postnatal undernourishment, a condition that also results in the reduction of myelin synthesis [61].

In vitro studies have shown that cells in the oligodendroglial lineage express the BDNF receptors p75NTR, as well as full-length and truncated forms of TrkB [62, 63]. Interestingly, while BDNF does not support the survival or proliferation of OPCs derived from the optic nerve [64], it appears to induce these activities amongst OPCs derived from the basal forebrain [65]. In addition, the role BDNF plays in the differentiation of basal forebrain-purified post-mitotic oligodendrocytes in vitro has been studied in some detail, with BDNF eliciting increased expression of MBP, PLP and myelin-associated glycoprotein amongst oligodendrocytes. This is a full-length TrkB-dependent effect, requiring activation of the mitogen-activated protein kinase (MAPK)-signalling pathway [66, 67]. While these results could be indicative, the relation-

ship between the cellular events required for oligodendrocytes to differentiate into elaborate stellate-shaped, myelin-expressing cells in vitro and for them to co-ordinate ensheathment and generate compact myelinated axonal segments is far from clear. Interestingly, nestin-targeted deletion of B-Raf, one of the Raf kinases that relays signals to MAPK, results in marked CNS hypomyelination in vivo. In this model, nestin drives B-Raf deletion in CNS neural precursor cells that give rise to neurons, oligodendrocytes and astrocytes, and while the brains of these mice did not show any gross neuronal abnormalities, not only was myelination reduced significantly, but the mutant oligodendrocytes failed to differentiate both in vivo and in vitro [68]. While MAPK activation in oligodendrocytes in vitro was attenuated in response to PDGF, FGF and EGF (BDNF was not directly investigated), one could speculate that at least part of this phenotype could be a consequence of deficient BDNF-induced signalling via B-Raf, which might otherwise drive myelination in oligodendrocytes. In support of this, in a separate experiment, nestin-targeted deletion of TrkB also resulted in a hypomyelinating phenotype, although this was also associated with deficits in neuronal migration and differentiation [69]. Identifying the cellular mechanism underpinning the hypomyelinating phenotype in these studies is unclear, as neurons, oligodendrocytes, and astrocytes are all subject to deletion. It is important to note, however, that there are regional differences in the responsiveness of oligodendrocytes, and whilst oligodendrocytes purified from the basal forebrain express TrkB and differentiate in response to BDNF, oligodendrocytes purified from the cortex do not express this receptor [63]. It is also important to note that our investigation into the effects that the neurotrophins exert upon peripheral myelination indicate that the above observations are insufficient to conclude the effects of BDNF in this context are mediated directly via the oligodendrocyte. Thus, this opens up the possibility for the utilisation of in vitro myelinating co-cultures to further investigate the effect that BDNF can exert in this context. Utilising reductionist approaches, such as those employed for the study of Schwann cell myelination [25, 32], will provide an opportunity to rigorously investigate the identity of the cell type and receptor that BDNF acts through to induce these putatively promyelinating effects. Once armed with this knowledge, it would be most instructive to verify the veracity of these in vitro data by generating a transgenic mouse, targeting conditional deletion of the receptor in the cell type through which BDNF appears to be acting.

NT-3 – Also a Positive Regulator of CNS Myelination?

NT-3 is required for the survival of substantial numbers of DRG, sympathetic and spinal propioceptive afferent neurons [70]. It is also essential for normal cardiac development [71], and as a result most NT-3–/– mice die shortly after birth, with the few surviving up to 2–3 weeks exhibiting marked growth retardation and substantial decreased body weight [72]. Despite this, the CNS appears morphologically normal. Interestingly, the expression of NT-3 in the normal brain displays a highly regulated profile, with the highest levels occurring during the late prenatal period and the first few weeks of postnatal development, after which levels decline markedly [73]. Superficially, these data could suggest a role for NT-3 in regulating CNS myelination, as this coincides with critical stages in oligodendrocyte precursor cell proliferation and myelination [74]. This is supported in vitro, as NT-3 was one of the first growth factors identified to support the survival of OPCs [64]. The OPCs in this study were purified from developing rat optic nerve, were identified to express the receptor tyrosine kinase TrkC, and to undergo a proliferative response to NT-3 [75–77]. NT-3 was later found to exert the same influence on cortical OPCs [78]. The effect NT-3 exerts on OPCs is context-dependent and extends beyond survival, as OPCs transfected to over-express NT-3 and co-cultured with hippocampal neurons demonstrate enhanced myelinogenic potential, display greater MBP expression and an increase in the number of myelinated segments in vitro, compared to control transfected OPCs [79]. In addition, exogenous NT-3 increases DRG neuron ensheathment with myelin in vitro [80]. Together, these data suggest that NT-3 could exert a survival/proliferative response in OPCs when cultured alone, or a differentiative response in the presence of neurons. Indeed, it has been demonstrated that OPCs can activate an integrin-regulated switch when they contact axonal laminins that alter the nature of growth factor signalling [81], which could account for how NT-3 exerts these apparently disparate effects.

In vivo, injection of hybridoma cells secreting a neutralising anti-NT-3 monoclonal antibody into the myelinating optic nerve results in a reduction in the proliferation of OPCs, as well as fewer OPCs and mature oligodendrocytes, but no change in the number of type-1 astrocytes [77]. This suggested that, as observed in vitro, the primary effect of NT-3 could be on OPC proliferation, rather than myelination per se. Examination of NT-3–/– mice also supports a promyelinating role for this growth factor. While myelination in the cortex does not occur

until later in the postnatal period, myelination in the ventral regions of the spinal cord can be detected by P0 [74]. Utilising PDGFR-α expression as a marker for OPCs, Kahn et al. [82] found that in P0 NT-3–/– mice, while the regional distribution of PDGFR-α mRNA+ cells in spinal cord was similar to wild-type mice, the NT-3–/– mice exhibited a 35% reduction in the number of OPCs. In addition, quantification of more mature cells in the oligodendrocyte lineage revealed significant reductions in the number of galactocerebroside-positive (28% reduction) and MBP mRNA+ (27% reduction) oligodendrocytes in the spinal cord of NT-3–/– mice, which also exhibited less complex process formation [82]. Analysis of the brain also suggested that the numbers of PDGFR-α mRNA+ OPCs and galactocerebroside-positive oligodendrocytes were reduced. These data support the in vitro data mentioned above, suggesting that NT-3 could play an important role in regulating OPC survival and proliferation in vivo. Interestingly, in the NT-3–/– brain not only were cells of the oligodendrocyte lineage affected, but numbers of astrocytes (s100α+) and microglia (IB4+) were also reduced, suggesting a wider role for NT-3 in regulating early gliogenesis and glial cell proliferation. In support of this, the cytoarchitecture of the subventricular zone in the NT-3–/– mice appeared attenuated, suggesting fewer glial cells were being generated. Interestingly, in vitro treatment of embryonic neurospheres with NT-3 also promotes glial fates [83]. Thus, the primary effect that NT-3 could be playing in the myelinating context in vivo is in the generation and proliferation of OPCs, rather than a bona fide promyelinating role; however, this has yet to be definitively established. Studies have implicated that OPC number is regulated by axonally derived growth factors [64], suggesting that axons themselves could determine CNS oligodendrocyte number, so at what precise stage NT-3 exerts its effect also remains an open question. As analysis of CNS myelination in NT-3–/– mice can only be undertaken in the early postnatal period, it also remains uncertain whether NT-3 deficiency would result in a permanent hypomyelinating phenotype as opposed to a simple delay in the normal development of CNS myelin. Interestingly, exogenous NT-3 can also exert a differentiative influence upon oligodendrocyte progenitor cells in vitro [84]; however, as questions remain as to the precise role that NT-3 is playing in oligodendrocyte myelination, its influence is also open to further reductionist investigation in vitro, and ultimately translation in vivo, to identify precisely how it achieves this.

A Role for the Proneurotrophins in Regulating Myelination?

Identification by Lee et al. [85] in 2001 that the precursor form of the NTs, the proneurotrophins, are biologically active invited a reappraisal of the precise roles that the NTs are playing, and whether some of the effects attributed to NT signalling could, in fact, be mediated by the proneurotrophins. The observation that the pro-form of both NGF [86] and BDNF [87] are the predominant form in brain tissue certainly fuelled this speculation, and raises the possibility that at least some of the deficits observed in the NT knockout mice could derive from proneurotrophin activity. Proneurotrophin signalling is mediated by a receptor complex consisting of p75NTR and sortilin [85, 88], and pro-apoptotic roles for this ligand-receptor complex were almost immediately identified in a number of primary cells, including oligodendrocytes [89]. However, these studies have by and large utilised in vitro techniques and injury models in vivo, so what of the role of proneurotrophins in the developmental context? Certainly non-apoptotic roles are emerging for the proneurotrophins: for example, it has been found that proBDNF could play a role in modulating synapse formation, facilitating long-term depression [90]. In the case of BDNF at least, studies have shown that proBDNF can be anterogradely transported [91] and secreted from central neurons [92]. Interestingly, the firing rate of the neuron can influence whether the mature or pro-form is predominantly secreted [93]; however, it should also be noted these data have been disputed [94]. The pro-region of the neurotrophins is readily cleaved [95]; however, in the case of myelination this may not be a major factor, as in this instance the proneurotrophins would not be acting as diffusible growth factors, but within the relatively protected environment of the axo-glial interface. So, in this context, do proneurotrophins play a role in regulating myelination? Analyses of proneurotrophin receptor knockout mice do not provide direct evidence. Sortilin–/– mice have recently been generated, and there was no report of a myelinating phenotype in these mice [96]. Sortilin is a member of a larger family of receptors [97], and p75NTR also has a related homolog, NRH2 [98], and whether there is promiscuity in the interaction between these receptors is unresolved. NRH2 can interact with Trk receptors and regulate their signalling [99, 100]; however, whether it also interacts with sortilin to mediate proneurotrophin signalling is unclear. Certainly, it is established that p75NTR is expressed by both Schwann cells and oligodendrocytes during the myelinating period, and that sortilin can at least be expressed by these

cells in vitro [101, 102]. Thus, given that proneurotrophins can be axonally secreted and that their receptors are expressed by myelinating glial cells, it remains plausible that the proneurotrophins could influence myelination, a possibility that warrants further study.

Concluding Remarks

Our ability to conclusively identify the influence that the neurotrophins exert on both CNS and PNS myelin development is compromised by the fact that, in most cases, knockout of either the neurotrophins or their receptors results in substantial peripheral neuronal loss and early postnatal death. While the analysis of myelination in vitro clearly has limitations, there are important insights to be gained by utilising these methods. Such experiments have proven pivotal in identifying the cell type and receptors that both NGF and BDNF are utilising to regulate Schwann cell myelination, although this too must be taken in context. Myelination in vivo is regulated by the combination of an unknown number of growth factors, and it is the additive impact that both positive and negative influences, most likely limited to strictly defined developmental windows, which will ultimately decide whether myelination proceeds. It has proven useful to identify the cellular and molecular basis of how the neurotrophins regulate myelination in vitro, and based on these findings it is now important to translate this to the specific conditional targeting of neurotrophins or their receptors. With careful examination of myelin development in vivo we should be able to unambiguously define the roles this family of molecules is playing in regulating both central and peripheral myelination. These analyses need not only be restricted to development, as with the ability to generate mice with inducible conditional deletion of the neurotrophins or their receptors, it may also be possible to question the role that the neurotrophins play in the maintenance of the myelinated nervous system. In addition, the induction of demyelination in these mice may also give insight into the roles that this family of growth factors are playing in the process of remyelination, which ultimately could pave the way for developing new therapeutic approaches for the treatment of demyelinating disease.

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