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A molecular mechanism of optic nerve regeneration in fish: The retinoid signaling pathway

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ABSTRACT

The fish optic nerve regeneration process takes more than 100 days after axotomy and comprises four stages: neurite sprouting (1–4 days), axonal elongation (5–30 days), synaptic refinement (35–80 days) and functional recovery (100–120 days). We screened genes specifically upregulated in each stage from axotomized fish retina. The mRNAs for heat shock protein 70 and insulin-like growth factor-1 rapidly increased in the retinal ganglion cells soon after axotomy and function as cell-survival factors. Purpurin mRNA rapidly and transiently increased in the photoreceptors and purpurin protein diffusely increased in all nuclear layers at 1–4 days after injury. The purpurin gene has an active retinol-binding site and a signal peptide. Purpurin with retinol functions as a sprouting factor for thin neurites. This neurite-sprouting effect was closely mimicked by retinoic acid and blocked by its inhibitor. We propose that purpurin works as a retinol transporter to supply retinoic acid to damaged RGCs which in turn activates target genes. We also searched for genes involved in the second stage of regeneration. The mRNA of retinoid-signaling molecules increased in retinal ganglion cells at 7–14 days after injury and tissue transglutaminase and neuronal nitric oxide synthase mRNAs, RA-target genes, increased in retinal ganglion cells at 10–30 days after injury. They function as factors for the outgrowth of thick, long neurites. Here we present a retinoid-signaling hypothesis to explain molecular events during the early stages of optic nerve regeneration in fish.

Contents

Abbreviations: AEC, 3-amino-9-ethylcarbazole; cFXIII, cellular factor XIII; cGMP, cyclic GMP; CNS, central nervous system; CRABPs, cellular retinoic acid-binding proteins; CYP26a1, cytochrome P450/26a1; FXIII, factor XIII; GAP43, growth-associated protein 43; GCL, ganglion cell layer; HRP, horse radish peroxidase; HSF, heat shock factor; HSP70, heat shock protein 70; HSPs, heat shock proteins; IGF-1, insulin-like growth factor-1; INL, inner nuclear layer; NADPHd, NADPH diaphorase; nNOS, neuronal NOS; NO, nitric oxide; NOS, nitric oxide synthetase; OKR, optokinetic response; OMR, optomotor response; ONL, outer nuclear layer; PI3K, phosphatidylinositol-3-kinase; p-Akt, phospho-Akt; p-Bad, phospho-Bad; PKG, protein kinase G; RA, retinoic acid; RAGs, regeneration-associated genes; RALDH, retinaldehyde dehydrogenase; RARs, retinoic acid receptors; RBP, retinol-binding protein; RGCs, retinal ganglion cells; SNAP, S-nitroso-N-acetyl penicillamine; TG, transglutaminase; TG_R, retinal tissue type TG; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.

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1. Introduction

Generally, mammalian central nervous system (CNS) neurons cannot regrow their axons and therefore become apoptotic after nerve injury. In contrast, since the pioneering works of Sperry (Attardi and Sperry, 1963; Sperry, 1948), it is well known that fish CNS neurons can regrow their axons and restore their function after nerve injury. The fish visual system (retina, optic nerve and tectum) has been used as a CNS nerve regeneration model. Many conceptual theories of nerve regeneration have been developed using the goldfish visual system (Murray and Grafstein, 1969; Stuermer, 1988), mainly through morphological studies. Since the 1980s, there have been an increasing number of studies looking for factors or molecules that are involved in optic nerve regeneration in goldfish (Benowitz et al., 1981; Perrone-Bizzozero and Benowitz, 1987). Through these studies, the disparity in our understanding of CNS neurons in non-mammals (fish) and mammals has become apparent. The environmental conditions surrounding regenerative fish CNS neurons are quite different from those surrounding the un-regenerative mammalian CNS, such as the presence of oligodendrocytes and extracellular matrix proteins (Pizzi and Crowe, 2007). The glial environment of the fish CNS is similar to that of the mammalian peripheral nervous system (where neurons can regenerate after injury) and there are fewer extracellular matrix proteins in the CNS than in the peripheral nervous system (Richardson et al., 1980). Furthermore, optic nerve crush increases extracellular matrix protein such as tenascin-R, chondroitin sulfate proteoglycan and laminin in fish optic nerve tract during nerve regeneration (Becker et al., 2004; Hoffman and O'Shea, 1999; Hopkins et al., 1985). In 1993, myelin-inhibitory-molecules, which are impermissible for CNS nerve regeneration, were discovered in the mammalian CNS (Schwab et al., 1993). Such myelin-inhibitory properties in rat oligodendrocytes are not found in fish oligodendrocytes (Caroni and Schwab, 1988). Since then, many researchers are investigating nerve regeneration-associated molecules in the fish visual system that can be used for mammalian CNS regeneration.

We investigated regeneration-associated genes (RAGs) in fish optic nerve regeneration after injury using molecular cloning techniques (Liu et al., 2002; Matsukawa et al., 2004a; Sugitani et al., 2006). We constructed a cDNA library made from fish optic nerve or retinas prepared at various times after optic nerve injury. We determined a precise time course of fish optic nerve regeneration and isolated RAGs from each phase of the process. In this review, we characterize RAGs isolated from fish axotomized retinas and optic nerve and demonstrate their role during regeneration. We propose a retinoid-signaling pathway to explain optic nerve regeneration in fish.

2. Functional recovery of regrowing optic axons

Before describing RAGs in the fish retina and optic nerve during optic nerve regeneration, we wanted to know the time course of the regeneration process after injury. Using a combination of morphological, physiological, biochemical and behavioral methods, we determined the onset and offset of each event during optic nerve regeneration in goldfish or zebrafish. This was necessary to make a specific cDNA library for each stage of the process.