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Retinal regional differences in photoreceptor cell death and regeneration in light-lesioned *albino* zebrafish

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Abstract

Teleost fish regenerate retinal cells from a population of inner nuclear layer (INL) stem cells. To characterize photoreceptor regeneration in zebrafish (*Danio rerio*), adult *albino* fish were subjected to constant intense light to cause photoreceptor cell death. Retinal morphometry was performed on histological sections of control and light-lesioned *albino* retinas to compare the extent of light damage in the ventral, central and dorsal retinal regions. In addition, opsin immunohistochemistry and TUNEL were used to compare photoreceptor cell death in these different retinal areas, while PCNA immunolabeling quantified the cell proliferation that precedes the photoreceptor regeneration. Transgenic *albino*; Tg(*α1-tubulin:egfp*) zebrafish were also exposed to the intense light in order to examine regeneration-related gene expression changes.

The light-lesioned retinas are characterized by extensive rod and cone photoreceptor cell death in the central and dorsal regions. In contrast, many of the rods and cones survive in the ventral retina. The highest levels of INL cell proliferation, which occurs subsequent to photoreceptor death, correspond to the retinal regions that suffer the greatest levels of photoreceptor damage. In the ventral retina, where photoreceptor cell death is minimal, cell proliferation is confined to the ONL. In addition, EGFP expression from the α1-tubulin promoter is increased in Müller glial cells in the light-damaged central and dorsal retina, while transgene expression in the ventral retina is restricted to small, round INL cells. Furthermore, expression of the HuC/D neuronal antigen is detected in a subpopulation of the Müller cells in the light-damaged superior retinal region. These data demonstrate that adult *albino* zebrafish display retinal regional differences in photoreceptor cell death and in the regeneration-related INL cell proliferation response. The high levels of INL cell proliferation and *α1-tubulin:egfp* transgene expression in the Müller cells may be graded in response to the degree of photoreceptor cell death. This suggests that the levels of photoreceptor damage may directly influence cell responses in the underlying retinal layers.

Keywords: light-damage; photoreceptor regeneration; retinal stem cell; cell proliferation

1. Introduction

Light-induced photoreceptor cell death is a valuable experimental model for studying the cellular mechanisms and molecular pathways of neural cell degeneration and regeneration. Light treatment of rodents results in the activation of multiple independent pathways leading to photoreceptor apoptosis [reviewed in (Wenzel et al., 2005)]. Consecutive rhodopsin bleaching is necessary to initiate this process and photo-oxidative stress may further contribute to the light damage pathology since several antioxidant genes are up-regulated in the retina following the light-induced injury (Chen et al., 2004; Kutty et al., 1995; Ohira et al., 2003; Sieving et al., 2001; Tanito et al., 2002; Wenzel et al., 2001). Genetic background, including pigmentation of the iris and retinal pigmented epithelium, dietary factors and light exposure history can all affect the severity of the light-induced damage (Chen et al., 1999; LaVail and Gorrin, 1987; LaVail et al., 1987; Organisciak et al., 1998; Rapp and Williams, 1980a). Increased levels of light exposure accelerate the degeneration in some hereditary forms of retinal disease; therefore, the light treatment models utilize

Abbreviations ROS, rod outer segment; CC, cone cell layer; ONL, outer nuclear layer; INL, inner nuclear layer; OPL, outer plexiform layer; IPL, inner plexiform layer; GCL, ganglion cell layer; optic n, optic nerve.

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an environmentally relevant stimulus to study photoreceptor cell death and the development of neuroprotective strategies (Wenzel et al., 2005).

Unlike mammals, adult teleost fish and amphibian species regenerate retinal cells that are lost due to various types of damage including surgical and neurotoxic injury (Moshiri et al., 2004). The ability to regenerate photoreceptor cells in zebrafish is likely related to the continual retinal growth displayed by fish (Hitchcock et al., 2004; Johns, 1977; Johns and Easter, 1977). The fish retina harbors two spatially and functionally distinct populations of self-renewing stem cells. The first stem cell population is located in the inner nuclear layer (INL) and their retinal progenitors migrate to the outer nuclear layer (ONL) to become rod precursors, which differentiate into new rod photoreceptors during normal retinal growth (Otteson et al., 2001). The second population of stem cells is located in the circumferential germinal zone at the retinal margin and produces the remaining retinal cell types (Hitchcock et al., 2004). Selective damage to specific retinal cell types by toxin injection or laser-induced photocoagulation demonstrates the retinal regeneration response in fish is stimulated by destruction or significant damage to the photoreceptor cells, while damage restricted to INL cells fails to engage the regeneration machinery (Braisted et al., 1994; Braisted and Raymond, 1992). Thus, some form of photoreceptor-related signaling may be responsible for the increased INL stem cell mitotic activity. In some model systems, Müller glial cells become proliferative subsequent to retinal damage, display gene expression patterns characteristic of neural progenitor cells and may be a source of the regenerated retinal neurons (Fischer et al., 2002; Fischer and Reh, 2001; Senut et al., 2004; Wu et al., 2001; Yurco and Cameron, 2005).

Previously, we demonstrated that intense light treatment of *albino* zebrafish causes photoreceptor cell death in the central region of the retina surrounding the optic nerve (Vihtelic and Hyde, 2000). Subsequent to the cell death, proliferating cells within the INL migrate to the ONL and differentiate into new rod and cone photoreceptors (Vihtelic and Hyde, 2000). The studies presented here were designed to further characterize the light-induced retinal changes in *albino* zebrafish. We demonstrate that while extensive photoreceptor cell death occurs in the central and dorsal retina, many rods and cones in the ventral retina survive the light treatment. Large numbers of apoptotic photoreceptor cells are identified by TUNEL in the central and dorsal retina, while very few TUNEL-positive cells are observed in the ventral region. In addition, PCNA and rhodopsin immunohistochemistry reveal the cell proliferation in the INL is greatest in the retinal regions that experience the highest levels of photoreceptor cell death, while cell proliferation in the ventral retina is mostly confined to the ONL. Furthermore, expression of the *α1-tubulin:egfp* transgene is activated in Müller glial cells located in the central and dorsal retina, but not in the ventral retina. These results demonstrate there are regional differences in the photoreceptor cell death and cell proliferation responses that precede regeneration in light-lesioned *albino* zebrafish. In this paradigm of retinal injury, the amount and location of cell proliferation corresponds to the extent of photoreceptor cell death, which suggests that the INL cell proliferation response may be stimulated in a graded fashion by different levels of photoreceptor cell damage.

2. Methods

2.1. Animals

Adult zebrafish (*Danio rerio*) were raised at the University of Notre Dame under normal facility lighting (250 lux light intensity) and a 14-hr light:10-hr dark cycle using standard techniques (Westerfield, 1993). Experiments utilized either *albino* mutant or *albino*; Tg*[α1-tubulin:egfp]* transgenic fish, which were all 7–9 months post-fertilization (Goldman et al., 2001; Vihtelic and Hyde, 2000). A total of 20 *albino* fish were used for the experiments (Table 1). All animals were treated according to protocols approved by the university animal use committee.

2.2. Light lesion protocol

Fish were kept in constant darkness for 14 days prior to exposure to the constant intense light. All light exposures

Table 1
Experimental protocols

Experiment descriptions	Total fish/retinas/ Sections examined	Sampling methods
Retinal morphometry (controls)	5/5/17	Total retinal and INL thickness, ROS length, number of ONL nuclei quantified in defined retinal regions
Retinal morphometry (3 days L Tx)	4/4/11	Total retinal and INL thickness, ROS length, number of ONL nuclei quantified in the different regions of light-lesioned retinas
Rhodopsin-(+) nuclei; 4 days L Tx	2/2/55	ONL rhodopsin-(+) cell bodies counted in the ventral, central, and dorsal retinal regions
TUNEL; 24 hrs L Tx	4/6/156	TUNEL-(+) cells counted in the ventral, central and dorsal retina
PCNA detection; 4 days L Tx	3/3/81	PCNA-(+) INL cell clusters (3 or more PCNA-(+) cells) and individual ONL PCNA-labeled cells quantified in each retinal region
PCNA detection; 7 days L Tx	3/2/73	INL PCNA-(+) cell clusters and individual ONL PCNA-labeled cells

(+), Positive; L Tx, light treatment; ONL, outer nuclear layer; INL, inner nuclear layer