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The Scales and Tales of Myelination: using zebrafish and mouse to study myelinating glia

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Abstract

Myelin, the lipid-rich sheath that insulates axons to facilitate rapid conduction of action potentials, is an evolutionary innovation of the jawed-vertebrate lineage. Research efforts aimed at understanding the molecular mechanisms governing myelination have primarily focused on rodent models; however, with the advent of the zebrafish model system in the late twentieth century, the use of this genetically tractable, yet simpler vertebrate for studying myelination has steadily increased. In this review, we compare myelinating glial cell biology during development and regeneration in zebrafish and mouse and enumerate the advantages and disadvantages of using each model to study myelination.

Keywords

Myelin; Schwann cell; oligodendrocyte; zebrafish; mouse

1. Introduction

Over the course of evolutionary history, vertebrates experienced a dramatic increase in body size. Because the speed at which an action potential propagates along an axon is directly proportional to axon diameter, large increases in body size could be facilitated by a subsequent increase in axon diameter. Indeed, this compensation is observed in large invertebrate species including cephalopods, whose axons can reach several millimeters in diameter (Zalc and Colman, 2000). However, the emergence of larger, more complex vertebrate species with dermal skeletons encasing the nervous system (thereby restricting continued increase in axon diameter) required additional means for ensuring proper nerve conduction velocity (Zalc et al., 2008; Zalc, this issue). To facilitate this increase in body

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size, the vertebrate nervous system adapted to produce specialized glial cells that could insulate regular intervals (internodes) along large caliber axons. This insulation, in the form of myelin generated by Schwann cells (SCs) in the peripheral nervous system (PNS) and oligodendrocytes (OLs) in the central nervous system (CNS), prompted saltatory conduction between ion channels clustered at the nodes of Ranvier rather than continuous membrane depolarization, which is both energetically unfavorable and slow (Nave, 2010). Therefore, the development of myelin was likely essential for the expansion and evolutionary success of vertebrates by enabling rapid nerve conduction velocity in a confined space.

Although myelin is an innovation of the jawed-vertebrate lineage, myelin biology has been primarily studied in mammalian model systems with particular emphasis on rodent models (Bullock et al., 1984; Zalc et al., 2008; Schweigreiter et al., 2006). In the past few decades, zebrafish (*Danio rerio*) have emerged as a powerful vertebrate model system; external fertilization, large brood size, and the optical clarity of zebrafish embryos are just a few of the advantages of using this model to study development (Driever et al., 1994). Zebrafish belong to the jawed-vertebrate lineage and therefore represent a more simple and accessible genetically tractable organism for studying the development of myelinating glial cells. Indeed, within the past ~10 years, numerous studies have demonstrated that zebrafish can be used to elucidate essential and evolutionary conserved pathways that regulate both SC and OL myelination (Lyons and Talbot, 2015; Preston and Macklin, 2015). In this review, we will summarize similarities and differences between SC and OL development and myelination in zebrafish and mouse, discuss how these differences impact CNS remyelination, and highlight the strengths and weaknesses of each model system for the study of myelinating glia.

2. Schwann cell development and myelination

In the PNS, myelin is formed from neural crest-derived SCs that sort and associate with single axonal segments in a 1:1 SC:axon segment relationship (Jessen and Mirsky, 2005; Monk et al., 2015; Feltri et al., 2015). The timing and expression of essential molecular markers governing each stage of SC development are well described and are remarkably conserved between mouse and zebrafish (Figure 1). In both species, neural crest cells expressing *Sox9* and *Sox10* delaminate from the neural tube during early embryonic development (embryonic day (E) 8.5 in mouse, 12.5 hours post-fertilization (hpf) in zebrafish) and migrate into the periphery (Kelsh 2006; Klymkowsky et al., 2010; Erickson and Weston, 1983). SC precursors expressing *ErbB2* and *ErbB3* develop from a subset of migrating neural crest cells (E12–13 in mouse, 18–48 hpf in zebrafish) and continue to co-migrate along pathfinding axons (Jessen and Mirsky, 2005; Gilmour et al., 2002; Lyons et al., 2005). As SC precursor migration terminates, the newly formed immature SCs encompass bundles of axons, interdigitate cytoplasmic processes into the bundle, and separate axons according to their size in a process called radial sorting (initiating ~E13–15 in mouse and ~48 hpf in zebrafish) (Jessen and Mirsky, 2005; Raphael et al., 2011; Feltri et al., 2015). Small caliber axons that express low levels of axon-derived Neuregulin I type III remain unmyelinated; however, bundles of small caliber axons are encompassed by the cytoplasm of non-myelinating (Remak) SCs in mature nerves. Conversely, segments of large caliber axons, approximated by high levels of Neuregulin I type III, are sorted into a 1:1

relationship with a promyelinating SC expressing *Oct6/Pou3f1* and *Krox20/Egr2* (initiating ~E15 in mouse and ~48 hpf in zebrafish). At this point, the future internode is wrapped 1–1.5 times by the promyelinating SC, but is not yet myelinated (Michailov et al., 2004; Taveggia et al., 2005; Jessen and Mirsky, 2005; Raphael et al., 2011). Once sorted, terminal differentiation of promyelinating SCs into myelinating SCs ensues (initiating perinatally in mouse and ~60 hpf in zebrafish), accompanied by vast morphological changes, upregulation of key myelin genes, and a dramatic increase in lipid synthesis (Jessen and Mirsky, 2005; Brösamle and Halpern, 2002; Raphael et al., 2011; Chrast et al., 2011; Monk et al., 2015).

One essential regulator of SC development is the adhesion class G protein-coupled receptor (aGPCR) Gpr126/Adgrg6, which was first discovered in zebrafish. Starting with a forward genetic screen for mutations that affect the development of myelinated axons (Pogoda et al., 2006), it was determined that Gpr126 is an evolutionarily conserved protein required for the terminal differentiation of SCs in zebrafish and in mammals (Monk et al., 2009; Monk et al., 2011; Glenn and Talbot 2013; Mogha et al., 2013). Interestingly, comparisons between zebrafish and mouse mutant phenotypes, coupled with experimental approaches harnessing specific advantages of the two systems, have begun to illuminate how Gpr126 controls SC development.

As an aGPCR, like all GPCRs, Gpr126 possess a 7-transmembrane domain that couples to heterotrimeric G proteins (Mogha et al., 2013; Liebscher et al., 2014). The aGPCR class is further defined structurally by a long N-terminus, which often contains functional domains that can mediate cell-cell or cell-matrix adhesion as well as a GPCR autoproteolysis-inducing (GAIN) domain. Within the GAIN domain, receptor autoproteolysis occurs at the GPCR proteolytic site (GPS) motif during the secretory pathway, such that a mature aGPCR exists as an N-terminal fragment (NTF) and a C-terminal fragment (CTF), which are thought to traffic together and remain non-covalently attached at the cell membrane (Arac et al., 2012; Hamann et al., 2015). Although SCs from both zebrafish and mouse *Gpr126* mutants ultimately arrest at the promyelinating stage, initial characterizations revealed that mouse mutant SCs had severe defects in radial sorting, while zebrafish mutant SCs did not (Monk et al., 2009; Monk et al., 2011; Glenn and Talbot, 2013; Mogha et al., 2013). Rather than representing a difference in Gpr126 function between the two species, however, recent work suggests that these phenotypic differences may be due to the nature of the genetic lesions utilized in the studies. Both *gpr126* mutants uncovered in the original screen (Pogoda et al., 2006) are predicted to possess a transcript that encodes the NTF of Gpr126 protein, while *Gpr126* mouse mutants are likely null alleles (Monk et al., 2009; Monk et al., 2011; Mogha et al., 2013). Using targeted genome editing in zebrafish, Petersen and colleagues recently proposed that Gpr126-NTF is necessary and sufficient for radial sorting of axons by SCs (Petersen et al., 2015), consistent with previous observations that the NTFs and CTFs of aGPCRs can have distinct biological functions (Prömel et al., 2012; Patra et al., 2013).

Targeted genome editing in zebrafish (see Section 4.1 for more detail) has also illuminated how Gpr126 signals to control myelination. While Gpr126-NTF drives radial sorting, Gpr126-CTF couples to Gs proteins to elevate 3',5'-cyclic monophosphate (cAMP), activate Protein kinase A (PKA), and initiate transcription of genes that drive terminal differentiation (Mogha et al., 2013; Glenn and Talbot 2013; Liebscher et al., 2014). Using pharmacologic

approaches, Liebscher and colleagues recently demonstrated that GPR126 receptor autocleavage generates a cryptic tethered agonist ligand termed the *Stachel* (German word for “stinger”) sequence buried within the GAIN domain. By targeting two key amino acids in the *Stachel* sequence of *gpr126* and generating new zebrafish mutants, Liebscher and colleagues also demonstrated that signaling via the tethered agonist is required for SC myelination *in vivo* (Liebscher et al., 2014).

Comparisons of zebrafish and mouse mutants in the Nrg-1/ErbB signaling network have been similarly complementary and have greatly enhanced our understanding of how this receptor-ligand pair regulate nearly all aspects of SC development. Neuregulin (Glial Growth Factor (GGF)) was first identified using rat cell culture techniques as a neuronally-derived SC mitogen (Lemke and Brockes, 1984), and many of the original studies focusing on Neuregulin’s function in SCs utilized *in vitro* techniques (Levi et al., 1995; Morrissey et al., 1995; Mahanthappa et al., 1996; Syroid et al., 1996). Using *in vivo* models, it has been shown that Nrg-1 together with its receptor pair (ErbB2/ErbB3) is required for numerous facets of SC biology: SC precursor migration (Lyons et al., 2005; Perlin et al., 2011; Torii et al. 2014), amplification of the SC progenitor pool (Garratt et al., 2000; Lyons et al., 2005), SC precursor survival (Riethmacher et al., 1997; Garratt et al., 2000; Lyons et al., 2005), radial sorting independent of proliferation (Raphael et al., 2011), and regulation of myelin thickness (Michailov et al. 2004). Given the many aspects of SC development that are regulated by the Nrg-1/ErbB signaling network (for more, see Newbern and Birchmeier, 2010; Mei and Nave, 2014), here we will focus on the relative contributions of mouse and zebrafish models to our understanding of SC precursor survival and migration.

As aforementioned, the Nrg-1 pathway first gained the attention of the glial community for its mitogenic effects on SC *in vitro* (Lemke and Brockes, 1984) and was likewise shown to regulate the survival (Dong et al. 1995) and migration (Meintanis et al. 2001) of rat SC precursors in culture. Loss of *Nrg1* results in early embryonic lethality in mice (Meyer and Birchmeier, 1995), prohibiting thorough analysis of nervous system development in *Nrg1*^{-/-} mice. In a landmark paper from the Birchmeier group in 1997, Riethmacher and colleagues used a targeted deletion strategy to generate null alleles of *ErbB3* in mice, providing the first *in vivo* evidence that the Nrg-1/ErbB pathway is required for SC development. Analysis of peripheral nerves from *ErbB3*^{-/-} embryos revealed that in the absence of ErbB signaling, SC precursors are completely lost. Using a chimeric approach, the group demonstrated ErbB signaling functions cell autonomously in SC precursors to promote survival, but the severe phenotypes resulting from loss of *ErbB3* prohibited further investigation of the role of ErbB signaling in SC development. Using four hypomorphic mutant alleles of the zebrafish ErbB2/3 receptors (*st14* and *st48* affecting *erbb3b* and *st50* and *st61* affecting *erbb2*) isolated from forward genetic screens, Lyons et al. first demonstrated that ErbB signaling was also required for SC precursor proliferation *in vivo* (as had previously been shown *in vitro* (Lemke and Brockes, 1984)), as well as for directed migration of SC precursors. Using a stable transgenic reporter line (*foxd3::gfp*), which is expressed in crest derivatives through the mature SC stage, Lyons et al. found that although neural crest migration from the dorsal neural tube to the posterior lateral line ganglia was unaffected in *erbb3b*^{st48/st48} mutants, SC precursors failed to migrate out of the ganglia along growing axons. Interestingly, SC precursor survival was not impaired in the mutant alleles characterized in this study, perhaps

reflecting a species difference, the hypomorphic nature of the zebrafish alleles used, and/or the teleost- and zebrafish-specific genome expansion events that result in multiple copies of some genes in the present day zebrafish genome (Taylor et al., 2003; Harty et al., 2015; Figure 1). Recently, work from the Yamauchi lab demonstrated that knockdown of *ErbB3* *in vivo* in mouse likewise results in impaired SC precursor migration from dorsal root ganglia into ventral roots (Torii et al. 2014), underscoring the notion that a forward genetic screen in zebrafish can identify novel protein functions that are conserved in mammals.

Although the progression of SC development from neural crest to myelinating SC is conserved in both species, there are some key morphological differences between developing zebrafish and mouse peripheral nerves that are important to address, two of which we address here: the basal lamina and myelin compaction.

2.1 The Basal Lamina

In both mouse and zebrafish, as SC precursors cease migrating and transition to the immature SC stage, they begin to secrete proteins to build their own basal lamina (BL) and this BL is essential for both proper radial sorting of axons by immature SCs and for normal myelination (Billings-Gagliardi et al., 1974, Chen and Strickland, 2003; Yu et al., 2005; Saito et al., 2003; Feltri et al., 2015; Petersen et al., 2015). Ultrastructural analyses of mouse and other mammalian peripheral nerves have determined that during radial sorting of axons by immature SCs, the BL is discontinuous and patchy. As the SC develops, the immature BL transforms into a very compact, electron dense structure surrounding the abaxonal (non-axon facing) side of the SC during myelination. Because the endoneurium, or the interstitial connective tissue that separates axons and axon:SC units within the nerve, is spacious in both newborn rodents and in adults, it is straightforward to image the BL and thus the maturation of the BL throughout peripheral nerve development using standard ultrastructural approaches (Webster, 1974). In zebrafish, a common nerve used to study SC development and myelination is the posterior lateral line nerve (pLLn), a purely sensory nerve that runs along the flank of the fish on both sides and aids in shoaling behaviors (Ghysen and Dambly-Chaudière, 2007; Chitnis et al., 2011; Lyons et al., 2005; Monk et al., 2009; Raphael et al., 2011; Glenn and Talbot, 2013; Petersen et al., 2015). The majority of studies using zebrafish to study SC development and myelination have focused on larval stages, during which the pLLn is very compact and the endoneurial space is so small that the BL cannot be visualized. Not until the nerve is fully mature is the endoneurium large enough to observe the BL in the zebrafish pLLn (Figure 2). Thus, research aiming to study BL maturation during SC development at the ultrastructural level is currently better suited for mouse.

2.2 Myelin Compaction

As noted in section 2, the expression of key markers of each stage of SC development is well conserved from zebrafish to mouse; however, expression of key myelin genes including *myelin protein zero* (*mpz*) and *proteolipid protein* (*plp*) is absent from early stage myelinating SCs in zebrafish (Brösamle and Halpern, 2002). Consequently, compaction of the myelin sheath (which occurs continuously in mouse SCs) may be delayed in zebrafish (Figure 3) (Martini and Schachner, 1997). Moreover, common and accessible chemical

fixation techniques are inferior to high-pressure freezing techniques for the preservation of myelin in any species (see Möbius, this issue). As a consequence, studying the importance of a candidate gene in more subtle roles for in SC myelination such as myelin compaction would be more appropriate for mouse models that utilize high-pressure freezing approaches. Similarly, morphological domains of myelin like Schmidt-Lanterman incisures, Cajal bands, and appositions have not been well-described in zebrafish (Münzel et al. 2012). In mice, these features form during postnatal development as the nerve matures (Webster, 1971; Sherman & Brophy, 2005). Given that the majority of studies using the zebrafish pLLn to study SC myelination focus on early larval development before *mpz* and *plp* are expressed, analysis of adult zebrafish peripheral nerves is likely required to determine whether these subdomains are also formed by zebrafish SCs. For further information regarding myelin ultrastructure in the PNS and CNS of zebrafish versus mouse, see Avila et al. 2007.

3. Oligodendrocyte development and myelination

OLs derive from Olig2+ neural precursor cells within the pMN domain of the neural tube during early embryonic development (E9.5 in mouse and 10.5 hpf in zebrafish) (Sun et al., 2006; Park et al., 2002; Mitew et al., 2014). Proliferative OL precursor cells (OPCs, Olig2+ and Sox10+) begin to migrate dorsally from the pMN domain around E12.5 in mouse and 30 hpf in zebrafish. Unlike SC precursors, both mouse and zebrafish OPCs do not need to follow axonal projections for their migration and instead require morphogen and extracellular matrix (ECM) molecule signaling (Mitew et al., 2014; Ackerman et al., 2015; Colognato and Tzvetanova, 2011). When OPC migration and proliferation terminates, some OPCs transition into pre-myelinating OLs (Nkx2.2+, initiating at E16.5 in mouse and 48 hpf in zebrafish), which extend processes and ensheath axons, but do not yet form mature myelin (Mitew et al., 2014; Zhu et al., 2014; Kucenas et al., 2008; Kirby et al., 2006; Snaidero et al., 2014). As noted in Section 2, in both species, immature SCs select axons to myelinate based on interactions between heterodimerized ErbB2 and ErbB3 receptors on the SC with axon-derived Neuregulin I type III (Michailov et al., 2004; Taveggia et al., 2005; Lyons et al., 2005). An analogous ligand-receptor pair that functions to determine which axons will be myelinated is unknown in the CNS, and recent studies in both mouse and zebrafish demonstrate that neuronal activity can be instructive in this process (Gibson et al., 2014; Hines et al., 2015; Mensch et al., 2015). After individual axons have been chosen, terminal differentiation of myelinating OLs (postnatal development in mouse, initiating ~72 hpf in zebrafish) results in vast morphological changes requiring massive expansion of membrane — each OL can myelinate up to 40 axonal segments (Pfeiffer et al., 2003), in addition to the expression of a variety of myelin genes, including *Mbp* and *Mag* (Figure 4) (Mitew et al., 2014; Almeida et al., 2011; Hu et al., 2003; Matthews et al., 2014; Snaidero et al., 2014).

The many similarities between zebrafish and mouse OL development have led to an increasing number of studies using zebrafish to study CNS myelination (Preston and Macklin, 2015). Nevertheless, here we discuss two major difference between mature zebrafish and mouse OLs: *1. myelin protein composition and 2. the ability to robustly remyelinate.*

3.1 Major myelin protein composition of zebrafish and mouse OLs

Though the transcription factor cascades that govern OL development remain largely conserved from zebrafish to mouse, the major protein constituents of OL myelin have diverged over the course of evolutionary history. In both species, MBP and CNP comprise a large component of CNS myelin (Avila et al. 2007, Möbius et al. 2008). However, analyses of purified CNS myelin have identified several proteins that are abundant in zebrafish and other teleost species, but not detected in mammalian CNS myelin (Morris et al. 2004, Schaefer and Brösamle 2008, Möbius et al. 2008). For example, Zwilling-A and Zwilling-B are two related proteins that originate from a single bi-cistronic transcript and resemble the structure of Mbp, and both proteins are highly conserved throughout the teleost clade but absent from mammals (Schaefer and Brösamle 2008). Similarly, the protein 36K is a teleost-specific myelin protein that was first identified in trout myelin and has since been further characterized in zebrafish (Jeserich and Waehneldt 1986, Morris et al. 2004). Protein 36K is one of the most abundant proteins in CNS myelin of teleost species, and sequence analysis revealed that 36K belongs to the short-chain dehydrogenase/reductase (SDR) family, a large protein family implicated in many biological processes including lipid synthesis (Oppermann et al. 2003). Although 36K mRNA is expressed in human brain, the protein has not been shown to form a major component of mammalian CNS myelin (Morris et al. 2004). It is hypothesized that the presence of these additional, teleost-specific myelin proteins reflects the fact that teleosts are poikilothermic (cold-blooded) meaning that their body temperatures can vary considerably during the course of the day, requiring their cellular machinery to adapt with changing external temperatures. Membrane fluidity and maintaining proper anchoring of myelin-associated proteins to the membrane is essential for proper myelin function (Hu et al. 2004); thus, it is possible that teleost-specific myelin proteins facilitate myelin stability throughout changing environments. In addition to the expansion of teleost-specific CNS myelin proteins, the most prominent myelin protein constituent changed during the evolutionary transition of the teleost-clade to mammals from MPZ (an adhesion protein) to PLP (a lipid-associated transmembrane-tetraspan), likely to allow for increased membrane flexibility. For a more exhaustive review, see Möbius et al. 2008).

3.2 Oligodendrocyte remyelination

Zebrafish, exhibiting the remarkable ability to regrow entire organs (*e.g.* heart and fin), represent an excellent vertebrate model organism for studying regeneration (Shi et al. 2015). Recently, several groups have taken advantage of the elevated regenerative capacity of zebrafish to understand repair after injury in the nervous system (McCurley et al., 2010; Ma et al. 2012; Moore et al., 2012; Münzel et al., 2012; Chung et al. 2013). In the PNS of zebrafish and mouse, axons are readily remyelinated by SCs after injury (Wang and Jin, 2011; Moore et al., 2012; Jessen et al., 2015). In the CNS of mammals, however, repair is frequently impeded after axon injury due to both defects in axon regrowth through the glial scar that surrounds injury site (Huebner and Strittmatter, 2009) as well as impaired remyelination (the process by which OLs generate new myelin to ensheath demyelinated axons). In contrast, the majority of axons fully regenerate after spinal cord injury or optic nerve crush in both larval and adult zebrafish (Becker and Becker, 2008; März et al., 2011; Vajn et al., 2013). In the CNS of patients who suffer from inflammatory disorders that

damage OLs and/or induce demyelination, (*e.g.* multiple sclerosis, MS), failed remyelination as the disease progresses represents a major challenge to clinicians (Prineas and Connell, 1979; Franklin and French-Constant, 2008; Franklin and Goldman, 2015). While MS patients can experience a high degree of remyelination during early stages of the disease, remyelination frequently fails as the disease progresses, leading to permanent axon loss in the absence of metabolic and functional support by OLs (Franklin and French-Constant, 2008). At present, several different mouse models of MS exist, and though none are an exact replica of the human disease (Ransohoff, 2012), these paradigms have been widely used to test different genetic and pharmacological treatments to improve OL remyelination in the context of disease (Simmons et al., 2013). Of these, experimental autoimmune encephalomyelitis (EAE) mouse models have been used to mimic the relapsing-remitting phases of MS in which patients experience periods of recovery followed by periods of more severe symptoms, and remyelination is likewise inadequate in EAE models (Skundric 2005). Interestingly, recent studies in larval and adult zebrafish have shown that OLs robustly remyelinate after demyelination achieved through metronidazole-induced death of OLs, or through chemical administration of Lysophosphatidylcholine (LPC) to optic nerve (Chung et al., 2013; Münzel et al., 2014). It is not completely understood why remyelination occurs so robustly in the periphery and so poorly in the CNS, though one hypothesis is that the environment after injury is hostile to OL terminal differentiation. In accordance with this hypothesis, a common feature of CNS lesions from patients with multiple sclerosis is an abundance of pre-myelinating OLs that fail to terminally differentiate (Huang et al., 2011). It is exciting to speculate on the use of both existing and to-be-developed zebrafish demyelination and remyelination models in genetic and small molecule screens, which could prove to be very important tools for elucidating molecular mechanisms that can promote or inhibit regeneration in the CNS of mammals (Buckley et al., 2008).

Zebrafish and Mouse, Advantages and Disadvantages

Although there is clear conservation of the molecular mechanisms governing glial cell development and myelination in zebrafish and mouse, it is always important to choose the model that will best address the research question at hand. Below, we enumerate some advantages and disadvantages of using zebrafish and/or mouse to highlight which types of experiments are currently best suited for each model system (summarized in Table 1).

4.1 Zebrafish

In the last several decades, zebrafish have emerged as a powerful vertebrate model system for multiple reasons: external fertilization giving rise to large brood sizes (each female can give rise to hundreds of offspring at a given time), rapid development, and optical clarity of the embryos facilitating visualization of key developmental processes *in vivo* (Driever et al., 1994). As zebrafish represent the simplest genetically tractable model organism for studying myelination, they have recently garnered much attention from both the glial and broader neuroscience communities (Zalc et al., 2008; Lyons and Talbot, 2015; Stewart et al., 2014). One of the greatest advantages of using the zebrafish model system for studying myelination (or any area of interest) is the ability to perform both forward genetic screens and small-molecule chemical screens on thousands of individuals to define genetic and

pharmacological modulators of neurodevelopment (e.g., Driever et al., 1996; Podoga et al., 2006; Kazakova et al., 2006; Rihel et al., 2010; Mathews et al., 2014; Miller et al., 2015; Kokel and Peterson, 2011). This feature of the zebrafish is not only the consequence large brood size and short generation time, but also reflects the ability to quickly screen for phenotypes *in vivo* in the developing embryo given their optical clarity and the rapid development of the nervous system (Figures 1 & 4) (Snyder et al., 2012). Underscoring the power of forward genetics approaches in zebrafish, we note that the function of Gpr126 in SC myelination was discovered from a genetic screen in zebrafish (Pogoda et al., 2006; Monk et al., 2009). Excitingly, a recent report suggests that Gpr126 is also required for myelination in humans (Ravenscroft et al., 2015); thus, genetic screens in zebrafish can define novel regulators of myelination in humans.

Although forward genetic screens are very useful tools for gene discovery, a major disadvantage of the zebrafish model system has historically been the inability to target specific genes for mutagenesis coupled with the non-specificity of available knockdown approaches (Kok et al., 2015). With the advent of transcription activator-like effector nucleases (TALENs) in 2011 and more recently, clustered regularly interspaced short palindromic repeats-CRISPR associated (CRISPR-Cas) technology, targeted-generation of mutations in the zebrafish genome is both rapid and highly efficient (Dahlem et al., 2012; Hwang et al., 2013). Briefly, TALENs consist of two engineered fusion proteins that recognize specific DNA sequences each fused to a Fok1 nuclease. CRISPR-Cas technology entails generation of a guide RNA that is co-injected with Cas9 nuclease. Both technologies cause DNA double-strand breaks, and imperfect repair leads to insertion and deletion (indel) mutations. In zebrafish, external fertilization and development allows for direct injection of TALEN or CRISPR-Cas reagents into fertilized, one-cell stage zygotes, which can then be raised and screened in the next generation for heritable mutations. We recently used TALENs to test the role *gpr56*, an aGPCR enriched in OPCs, in OL development and myelination, and found that this aGPCR is an evolutionarily conserved regulator of OPC development (Ackerman et al., 2015; Giera et al., 2015). Given the ease of genome editing approaches in zebrafish, many labs now routinely use both TALENs and CRISPRs to target genes for mutagenesis (Ackerman et al., 2015; Shiao et al., 2015; Gagnon et al., 2014) or in high-throughput discovery platforms (Shah et al., 2015). Although these technologies are very efficient for inducing indels in target regions of the genome, use of CRISPRs and TALENs in site-directed homologous recombination for transgene insertion or for gene replacement is also possible, though it is currently less efficient than generating simple indels (Auer and Del Bene, 2014; Shin et al., 2014). Even so, the fast generation time of zebrafish (6–8 weeks to adulthood under ideal conditions) coupled with the rapid generation of TALENs and CRISPRs results in the ability to screen homozygous animals within 4–5 months.

Analysis of glial cell development and myelination in zebrafish requires many of the same techniques used to study these processes in mouse, including *in situ* hybridization and electron microscopy. However, one major utility of the zebrafish, which should be underscored, is the ability to image these processes *in vivo* in a live, intact organism. Although some aspects of glial cell development can be very elegantly imaged in mouse (e.g., Hughes et al., 2013), *in vivo* time-lapse imaging is far more straightforward in

zebrafish. Because zebrafish larvae are transparent, and because several mutant lines exist in which the adults are likewise transparent, it is possible to image the entire process of glial development and myelination, and even axon regrowth and remyelination after injury, *in vivo* (e.g., Kirby et al., 2006; Kucenas et al., 2008; Almeida et al., 2011; Rosenberg et al., 2012; Czopka et al., 2013; Snaidero et al., 2014; Ceci et al., 2014; Rosenberg et al., 2014; Smith et al., 2014). Indeed, many studies have used live imaging in the zebrafish embryo to address key biological questions regarding the timing and the progression of OL myelination and recently, exciting work from the Lyons and Appel labs have used zebrafish to demonstrate that OLs “choose” which axons they will myelinate in part based on neuronal activity (Hines et al., 2015; Mensch et al., 2015). These studies demonstrate the advantage of using zebrafish for imaging dynamic processes such as glial cell development and myelination, which cannot be easily visualized in mouse.

4.2 Mouse

Though there are many benefits of using zebrafish for studying glial cell development and myelination, the tools available to zebrafish biologists lag behind those available to other researchers because the system is much younger than other animal models, including mouse. One major disadvantage of using zebrafish resulting from this deficit of proper tools is the inability to easily address questions of cellular autonomy. In contrast, use of the well-established Cre-Lox system in mouse enables generation of cell-type specific knockouts, which are crucial for understanding whether the function of a gene in SC or OL myelination is required cell- or non-cell autonomously. Further, the ability to temporally remove gene function in a cell-type specific manner with inducible Cre drivers is useful for testing whether a specific gene is required at different stages of glial cell development, for myelin maintenance, or required post-injury (e. g. Sauer, 1998; Lewandoski, 2001; Leone et al., 2003; Grove et al., 2007; Nodari et al., 2007; Fancy et al., 2009; Fünfschilling et al., 2012; Lee et al., 2012; Arthur-Farraj et al., 2012; Mogha et al., 2013; Stassart et al., 2013; Bercury et al., 2014). While these genetic strategies are commonplace in mouse, similar tools are only just emerging in the zebrafish community (Ablain et al., 2015). Moreover, basic reagents such as antibodies and tools including cell culture techniques that are readily available to the research community for studying glial cell development in mouse are limited (though improving) for use in zebrafish (Staudt et al., 2015; Tapanes-Castillo et al., 2014; Hong et al., 2014; Grunow et al., 2015).

One major factor that is important to address when testing the necessity of a given gene for proper myelination is how loss of that gene’s function affects the overall behavior and wellbeing of the individual. Loss of myelin and myelinating glia in the CNS and PNS of patients with diseases such as multiple sclerosis and Charcot-Marie-Tooth disease results in devastating symptoms including chronic pain and paralysis (Runia et al., 2012; Azzedine et al., 2012). It is therefore important to be able to assess behavioral defects in animal models that attempt to represent these complex neurological diseases in order to test how well the animal model mimics the human disease state. In the past few decades, there has been a major push for developing behavioral tests that examine motor function (and therefore neurological function) in mouse, with the rotarod being the most prevalently used test for behavioral deficits in myelin mutants (Brooks and Dunnet, 2009; Kuhn et al., 1995). Poor

performance in these assays, coupled with reduced nerve conduction velocity, is a strong indication that the animal model accurately reflects the human myelinopathy (Court et al., 2004). Though some behavioral tests do exist to test motor function in zebrafish larvae, the effects of myelin loss on behavior in zebrafish myelin mutants has not been evaluated (Rihel et al., 2010; Kokel and Peterson, 2011). Further, nerve conduction velocity assays are not widely employed in zebrafish, thus it is less feasible to directly measure the effects of myelin loss on nerve function in this model. The current lack of myelin-specific behavioral and electrophysiological assays is a disadvantage of using zebrafish for modeling neurological disorders, rendering the mouse a more appropriate system for these types of experiments.

5. Conclusions

The emergence of the zebrafish model system in the glial cell community over the past decade has driven the field into new exciting areas of research (Hines et al., 2015; Mensch et al., 2015). The amenity of the zebrafish model system to large-scale, forward genetics approaches has identified new, essential regulators of myelination. In addition, the ability to image nervous system development *in vivo* using zebrafish has increased our understanding of the timing and mechanics of SC and OL glial cell development and myelination (Lyons et al., 2005; Kirby et al., 2006; Perlin et al., 2011; Almeida et al., 2011; Czopka et al., 2013). Despite these advances, zebrafish is still a young model system and as such, tools for addressing cellular autonomy and behavioral/electrophysiological function that are well established in mouse are underdeveloped in zebrafish (Ablain et al., 2015). Thus, by taking advantage of the unique strengths of both model systems, the field will more quickly gain a complete picture of glial cell development and myelination.

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Abbreviations

aGPCR	adhesion G protein-coupled receptor
BL	basal lamina
cAMP	3',5'-cyclic monophosphate
CRISPR-Cas	clustered regularly interspaced short palindromic repeats-CRISPR associated
CTF	C-terminal fragment
dpf	days post-fertilization
E	embryonic day
GAIN	GPCR autoproteolysis-inducing

GPS	GPCR proteolytic site
hpf	hours post-fertilization
indel	insertion and deletion
mpf	months post-fertilization
NTF	N-terminal fragment
OL	oligodendrocyte
OPC	oligodendrocyte precursor cell
PKA	protein kinase A
pLLn	posterior lateral line nerve
SC	Schwann cell
TALENs	transcription activator-like effector nucleases

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Highlights

Myelin is an evolutionary innovation of the jawed-vertebrate lineage.

Many studies of myelin use mouse, but zebrafish are also a powerful model organism.

This review compares myelinating glial cell biology during development and regeneration in zebrafish and mouse.

Figure 1. Schwann cell development. Descriptions follow above images from left to right. Neural crest (NC)/Schwann cell (SC) lineage cells are shown in blue, axons/neuronal tissues are shown in green, and maturing SC basal lamina is shown in orange. NC cells delaminate from the roof plate (RP) of the neural tube (shown in cross section) during embryonic development and migrate into the periphery where SC precursors are specified. SC precursors then co-migrate along growing axons as they pathfind (lateral view shown). Once this migration has ended, SC precursors transition into immature SCs, which surround bundles of axons, interdigitate cytoplasmic processes into the bundle, and sort the axons within by diameter in a process called radial sorting (these and all subsequent diagrams shown in cross section). Small caliber axon segments are sorted into a 1:1 relationship with a pro-myelinating SC; which, upon terminal differentiation signals, successively spirals its plasma membrane around the sorted axon to form myelin. Accompanying table shows the approximate age (hours post-fertilization (hpf) or embryonic day (E)) and key molecular markers for each stage of SC development in zebrafish and mouse. An “a” or a “b” following zebrafish markers indicates that the gene is duplicated in the zebrafish genome. We note that the ages indicated for zebrafish development refer specifically to the posterior lateral line nerve (pLLn) at a stereotyped position, between segments 5–7, along the anterior-posterior axis. FP, floor plate.

Figure 2.

Basal lamina observations in zebrafish and mouse. (A–C) Representative transmission electron microscopy (TEM) images of cross sections through the pLLn in a 5 day post-fertilization (dpf) zebrafish larva (A–B') and a 6 months post-fertilization (mpf) adult zebrafish (C). (A) The basal lamina (BL) is not readily detected around SCs (pseudocolored blue in all images) in the zebrafish pLLn during larval stages when the nerves are fixed well by microwave-assisted chemical techniques (e.g., Czopka and Lyons, 2011). (B–B') Suboptimal fixation of the pLLn causes gaps between individual SCs such that the immature BL is detected (white arrowhead). (C) Mature BL can be more readily observed as an electron dense structure surrounding mature SCs at 6 mpf (red arrowheads). (D–E) Representative TEM images of cross-sections through mouse sciatic nerve at postnatal (P) day 3 (D) and P14 (E). At P3, immature BL (white arrowheads) is detected surrounding an immature SC as it sorts multiple axons (D). By P14, an electron dense, mature BL (red arrowheads) is seen surrounding a myelinating SC (E). Scale bars, 500 nm.

Figure 3. Peripheral myelin compaction. (A–D) Representative TEM images of cross sections through the pLLn in 3 dpf (A), 5 dpf (B), 21 dpf (C), and 6 mpf zebrafish to show myelin compaction during early larval, larval, juvenile, and adult stages of zebrafish development, respectively. Myelin appears as loose wraps (red arrowheads) through juvenile stages in zebrafish and does not fully compact until adulthood. We note that TEMs were obtained from animals processed by microwave-assisted chemical fixation methods (e.g., Czopka and Lyons 2011). (E–H) Representative TEM images of a cross-section through mouse sciatic nerve at P3, P14, P21, and P180. At all stages of development, mouse peripheral myelin appears fully compact. Scale bars, 1 μ m.

Figure 4. Oligodendrocyte development. Descriptions follow above images from left to right. Neural precursor (NP)/oligodendrocyte (OL) lineage cells are shown in purple and axon/neuronal tissues are shown in green. NP cells born within the pMN domain of the embryonic neural tube (shown in cross-section) give rise to OL precursor cells (OPCs). OPCs proliferate as they migrate through the developing CNS (image and all subsequent images shown as lateral views). Following migration, OPCs transition into pre-myelinating OLs, which extend cytoplasmic processes towards and associate with axons, but do not yet form myelin. Upon terminal differentiation, pre-myelinating OLs become myelinating OLs and iteratively wrap their plasma membranes around multiple axon segments to form myelin sheaths. Accompanying table indicates approximate age in zebrafish (shown in hpf) and mouse (shown in embryonic (E) days) in addition to key molecular markers characteristic of each stage of OL development. An “a” or a “b” following zebrafish markers indicates that the gene is duplicated in the zebrafish genome. Ages indicated for zebrafish development refer specifically to the spinal cord at a stereotyped position along the anterior-posterior axis (between segments 5–7). RF, roof plate. FP, floor plate.

Table 1

Some advantages of the zebrafish and mouse model systems. This table lists some key advantages of using either zebrafish or mouse for studying myelin with regards to the experimental tools available for each model system.

Zebrafish	Mouse
<ul style="list-style-type: none">• <i>low cost relative to mouse husbandry</i>• <i>external fertilization & large clutch size</i>• <i>forward genetic screens</i>• <i>emergence of reverse genetics techniques (CRISPR-Cas & TALEN technology)</i>• <i>chemical screens</i>• <i>in vivo imaging</i>	<ul style="list-style-type: none">• <i>cell-type specific KOs (Cre-Lox and inducible Cre-Lox technology)</i>• <i>long established reverse genetics & mutant availability</i>• <i>cell culture studies</i>• <i>antibody availability</i>• <i>electrophysiology and behavioral studies</i>• <i>well-established aging and injury paradigms</i>