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Review Article

Learning to swim, again: Axon regeneration in fish

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ABSTRACT

Damage to the central nervous system (CNS) of fish can often be repaired to restore function, but in mammals recovery from CNS injuries usually fails due to a lack of axon regeneration. The relatively growth-permissive environment of the fish CNS may reflect both the absence of axon inhibitors found in the mammalian CNS and the presence of pro-regenerative environmental factors. Despite their different capacities for axon regeneration, many of the physiological processes, intrinsic molecular pathways, and cellular behaviors that control an axon's ability to regrow are conserved between fish and mammals. Fish models have thus been useful both for identifying factors differing between mammals and fish that may account for differences in CNS regeneration and for characterizing conserved intrinsic pathways that regulate axon regeneration in all vertebrates. The majority of adult axon regeneration studies have focused on the optic nerve or spinal axons of the teleosts goldfish and zebrafish, which have been productive models for identifying genes associated with axon regeneration, cellular mechanisms of circuit reestablishment, and the basis of functional recovery. Lampreys, which are jawless fish lacking myelin, have provided an opportunity to study regeneration of well defined spinal cord circuits. Newer larval zebrafish models offer numerous genetic tools and the ability to monitor the dynamic behaviors of extrinsic cell types regulating axon regeneration in live animals. Recent advances in imaging and gene editing methods are making fish models yet more powerful for investigating the cellular and molecular underpinnings of axon regeneration.

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1. Introduction

Fish, our distant vertebrate cousins, are at least as vulnerable as we are to injuries, but their nervous systems have a greater capacity to regrow axons, repair circuits, and recover function. Despite the difference in regenerative ability between mammals and fish, many of the molecular and cellular pathways that regulate axon regeneration are conserved. Fish models have already provided insight into shared mechanisms of axon regeneration and new techniques promise to make them even more powerful systems for investigating how molecules and cells regulate neural repair.

Adult fish regeneration models, which have been established for decades, and the more recently developed larval zebrafish model, have distinct experimental advantages (Table 1). The robust regeneration of optic nerve (ON) and spinal cord axons in larval lamprey and adult goldfish and zebrafish has been exploited to identify factors that promote successful regeneration in the central nervous system (CNS). By contrast, most studies using the larval zebrafish model have focused on axon regeneration in the peripheral nervous system (PNS). The amenability of larval zebrafish to live imaging and genetic manipulation makes them ideal for studying dynamic behaviors of regenerating axons and extrinsic cell types. Adult and larval fish both have well-defined circuits and stereotyped behaviors, facilitating studies of the cell biology underlying axon regrowth and synapse reestablishment, and making it possible to address how anatomical regeneration relates to functional recovery.

Here we review four aspects of axon regeneration studies in adult and larval fish models. First, we discuss efforts to answer one of the most fascinating questions about axon regeneration in the adult fish CNS—why is it so much more successful than axon regeneration in the mammalian CNS? Second, we describe studies of intrinsic growth pathways in fish, which have demonstrated that the molecular basis of axon growth is conserved between fish and mammals. These studies have also identified new molecules associated with regenerative axon growth, providing candidate targets for therapeutic interventions. Third, we review studies in both adults and larvae that assessed the success of functional recovery and mechanisms of circuit re-establishment. Finally, we discuss what has been learned from fish models about interactions of non-neuronal cells with regenerating axons. These studies, many using live imaging in larval zebrafish, have uncovered new roles for extrinsic cell types in PNS axon regeneration and have the potential to reveal much more about dynamic cell behaviors during axon regeneration in both the PNS and CNS.

2. Why do axons regenerate so well in the fish CNS?

What underlies the disparate regenerative abilities of mammalian and fish CNS axons? Exposing mammalian axons to cells of the fish CNS, or fish axons to cells of the mammalian CNS, can distinguish whether differences in regeneration are attributable to neurons themselves or to their surrounding environment. Regenerating axons of both mammalian and fish neurons are repelled by mammalian oligodendrocytes and myelin (Bandtlow et al., 1990; Bastmeyer et al., 1991; Fawcett et al., 1989) but both can grow in the presence of fish oligodendrocytes or fish CNS conditioned media (Bastmeyer et al., 1991, 1993; Schwalb et al., 1995; Schwartz et al., 1985; Wanner et al., 1995). Thus, just as factors in the environment of the mammalian CNS and PNS account for differences in the success of axon regeneration (David and Aguayo, 1981), distinct factors in the mammalian and fish CNS environment account for much of their difference in axon regeneration. Three explanations for the more growth-permissive environment of the fish CNS have been proposed: the absence of inhibitory cues, the presence of factors that block inhibitory cues, and the presence of pro-growth factors.

2.1. Are inhibitory factors present in the fish CNS?

The adult mammalian CNS contains multiple molecules inhibitory to axon growth, including myelin proteins, ECM proteins such as chondroitin sulfate proteoglycans (CSPGs) and Tenascins, and chemorepulsive guidance cues (reviewed by Giger et al., 2010). The mammalian myelin proteins RTN4-A/Nogo-A, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp)—all of which have homologs in the zebrafish genome (Lehmann et al., 2004; Shypitsyna et al., 2011; our unpublished observations)—activate the growth-inhibiting Nogo receptor (NgR) complex on regenerating axons (Giger et al., 2010). Although functional studies of fish MAG and OMgp have not been reported, zebrafish Nogo-A homologs have been studied biochemically and genetically. Purified mammalian Nogo and MAG inhibit the growth of fish axons (Abdesselem et al., 2009; Chen et al., 2013), but the fish homolog of the mammalian Nogo-66 domain, which is responsible for growth inhibition in mammals, does not affect growth of fish or mammalian axons (Abdesselem et al., 2009). Zebrafish Nogo-66 can bind to mammalian NgR but fails to activate downstream signaling (Abdesselem et al., 2009), suggesting that zebrafish Nogo-A and its mammalian counterpart may have functionally diverged from each other (Abdesselem et al., 2009; Shypitsyna et al., 2011). Further evidence for functional divergence comes from studies of Rtn4b, a fish

Table 1
Fish models to study axon regeneration.

Organism	Axon models	Pros	Cons
Larval sea lamprey (*Petromyzon marinus*)	Spinal cord	● Basal vertebrate lacking myelin ● Flat, translucent spinal cord facilitates microscopy ● Large, easily identifiable neurons ● Quantifiable swimming behaviors	● Lack of genetic tools ● Lack of live imaging ● "Developmental" environment
Adult goldfish (*Carassius auratus*)	Optic nerve, spinal cord	● Well-described anatomy, electrophysiology ● Well-characterized behaviors	● Lack of genetic tools ● Live imaging difficult
Adult zebrafish (*Danio rerio*)	Optic nerve, spinal cord, motor neurons, posterior lateral line nerve (pLLn)	● Increasing number of transgenic tools ● Genetic models possible (mutants, inducible transgenes, implantable morpholinos)	● Live imaging difficult
Larval zebrafish (*Danio rerio*)	Optic nerve, spinal cord, motor neurons, pLLn, somatosensory neurons	● Numerous transgenes ● Many genetic tools ● Live imaging ● Easily quantifiable behaviors	● "Developmental" environment