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BRAIN RESEARCH

Research Report

Purpurin is a key molecule for cell differentiation during the early development of zebrafish retina

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ABSTRACT

Recently, we cloned purpurin cDNA as an upregulated gene in the axotomized fish retina. The retina-specific protein was secreted from photoreceptors to ganglion cell layer during an early stage of optic nerve regeneration in zebrafish retina. The purpurin worked as a trigger molecule for axonal regrowth in adult injured fish retina. During zebrafish development, purpurin mRNA first appeared in ventral retina at 2 days post-fertilization (dpf) and spread out to the outer nuclear layer at 3 dpf. Here, we investigated the role of purpurin for zebrafish retinal development using morpholino gene knockdown technique. Injection of purpurin morpholino into the 1–2 cell stage of embryos significantly inhibited the transcriptional and translational expression of purpurin at 3 dpf. In the purpurin morphant, the eyeball was significantly smaller and retinal lamination of nuclear and plexiform layers was not formed at 3 dpf. Retinal cells of purpurin morphants were still proliferative and undifferentiated at 3 dpf. The visual function of purpurin morphant estimated by optomotor response was also suppressed at 5 dpf. By contrast, the control morphants with random sequence morpholino showed retinal lamination with distinct layers and differentiated cells at 3 dpf. These results strongly suggest that purpurin is a key molecule for not only optic nerve regeneration in adult but also cell differentiation during early development in embryo.

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Abbreviations: hpf, hours post-fertilization; dpf, days post-fertilization; MO, morpholino; ONL, outer nuclear layer; INL, inner nuclear layer; GAP-43, growth associated protein-43; PKC, protein kinase C; GFAP, glial fibrillary acidic protein; BrdU, bromo-deoxyuridine; AO, acridine-orange; FGF, fibroblast growth factor; HDAC, histone deacetylase; RA, retinoic acid; HSPG, heparan sulfate proteoglycan

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1. Introduction

Purpurin, a retina-specific adhesion molecule, was originally isolated from cultured neural cells of developing chicken retina by Schubert et al. (Schubert and LaCorbiere, 1985; Schubert et al., 1986). Classical works demonstrated that the 22-kDa retinol-binding secretory protein stimulates retina cell-substratum adhesion and prolongs the survival of neural retina cells in culture. The cellular localization of chicken purpurin mRNA is limited to photoreceptors. The expression of chicken purpurin is maximal at embryonic 6–20 days in chicken retina and becomes greatly reduced in adulthood (Berman et al., 1987). We accidentally isolated a goldfish purpurin cDNA clone as an upregulated gene after optic nerve transection in adult goldfish retina (Matsukawa et al., 2004). Goldfish purpurin mRNA expression rapidly increased in the photoreceptors 2–5 days and then rapidly decreased to reach control level by 10 days after optic nerve injury (Matsukawa et al., 2004). This rapid and transient increase of purpurin mRNA expression at the early stage of optic nerve regeneration strongly indicated a trigger action of purpurin on axonal regrowth after optic nerve injury in adult goldfish. Recombinant purpurin protein with retinol has been reported to actually promote neurite outgrowth in cultured adult goldfish retina (Matsukawa et al., 2004). We further identified zebrafish purpurin cDNA, which was highly homologous to that of goldfish (Tanaka et al., 2007).

Zebrafish is a more popular animal model in developmental biology as compared to goldfish. They breed throughout the year, and they have a very short time for hatching and maturation. Because the database for zebrafish cDNA or genomic DNA is updated daily, various genetic approaches such as gene knockdown or transgenic experiments have become easier than those used with goldfish. In the present study, we investigated the role of the nerve regeneration-associated protein, purpurin, in the early development of the zebrafish retina using morpholino gene knockdown technique and computational probing system. In zebrafish embryos, which were injected with morpholino antisense oligonucleotides against zebrafish purpurin, cell differentiation of the developing retina was largely suppressed as compared to control retinas. In addition, the visual-guided behavior of the treated fish measured by a computer image processing system

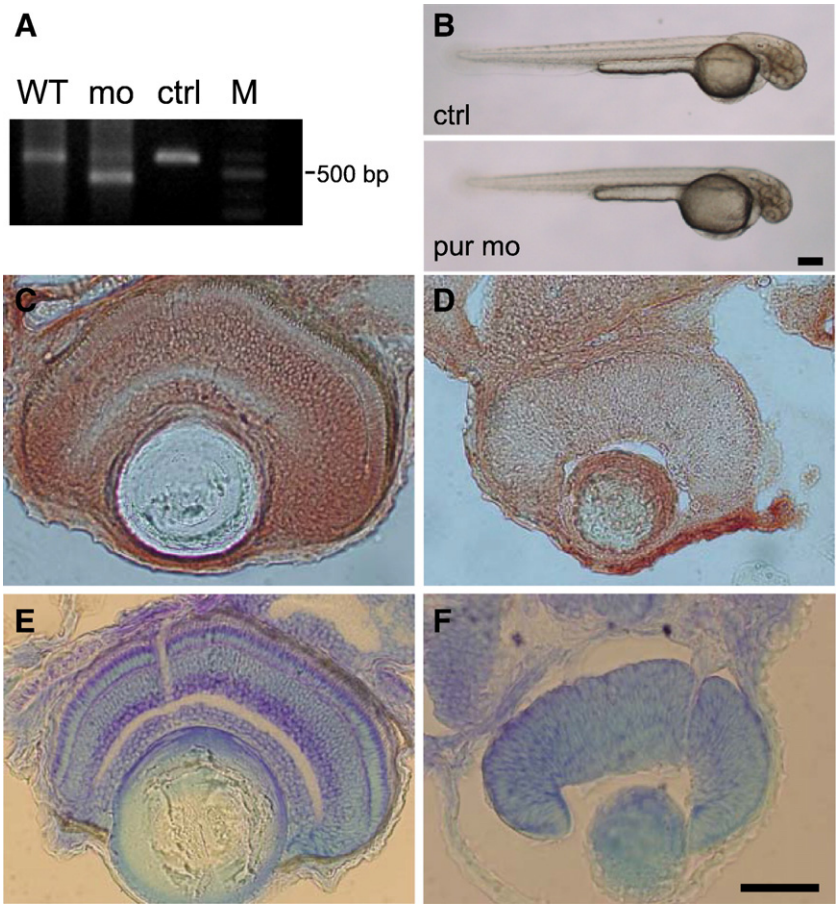

Fig. 1 – Purpurin gene knockdown with morpholino antisense oligonucleotide. (A) RT-PCR using exon 1 and exon 5 primers at 3 dpf. Purpurin morphants (mo) showed about 100 bp shorter than wild-type (WT) and control embryos which was injected with random sequence morpholino (ctrl). The bar indicated the 500-bp marker. (B) Control and purpurin morphant embryos injected with 0.5 mM control (upper panel) and 0.5 mM MO1 (lower panel), respectively. Scale bar = 200 μm. (C, D) Immunohistochemistry of purpurin in the control (C) and purpurin morphant (D) retinas at 3 dpf. Immunoreactivity of purpurin was drastically decreased in the morphant retina. (E, F) Toluidine blue staining of the control (E) and purpurin morphant (F) retinas at 3 dpf. Morphants had a small eye and no retinal lamination. Scale bar = 50 μm.