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Abstract

Myelin is essential for rapid and accurate conduction of electrical impulses by axons in the central and peripheral nervous system. Myelin is formed in the early postnatal period, and developmental myelination in the peripheral nervous system (PNS) depends on axonal signals provided by Nrg1/ErbB receptors. In addition, Nrg1 is required for effective nerve repair and remyelination in adulthood. We discuss here similarities and differences in Nrg1/ErbB functions in developmental myelination and remyelination after nerve injury.

Introduction

Communication between cells is fundamental for formation and regeneration of organs. We know today that only a handful of important signaling systems are used for cell communication during development, and receptor tyrosine kinases are prominent among them. Many receptor tyrosine kinases were identified because of their oncogenic potential when mutated, and the non-mutant variants (the proto-oncogenes) function in development (Schlessinger, 2000). Recent evidence demonstrates that these receptors are frequently reused in the adult where they orchestrate regeneration.

Neuregulins (Nrgs, in humans called heregulins HRGs) are a family of growth factors that display sequence similarities with epidermal growth factor (EGF) (Falls, 2003). The factors signal via tyrosine kinase receptors of the ErbB (in humans called HER) family (Yarden and Sliwkowski, 2001). Nrgs/ErbBs were identified and intensely studied because of their functions in cancer. Antibodies against the receptors or small molecular weight inhibitors that interfere with their activity are used in cancer therapy and provide excellent tools for functional studies (Hynes and Lane, 2005). Among the different members of the family, Neuregulin-1 (Nrg1) is the most important and the best-studied. Nrg1 is one of the largest genes in the human genome and spans almost two megabases. From this gene, many different isoforms are produced by alternative splicing and the usage of different promoters (Falls, 2003). All Nrg1 isoforms contain an EGF-like domain embedded in otherwise divergent sequences, and the EGF-like domain is sufficient to bind and activate ErbB receptors. Major classes of Nrg1 isoforms use different promoters and are expressed in a characteristic and distinct pattern, for instance type III mainly by neuronal cells, and type I Nrg1 by a few restricted neuronal cell types and by mesenchymal cells of many organs (Meyer and Birchmeier, 1995). Interestingly, different isoforms take over distinct functions in vivo. For instance type III and type I Nrg1 are produced by all or just a subset of sensory neurons, respectively, and have distinct functions in myelination and

muscle spindle induction (Meyer and Birchmeier, 1995; Hippenmeyer et al., 2002; Perlin et al., 2011; Cheret et al., 2013). Nrg1 isoforms are produced as soluble membrane-bound proteins. Membrane-bound variants can be released by proteases, or they remain bound to the cell surface after proteolysis (Falls, 2003). Because Nrg1 can be shed or remain membrane-bound, the receptors detect signals provided by distant or directly neighboring cells (paracrine signaling). In addition, occasionally the same cell produces and receives the signal (autocrine signaling). Thus, the diversity of the Nrg1 isoforms contributes to the versatility of the signaling system.

Important Nrg1 functions were identified by genetics, mainly in mice but also in zebrafish. Responses to Nrg1 are astonishingly diverse, and encompass migration, cell fate decisions, morphogenesis, proliferation and the control of cell size. Moreover, glial cells, neurons, muscle and epithelial cells respond to Nrg1. Prominent among the Nrg1 functions is its role in PNS development and regeneration. In particular, the myelinating glia of the PNS, Schwann cells, depend on Nrg1, and we concentrate on discussing this function here. In Schwann cells, the Nrg1 signal is mediated by two receptors, ErbB2 and ErbB3 (Woldeyesus et al., 1999), and these receptors are unusual in the respect that ErbB3 lacks tyrosine kinase activity, whereas ErbB2 is a ligand-less receptor (Citri et al., 2003). They function as heterodimers where ErbB2 and ErbB3 provide the tyrosine kinase and the ligand binding activity, respectively. Nrg1 binding to ErbB2/3 results in receptor tyrosine phosphorylation, which triggers recruitment of adaptors and enzymes and culminates in the activation of signaling cascades (Schlessinger, 2000; Yarden and Sliwkowski, 2001).

Schwann cell precursors

Schwann cells depend on Nrg1 signaling for their development. Schwann cells derive from neural crest and Schwann cell precursors are defined by their

anatomical position, i.e. they associate with axons of peripheral nerves. Schwann cells depend on Nrg1/ErbB signaling for migration along the axon tracts and for proliferation, and are basically absent in mice or zebrafish that lack Nrg1, ErbB2 or ErbB3 (Meyer and Birchmeier, 1995; Riethmacher et al., 1997; Woldeyesus et al., 1999; Lyons et al., 2005; Perlin et al., 2011). The Nrg1 signal that drives Schwann cell development is provided by neurons, and presented in the axolemma. Thus, axonal membranes are mitogenic for Schwann cell precursors, and the mitogenic agent is Nrg1 (Dong et al., 1995; Morrissey et al., 1995; Schroering and Carey, 1998). Genetic evidence demonstrates that type III Nrg1 drives Schwann cell development (Meyer et al., 1997); this isoform can remain membrane-bound after proteolytic cleavage (Cabedo et al., 2002).

Schwann cell precursors retain characteristics of neural crest cells and remain pluripotent (Morrison et al., 1999). They generate immature Schwann cells, endoneurial fibroblasts of fetal nerves as well as melanocytes, parasympathetic neurons and odontoblasts (Joseph et al., 2004; Adameyko et al., 2009; Dyachuk et al., 2014; Espinosa-Medina et al., 2014; Kaukua et al., 2014). Schwann cell progenitors give rise to immature Schwann cells around E16 (Jessen et al., 2015). This transition is associated with axon wrapping, appearance of a basal lamina around Schwann cells, and formation of the perineural sheath. Subsequently, Schwann cells sort and wrap axons, and they generate the multilayered myelin sheaths.

Entry into the myelination program

Myelination is essential for neuronal function, and deficits in myelination cause devastating disease (Suter and Scherer, 2003; Quarles et al., 2006; Nave and Trapp, 2008). Myelin electrically insulates axons and allows fast propagation of nerve impulses by saltatory conduction. The overall conduction velocity of the myelinated axon is determined by myelin and axonal thickness, internodal length and myelin integrity (Waxman, 1980; Court et al., 2004). The overall organization

of the myelin sheath is similar in the PNS and CNS, but substantial differences exist in development, assembly and molecular composition of the myelin, or in the extrinsic signals and transcription factors that drive myelination (Quarles et al., 2006; Brinkmann et al., 2008; Emery, 2013; Hornig et al., 2013). We discuss here the molecular processes active during peripheral myelination.

Myelination is tightly controlled by axons. Only medium and large diameter axons are myelinated, and thin axons (smaller than 1 μm) are ensheathed by non-myelinating Schwann cells and are organized in bundles (called Remak bundles; Hillarp and Olivecrona, 1946). Thin and thick myelinated axons have thin and thick myelin sheaths, respectively. This leads to the constant G-ratio (defined as ratio of axonal diameter to diameter of the myelinated fiber) of 0.67 (Donaldson and Hoke, 1905). Thus, the axon provides cues that determine its myelination fate and the thickness of its myelin sheath. Conversely, Schwann cell-derived signals allow neuronal survival by providing trophic signals and/or metabolic support (Riethmacher et al., 1997; Viader et al., 2013). Interactions between Schwann cells and axons also guide cell adhesion molecules and ion channels into distinct axonal domains, the nodes of Ranvier and internodes, a pre-requisite for efficient saltatory conduction (Eshed-Eisenbach and Peles, 2013). Finally, intact myelin provides signals for radial axonal growth and enhances axonal transport, which is impaired in demyelinating diseases (de Waegh et al., 1992; Watson et al., 1994; Kiryu-Seo et al., 2010; Saxton and Hollenbeck, 2012).

The surface membrane of Schwann cells was estimated to increase during myelination up to several thousand-fold. Therefore, myelination is accompanied by the production of huge amounts of myelin proteins and lipids. The entry into the myelination program in Schwann cells is controlled on the transcriptional level by factors like Egr2 (Krox-20), Pou3f1 (Oct-6/Scip/Tst-1) and Pou3f2 (Brn2) that interact with Sox10 to drive expression of myelin genes (Topilko et al., 1994; Bermingham et al., 1996; Jaegle et al., 2003; Finzsch et al., 2010; see also Svaren and Meijer, 2008, and the references herein). In addition, lipid

biosynthesis genes are coordinately regulated, and the Srebp/Scap transcription factors and the control of their expression play important roles in lipid production (Verheijen et al., 2009; Norrmen et al., 2014). Extrinsic signals that trigger entry into myelination are provided by Nrg1/ErbB2/3, GPR126, integrins, extracellular matrix components and ADAM22/Lgi4 (Bermingham et al., 2006; Nave and Salzer, 2006; Ozkaynak et al., 2010; Taveggia et al., 2010; Raphael and Talbot, 2011). Thus, the Nrg1/ErbB2/3 system that regulates expansion of the progenitor pool and migration in early Schwann cell development is unexpectedly reused for myelination. It was therefore discussed whether Nrg1 provides instructive or permissive signals during myelination (Lemke, 2006; Nave and Salzer, 2006).

Nrg1 and other extrinsic signals that control myelination

Nrg1 activates Ras/MAPK/Erk1/2, PI3K/Akt, PLC$_\gamma$, focal adhesion kinase (FAK), Rho-GTPases and JNK, and these pathways have been implicated in myelination using mouse genetics or cell culture experiments (Newbern and Birchmeier, 2010). Cell culture models are frequently used to study entry into myelination and are combined with pharmacological inhibitors, overexpression through viral transduction and interfering RNAs. Results obtained with such methods can vary according to the exact culture conditions. Such studies mainly implicated PI3K/Akt as key signals in myelination, and suggested that MAPK/Erk played a limited role or even drive Schwann cells into dedifferentiation (Maurel and Salzer, 2000; Harrisingh et al., 2004; Ogata et al., 2006). Unexpectedly, recent genetic experiments have now shown that MAPK/Erk signaling is a major positive regulator of myelination in vivo, indicating that this notion has to be revisited (Grossmann et al., 2009; Newbern et al., 2011; Ishii et al., 2013; Sheean et al., 2014).

Erbin is an intracellular molecule that binds ErbB2 and modulates and promotes Nrg1 signaling and is essential for myelination (Tao et al., 2009). Shp2 mediates Nrg1 signals downstream of ErbB2/3 and is required for sustained activation of

MAPK/Erk1/2 as well as for Nrg1-evoked cellular responses like proliferation, migration or myelination, which are impaired when Shp2 is ablated in vivo (Grossmann et al., 2009). Furthermore, ablation of Erk1/2 in Schwann cells causes deficits that are remarkably similar to those observed in Shp2 or Nrg1/ErbB2/ErbB3 mutants (Newbern et al., 2011). Interestingly, mild activation of MAPK signaling releases Schwann cells from their dependence of Nrg1 signaling during all stages of Schwann cell development and myelination (Sheean et al., 2014). In particular, MAPK/Erk positively regulate entry into myelination but a strong and sustained activation of Raf (and thus the Ras/MAPK/Erk cascade) results in demyelination, indicating that signaling strength determines the cellular response (Napoli et al., 2012).

Pharmacological inhibition of PI3K/Akt in cultured Schwann cells enhances myelin gene expression and myelination in neuron/Schwann cell co-cultures (Maurel and Salzer, 2000; Ogata et al., 2006). PTEN is a negative regulator of the PI3K/Akt pathway and, accordingly, loss of PTEN results in Akt activation. This leads to mild hypermyelination of small peripheral axons, and to locally restricted hypermyelination close to the nodes of Ranvier (Goebbels et al., 2010; Goebbels et al., 2012).

The activator of adenylate cyclase, forskolin, has long been known to stimulate myelination in culture (Lemke and Chao, 1988; Morgan et al., 1991). In vivo, cAMP-mediated signals provided by the G protein-coupled receptor GPR126 are essential for myelination, and mutations of GPR126 preclude peripheral myelination in zebrafish and mice (Monk et al., 2009; Monk et al., 2011). Myelination in GPR126 mutant fish is rescued by forskolin in the swimming water (Monk et al., 2009). GPR126 is activated by binding to laminin and collagen, providing evidence that the extracellular matrix formed by immature Schwann cells provides signals that control entry into myelination (Paavola et al., 2014; Petersen et al., 2015). Integrin signaling also provides cues for myelination. Integrins are important adhesion and signaling receptors that recognize

extracellular matrix components like laminin, and upon binding to matrix they recruit intracellular molecules like FAK. Laminin, integrin and FAK provide essential cues for axonal sorting and myelination in vivo (Feltri et al., 2002; Chen and Strickland, 2003; Grove et al., 2007; Berti et al., 2011; Pellegatta et al., 2013). Thus, GPR126 and integrins provide links to the extracellular matrix and signal in a matrix-dependent manner to control axonal sorting and myelination.

Proteolytic processing is rate limiting for Nrg1 activity and function

Many Nrg1 isoforms are synthesized as membrane-bound proteins and require proteolytic processing for function. Type I and type III Nrg1 are processed by Bace1, an aspartic-acid protease, and by members of the disintegrin and metalloproteinase (Adam) family (Horiuchi et al., 2005; Hu et al., 2006; Willem et al., 2006; Yokozeki et al., 2007; La Marca et al., 2011; Fleck et al., 2013). A single Bace1-cleavage of type I Nrg1 releases a soluble EGF-domain containing fragment, whereas cleavage of type III Nrg1 at a similar site generates an EGF-domain containing fragment that remains associated with the membrane (Hu et al., 2006; Willem et al., 2006; Fleck et al., 2013). Systematic in vitro mapping demonstrates that protease cleavage sites exist that surround the EGF-like domain in type III Nrg1 (Hu et al., 2006; Willem et al., 2006; Fleck et al., 2013). Thus, small soluble EGF-domain containing peptides can be produced that are biologically active in vitro and in the zebrafish in vivo (Fleck et al., 2013). In addition, ADAM17 might cleave type III Nrg1 in the EGF-domain and thus destroy its activity but this cleavage site was not found in all reported mapping experiments (La Marca et al., 2011; Fleck et al., 2013).

Genetic models demonstrated that Bace1-dependent cleavage of Nrg1 is physiologically important for its function. Thus, Bace1 mutant mice and zebrafish display deficits in developmental myelination. In addition, remyelination of peripheral nerves and formation and maintenance of muscle spindles are impaired in mice, and these phenotypes resemble the well-characterized ones

observed when Nrg1/ErbB signaling is ablated (Hu et al., 2006; Willem et al., 2006; Cheret et al., 2013; Fleck et al., 2013). It should be noted that phenotypes in Bace1 mutants are not as strong as the ones observed in Nrg1 signaling mutants, indicating that other proteases participate in shedding. However, overexpression of type I Nrg1 in transgenic mice induces supernumerary muscle spindles in a strictly Bace1-dependent manner, indicating that this isoform requires Bace1 processing for its function (Cheret et al., 2013). The finding that Bace1 cleaves Nrg1 might be of interest for new therapies that are being developed for Alzheimer's disease. Bace1 is essential for APP processing and amyloid peptide formation, and Bace1 inhibition is a strategy for treatment of Alzheimer's disease. Adverse effects due to inhibition of other Bace1 substrates including Nrg1 must be considered (Fleck et al., 2012).

Rates of protein synthesis control the end of myelination and myelin thickness

During normal development, myelin is formed rapidly during the postnatal period. In particular, radial myelin growth is fast during the first two postnatal weeks in mice, slows in the maturing animal and ceases in adulthood. Recent data indicate that translational control mechanisms are important determinants that end myelination and thereby control myelin thickness (Pereira et al., 2010; Sheean et al., 2014).

The control of protein synthesis in vivo is difficult to analyze due to technical limitations, and synthesis of myelin components have been little investigated. However, new methods are now available that are based on a pulse-labeling of proteins with stable (nonradioactive) isotope-labeled amino acids and on quantification of labeled and unlabeled proteins by mass spectroscopy (Selbach et al., 2008; Doherty et al., 2009; Schwanhausser et al., 2011; Sheean et al., 2014). With these techniques it was possible to assess translational control mechanisms during myelination in the PNS and their dependence on MAPK/Erk

(Sheean et al., 2014). Similar mechanisms appear to be operative in myelination of the CNS (Michel et al., 2015).

Protein synthesis rates are highly regulated. A rate-limiting step is the initiation of translation that is strongly enhanced by growth factor signaling mediated by mTor and MAPK. Sustained activation of MAPK overcomes the signals that end myelination and results in continuous myelin growth achieved by enhanced protein synthesis rates. MAPK exerts this effect in part by mTor-dependent signaling, possibly by regulating the negative regulator of mTor, TSC2, but also by mTor-independent mechanisms (Sheean et al., 2014). Further supporting evidence derives from the analysis of mTor mutant mice that arrest myelin growth prematurely and that form thin myelin (Sherman et al., 2012). Dlg1-PTEN interactions were also reported to control the end of myelination and thus myelin thickness, and might impinge on the control of protein synthesis (Cotter et al., 2010).

Nrg1 and remyelination after injury

The potent role of Nrg1 as an axonal signal that drives virtually all stages of Schwann cell development and myelination provides a rationale for studying its function in peripheral nerve injury. Despite its importance in developmental myelination, Nrg1/ErbB signaling is not required for the maintenance of the myelin sheath in adulthood (Atanasoski et al., 2006; Fricker et al., 2011; Fricker et al., 2013). Injury radically alters the communication between axons, glia and immune cells, and effective signaling between these cells is crucial for successful nerve repair (Fawcett and Keynes, 1990; Scheib and Hoke, 2013). There is growing evidence that Nrg1 is important for repair and remyelination after injury, but signaling requirements in repair do not merely recapitulate requirements in development. Instead, differences exist in usage of Nrg1 isoforms and co-operating signaling systems.

Peripheral nerve injury has many causes ranging from traumatic damage to metabolic disturbances in diabetes mellitus. The primary pathology can affect axons (nerve transection) or Schwann cells, and their inter-dependence means that injury to one will ultimately affect the other. Fortunately, the PNS has a significant capacity for repair, and axons are able to regenerate over long distances and to remyelinate. This repair process leads to appreciable recovery of function after damage, but significant challenges remain: The clinical outcome after nerve injury often remains poor and there is no licensed therapeutic (Scheib and Hoke, 2013). The outcome depends on the injury type: Complete nerve transection has a worse outcome than nerve crush due to mis-directed axon growth, and proximal injuries also have a poorer outcome than distal ones due to the slow (1 mm/day) rate of axon regrowth (Fu and Gordon, 1995). In addition, aged Schwann cells are less supportive of axon outgrowth than young Schwann cells and might produce less efficiently beneficial growth and metabolic factors (Painter et al., 2014).

Traumatic nerve injury is followed by stereotypic events called Wallerian degeneration, i.e. axonal degeneration, Schwann cell dedifferentiation and proliferation. Subsequently, axons begin to regenerate, Schwann cells redifferentiate and remyelinate and targets are reinnervated. Although similarities between development and repair of myelin are apparent, key differences exist, and for instance remyelinated axons have shorter internodes and thinner myelin sheaths (Fancy et al., 2011). Nevertheless, the reformed myelin enables rapid saltatory conduction.

Traumatic nerve injury provides a model for the analyses of biological pathways important for nerve degeneration and repair, and the stereotypic events allow the quantitation of degeneration/regeneration (Conforti et al., 2014). Genetic mutants in invertebrates and vertebrates demonstrated that injury induces an active axon destruction program (Mack et al., 2001; McDonald et al., 2006; Osterloh et al., 2012; Gilley et al., 2015). Schwann cells in the distal nerve stump lose their

axonal attachment, proliferate and resorb myelin. Autophagy of myelin by Schwann cells provides the rate-limiting mechanism for myelin clearance and depends on JNK/c-Jun (Gomez-Sanchez et al., 2015). The initial signal for the Schwann cell response appears to be provided by axons, as Wallerian degeneration slow (Wlds) mouse mutants whose axon destruction is delayed also show gross delays in myelin removal and nerve regeneration. The remaining myelin was proposed to inhibit axon outgrowth (Brown et al., 1994).

An interesting concept that emerged over the last decade is that Schwann cells in the distal nerve stump display unique characteristics (they were also called 'repair cells') and do not simply revert to an earlier developmental stage (Arthur-Farraj et al., 2012; Jessen et al., 2015). These 'repair cells' are essential for nerve repair that requires axoglial signaling: Axon-derived signals promote differentiation of repair cells and growth factors released by them stimulate axon growth (Fawcett and Keynes, 1990; Jessen et al., 2015).

Traumatic nerve injury results in the dysregulated expression of many components of the Nrg1 signaling pathway like increased expression of ErbB2/ErbB3 receptors and enhanced receptor activation in the distal nerve (Cohen et al., 1992; Carroll et al., 1997; Kwon et al., 1997). Neuronal type III Nrg1 expression declines after axon transection, but is restored as axons reinnervate their targets and may therefore depend on target-derived growth factors (Bermingham-McDonogh et al., 1997). After axon destruction, type I Nrg1 expression in Schwann cells is transiently upregulated (Stassart et al., 2013).

Remyelination depends on juxtacrine Nrg1 derived from the axolemma. Mice in which Nrg1 is genetically ablated in a subpopulation of axons (and not from Schwann cells) show absent or grossly impaired remyelination, and the deficit is restricted to those axons that lack Nrg1 (Fricker et al., 2011). Thus, axon derived type III Nrg1 plays a key role in remyelination. Schwann cell derived type I Nrg1 modulates remyelination, and selective ablation of Nrg1 in Schwann cells does

not impair developmental myelination but impinges on remyelination and functional recovery after injury (Stassart et al., 2013). Thus, developmental myelination exclusively depends on axonal type III Nrg1, whereas Schwann cell-derived type I Nrg1 contributes to remyelination. The protease Bace1 is required for full Nrg1 activity and remyelination is impaired in mice lacking Bace1 (Hu et al., 2008). Erbin expression increases in distal nerve after injury, and Erbin mutant mice display impaired remyelination and functional recovery (Liang et al., 2012). The early phases of the injury response are not Nrg1 dependent: Myelin clearance is not delayed when Nrg1 is lacking, and Schwann cell proliferation during regeneration was not impaired in the absence of Nrg1 signaling (Atanasoski et al., 2006; Fricker et al., 2013). Finally, the inflammatory response is not Nrg1-dependent (Fricker et al., 2013).

A further important difference between Nrg1 functions in development and repair exists: remyelination and functional recovery are greatly delayed when Nrg1 is absent, but ultimately large or medium diameter axons are remyelinated, whereas small diameter axons are wrapped but not myelinated (Fricker et al., 2013). Therefore, Nrg1 determines the rate of remyelination after injury, but it neither decides the myelination fate (i.e. selects the axons that are remyelinated) nor the final outcome of remyelination. This is distinct from its developmental function where even a reduction in Nrg1 signaling results in reduced myelin thickness that is not compensated with time (Garratt et al., 2000; Michailov et al., 2004). This result implies that signals cooperate with Nrg1 during repair, which are not operative during development (Fricker et al., 2011). Nrg1 might not only modulate Schwann cell function during nerve repair, since the rate of axon regeneration was also reduced when Nrg1 was lacking. 'Back signaling' (i.e. the release of the intracellular domain of type III Nrg1 by gamma secretase; Hancock et al., 2011) or alternatively, indirect effects due to changed release of glial factors might contribute to this.

Can exogenous Nrg1 promote nerve repair and functional recovery after injury and thus be of therapeutic use? A successful outcome of a Nrg1 therapy may require just the right level of signaling, since excessive stimulation of Schwann cells is known to cause proliferation, demyelination, and formation of myelin that is too thick and possibly unstable (Zanazzi et al., 2001; Sheean et al., 2014). Moreover, Nrg1-dependent activation of ErbB receptors on Schwann cells (and other cell types exposed to Nrg1) could cause oncogenic transformation (Huijbregts et al., 2003). The final outcome of such a therapy is therefore likely to depend on the exact mode of Nrg1 delivery including timing of the treatment and dose. Despite these caveats, encouraging findings have emerged. For the treatment of cardiovascular disease, Nrg1 is already being tested in clinical trials and the treatment has a good safety profile (Jabbour et al., 2011). Transgenic over-expression of type I or type III Nrg1 in neurons promotes remyelination after traumatic nerve injury (Stassart et al., 2013). Furthermore, exogenous administration of type I and II Nrg1 protein or delivery of type I Nrg1 by viral vectors promotes axon outgrowth and nerve repair in experimental settings (Mahanthappa et al., 1996; Cai et al., 2004). Thus, the effects were not restricted to improved remyelination but also enhanced axonal outgrowth, which represents probably the critical factor in functional repair.

Charcot-Marie-Tooth is an inherited neuropathy that affects approximately 1 in 2500 people (Rossor et al., 2013). CMT1A, the most common variant of CMT, is caused by the duplication of a large region of chromosome 17 that includes the PMP22 gene, i.e. an excess of the myelin protein PMP-22. Thus, CMT1A affects primarily the myelin sheath and is associated with aberrant Schwann cell differentiation, myelination and demyelination. Administration of exogenous Nrg1 during early postnatal development did overcome impaired peripheral myelination, prevented axon loss and ameliorated the impairment of nerve function (Fledrich et al., 2014).

Nrg1 also promotes the survival of terminal Schwann cells that envelop the neuromuscular junction, and these cells contribute to remodeling of the neuromuscular synapse after injury and to reinnervation of denervated synapses (Kang et al., 2014). The survival of terminal Schwann cells after neonatal denervation can be improved through Nrg1 administration, and they remain responsive to Nrg1 in adulthood (Trachtenberg and Thompson, 1996).

In summary, Nrg1 is required for effective nerve repair in adulthood and acts during remyelination. Both type I and type III Nrg1 isoforms contribute to myelin repair, in contrast to the developmental myelination, which appears to be strictly type III dependent (Fig. 1). In contrast to developmental myelination, during repair the loss of the Nrg1 signal is partly compensated by other mechanisms. Exogenous Nrg1 helps to promote nerve repair in a number of different animal models ranging from traumatic nerve injury to hereditary neuropathy, and promotes axon outgrowth as well as myelin repair. It is thus an encouraging therapeutic candidate. Complex decisions will need to be made prior to its use in clinical trials in regards to optimal isoform/formulation, dose and timing of administration.
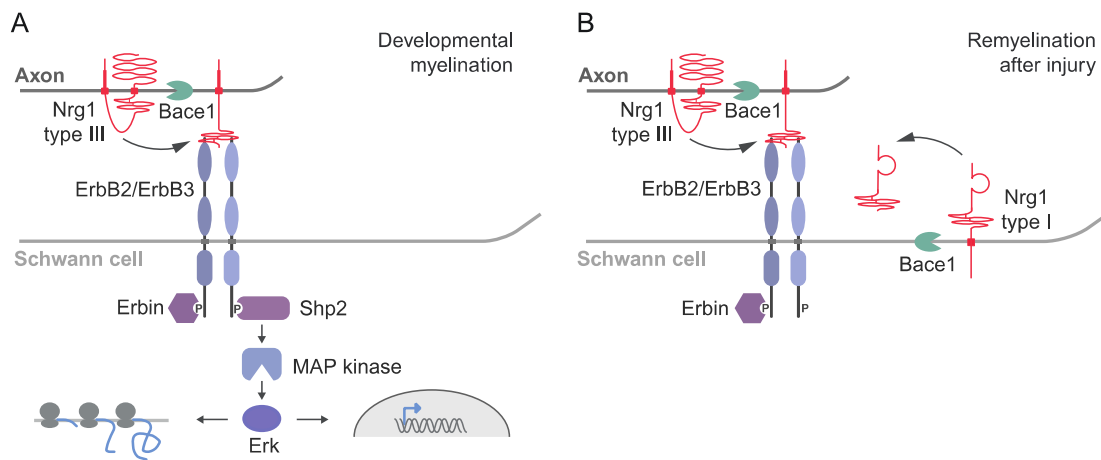
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Fig. 1: Nrg1 signaling during developmental myelination and remyelination.

Developmental myelination **(A)** depends on type III Nrg1 that is produced by peripheral sensory and motoneurons and presented in the axonal membrane. Nrg1 needs to be processed by Bace1 for full signaling activity. ErbB2/ErbB3 receptors on Schwann cells recognize this signal and transmit it. Erbin, an intracellular ErbB2 interacting protein, is important for full activation of ErbB receptors. Many signaling components act downstream of Nrg1/ErbB, and we display here Shp2 and Erk1/2, because mutations of Nrg1/ErbB, Shp2 and Erk1/2 all produce very similar or even identical changes in Schwann cell development and myelination. Activation of MAPK, i.e. expression of an activated variant, can substitute for Nrg1/ErbB3/Shp2 during Schwann cell development and myelination. MAPK represents thus an important readout of Nrg1/ErbB signaling. Transcriptional and translational responses to the Nrg1 signal are observed in developmental myelination. Remyelination after injury **(B)** reuses the Nrg1 signaling network, and remyelination and nerve repair are severely

delayed in the absence of Nrg1. Two Nrg1 isoforms participate in remyelination, axonal type III and Schwann cell-derived type I. Full ErbB activation and efficient regeneration depend again on Bace1 and Erbin.

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