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# Nrg1/ErbB signaling networks in Schwann cell development and myelination

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## Abstract

Neuregulin-1 (Nrg1) provides a key axonal signal that regulates Schwann cell proliferation, migration and myelination through binding to ErbB2/3 receptors. The analysis of a number of genetic models has unmasked fundamental mechanisms underlying the specificity of the Nrg1/ErbB signaling axis. Differential expression of Nrg1 isoforms, Nrg1 processing, and ErbB receptor localization and trafficking represent important regulatory themes in the control of Nrg1/ErbB function. Nrg1 binding to ErbB2/3 receptors results in the activation of intracellular signal transduction pathways that initiate changes in Schwann cell behavior. Here, we review data that has defined the role of key Nrg1/ErbB signaling components like Shp2, ERK1/2, FAK, Rac1/Cdc42 and calcineurin in development of the Schwann cell lineage *in vivo*. Many of these regulators receive converging signals from other cues that are provided by Notch, integrin or G-protein coupled receptors. Signaling by multiple extracellular factors may act as key modifiers and allow Schwann cells at different developmental stages to respond in distinct manners to the Nrg1/ErbB signal.

### Keywords

Neuregulin; Shp2; Erk; Erbin; myelination

## Introduction

The ErbB receptors were originally identified by virtue of their oncogenic potential [1–3]. Due to their important role in cancer, the structure and activity of ErbB receptors was extensively studied, and consequently the mechanism of ErbB receptor signaling is well understood today [4,5]. Ligand binding to the extracellular domain of ErbB receptors promotes receptor dimerization and activation of the intracellular tyrosine kinase domain. Activated receptors phosphorylate each other on a number of tyrosine residues, which serve as docking sites for the downstream enzymes or adaptor proteins that mediate further intracellular signal transduction [6,7]. ErbB receptors activate various signaling cascades, among them the Ras/Extracellular Signal Regulated Kinase 1/2 (Erk1/2) and Phosphatidylinositol-3-Kinase (PI3K)/Akt pathways, the mobilization of $Ca^{2+}$, and the

regulation of $Ca^{2+}$-dependent Protein Kinase C (PKC) and NFAT activity (Fig. 1) [4]. Activation of these pathways elicits, alone or in concert, cellular responses like proliferation, differentiation, motility and cell survival. The signaling cascades that act downstream of ErbB receptors have been primarily analyzed in cultured fibroblasts or epithelial and carcinoma cells, and the results obtained provide a paradigm for ErbB signaling in other cell types.

ErbB receptors take over the essential functions of many cell types and organs during development. Here, we review an extensively studied and well-understood example of ErbB receptor function *in vivo*, their role during Schwann cell development [8]. Both ErbB2 and ErbB3 receptors are required, but the two receptors exhibit dissimilar roles: ErbB3, but not ErbB2, binds extracellular ligands with high affinity, however ErbB3 is catalytically inactive, and it is ErbB2 that contributes the tyrosine kinase activity essential for signaling [9]. A neuronally-produced ligand, Nrg1, is recognized by ErbB2/3 during Schwann cell development [10]. Data obtained in cell culture indicate that peripheral axons present Nrg1 to the accompanying Schwann cells [11]. During early Schwann cell development, the Nrg1/ErbB signal promotes proliferation of the Schwann cell precursor pool, stimulates migration of Schwann cell precursors along the axon, and during late stages controls myelination. Thus, depending on the developmental stage, Schwann cells respond in distinct manners to Nrg1/ErbB signals.

## Nrg1 in Schwann cell development

Schwann cells are generated from neural crest cells, and undergo extensive migration, proliferation and maturation before they terminally differentiate [12]. During these events, Schwann cells are in continuous contact with axons, and axonal cues, especially Nrg1, are driving forces for Schwann cell development. In cell culture, many aspects of Schwann cell biology are affected by Nrg1: (i) Nrg1 suppresses neuronal differentiation of neural crest stem cells while promoting or allowing glial differentiation; (ii) Nrg1 is required for survival of cultured Schwann cell progenitors; (iii) Nrg1 promotes proliferation and migration of Schwann cell precursors; (iv) Nrg1 provides signals essential for myelination [11,13–15]. *In vivo*, early stages of Schwann cell development depend upon Nrg1/ErbB signaling, as evidenced by the near complete absence of Schwann cell progenitors in the developing peripheral nerve of *ErbB2*, *ErbB3*, or *Nrg1* mutant mice [16–20]. This phenotype is specific to the Schwann cell lineage since the formation of the satellite glia within dorsal root ganglia is not impaired. Dissection of the cellular defects in *ErbB2/3* mutant mice and zebrafish suggests that the underlying cellular mechanism involves restriction of the migratory and proliferative abilities of Schwann cell progenitors [21,22].

Shortly after birth, immature Schwann cells have a choice of two fates which is dictated by the type and diameter of the axons they ensheath: (i) Schwann cells myelinate a single, large caliber axon or (ii) they ensheath multiple, small caliber axons to form a Remak bundle. Myelination insulates the axonal membrane and increases the speed at which electrical nerve impulses propagate, whereas unmyelinated axons lack this insulation and propagate action potentials at much slower rates. Interestingly, the level of axon-derived Nrg1-type III has been linked to Schwann cell commitment to a myelinating fate *in vitro* [11,23]. Several transcription factors are known to control Schwann cell myelination, and Nrg1 promotes the expression of some of these, for example Oct6/SCIP and Egr2/Krox-20 [23,24]. Thus, Nrg1 controls Schwann cell differentiation during the early postnatal period.

The subsequent extent of myelination is also dependent upon the precise levels of Nrg1. Schwann cell-specific disruption of *ErbB2* leads to hypomyelination (Fig. 2A,B). Similarly, heterozygous *Nrg1* deletion results in significant hypo-myelination, while neuronal

overexpression of *Nrg1* has the converse effect and leads to hypermyelination [25–27] . How axonal diameter is linked to *Nrg1* expression levels remains open. Myelinated and non-myelinated sensory neurons might express different levels of Nrg1, or they might differ in their ability to process and present Nrg1 in the axonal membranes. It should be noted that the volume of myelin produced by a Schwann cell correlates tightly with the surface area of the associated axon [28]. Thus, Nrg1 density on different axons might be similar, and a doubling of the axonal surface area would therefore double the amount of Nrg1 presented to the Schwann cell. Lastly, Nrg1 signaling coordinates the interaction between non-myelinating Schwann cells and small diameter axons in Remak bundles [29, 30]. These data indicate that axonal Nrg1 signals functionally modulate both myelinating and non-myelinating Schwann cells.

Nrg1 and ErbB2/3 are expressed well into adulthood, suggesting a potential role in mature Schwann cell function [31,32]. Indeed, early work on axotomized nerves indicated exogenous Nrg1 rescues apoptosis of Schwann cell associated with axons or neuromuscular nerve terminals [33,34]. Furthermore, overexpression of a transdominant ErbB4 receptor in non-myelinating Schwann cells was reported to affect Schwann cell survival [30], whereas the inducible loss of Nrg1 in sensory neurons did not impair Schwann cell survival, but affected Remak bundle morphology and the thickness of the myelin sheath [29]. The inducible loss of *ErbB2* in adult myelinating glia appears to have a limited effect on the maintenance of peripheral myelin or injury-induced Schwann cell proliferation and survival; if Nrg1 is required for re-myelination following injury has yet to be tested [35]. Interestingly, leprosy bacteria bind to and activate ErbB2 in myelinating Schwann cells, which causes demyelination [36].

## Nrg1 isoforms and proteolytic processing

The *Nrg1* gene spans more than 1.2 megabases of DNA, and it is thus one of the largest genes in the mammalian genome. Many different mRNA isoforms are produced from the gene by the use of distinct promoters and by alternative splicing. These messenger variants encode protein isoforms of distinct structure [8]. Each of the major isoform classes are encoded by mRNAs with distinct 5′ ends, indicating that they are generated by the use of different promoters. In accordance, a comparative expression analysis in the developing rat and mouse showed that the major isoform classes are expressed in different temporal and spatial patterns, and genetic experiments show that they take over distinct functions [10,21]. All of these isoforms contain an EGF-like domain that alone is sufficient to bind and activate the receptor. The raison d'être for the isoform diversity is starting to emerge: genetic evidence suggests that different isoforms have distinct functions and, possibly, that they are also presented in a distinct manner to target cells.

Sensory neurons project to the periphery, convey nociceptive, mechanoreceptive, and proprioceptive information, and their axons are ensheathed by Schwann cells. Sensory neurons produce types I and III Nrg1. Type III Nrg1 is expressed by most, if not all sensory neurons, whereas type I is mainly expressed by proprioceptive neurons [10]. Analysis of isoform-specific Nrg1 mutations in the mouse demonstrated that the presence of the type III isoform suffices to prevent the early deficits in Schwann cell development present in *Nrg1* null mutant mice, suggesting that it is type III Nrg1 that drives proliferation and survival of Schwann cell precursors [10]. Furthermore, hypermyelination of peripheral nerves can be induced by neuronal overexpression of type III *Nrg1*, whereas overexpression of type I *Nrg1* does not affect peripheral myelination [26]. Thus, type III Nrg1 plays an important role during Schwann cell development and myelination, indicating this isoform is presented by the axon to the Schwann cells. In contrast, *Nrg1* null mutation in sensory neurons, but not type III *Nrg1*-specific mutations, interferes with muscle spindle formation, indicating that

the type I isoform induces formation of muscle spindles [37]. Type I and type III Nrg1 might locate to specific sub-cellular membrane compartments and therefore be accessible to the two distinct target cell types, Schwann cells and skeletal muscle. Alternatively, the release of the two isoforms by proteolysis might be controlled in such a manner that one isoform preferentially signals to glia, and the other to muscle. Indeed, proteolytic processing is an important aspect for Nrg1 function.

Bace1, a type I transmembrane aspartyl protease, is required for processing of the beta-site of amyloid precursor protein, and is essential for the generation of amyloid beta peptide in Alzheimer's disease. *Bace1* is preferentially expressed in neurons, and Bace1 activity can release protein fragments from neuronal membranes [38]. The major phenotype observed in *Bace1* mutant mice is a hypomyelination of peripheral nerves and aberrant sorting of sensory axons, very similar to the changes seen in mice with heterozygous mutations in *Nrg1* or Schwann cell-specific *ErbB2* mutations [39]. In fact, Bace1 processes Nrg1 fragments in the stalk region in cell culture, and unprocessed Nrg1 accumulates in the brains of *Bace1* mutant mice [39,40]. Neural crest migration and heart development, two further Nrg1-dependent developmental processes, are apparently not impaired in *Bace1* mutant mice. Also, despite the pronounced hypomyelination, early Schwann cell development appears to be normal. Bace1 therefore might not be the only protease that processes Nrg1 or alternatively, neural crest migration and heart development might not depend on the presence of cleaved Nrg1. Plausible too, is that reduced levels of processed Nrg1 can still drive these developmental events but later not suffice for normal myelination.

A large family of membrane-anchored zinc-dependent proteases, known as 'a disintegrin and metalloprotease' (ADAM) family, are key components in the ectodomain shedding of membrane-anchored proteins. ADAMs process several members of the EGF ligand family, like EGF itself, TGFalpha, amphiregulin, and HB-EGF [8,41]. ADAMs are expressed in complex spatiotemporally controlled patterns in the organism, and are furthermore regulated post-transcriptionally, for instance by G-protein coupled receptor (GPCR) signaling [42]. Several lines of evidence that rely on co-transfection of Nrg1 and ADAM cDNAs indicate that ADAMs participate in Nrg1 processing [43,44]. Different proteases might preferentially process different Nrg1 isoforms, for instance PMA (phorbol-12-myristate-13-acetate), an activator of PKC, increases the proteolysis only of type I, but not of type III in COS cells [45]. Furthermore, mutation of *Nardilysin*, which encodes a zinc-peptidase that cleaves selectively dibasic sites, causes hypomyelination. The underlying mechanism was attributed to an enhanced Bace1- or ADAM-dependent ectodomain shedding of Nrg1 [46] .

## Receptor trafficking and localization: Erbin and ErbB2 signaling

Myelination is a complex morphogenetic event, and requires the spiral wrapping of the Schwann cell membrane around the axon. It is accompanied by a huge increase in the size of a Schwann cell, particular of its membrane area. During this process, only a small portion of the Schwann cell, the adaxonal membrane, contacts the axon, and only in that small area axonal signals can be received. Therefore, mechanisms must exist that ensure that ErbB receptors are present in the adaxonal membrane. The mechanism used to locate ErbB receptor to a particular membrane compartment has been extensively studied in *C. elegans*, where appropriate receptor localization is essential for signaling.

Signaling of the EGF receptor during development of *C. elegans* depends on a ternary complex consisting of three PDZ domain proteins. The complex (LIN-7, LIN-2, LIN-10) localizes the EGF receptor to the baso-lateral compartment of the epithelial vulval precursor cells [47]. The neighboring anchor cell provides the EGF-like ligand, and receptor activation depends on the location of the receptor in the membrane that faces the anchor cell. Many

PDZ proteins exist in the mammalian genome, and one of these, Erbin, interacts with ErbB2 but not other ErbB receptors, and co-localizes with ErbB2 in the baso-lateral surface of epithelial cells [48]. *In vitro* studies suggested that Erbin may have a broad range of interaction factors, binding not only to ErbB2, but also to other proteins, like integrin-beta4, SMADs, ion channels or delta-catenin [49-52]. Early data emphasized the role of Erbin in locating the receptor to the baso-lateral membrane compartment in epithelial cells, but recent data implicate Erbin in stabilization and internalization of the ErbB2 protein. The first genetic analysis of *Erbin* revealed a role for Erbin in the myelination of peripheral nerves. *Erbin* null mutant mice displayed hypomyelination of peripheral nerves and aberrant axonal segregation of small-diameter afferent fibers, very similar to that seen in mice with mutations in type III *Nrg1* or Schwann cell-specific *ErbB2* knockouts [53]. When the PDZ domain of Erbin is selectively removed, precluding the interaction between Erbin and ErbB2, identical changes were observed. ErbB2 protein, but not mRNA levels were reduced in the peripheral nerves of such mutant mice, indicating that ErbB2 is unstable and that the reduced ErbB2 levels compromise Nrg1 signaling.

## Nrg1 signal transduction in Schwann cells

Nrg1 activation of ErbB2/3 triggers a complex sequence of molecular interactions that result in the recruitment of adaptor proteins and enzymes to phospho-tyrosine residues on the receptors. Among these are Grb2, Shc, Sos, PLCγ, PI3K, and Src, and their binding and/or phosphorylation results in the activation of canonical signaling cascades, such as PI3K/Akt, Ras/Erk1/2, PLCγ, and focal adhesion kinase (FAK), which in turn direct changes in cytoplasmic processes and gene expression (Fig. 1) [54–59]. Progress continues to be made in identifying the signal transduction elements that mediate the effects of Nrg1 in Schwann cells. A link between Nrg1, PLCγ, and calcineurin/NFAT signaling in the control of myelination was recently identified: neural crest specific mutation of calcineurin caused a severe disruption of myelination without affecting early Schwann cell development (Fig. 1) [59]. FAK lies downstream of Nrg1 activation in Schwann cells [58] and conditional mutation of FAK in Schwann cells resulted in impaired axonal sorting and myelination [60]. Recent analyses implicate the cytoplasmic tyrosine kinase Src and Erk1/2 as major players downstream of Nrg1-ErbB in Schwann cells (see below).

Despite its enzymatic activity, the protein tyrosine phosphatase Shp2 functions as an *activator* of signaling, and this molecule is of particular importance for the regulation of the Ras/Erk1/2 pathway [61]. Mutation of the *Drosophila* homologue of *Shp2*, *corkscrew*, reproduces phenotypes observed in flies mutant for genes encoding tyrosine kinase receptors like *torso* or *sevenless* [62,63]. In the mouse, null-mutations of *Shp2* cause early embryonic lethality, and have been linked to deficits in FGF receptor signaling [64]. In humans, germline mutations in *Shp2* have been found in patients suffering from Noonan and LEOPARD syndromes, two multisymptomatic developmental disorders, and also occur in several types of malignancies, such as the most common type of juvenile leukaemia, JMML [61,65]. Neural crest specific mutation of S*hp2* in mice results in a striking loss of Schwann cell progenitors in the peripheral nerve, while *Shp2* inactivation in immature Schwann cells results in major deficits in myelination (Fig. 2C)[66]. These phenotypes are very similar to those observed in *Nrg1/ErbB2/3* mutant mice (Fig. 2). Indeed, Nrg1-evoked cellular responses like proliferation and migration were virtually abolished in cultured Schwann cells lacking *Shp2* [66].

Biochemical analyses demonstrated that *Shp2* mutation in Schwann cells results in impaired sustained Erk1/2 activity *in vivo* and *in vitro*, but leaves PI3K/Akt activation unchanged [66]. Previous studies using pharmacological inhibitors implicated PI3K/Akt signaling as key in Schwann cell development and myelination, and suggested that Erk1/2 played a

limited role [40,56,57,67]. Recent genetic evidence that relies on the conditional mutation of *Erk1/2* indicates that this notion has to be revisited [68]. Similar to the *Shp2* mutation, inactivation of *Erk1/2* in neural crest results in a lack of Schwann cell progenitors in the peripheral nerve, while Schwann cell specific mutation inhibits myelination (Newbern and Snider, unpublished data). These results suggest that Shp2-dependent, sustained activation of Erk1/2 is critical for the Nrg1-evoked effects *in vivo*. It will be interesting to further delineate the mechanisms by which the Shp2-Erk1/2 module controls Schwann cell myelination.

Further molecular analysis of cultured *Shp2* mutant Schwann cells revealed a reduction in Src and FAK activity in response to Nrg1 [66]. Pharmacological inhibition of Src was able to reproduce the effects of the loss of *Shp2* on Erk1/2 and FAK activity. Similarly, FGF receptor signaling was previously shown to depend on Shp2 for Src and sustained Erk1/2 activation [64]. The precise mechanism by which Shp2 regulates Src and sustained Erk1/2 activity remains unclear. The second kinase that is regulated via Nrg1/Shp2, FAK, is known to be required for Schwann cell proliferation *in vivo* [60]. In sum, it appears that Shp2 regulates the output of several signaling molecules, including FAK, Src, and Erk1/2, to elicit the proliferation, migration and myelination of Schwann cells *in vivo* (Fig. 1).

In Schwann cells, Nrg1 also regulates the activity of members of the Rho-GTPase family, Cdc42 and Rac1, which have been shown to control Schwann cell migration and proliferation *in vivo* [69–71]. Activation of Rac1 and Cdc42 has been linked to the c-Jun N-terminal kinase (JNK) and the p38 mitogen-activated protein kinase pathways (MAPK) [72,73]. This connection is interesting since inhibition of JNK or p38 MAPK interferes with myelination *in vitro* [74–76]. Thus, ErbB signaling through the Rho GTPase family may directly modulate Schwann cell differentiation.

## Network dynamics and the control of myelination: A summary and outlook

During Schwann cell development, axonal Nrg1 signals regulate the distribution of progenitors along the nerve, the size of the progenitor pool and the generation of myelinating or non-myelinating cells with a high degree of fidelity. Recent genetic analyses have correlated particular Nrg1/ErbB signaling cascades with Schwann cell growth, migration and myelination. However, the intrinsic mechanisms that couple Nrg1/ErbB signaling to proliferation and migration during early development, and to myelination at later stages remain open.

Input from multiple neuronal cues may modulate the outcome of Nrg1 signaling in Schwann cells. Among these are signals derived from Notch receptors, integrins or other growth factor receptors [77–80]. Notch signaling elevates levels of ErbB2 receptors and responsiveness to Nrg1 [77]. FAK, Src, Cdc42, and Rac1 have prominent roles in the signal transduction pathways downstream of integrins, which might alter convergent input arising from Nrg1. cAMP has long been known to drive myelination [81,82], but the ligand/receptor complex that raises cAMP levels at onset of myelination was unknown. Work performed in zebrafish has recently identified a key role for a GPCR, Gpr126, in myelination. In *Gpr126* mutant fish, Schwann cell development was arrested at a promyelinating state, and myelination was restored by the addition of the adenylate cyclase activator forskolin [83]. Thus, zebrafish genetics has finally identified the elusive receptor that drives myelination by raising cAMP levels, but the molecular nature of the ligand that activates Gpr126 remains unknown. In summary, Nrg1, cell adhesion components, and ligands of GPCRs act in conjunction to control Schwann cell development and myelination (Fig. 3). Much remains to be learned about the integration of the signals provided by these distinct molecules, and

about the dynamic interaction of the downstream signaling cascades that shape development and differentiation of Schwann cells.

The Schwann cell lineage provides an example of a cell type that strongly depends on Nrg1/ErbB signaling. Oligodendrocytes, the myelinating glia of the central nervous system, express ErbB receptors and respond to Nrg1 *in vitro* and *in vivo*. Recent experiments that relied on conditional mutations of all receptors known to bind Neuregulins did not reveal myelination deficits in the oligodendrocyte lineage, suggesting that the axonal control of myelination *in vivo* is different in the central and peripheral nervous system [84]. Nrg1/ErbBs also take over important roles in the physiology and pathophysiology of other organs like the heart or skeletal muscle [37,85,86]. Particularly interesting is recent work that correlates certain haplotypes of Nrg1 and the ErbB4 receptor with schizophrenia, a common mental disease in man [87,88]. These findings triggered a wide interest for Nrg1 functions in the central nervous system [89]. Evidence emerged that assigns a role to Nrg1/ErbB4 in the migration of cortical inhibitory neurons and the formation of thalamocortical projections during development, as well as in synaptic functions of inhibitory and excitatory neurons [90–92]. Thus, understanding functions of Nrg1/ErbB signaling in the central nervous system is expected to define one mechanism responsible for schizophrenia, and might provide new avenues for therapeutic intervention.

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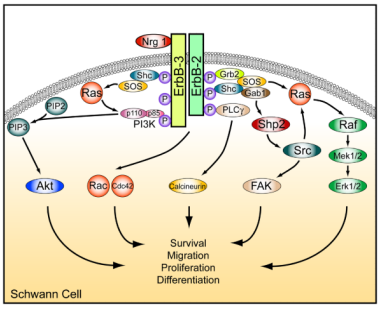


**Figure 1. Nrg1/ErbB signaling**

Following Nrg1 binding, heteromeric ErbB2/3 receptors auto- and trans-phosphorylate specific tyrosine residues, and to these phosphorylated residues specific adaptor proteins and enzymes are recruited. This results in the activation of a number of downstream signaling pathways, including PI3K/Akt, Erk1/2, FAK, and Rac/Cdc42, and $Ca2^{+}$ regulated pathways downstream of PLCγ lead to activation of calcineurin. Shp2 and Src are thought to regulate Erk1/2 signaling by modulating Ras, although other mechanisms also have been observed. These pathways regulate the activity of cytoplasmic and nuclear signal transduction molecules that mediate specific Schwann cell responses to the Nrg1 signal.

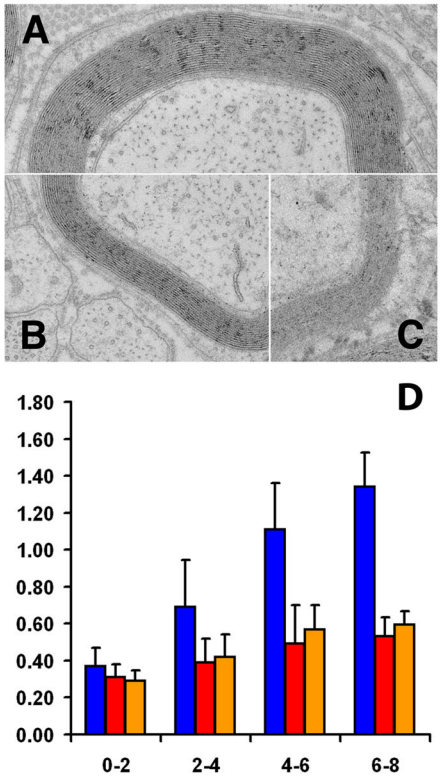


**Figure 2. Hypomyelination of ErbB2 and Shp2 mutant peripheral nerves**

Electron microscopic analysis of peripheral nerves from control mice (A), and from mice that carry Schwann cell-specific mutations of *ErbB2* (B) or *Shp2* (C) that were introduced using a Krox20cre allele. Note that the conditional ErbB2 and Shp2 mutant mice display a marked hypomyelination in the adult. (D) Quantification of myelin thickness in adult control (blue bars), conditional ErbB2 (red bars) and Shp2 (yellow bars) mutant mice. Displayed is a plot of the myelin thickness versus axon diameter. Note the similarity of the effects of the ErbB2 and Shp2 mutations.

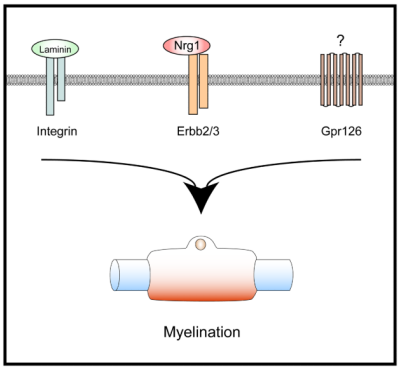


**Figure 3.**

Nrg1/ErbB signaling acts cooperatively with other extracellular cues to control myelination. Amongst many potentially important ligand/receptor complexes, key roles have been attributed to laminin/integrin and Gpr126 signaling in Schwann cell myelination *in vivo*.