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New insights into signaling during myelination in zebrafish

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Abstract

Myelin is a vertebrate adaptation that allows for the rapid propagation of action potentials along axons. Specialized glial cells – oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS) – form myelin by repeatedly wrapping axon segments. Debilitating diseases result from the disruption of myelin, including Multiple Sclerosis and Charcot-Marie-Tooth peripheral neuropathies. The process of myelination involves extensive communication between glial cells and the associated neurons, and the last few years have seen important progress in understanding the molecular basis of the signals that coordinate the development of these fascinating cells. This review highlights recent advances in myelination deriving from studies in the zebrafish model system, with a primary focus on the PNS. While Neuregulin1-ErbB signaling has long been known to play important roles in peripheral myelin development, work in zebrafish has elucidated its roles in Schwann cell migration and radial sorting of axons sorting *in vivo*. Forward genetic screens in zebrafish have also uncovered new genes required for development of myelinated axons, including *gpr126*, which encodes a G-protein coupled receptor required for Schwann cells to progress beyond the promyelinating stage. In addition, work in zebrafish uncovered new roles for Schwann cells themselves, including in regulating the boundary between the PNS and CNS and positioning a nerve after its initial outgrowth.

Introduction

The myelin sheath increases axonal conduction velocity by reducing capacitance of the axonal membrane and allowing saltatory conduction (Hodgkin, 1964; Stampfli, 1954). Thus, myelinated axons of small diameter can transmit information as rapidly as much larger unmyelinated axons. Myelin therefore is an evolutionary innovation that allows the nervous system to increase in speed and complexity without a corresponding increase in size and energy requirements. Although some invertebrate species have myelinated axons, myelin is ubiquitous among the gnathostomes (jawed vertebrates), and this adaptation has surely been essential for the formation of the large, complex nervous systems that distinguish the vertebrates from other groups (Bunge, 1968; Hartline and Colman, 2007).

Disruption of the myelin sheath underlies many debilitating diseases including Multiple Sclerosis, Charcot Marie Tooth disease, and others (Berger *et al.*, 2006; McQualter and

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Bernard, 2007). Specialized glial cells, oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS), wrap their membranes many times around a segment of an axon to form the myelin sheath (Bunge, 1968; Geren and Raskind, 1953; Peters, 1964). Along its length, each axon is ensheathed by multiple myelin segments, which are separated by unmyelinated gaps called nodes of Ranvier (Bunge, 1968; Stampfli, 1954; Tasaki, 1959). Oligodendrocytes interact with and elaborate myelin sheaths around many different axons; in contrast, Schwann cells myelinate only one segment of one axon (Bunge, 1968).

Schwann Cell Development

As extensively documented in mammals, Schwann cells originate from the neural crest and undergo a series of developmental transitions that culminates with myelination (Jessen and Mirsky, 2005). Schwann cell precursors form from neural crest progenitors that have delaminated from the dorsal neural tube and associated with axons in peripheral nerves (Jessen *et al.*, 1994; Sauka-Spengler and Bronner, 2010). Schwann cell precursors migrate with axons as they grow towards their targets (Dong *et al.*, 1999). Once migration is complete, Schwann cell precursors differentiate into immature Schwann cells, which associate with many axons organized as a bundle (Jessen and Mirsky, 2005). Through a process termed radial sorting, Schwann cells progressively sort axons from the main bundle, such that a Schwann cell associates with a single axonal segment that is to be myelinated (Webster *et al.*, 1973). At this point the immature Schwann cells associated with a single large caliber axon will become promyelinating Schwann cell, initiate the myelination transcription pathway, and begin to wrap their cytoplasm around the axon to create a myelin sheath (Jessen and Mirsky, 2005; Webster *et al.*, 1973). In contrast, immature Schwann cells associated with several small caliber axons will become non-myelinating Schwann cells, ensheathing each axon in a pocket of its cytoplasm, forming a Remak bundle (Aguayo and Bray, 1975; Aguayo *et al.*, 1976; Hahn *et al.*, 1987; Jessen and Mirsky, 2005; Peters and Muir, 1959).

Much of our current understanding of myelination comes from mammalian studies, and a number of genes essential for Schwann cell development are known. *sox10*, *erbb2*, *erbb3*, and *nrg1* are required at multiple steps of Schwann cell development, regulating neural crest migration and specification, as well as Schwann cell proliferation, survival, specification, and myelination (Britsch *et al.*, 2001; Chen *et al.*, 2006; Dong *et al.*, 1995; Finzsch *et al.*, 2010; Garratt *et al.*, 2000b; Jessen and Mirsky, 2005; Kuhlbrodt *et al.*, 1998; Michailov *et al.*, 2004; Morrissey *et al.*, 1995; Newbern and Birchmeier, 2010; Riethmacher *et al.*, 1997; Schreiner *et al.*, 2007; Woldeyesus *et al.*, 1999). Other genes are necessary at discrete stages - *oct6*, *brn2*, and *krox20* are required for Schwann cells to progress beyond the promyelinating stage and make myelin (Svaren and Meijer, 2008). Components of the extracellular matrix and cytoskeletal regulators are required for radial sorting, including *rac1*, *cdc42*, *FAK*, *ILK*, *β1-integrin*, *laminin-λ1*, *laminin-2*, and *laminin-8* (Benninger *et al.*, 2007; Chen and Strickland, 2003; Feltri *et al.*, 2002; Grove *et al.*, 2007; Nodari *et al.*, 2007; Pereira *et al.*, 2009; Yu *et al.*, 2009; Yu *et al.*, 2005).

Despite important progress in defining key genes that regulate Schwann cell and oligodendrocyte development and myelination, our understanding of the pathways that regulate glial development and myelination is still incomplete (Emery, 2010; Jessen and Mirsky, 2005). In this review, we seek to highlight advances in understanding myelination in the zebrafish model system, which combines powerful genetics with exquisite *in vivo* imaging. We will focus mainly on the peripheral nervous system, where a more complete pathway of Schwann cell development has emerged, and in particular on the roles of Neuregulin1/ErbB signaling and Gpr126. In addition, we will highlight new roles for Schwann cells that have been discovered in zebrafish.

Neuregulin1/ErbB Signaling

Neuregulin1 (Nrg1) is an EGF-related signal that activates ErbB receptor tyrosine kinases on Schwann cells to regulate several aspects of Schwann cell development, including proliferation, survival, myelination and the formation of Remak bundles (Birchmeier and Nave, 2008; Dong *et al.*, 1995; Garratt *et al.*, 2000b; Lyons *et al.*, 2005; Michailov *et al.*, 2004; Morrissey *et al.*, 1995; Riethmacher *et al.*, 1997; Taveggia *et al.*, 2005; Woldeyesus *et al.*, 1999). ErbB2 and ErbB3 are the main receptors for Nrg1 ligands in Schwann cells (Citri *et al.*, 2003; Newbern and Birchmeier, 2010). There are over 15 different isoforms of Nrg1, but a single isoform, Nrg1 type III, has emerged as a primary regulator of Schwann cell development (Falls, 2003; Michailov *et al.*, 2004; Taveggia *et al.*, 2005). For example, the amount of Nrg1 type III expressed on an axon determines whether the associated Schwann cell will become a myelinating or non-myelinating Schwann cell, as well as the thickness of the myelin sheath (Michailov *et al.*, 2004; Taveggia *et al.*, 2005). Nrg1 can also induce demyelination (Syed *et al.*, 2010; Zanazzi *et al.*, 2001), however, suggesting that the level or location of the signal may help determine how a Schwann cell will respond to it. Additionally, the signaling pathways downstream of Nrg1/ErbB signaling may modulate the response to the signal at different stages; these pathways include PI3K/Akt, MEK/ERK, Calcineurin/NFAT, Cdc42, Shp2, and others (Benninger *et al.*, 2007; Cotter *et al.*, 2010; Goebbels *et al*., 2010; Grossman *et al.*, 2009; Kao *et al.*, 2009; Ogata *et al.*, 2010; for a more extensive review of Nrg1/ErbB signaling in Schwann cells see Newbern and Birchmeier, 2010).

Analyses in zebrafish have investigated the role of Nrg1/ErbB signaling in Schwann cell migration and subsequent steps in Schwann cell development using mutants, small molecule inhibitors, and timelapse imaging *in vivo*. A genetic screen for mutants with defects in *myelin basic protein (mbp)* expression isolated mutations in *erbb2* and *erbb3*, among other genes (Lyons *et al.*, 2005; Pogoda *et al.*, 2006). Similar to previous studies of mouse mutants in *ErbB2* and *ErbB3*, the zebrafish *erbb2* and *erbb3* mutants lack Schwann cells along peripheral axons, although some Schwann cells do associate with neuronal cell bodies at cranial ganglia (Lyons *et al*., 2005; Pogoda *et al*., 2006; Reithmacher *et al.*, 1997; Woldeyesus *et al*., 1999). BrdU incorporation studies revealed that Schwann cell proliferation is reduced (but not eliminated) in erbb mutants, consistent with many mammalian studies showing that Nrg1 is a Schwann cell mitogen (Garratt *et al.*, 2000b; Lyons *et al.*, 2005; Morrissey *et al.*, 1995; Newbern and Birchmeier, 2010). The use of timelapse imaging combined with chemical inhibitors of ErbB activity also showed that

ErbB signaling is required continuously during Schwann cell migration, in addition to its role in Schwann cell proliferation (Lyons *et al.*, 2005). When the ErbB inhibitor AG1478 was applied after the start of migration, some Schwann cells continued to move, but in a misdirected fashion – in some instances even switching nerves. These studies support the possibility that ErbB signaling is important for directed migration of Schwann cells, rather than simply promoting the motility of these cells. Future studies are required to determine the identity of the axonal signal that directs Schwann cell migration, but, given the involvement of ErbB receptors, it is likely that Nrg1 is involved.

A recent study has added to the understanding of ErbB signaling in radial sorting, by analyzing the effects of ErbB inhibitors added after Schwann cell migration is complete (Raphael *et al.*, 2011). Migrating Schwann cells associate with bundles of many axons, but shortly after the end of migration, radial sorting begins (Figure 1). Radial sorting occurs similarly in zebrafish as in mammals, with immature Schwann cells surrounding a bundle of axons, extending processes into the axon bundle and "radially" sorting axons to the periphery of the bundle, at which point one Schwann cell is interacting with one axon and the myelination program can begin (Raphael *et al.*, 2011; Webster *et al.*, 1973). During radial sorting, Schwann cells proliferate extensively, so that they are present in numbers corresponding to the many axonal segments that will be myelinated (Jessen and Mirsky, 2005; Webster *et al.*, 1973). Inhibitors of cell division interfere with radial sorting and the onset of myelination (Lyons *et al.*, 2005; Raphael *et al.*, 2011). Inhibition of ErbB signaling also disrupted radial sorting, as expected in light of the role of Nrg1-ErbB signaling in Schwann cell proliferation (Raphael *et al.*, 2011). Supporting a previous study of mammalian Schwann cells in culture (Taveggia *et al.*, 2005), ErbB signaling also has a role that is independent of Schwann cell number: Schwann cells processes do not extend into axon bundles in fish treated with ErbB inhibitors, in contrast to fish treated with inhibitors of cell division or untreated controls (Raphael *et al.*, 2011). This indicates that, in addition to regulating Schwann cell proliferation during radial sorting, ErbB signaling is also required for Schwann cell process extension. It will be interesting to learn how Nrg1 signals from axons are coordinated with signaling from the basal lamina, which is generated by the Schwann cells themselves, to bring about radial sorting and myelination. In addition, timelapse imaging of the interaction of Schwann cells and their axons in living zebrafish may generate new insights into radial sorting, a dynamic process that is currently understood from electron micrograph time courses.

Combined, the zebrafish and mammalian studies have revealed roles for Nrg1-ErbB signaling in migration, survival, proliferation, Remak bundle formation, radial sorting, and myelination (Birchmeier and Nave, 2008; Dong *et al.*, 1995; Garratt *et al.*, 2000b; Lyons *et al.*, 2005; Michailov *et al.*, 2004; Morrissey *et al.*, 1995; Raphael *et al.*, 2011; Riethmacher *et al.*, 1997; Taveggia *et al.*, 2005; Woldeyesus *et al.*, 1999). Future investigation is required to understand how one signal controls so many different aspects of Schwann cell development, but important factors likely include the concentration and source of the ligand, the developmental stage of the Schwann cell receiving the signal, and process specific downstream factors, such as Calcineurin/NFAT, PI3K/Akt, MEK/ERK, Shp2, and Cdc42

(Benninger *et al.*, 2007; Cotter *et al.*, 2010; Goebbels *et al.*, 2010; Grossmann *et al.*, 2009; Kao *et al.*, 2009; Newbern and Birchmeier, 2010; Ogata *et al.*, 2004; Syed *et al.*, 2010).

Gpr126

In addition to finding new roles for genes initially characterized in mammals, zebrafish screens have also uncovered novel genes that regulate myelin formation. A screen for mutants with abnormal expression of *mbp* identified two mutations in gpr126 (Monk *et al.*, 2009; Pogoda *et al.*, 2006). Gpr126 is an orphan member of the adhesion subfamily of GPCRs, which are characterized by long, extracellular segments N-terminal to the 7-pass transmembrane domain (Bjarnadottir *et al.*, 2004; Fredriksson *et al.*, 2003). In gpr126 mutants, expression of the early Schwann cell marker *sox10* is normal but markers of later stages are significantly reduced, including the promyelinating genes *oct6* and *krox20* (Monk *et al.*, 2009). These marker studies suggested that gpr126 is dispensable for early stages of Schwann cell development, but essential for the onset of myelination. Transmission electron microscopy analysis revealed that Schwann cells arrest at the promyelinating stage in gpr126 mutants, with no more than one and a half wraps of Schwann cell cytoplasm around axons in peripheral nerves. This phenotype is reminiscent of *krox20* mutants in both mammals and zebrafish, where mutant Schwann cells also arrest with only one and a half wraps of cytoplasm around an axon (Monk *et al.*, 2009; Topilko *et al.*, 1994). It had been known for many years that cAMP is an important second messenger during myelination and that the addition of forskolin, which elevates cAMP, could initiate myelination in cultured Schwann cells and mimic the presence of an axon (Jessen *et al.*, 1991; Monuki *et al.*, 1989). The endogenous regulation of cAMP, however, was not understood. Many G-protein coupled receptors signal through cAMP (Jalink and Moolenaar, 2010), raising the possibility that Gpr126 activates myelination by elevating levels of cAMP. Application of forskolin rescues myelination in *gpr126* mutants, but not in *krox20* mutants (Monk *et al.*, 2009). These results support the possibility that Gpr126 acts upstream of cAMP, so that adding forskolin to artificially increase cAMP bypasses the requirement for Gpr126 (Monk *et al.*, 2009). In contrast, *krox20* is downstream of cAMP signaling, and these mutants cannot be rescued by elevating the levels of cAMP (Monk *et al.*, 2009). Future studies are required to investigate the pathway downstream of gpr126, the interaction of gpr126 and other key regulators such as Nrg1, and the identity of ligands that may activate gpr126.

Characterization of a *gpr126* mutant mouse revealed conservation of its function in myelination, and also new roles for gpr126 in the regulation of other aspects of peripheral nerve development (Monk *et al*., in press). *gpr126* mutant mice have severe congenital hypomyelinating peripheral neuropathy. Similar to the situation in zebrafish, mouse gpr126 mutants have decreased expression of *oct6*, *krox20*, and *mbp*, and the mutant Schwann cells arrest at the promyelinating stage (Monk *et al*., 2009; Monk *et al*., in press). The analysis also revealed that there is a delay in radial sorting and a loss of Remak bundles, and thus non-myelinating Schwann cells (Monk *et al*., in press). Additionally, ectopic perineurial fibroblasts were found inappropriately within the nerve and these fibroblasts segregated the axon fibers in small bundles, forming “minifascicles.” Several aspects of the *gpr126* mutant phenotype are similar to mutants for *Adam22* and *lgi4/claw paw*, which also have Schwann cells arrested at the promyelinating stage and axons organized into minifascicles (Darbas *et*

al., 2004; Henry *et al.*, 1991; Nishino *et al.*, 2010; Ozkaynak *et al.*, 2010). Adam22 binds Lgi4, and it is possible that the functions of these proteins are in some way related to Gpr126.

New Roles for Schwann Cells

Mutations in genes that have critical functions in Schwann cells, including *erbb2*, *erbb3*, and *sox10*, have led to important progress in the understanding of the pathways that regulate Schwann cell development. In addition, these mutants have provided useful tools to analyze roles of Schwann cells in peripheral nerve development. The study of zebrafish mutants lacking Schwann cells has defined several new roles for these cells, including preventing the ectopic accumulation of axonal sodium channels in internodal axonal segments, preventing the premature differentiation of sensory organs, and in the proper fasciculation of the nerve (Gilmour *et al.*, 2002; Grant *et al.*, 2005; Voas *et al.*, 2009). Additionally, more recent studies have uncovered roles for Schwann cells in preventing oligodendrocytes from improperly exiting the spinal cord, and repositioning a peripheral nerve across a basement membrane (Kucenas *et al.*, 2009; Raphael *et al.*, 2010).

Timelapse imaging of oligodendrocytes in the absence of Schwann cells revealed that oligodendrocytes can inappropriately exit the spinal cord in the absence of Schwann cells, suggesting that Schwann cells are required to keep oligodendrocytes from crossing the nerve root transition zones between the PNS and CNS (Kucenas *et al.*, 2009). Normally, only axons cross these zones, either exiting or entering the spinal cord, with oligodendrocytes forming a heminode of myelin on the CNS side and Schwann cells forming one on the PNS side (Fraher, 1999). Extensive time-lapse imaging revealed that in the absence of Schwann cells, oligodendrocyte processes project out of the spinal cord along the motor axons, and the cell bodies ultimately follow (Kucenas *et al.*, 2009). These data support the idea that the glial cells themselves regulate the transition zones and that in the absence of Schwann cells, oligodendrocytes are free to exit the spinal cord and myelinate peripheral axons.

Two recent studies in mammals also investigated the role of Schwann cells in restricting the migration of oligodendrocytes in peripheral nerves, suggesting that Schwann cells must progress beyond the promyelinating stage to limit ectopic migration of oligodendrocytes. In *krox20* mutant mice, where Schwann cells are arrested at the promyelinating stage, oligodendrocytes exited the CNS and enter peripheral nerves (Coulpier *et al.*, 2010). Similarly, in the study of the *gpr126* mutant mouse described above, oligodendrocytes were expanded into peripheral territory at the transition zone of the auditory nerve, despite the presence of Schwann cells arrested at the promyelinating stage (Monk *et al.*, in press). Interestingly, oligodendrocytes did not extend into the periphery in *Trembler*^{J}*/PMP20* mutant mice, which express Krox20 but lack PNS myelination (Coulpier *et al.*, 2010). This expansion of CNS glia into the PNS has also been observed in a human patient with congenital amyelinating neuropathy, which is characterized by deficits in Krox20 protein in Schwann cells (Coulpier *et al.*, 2011). These results suggest that Krox20, downstream of Gpr126, may play a role in mediating the transition zone and that oligodendrocytes may be sensitive to the myelination state of adjoining Schwann cells.

A new role for Schwann cells in the repositioning of a peripheral nerve has recently been described in zebrafish (Raphael *et al.*, 2010). The posterior lateral line nerve innervates sensory organs that sense changes in water currents and, in larvae and adults resides just below the basement membrane of the epidermis (Ghysen and Dambly-Chaudiere, 2007; Raphael *et al.*, 2010; Winklbauer, 1989). Recent work revealed that the nerve (both axons and Schwann cells) initially grows within the epidermis, and then rapidly transitions across the epidermal basement membrane to its mature location in the subepidermal space (Figure 2; Raphael *et al.*, 2010). Schwann cells are required for this process, as mutants lacking Schwann cells have the nerve improperly located within the epidermis, and transplantation of wildtype Schwann cells into these mutants is sufficient to restore the nerve to its correct position. Significant defects arise when the posterior lateral line nerve is mislocalized in the epidermis, including defasciculation of the nerve and mispositioning of the nerve along the dorsal-ventral axis. This is apparently the result of the nerve being pulled by the ventrally migrating sensory organs that it innervates. In wildtype animals, the epidermal basement membrane separates the main body of the nerve from the sensory organs; in mutants lacking Schwann cells, however, the nerve remains improperly located within the epidermis in close contact with its target sensory organs. This anatomical organization, with a sensory organ within an epidermal layer and the main nerve fascicle is located below a basement membrane, also occurs in many sensory tissues including the tongue, skin, nasal epithelium, and vestibular organ (Boulais and Misery, 2008; Fernandez *et al.*, 1990; Nedelec *et al.*, 2005; Northcutt, 2004; Oakley and Witt, 2004; Purcell and Perachio, 1997; Si *et al.*, 2003), suggesting that this may be a conserved method of protecting axons that do not easily regenerate from the remodeling or frequent turnover of their targets.

Oligodendrocytes

While this review has focused primarily on Schwann cell development, advances have also been made in the understanding of oligodendrocyte development. Forward genetic screens have uncovered new genes required during oligodendrocyte development and myelination, while detailed timelapse imaging studies have elucidated the complex behavior of oligodendrocytes *in vivo* (Kirby *et al.*, 2006; Larson *et al.*, 2010; Lyons *et al.*, 2009; Parichy and Turner, 2003; Parichy *et al.*, 2003; Pogoda *et al.*, 2006; Takada *et al.*, 2010). For example, two genes required for oligodendrocyte development that came out of forward genetic screens are *kif1b* and *tuba8l3a* – a kinesin molecular motor and a tubulin gene (Larson *et al.*, 2010; Lyons *et al.*, 2009; Parichy and Turner, 2003; Parichy *et al.*, 2003). Both mutants have defects in the localization of myelin specific mRNAs in glial processes. Furthermore, *kif1b* mutant oligodendrocytes have inappropriate, myelin-like membrane compaction in proximal processes and around the cell body, supporting the possibility that myelin mRNA localization within oligodendrocyte distal processes prevents ectopic membrane compaction in other parts of the cell (Lyons et al. 2009). *In vivo* timelapse imaging studies have also revealed new interactions between oligodendrocytes – oligodendrocyte precursor cells (OPCs) actively repel each other through contact inhibition (Kirby *et al.*, 2006), presumably to ensure the proper spacing of oligodendrocytes throughout the CNS. Many questions about oligodendrocyte development remain unanswered, including the axonal signals that regulate oligodendrocytes, the cell-cell contact

signals that OPCs use to repulse each other, and the cues that coordinate the organization of the cytoskeleton to make myelin. Future studies combining genetics with *in vivo* imaging studies in zebrafish will address these questions.

Conclusions and Future Directions

The zebrafish is a genetically tractable vertebrate model organism that is now being used to study the development of myelinating glial cells. Many of the genes required for myelination are conserved from zebrafish to mammals. Recent studies in zebrafish have uncovered new roles for previously known myelin genes and have found new genes that regulate Schwann cell and oligodendrocyte development. *In vivo* imaging is being used to study the behavior of developing Schwann cells and oligodendrocytes; advances in imaging techniques may soon allow for the study of glial cells in adult zebrafish (Blackburn *et al.*, 2011). Future studies will combine these powerful techniques to do high throughput screening using *in vivo* imaging – automated *in vivo* screening was used in a small molecule screen described in Box 1 (Buckley *et al.*, 2010). Improvements in sequencing techniques could be used as a screening method as well – mutations could be chemically induced and then the genome of the mutagenized fish would be sequenced looking for mutations in every single gene. This would rapidly generate many thousands of mutants and once mutations were found they could then be phenotypically assayed. Zebrafish will continue to contribute important information to the myelin field, as new forward genetic and small molecule screens uncover more myelin genes and pathways and *in vivo* imaging continues to improve.

Box 1

Experimental advantages of the zebrafish model system

The zebrafish model system offers several experimental advantages that facilitate the investigation of myelination and other aspects of vertebrate biology.

Forward Genetic Screens

Forward genetic screens in zebrafish are a standard tool in the field, using either chemicals or insertional mutagenesis (Amsterdam and Hopkins, 2006). Two forward genetic screens for myelin mutants have been reported – a screen for defects in the expression of *mbp* mRNA that led to the discovery of the *erbb2*, *erbb3*, and *gpr126* mutants discussed in the main text and a “shelf” screen, where known mutants with neural defects were similarly screened, that found four mutants, *neckless*, *motionless*, *iguana*, and *doc*, required for myelination (Kazakova *et al.*, 2006; Pogoda *et al.*, 2006). Forward genetic screens are unbiased in that they are not looking for a specific gene, but rather are looking for a specific phenotype that defines gene function. Additionally, because current screens have not yet reached saturation, future screening for myelin mutants will yield new genes (Pogoda *et al.*, 2006). Finally, with improvements in the zebrafish genome and rapid advances in sequencing technologies, positional cloning of mutated genes has become much easier and faster in recent years.

Reverse Genetics: Morpholinos, TILLING, and Zinc Fingers

Zebrafish also offers several approaches to study the function of a particular gene of interest, such as a human disease gene, in a developmental context. Overexpression of genes can be achieved by simply injecting RNA into the embryo or by generating transgenic fish with the Tol2 vector system (Kawakami, 2007; Xu *et al.*, 2008). Knockdown of a gene can be performed with antisense morpholino oligonucleotides that block either mRNA splicing or translation (Shestopalov and Chen, 2010). Two methods are available to generate a mutation in a gene of interest. TILLING (targeting induced local lesions in genomes) works by rapidly screening the sequences of many chemically generated mutations to find a mutation in the gene of interest (Stemple 2004). For example, the mutation in zebrafish *krox20* was generated using TILLING (Monk *et al.*, 2009). By contrast, zinc finger mutagenesis targets a gene of interest using zinc finger nucleases that generate targeted double stranded breaks; loss-of-function mutations often result from imprecise repair of these breaks (Doyon *et al.*, 2008; Meng *et al.*, 2008).

Small Molecules

Small molecules have been used extensively in zebrafish to activate or inactivate different signaling pathways (e.g. AG1478 and forskolin described in main text; Lyons *et al*., 2005; Monk *et al*., 2009; Raphael *et al*., 2011). Additionally, small molecule screens have been conducted to look for compounds with certain activities *in vivo* – these screens have uncovered compounds that rescue a cardiac defect, protect hair cells, and have behavioral effects, among others (Ou *et al.*, 2009; Peterson *et al.*, 2004; Rihel *et al.*, 2010). A recent small molecule screen combined *in vivo* imaging of oligodendrocytes precursor cells with high-throughput screening; several compounds were found that affected the number of *olig2:EGPF* expressing oligodendrocyte precursor cells, or the amount of *mbp* expression in the spinal cord (Buckley *et al*., 2010). Small molecule screens for compounds that ameliorate myelin-related mutant phenotypes could be an approach toward strategies for therapeutic remyelination.

In vivo imaging

Several examples in the main text demonstrate power of *in vivo* imaging for studying glial development in zebrafish. Future studies will take advantage of *in vivo* imaging to generate better probes to watch the localization of molecules during myelination and to follow the behavior of individual cells (Yoo *et al.*, 2010). A recent study used “brainbow” to label different neurons in the zebrafish brain with different combinations of red, green, and blue fluorescent proteins – this technique could adapted to address questions in myelination and many other areas (Pan *et al.*, 2011). Additionally, new microscopic techniques, such as “Selective Plane Illumination Microscopy” (SPIM), are allowing for even more detailed *in vivo* imaging studies (Arrenberg *et al.*, 2010; Huisken and Stainier, 2009). New imaging methods and transgenic reporter systems with continue to exploit the optical clarity of the zebrafish embryo.

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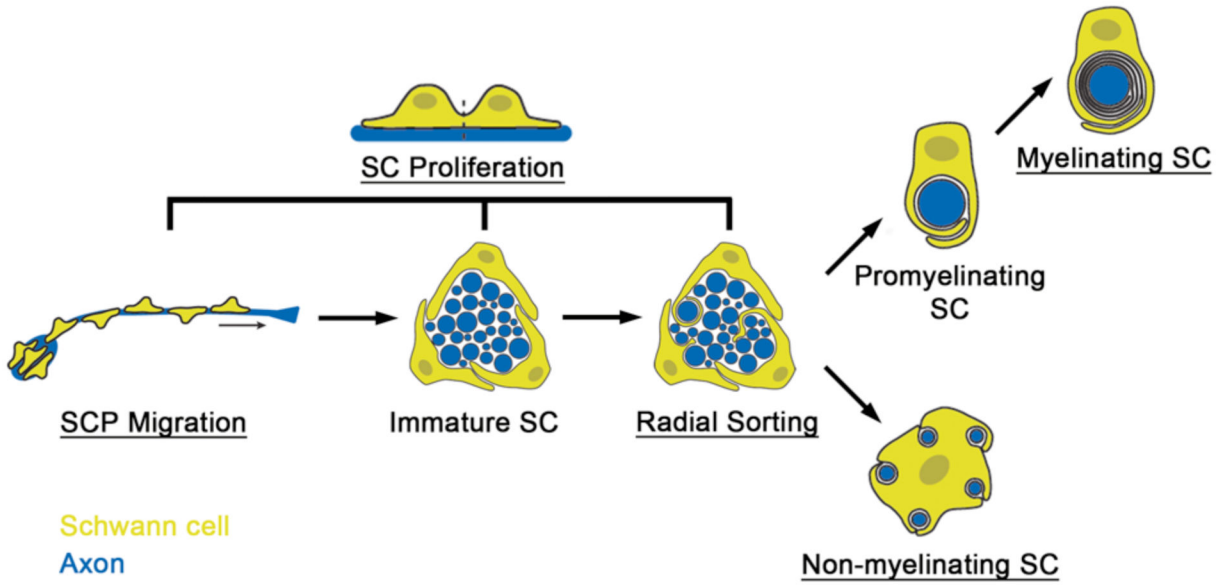

Figure 1. Multiple roles for Nrg1/ErbB signaling during Schwann cell development

Schwann cells (yellow) migrate along axons (blue) as Schwann cell precursors (SCP; Jessen and Mirsky, 2005). Once migration is complete, SCPs differentiate into immature Schwann cells (SC) and begin the process of radial sorting by inserting their processes into the axon bundle (Jessen and Mirsky, 2005; Webster *et al.*, 1973). Immature Schwann cells can become either promyelinating and then myelinating Schwann cells, if they are associated with one axon, or non-myelinating Schwann cells, which associate with multiple small axons (Nave and Salzer, 2006). Schwann cell proliferation occurs in SCPs and in immature Schwann cells and during radial sorting; however, once immature Schwann cells differentiate, they exit the cell cycle (Jessen and Mirsky, 2005; Martin and Webster, 1973; Webster *et al.*, 1973). Schwann cells divide parallel to the axons (Martin and Webster, 1973). Steps that require Nrg1/ErbB signaling are underlined and include Schwann cell migration, Schwann cell proliferation, radial sorting, myelination, and the formation of Remak bundles – non-myelinating Schwann cells ensheathing small caliber axons (Dong *et al.*, 1995; Garratt *et al.*, 2000a; Garratt *et al.*, 2000b; Lyons *et al.*, 2005; Michailov *et al.*, 2004; Morrissey *et al.*, 1995; Raphael *et al.*, 2011; Riethmacher *et al.*, 1997; Taveggia *et al.*, 2005; Woldeyesus *et al.*, 1999).

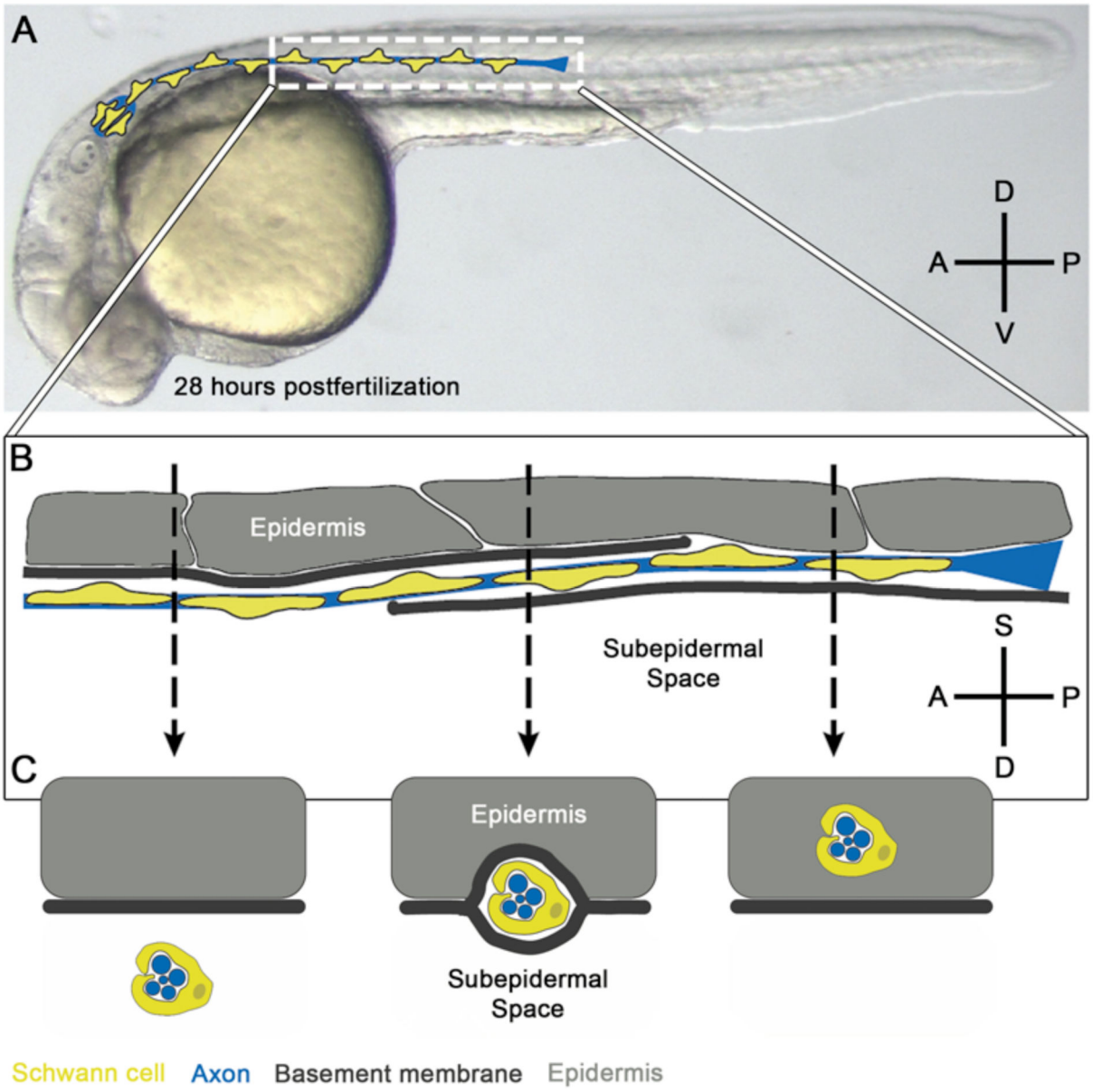

Figure 2. Schwann cells are required for repositioning a peripheral nerve

(**A**) The posterior lateral line nerve in cartoon superimposed on a 28 hours postfertilization zebrafish embryo. Schwann cells (yellow) co-migrate with axons (blue; Gilmour *et al.*, 2002). Anterior (A) is to the left, posterior (P) to the right, dorsal (D) up, ventral down (v). (**B**) Zoom of dashed region in (**A**), showing the transition of the posterior lateral line nerve across the epidermal basement membrane (dark grey) from the epidermis (light grey) into the subepidermal space (unlabeled), with anterior-most portions of the nerve transitioning prior to posterior portions closer to the outgrowing axonal growth cones (Raphael *et al.*, 2010). Dashed lines indicate cross sections shown in (**C**). (**C**) Cross sections through the posterior lateral line nerve showing the position of the nerve with respect to the basement membrane (Raphael *et al.*, 2010). Anterior (far left) the nerve has transitioned across the basement membrane and is embedded in the subepidermal space. Middle, the nerve is

transitioning, with basement membrane both superficial and medial to the nerve. Posterior (far right) the nerve is still within the epidermis, superficial to the basement membrane. In (**C**) superficial is up (S), deep (D) down, anterior left, posterior right. (**A, B**) adapted from Raphael *et al.*, 2010.