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Genetic dissection of myelinated axons in zebrafish

Kelly R. Monk and **William S. Talbot**
Department of Developmental Biology, Stanford University School of Medicine, Beckman Center B300, 279 Campus Drive, Stanford, CA 94305

Kelly R. Monk: kmonk@stanford.edu; William S. Talbot: wtalbot@stanford.edu

Abstract

In the vertebrate nervous system, the myelin sheath allows for rapid and efficient conduction of action potentials along axons. Despite the essential function of myelin, many questions remain unanswered about the mechanisms that govern the development of myelinated axons. The fundamental properties of myelin are widely shared among vertebrates, and the zebrafish has emerged as a powerful system to study myelination in vivo. This review will highlight recent advances from genetic screens in zebrafish, including the discovery of the role of *kif1b* in mRNA localization in myelinating oligodendrocytes.

Introduction

Myelin is a multilayered membrane formed by the wrapping of glial cells around axons that allows for efficient conduction of action potentials in the vertebrate nervous system [1,2]. In the central nervous system (CNS), oligodendrocytes form myelin, whereas Schwann cells myelinate axons in the peripheral nervous system (PNS). Myelinating glial cells are critical for the proper function of the vertebrate nervous system, not only in allowing rapid propagation of action potentials, but also in providing axons with trophic support [2]. At the gaps between adjacent myelin segments are the nodes of Ranvier, which contain abundant voltage-gated sodium channels. These channels propagate action potentials, and the specific localization of channels at the nodes is required for saltatory conduction in myelinated axons [3,4]. The importance of myelinating glia is underscored by human disorders in which myelinated axons are disrupted, including multiple sclerosis (MS) and Charcot-Marie-Tooth disease [5,6]. Studies in human patients and mammalian model organisms have identified key genes involved in myelination [6,7], but important questions about the genetic control of glial development and differentiation remain unanswered. The goal of this review is to summarize recent advances from genetic studies of myelination in zebrafish.

Zebrafish as a model organism to study myelination

In the last two decades, the zebrafish has become a well-established model system for the study of vertebrate biology [8]. The embryos are large, externally developing, and translucent, which is advantageous for timelapse imaging, cellular transplantation, and microinjection. The ability to perform timelapse imaging is particularly advantageous for the investigation of dynamic interactions between cells that occur during glial development, and several studies have

exploited this property to examine glial migration and behavior in zebrafish [9–11]. Additionally, the zebrafish is fast-growing and fecund, which facilitates genetic screens. These attributes have also allowed zebrafish embryos and larvae to be used to screen for small molecules affecting development or behavior in a whole animal [12]. In light of these experimental advantages, the zebrafish has emerged as a model to study glial cell development and myelination in vivo [9,10,13–15].

As in mammals, oligodendrocytes and Schwann cells form myelin in the zebrafish CNS and PNS, respectively [9,10,13]. Additionally, nodes of Ranvier have similar protein composition and ultrastructure in mammals and zebrafish [16,17]. Biochemical studies have described both similarities and differences between zebrafish and mammalian myelin. For example, Myelin basic protein (Mbp) and myelin proteolipid protein (PLP/DM20) are components of myelin in fish and mammals, but there are also teleost-specific myelin proteins in the CNS including 36K, Zwilling-A, and Zwilling-B [13,18–20]. Myelin Protein Zero (P_0), an Ig-like protein, is present in peripheral myelin of both tetrapods and teleosts, but only in fish is this protein prominently expressed in the CNS [13,21–23]. X-ray diffraction studies have shown that the structure of myelin is fundamentally similar in zebrafish and mammals, but that the periodicity of myelin varies slightly [23]. Thus, despite these differences, the properties of vertebrate myelin are overall highly conserved.

Many genes appear to have conserved functions in glial development of zebrafish and mammals. For example, *Olig1*, *Olig2*, and *Sox10* act in oligodendrocyte development in zebrafish and mouse [24,25]. Likewise, *Sox 10*, *ErbB2/ErbB3*, *Oct6*, and *Krox20* act in Schwann cells of both species [7,9,14,26]. These similarities provide evidence that genes identified by genetic approaches in zebrafish will also have essential functions in glial development and myelination in mammals.

Mutational screens for genes with essential function in glial development and myelination

In the mid-1990s, large-scale genetic screens in zebrafish identified mutations in more than 1000 genes that disrupt the morphology of the embryo and early larva [27,28]. These mutations fueled advances in many areas, including the genetic control of axis formation, gastrulation, and organogenesis. Because these initial screens identified mutants with readily observable morphological defects, many of the mutations disrupt global patterning of the embryo or otherwise affect the development of numerous different cell types. Nonetheless, examination of *mbp* expression in 39 previously identified mutations identified four mutants in which axons were present but *mbp* expression was reduced in the posterior lateral line nerve (PLLn, a prominent sensory nerve in zebrafish; [29]). Further analysis of mutations in the *aldh1a2* gene supported a role for retinoic acid signaling at an early step of development essential for the eventual differentiation of myelinating glia [29].

In another approach, two complementary screens were performed to find new mutations that affect the development of myelinated axons. In the first of these, Pogoda et al. [14] screened more than 1850 clutches of F3 larvae for mutations with specific defects in the expression of *mbp* mRNA. This screen identified 13 mutations in 10 genes that disrupted *mbp* expression in the CNS, in the PNS, or in both [14]. A second smaller screen expanded the scope of phenotypes examined by assaying sodium channel (NaCh) expression at nodes of Ranvier in myelinated axons [17]. It is important to note that neither screen was pursued to saturation, so that continued screening will surely yield mutations in genes that have not yet been defined. Most of the mutants have normal morphology, and were therefore not uncovered in previous large-scale screens. The absence of obvious morphological defects suggests that the mutated genes have relatively specific functions in the development of myelinating glia and the associated axons;

this also implies that the screens overlooked genes with interesting but more general functions in myelination and other processes. As summarized in Table 1, the available phenotypic studies indicate that the mutated genes act at many different steps in the development of myelinated axons, including glial fate specification, axon outgrowth, Schwann cell migration, and organization of the nodes of Ranvier. In addition, at least two of the genes have been associated with human disease, suggesting that analysis of these zebrafish mutants may yield important insights into diseases that disrupt myelinated axons.

Defining the functions of mutated genes at the molecular and cellular level

Recent studies have provided insight into the cellular and biochemical functions of several of the genes defined by the mutational screens discussed above [9,16,17,30–32]. In the following section, we discuss three examples of mutational studies in zebrafish that have identified genes that function in glial development and myelination, and describe how these studies have contributed new insights.

ErbB signaling

ErbB2/ErbB3 receptors and their Neuregulin ligands are critical for many steps in Schwann cell development including proliferation, ensheathment, and myelination [33]. Accordingly, the *mbp* and NaCh screens recovered mutations in *erbb2* and *erbb3* [9,14]. As in mammals [33], *erbb2* and *erbb3* mutant zebrafish lack Schwann cells in peripheral nerves. Phenotypic studies of the mutants showed that ErbB signaling is required for Schwann cell proliferation in vivo, supporting previous work in mammals [34]. Time-lapse imaging of the *erbb* mutants and embryos treated with ErbB inhibitors demonstrated that ErbB signaling is essential for directed migration of Schwann cells in growing nerves [9]. Thus, analysis of these mutants not only underscored the fundamental similarities between zebrafish and mammalian PNS myelination, but also provided new insight into a role for ErbB signaling during Schwann cell migration.

αII-spectrin

In myelinated axons, the cytoskeletal protein αII-spectrin is localized at paranodes, sites of axoglial adhesion immediately adjacent to the nodes of Ranvier [35,36]. The screen for mutants with aberrant sodium channel clustering at nodes of Ranvier uncovered a mutant allele of *αII-spectrin*, providing an opportunity to examine its function in vivo [17]. Mutational studies showed that *αII-spectrin* is essential to stabilize nascent sodium channel clusters in developing axons and to assemble mature nodes of the correct dimensions. These functional studies, together with previous biochemical analyses showing that αII-spectrin forms a complex with βII-spectrin and Ankyin B [35], have added important understanding to role of these cytoskeletal proteins in the organization of myelinated axons.

Kif1b

In the CNS of mammals and zebrafish, mRNAs encoding Mbp and a small number of other structural components of myelin are localized to processes of myelinating oligodendrocytes (Figure 1 [13,37]). Analyses in cultured cells have implicated microtubules and kinesin motors in the transport of *mbp* mRNA [38–40], but genes required for endogenous mRNA localization in vivo had not been identified until recently. Furthermore, the role of this specific mRNA localization process in oligodendrocyte differentiation was not understood.

Lyons et al. recently reported that mutations in *kif1b*, which encodes a member of the kinesin motor superfamily, cause defects in *mbp* mRNA localization in oligodendrocytes and disruptions of the outgrowth on long axons in the spinal cord and PNS [32]. Analysis of genetic

chimeras indicated that *kif1b* is required in neurons for axon outgrowth, perhaps to transport key cargo to the distal region of rapidly growing axons.

In the CNS, *kif1b* mutants display a striking phenotype: *mbp* mRNA is present in oligodendrocyte cell bodies, but not in myelinating processes [32]. Analysis of genetic chimeras indicated that *kif1b* is required autonomously in oligodendrocytes for proper localization of *mbp* mRNA. *Kif1b* mutant oligodendrocytes form myelinating processes, and Mbp protein is present in these processes despite the lack of *mbp* mRNA. The mRNA for another myelin protein, 36K, is also localized to oligodendrocyte processes in wildtype zebrafish [19], but not in *kif1b* mutants. These results indicate that Kif1b is essential to localize specific mRNAs within myelinating processes. Future studies will examine if the Kif1b motor acts directly to transport mRNA cargo into myelinating processes.

The finding that *mbp* mRNA is mislocalized in *kif1b* mutants provided the opportunity to investigate the role of mRNA localization in the differentiation of oligodendrocytes. Ultrastructural analyses showed that myelin forms in the CNS of *kif1b* mutants, although the number of myelinated axons and the thickness of the myelin are somewhat reduced [32]. The most striking observation from the ultrastructural studies was that that *kif1b* mutants possess marked ectopic myelin-like membrane compaction throughout the anterior spinal cord. Long stretches of compact myelin-like membranes were observed surrounding neuronal cell bodies and in processes that did not ensheath axons. Mbp and 36K protein are largely restricted to myelin in wild-type oligodendrocytes, but these proteins are present throughout the oligodendrocytes of *kif1b* mutants at stages when ectopic myelin-like membrane is evident [32]. These studies suggest that mislocalized myelin proteins contribute to aberrant membrane compaction in *kif1b* mutants. According to this model (Figure 1), the role of mRNA localization in oligodendrocytes is to restrict the localization of Mbp, 36K and some other myelin proteins to the myelin itself, thereby preventing the deleterious action of these proteins in other parts of the cell. An additional implication is that the subset of localized mRNAs encodes the proteins that bring about myelin membrane compaction. Interestingly, in a recent genome-wide association study, *KIF1B* was associated with susceptibility to MS [41]. Thus, the discovery of a function for Kif1b in oligodendrocytes may shed some light on its possible roles in MS, and the mutant zebrafish may represent a useful tool to explore the pathophysiology of MS in the future.

Conclusions

Mutational screens in zebrafish have begun to contribute to the genetic dissection of myelination in vertebrates. The cellular and molecular analysis of zebrafish mutations has revealed new insight into the functions of genes essential for glial development and myelination. Additional discoveries are expected, as existing mutants are characterized and new screens recover mutations in genes that have not yet been identified. Additionally, zebrafish myelination mutations could be utilized in future small molecule screens for chemicals that rescue or exacerbate phenotypes. These studies may further our understanding of pathways involved in diseases of myelinated axons and speed the development of human treatments.

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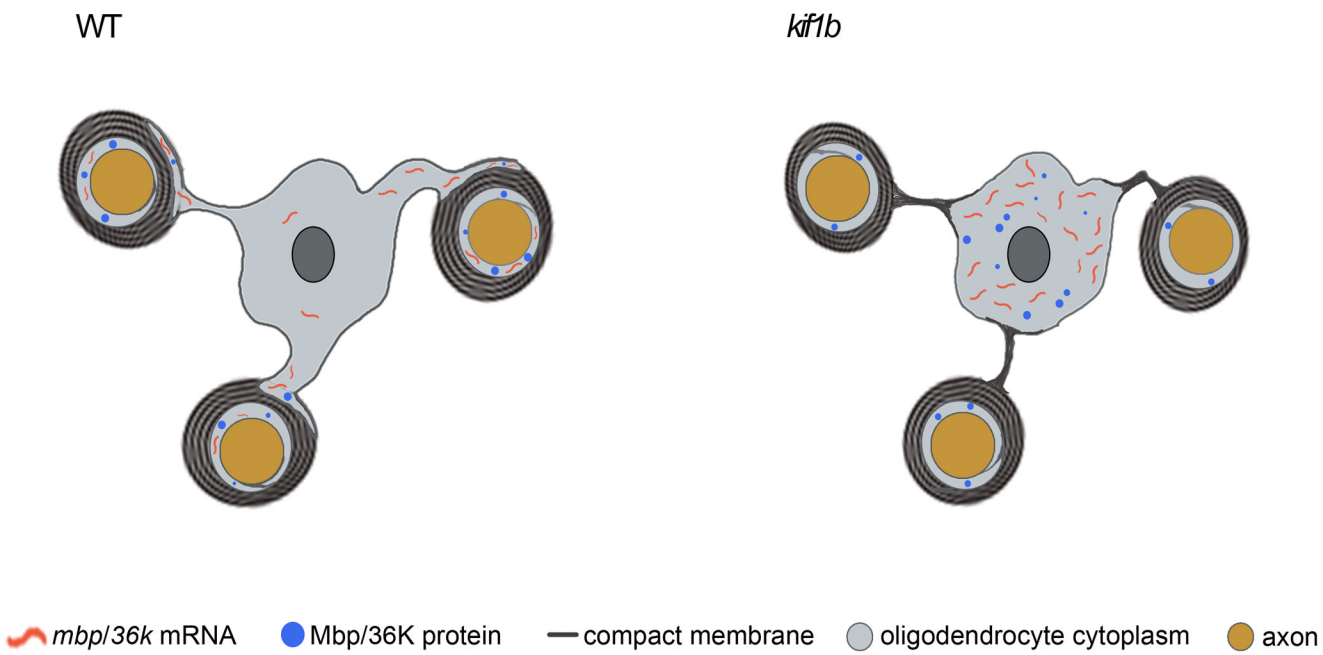

Figure 1. A model of defects in the CNS of *kif1b* zebrafish mutants

In WT zebrafish, axons (orange) are surrounded by compact myelin. Mbp and 36K mRNA and protein are localized to the uncompacted region of the myelinating process (gray), but are largely excluded from the cell body. In *kif1b* mutant zebrafish, *mbp* and *36k* mRNA are not transported into myelinating process, although proteins are localized to these regions. Mbp and 36K protein are aberrantly localized in oligodendrocyte cell body, coincident with mislocalization of compacted, myelin-like membrane. Adapted from [32].

Table 1

Mutated genes identified in screens for abnormal *mbp* expression and NaCh localization

Mutant allele	Gene (if reported)	Functions	Screen	Reference
st50, st61	*erbb2*	SC proliferation, migration, myelination	Both	[9]
st14, st48, st71	*erbb3*	SC proliferation, migration, myelination	Both	[9]
st20	*foxa2*	Development of midline OL precursors	*mbp*	[30]
st25, st53	*nsf*	Forming NaCh clusters at nodes	*mbp*	[16]
st60	*aII-spectrin*	Stabilizing NaCh clusters at nodes	NaCh	[17]
st23	*kbp**	Axon outgrowth in PNS and CNS	*mbp*	[31]
st43	*kif1b**	Axon outgrowth, CNS: mRNA localization	*mbp*	[32]
st49, st63	Unpubl.	SC development	*mbp*	[14]
st51	Unpubl.	Development of hindbrain OL	*mbp*	[14]
st64	Unpubl.	SC development	*mbp*	[14]
st67	Unpubl.	Myelin morphogenesis	NaCh	Unpubl.

SC: Schwann cell; OL: oligodendrocyte; NaCh: sodium channel

*Associated with human disease