

Genetics of photoreceptor degeneration and regeneration in zebrafish

Susan E. Brockerhoff · James M. Fadool

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Abstract Zebrafish are unique in that they provide a useful model system for studying two critically important problems in retinal neurobiology, the mechanisms responsible for triggering photoreceptor cell death and the innate stem cell-mediated regenerative response elicited by this death. In this review we highlight recent seminal findings in these two fields. We first focus on zebrafish as a model for studying photoreceptor degeneration. We summarize the genes currently known to cause photoreceptor degeneration, and we describe the phenotype of a few zebrafish mutants in detail, highlighting the usefulness of this model for studying this process. In the second section, we discuss the several different experimental paradigms that are available to study regeneration in the teleost retina. A model outlining the sequence of gene expression starting from the dedifferentiation of Müller glia to the formation of rod and cone precursors is presented.

Keywords Zebrafish · Photoreceptor · Degeneration · Regeneration · Retina

Photoreceptor degeneration

Retinal photoreceptors are the primary sensory cells responsible for detecting light. There are rods, responsible

for vision at night, and cones, responsible for color and daytime vision. These highly polarized neurons convert light information into an electrical signal that is ultimately transmitted to the vision detecting centers of the brain. The photoreceptor outer segment contains stacks of membranous discs in which the proteins involved in photodetection and transduction reside. The photoreceptor inner segment contains a high density of mitochondria to meet the intense energy demands of the cell, and it is also the site of protein production and sorting. The photoreceptor synapse contains a specialized structure known as a ribbon optimized for continual graded release of neurotransmitter (Fig. 1). The viability and maintenance of these specialized, compartmentalized neurons is critical for normal visual function. Thus, understanding the biology of normal and diseased photoreceptors is of fundamental importance.

The loss of photoreceptors through degeneration results in a devastating incurable form of blindness that represents a major fraction of inherited retinal disease. These disorders can affect either rods or cones individually, as in the case of retinitis pigmentosa or macular degeneration, respectively, or they can cause degeneration of both types of photoreceptors in diseases such as cone-rod dystrophy or Leber's congenital amaurosis. Defects in many different genetic loci are known to cause inherited photoreceptor degeneration, and unique mutations in a single gene can cause different degenerative disorders. These causal genetic loci encode proteins involved in all aspects of photoreceptor biology. For example, mutations in genes for proteins involved in phototransduction, protein transport, energy metabolism, outer segment formation, retinoid metabolism, RNA splicing, and gene transcription have all been identified as causing the various forms of photoreceptor degeneration found in humans (see RETNET for a

S. E. Brockerhoff (✉)
Department of Biochemistry, University of Washington,
357350, Seattle, WA 98195, USA
e-mail: sbrocker@uw.edu

J. M. Fadool
Department of Biological Science, Florida State University,
3018 Life Science Bldg, Tallahassee, FL 32306, USA
e-mail: jfadool@bio.fsu.edu

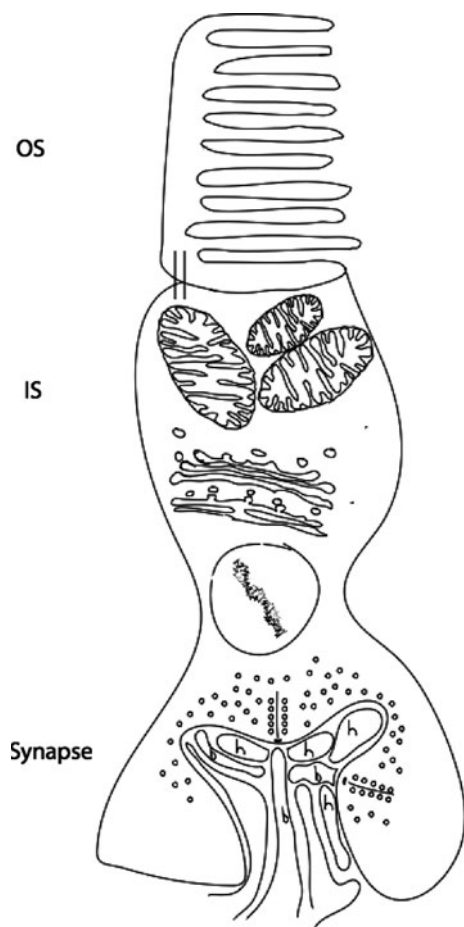


Fig. 1 Schematic of a cone photoreceptor. *OS* Outer segment, *IS* inner segment

comprehensive list of retinal disease-causing genes; <http://www.sph.uth.tmc.edu/Retnet/>).

Zebrafish are considered an outstanding model for studying photoreceptor degenerations. There are several distinct experimental advantages to the zebrafish model system. The ex utero rapid development, optical transparency, permeability to drugs, facile genetics, ease of making transgenics and mosaic animals, as well as the acute visual abilities of larvae, have secured zebrafish as a dominant model organism for studying the visual system. In addition, an exciting new development in recent years has been the identification of many gene mutations isolated from fish with photoreceptor degeneration phenotypes (Table 1). More than a dozen different genetic lesions causing photoreceptor degeneration are now known. These fish were identified in genetic screens that focused on identifying visual behavioral or morphology deficits, and subsequent cloning strategies have led to the identification of the mutated genes. The list provided in Table 1 presents the mutant name, causal gene, gene function and corresponding references. Importantly, this list only includes mutants with

known genetic mutations. The relevance of the zebrafish as a powerful model is further highlighted by the multiple categories of genes represented in this list. Proteins essential for phototransduction, protein/organelle transport, metabolism, and transcription have been isolated. Thus the molecular mechanisms triggering cell death due to unique primary lesions can be investigated using these zebrafish mutants. This provides a powerful scientific resource since the cascade of molecular events leading to cell death has not yet been determined in any organism even for a single mutation that causes photoreceptor degeneration.

To date, most of the photoreceptor degeneration mutants die within 2 weeks post-fertilization. This is not surprising since the majority of cloned mutants were isolated based on morphological criteria (Table 1). This indicates that these genes have multiple functions within the organism, and for some mutations the photoreceptor phenotype is at least partially a secondary consequence of defects in other cells. For example, in dynactin 1 and 2 mutants (*mok* and *ako*), the photoreceptor phenotype contains both cell-autonomous and non-cell-autonomous components [1–3]. Similarly, in *rep1* mutants, photoreceptor degeneration is caused by the presence of neighboring mutant retinal pigment epithelial cells [4]. In contrast, mutations isolated solely on the basis of visual behavioral defects are less likely to have pleiotropic effects. *pde6c* represents a gene in this category, and fish carrying mutations in this gene are viable as adults [5, 6]. Additional adult viable mutants with photoreceptor deficiencies were isolated in a large-scale behavioral screen [7], however, the genetic lesion responsible for the photoreceptor loss in these fish has not been determined. Behavioral screening strategies still represent the most efficient and likely successful strategy for identifying photoreceptor-specific lesions. The currently small number of behavioral mutations—the majority of which are single allele—indicates that many more mutations specifically affecting photoreceptor viability will be isolated in the future.

Many of the experimental advantages of the zebrafish for studying retinal degenerations are highlighted in a recent study using a cone-specific degeneration model with a mutation in the *pde6c* gene [8]. *pde6c* encodes a cone-specific version of phosphodiesterase essential for phototransduction in cone photoreceptors. A complete loss of Pde6c leads to the death of cone photoreceptors during the first week of zebrafish development [5]. An analogous mouse mutant containing a mutation in the rod-specific version of cGMP-phosphodiesterase has been available for decades, and yet the mechanism triggering apoptosis of photoreceptors due to phosphodiesterase deficiency is still unknown [9]. Using transgenic zebrafish expressing fluorescent reporter proteins in cones, photoreceptor cell death was visualized in real time in live *pde6c* mutant animals, and the sequence of morphological changes occurring

Table 1 Zebrafish photoreceptor degeneration mutants

Mutant name/ allele	Causal gene	Protein function	Phenotype	Gene/ mutation identification
<i>pob^{a1}</i>	Partial optokinetic response b; GeneID: 406826	Unknown	Red cone degeneration 4 dpf	[74]
<i>pde6c^{w59}</i> <i>els^{rw76a}</i> <i>ovl^{tz288b}</i>	Phosphodiesterase 6c; GeneID: 5146	Hydrolysis of cGMP during phototransduction	Cone degeneration 4 dpf onward	[5, 6]
<i>nrf1^{hi39a}</i>	Intraflagellar transport (Ift) 88; GeneID: 321855	Intraflagellar transport	Photoreceptor degeneration 4 dpf	[18]
<i>nrf1^{hi39a}</i>	Nuclear respiratory factor 1; GeneID: 64604	Transcription factor	Photoreceptor degeneration 3 dpf	[75]
<i>pwt^{hi1482}</i>	Tryptophan rich basic protein; GeneID: 335756	Unknown	Photoreceptor degeneration 2–3 dpf	[19]
<i>ift172^{hi2211}</i>	Ift172; GeneID: 432389	Intraflagellar transport	Photoreceptor degeneration 4 dpf	[19]
<i>ift57^{hi3417}</i>	Ift57; GeneID: 414929	Intraflagellar transport	Photoreceptor degeneration 4 dpf	[19]
<i>elipsa^{m649}</i> ; <i>elipsa^{lp49d}</i>	TNF receptor-associated factor 3 interacting protein 1; GeneID: 393572	Component of IFT particle B	Photoreceptor degeneration 4 dpf	[21]
<i>ako^{ji50}</i>	Dynactin 2 (dynamitin); GeneID: 394141	Intracellular organelle transport	Photoreceptor degeneration 3 dpf	[2]
<i>mok^{s309}</i> ; <i>mok^{m632}</i>	Dynactin 1a (p150); GeneID: 407638	Intracellular organelle transport	Photoreceptor degeneration 3 dpf	[1, 76]
<i>flr^{m477}</i>	Fleer, TPR repeat protein 30; GeneID: 797829	Tubulin polyglutamylolation	Photoreceptor degeneration 4 dpf	[20]
<i>syu^{td+/-}</i> ; <i>syu^{tbx+/-}</i>	Shha, sonic hedgehog a; GeneID: 30269	Maintenance/survival of the cones in the adult retina	Slow adult degeneration	[23]
<i>rep1^{ru848}</i>	Rab escort protein-1; GeneID: 402,935	Directs rab protein movement	Photoreceptor degeneration 4 dpf	[77]

dpf days post fertilization

during this process was categorized. Using two-photon microscopy, the authors found that the first sign of death was a retraction of synaptic processes within the outer plexiform layer. This was followed by shrinkage and collapse of the outer segment. These morphological changes provide the foundation for examining subcellular changes in fluorescent signaling molecules as photoreceptors are undergoing apoptosis.

The authors further exploit the ease of generating mosaic fish to examine whether mutant cone photoreceptors trigger neighboring wild-type (WT) cone death in a process known as bystander-associated cell death [8]. A well-known example of this is in the human disease retinitis pigmentosa (RP), where dying rods cause the secondary death of healthy cones [10]. The molecular mechanism causing the secondary death of healthy photoreceptors is unknown, and thus animal models are useful for studying this process [11]. For example, the underlying mechanism of cone death in rod dystrophies is actively being studied in the mouse [12]. Analysis in humans indicates that the extent of bystander cell death is not the same for all classes of retinal degeneration [13–15]. In

addition, the ability of dying cones to affect neighboring healthy cones is unknown. The cone dominance of the larval zebrafish retina made it possible using this model system to evaluate whether dying cones could cause the secondary death of neighboring healthy cones [8]. By generating zebrafish with heterogenous retinas containing mixtures of WT and *pde6c* mutant cones, parameters of transplant size and position were carefully evaluated for their influence on bystander-associated cone death. Surprisingly, although dying mutant cones could cause rod death in certain retinal regions [5], mutant cones did not adversely affect the viability of WT cones regardless of transplant size or position within the retina. This finding suggests that cones may be more likely to remain viable in transplant experiments aimed at rescuing vision in degenerating retinas. These findings with the *pde6c* mutant also suggest that this model may be useful for studying the ability of dying cones to cause neighboring WT rod death [5]. However, in contrast to the mammalian system, the ability of degenerating rods to cause neighboring WT cone death has not been observed in the zebrafish with the degeneration models that are currently available [16].

To date, the majority of mutants with photoreceptor degeneration have mutations in proteins critical for ciliary transport from the inner to the outer segment (*ovl*, *flr*, *ift57*, *ift172*, and *elipsa*). The process of ciliary transport is complicated, involving multiple large transport complexes and regulators, and this process is currently being actively studied by many investigators using a variety of model systems. A recent review nicely summarizes the current status of this field as it pertains to photoreceptor morphogenesis [17]. The zebrafish is contributing to this field through the identification of several essential components and through an analysis of genetic interactions between components.

The first cloned zebrafish mutation essential for ciliary transport of proteins to the outer segment was *ovl*, a gene encoding one of the intraflagellar transport proteins, IFT88 [18]. A subsequent rescreen of insertional mutants identified additional IFT components, IFT57 and IFT172, as causing photoreceptor degeneration [19]. Two other factors identified are the *fleer* gene, a modulator of tubulin post-translational modification [20], and most recently *Elipsa* [21], a polypeptide thought to link the IFT complex polypeptide IFT20 with Rabaptin 5, an endocytosis regulator. The ability to quickly analyze gene interactions using transient gene knockdown experiments has also been instrumental in connecting *elipsa* to *rabaptin 5* in ciliary transport [21]. All five of these mutants show deficiencies in outer segment formation accompanied by photoreceptor degeneration, and although the mutants have multiple defects, *fleer*, *elipsa*, and *ovl* are known to function cell autonomously in causing photoreceptor degeneration [22]. It is likely that IFT57 and IFT172 have cell autonomous photoreceptor functions as well. The abundance of mutations in this category and the many protein molecules involved in ciliary transport suggest that several additional genes in this pathway will be isolated using the zebrafish model system.

In addition to early-onset rapid degeneration, zebrafish offer the potential to study slower and late-onset degeneration. One study has evaluated photoreceptor abnormalities associated with postreproductive senescence due to sub-optimal levels of sonic hedgehog expression [23]. Sonic hedgehog (*shh*) is known to play an important role in zebrafish retinal development. *shh* regulates neurogenesis during embryonic development [24], and later, it regulates differentiation of retinal neurons, including photoreceptor cells [25]. In contrast, the role of *shh* in the adult retina, where it is also expressed, is unknown. Although lethal as a homozygous mutation, fish heterozygous for mutations in the sonic hedgehog gene survive to adulthood. By taking advantage of chronically reduced *shh* expression in heterozygous carriers, the investigators evaluated the effect of long-term *shh* deprivation [23]. Interestingly, by 2 years of

age dramatic disruptions in photoreceptor patterning and reductions in photoreceptor number were apparent in the heterozygous fish but not in control fish. These disruptions were detected with markers specific to blue, red or green cones. These studies highlighted both the continued role for sonic hedgehog in adult photoreceptor maintenance/renewal and the usefulness of zebrafish for studying degeneration in older fish.

Another strategy that is just starting to be exploited is the targeted expression of photoreceptor-specific genes in order to elicit degeneration. The identification of promoters to express genes specifically in rod photoreceptors [26], all cones [27], and cone subtypes [28, 29] makes these studies imminent. As an initial example of this type of study, the rod photoreceptor-specific promoter was used to derive expression of membrane-tagged cyan fluorescent protein to rod photoreceptors resulting in their apoptosis [16]. The combined use of photoreceptor-specific promoters with other knockout and gene expression strategies, reviewed in detail elsewhere [30–35], will provide additional experimental paradigms for studying the mechanisms of photoreceptor degeneration in both young and older retinas.

In summary, at least 13 mutations causing photoreceptor degeneration in zebrafish are now known. Many additional mutations causing degeneration will be identified through genetic screens and targeted gene knockout approaches. Transgenic approaches will enable investigators to examine hereditary eye disorders due to known mutations in photoreceptor specific genes. It is likely that zebrafish retinal disease models will continue to contribute significant new findings to the field of photoreceptor degeneration.

Regeneration

During the past decade, there has been tremendous resurgence in interest from both the scientific community and the general public in neural stem/progenitor cells and the prospect of novel therapies for trauma-induced injury and degenerative diseases of the retina. Several independent yet related lines of evidence have contributed to the optimistic outlook: the demonstration of continuous neurogenesis in discrete regions of the vertebrate retina, the ability to isolate and expand in culture cells with stem cell-like properties, and the demonstration that Müller glia can divide and give rise to neuroprogenitors [36–38]. In teleosts including zebrafish, mechanical damage, chemical toxicity, phototoxicity, and genetic lesions stimulated an increase in proliferation of cells in the inner nuclear layer (INL) and outer nuclear layer (ONL), regeneration of the damaged photoreceptors [39–42], and recovery of vision [43].

The regeneration of the retina shares features common to other regenerating tissues including the heart and fin [44]. To translate the recent advances into clinical treatments, three basic questions need to be answered more fully: What are the intrinsic properties of retinal stem cells? How do environmental signals direct regeneration of the photoreceptor cells? Can the intrinsic and extrinsic properties be manipulated to direct photoreceptor cell replacement? The regeneration of rod and cone photoreceptors in the teleost provides a model to systematically investigate the potential and the limitations of neural stem cells for replacement of lost photoreceptor cells.

Persistent neurogenesis and regeneration in the visual system of adult teleost fish have been valuable models of neural development. Zebrafish, like many teleosts, continue to grow throughout their life, and the increase in body mass is matched by an increase in the size of the eye and the area of the retina [45–49]. As the fish grows, new neurons, including photoreceptor cells, are added at the retinal margin by a population of mitotic progenitor cells located within the circumferential mitotic zone. The mitotic progenitors possess properties of stem cells, maintaining a balance between self-renewal and the generation of multipotent neuroblasts that differentiate into all classes of retinal neurons and glia. The temporal pattern of expression of transcription factors and signaling molecules in the retinal margin recapitulates a developmental program. The isolation of proliferative cells from the ciliary margin of rodents and identification of persistent, but limited neurogenesis at the margin of the hatchling chick retina suggest a conserved system, although further experimentation is warranted [36, 50–53].

Perhaps more relevant to this review is that, during postembryonic growth in teleosts, new rods are generated in the central retina from a second population of mitotic cells referred to as the rod progenitor lineage. As the retina is gradually stretched within the expanding optic cup, the addition of new rods maintains their constant density across the ONL, preserving scotopic sensitivity [45, 46, 49, 54]. Proliferating cells distributed across the ONL, termed rod precursors, were initially identified as the source of the newly generated rods [46, 47]. The rod precursors slowly divide followed by differentiation as rod photoreceptors. Subsequent studies using multiple injections of tritiated thymidine or exposure to thymidine analogues revealed groups of mitotically active cells arranged in radial arrays spanning the INL and ONL. In histological sections, these neurogenic clusters appeared to migrate along Müller glia. The authors proposed that the clusters of proliferating cells migrated to the outer retina and became the source of the rod precursors. The generation of functional photoreceptors from a population of mitotic precursors is precisely the goal of stem cell replacement therapy.

Several different models identified a subpopulation of Müller glia as the origin of both the rod progenitor lineage and the “neurogenic clusters” observed in regenerating fish retinas [55–61]. Müller glia–derived proliferating cells possess the capacity to regenerate virtually all classes of retinal neurons in teleosts. In the chick, the capacity of Müller glia regeneration is limited to small numbers of inner retinal neurons, and in the mouse, Müller glia proliferation and regeneration is even more severely limited [37, 38, 62, 63]. Double labeling experiments in zebrafish demonstrated that these proliferating cells colabel for definitive markers of Müller cells including glial fibrillary acid protein (GFAP), carbonic anhydrase, and glutamine synthetase. Barnados et al. [55, 64] used transgenic zebrafish in which regulatory elements of the zebrafish GFAP gene were used to drive expression of cytoplasmic or nuclear-targeted GFP specifically in Müller glia. The authors used the persistence of GFP fluorescence as a lineage tracer of cells derived from the Müller glia. Following BrdU labeling to identify mitotically active cells in the uninjured retina, the authors observed a small number of BrdU+ cells in the INL and ONL and some of those were GFP+. Following light damage, the number of mitotically active GFP+ cells increased considerably and could be traced as clusters extending from the INL to the ONL, where some expressed markers of differentiating photoreceptors. The authors concluded that the colabeled cells were dedifferentiated Müller glia and their progeny that migrate from the INL into the ONL, forming rod precursors and regenerated photoreceptors, respectively. It has also been demonstrated that constant intense light treatment of dark-adapted albino zebrafish selectively kills rod and cone photoreceptors in the central retina [41, 44], and induces approximately 50% of the Müller glia to colabel for mitotic markers such as PCNA [65]. Injection and electroporation of antisense morpholinos complementary to PCNA prior to retinal lesion led to a significant increase in the number of dying cells in the INL and reduced both the number of proliferating cells and the number of Müller glia in the region of the light damage [65]. These data suggest that, following retinal lesion, asymmetric cell division of Müller glia generates a mitotic progenitor that gives rise to the neurogenic cluster while maintaining a constant population of Müller glia.

The Müller glia–derived mitotic cells appear to initiate expression of retinal stem/progenitor cell markers, including *Ascl1a*, *Pax6*, *Rx1*, *Neurogenin1*, and *Chx10* [40, 66, 67]. *Ash1a*, a basic helix-loop-helix transcription factor and regulator of vertebrate neurogenesis, is expressed within 4 h post lesion, making it one of the earliest markers of the proliferating Müller glia. Knockdown of *Ascl1a* results in a reduction in divided cells following retinal lesions and inhibits regeneration, but unlike PCNA

knockdown, interfering with *Ascl1a* expression did not lead to cell death [40]. These data would suggest that activation of *Ascl1a* is an early step in the reprogramming of quiescent Müller glia. A putative downstream target of *Ascl1a* is *Pax6*, a paired box transcription factor with highly conserved roles in retinal and ocular development in species as diverse as flies and mammals. Previous studies in goldfish have shown that, in addition to expression by a subpopulation of inner retinal neurons in the mature retina, *Pax6* is expressed in a rare population of cells within the INL thought to be the source of the mitotic progenitors [68]. Following surgical removal of a region of the retina or after light lesion, *Pax6* immunoreactivity colocalized with BrdU in the regenerating tissue. As a vestige of the ancestral duplication of the teleost genome, zebrafish retained two *Pax6* co-orthologues, one encoded by the *pax6a* gene and the other encoded by the *pax6b* gene [67]. Knockdown of either *pax6a* or *pax6b* by intravitreal injection and electroporation of morpholinos that were complementary to either mRNA revealed subfunctionalization of the corresponding proteins during photoreceptor regeneration [67]. Loss of *Pax6b* expression did not affect Müller glial cell division but was required for subsequent division of the neuronal progenitors. Inhibition of the *Pax6a* protein blocked subsequent neuronal progenitor cell divisions and decreased the number of cells in the neurogenic cluster. Significantly fewer cones were identified in the *pax6b* and *pax6a/pax6b* double morphant retinas suggesting that INL proliferation is necessary for cone regeneration [67]. Based upon these and other results, a model of the sequence of gene expression leading from the activation of the Müller glia to the formation of photoreceptor precursors can be drawn (Fig. 2).

As regeneration progresses, the Müller-derived stem cells expressed numerous genes consistent with a photoreceptor developmental program. As the retinal progenitors migrate from the INL to the ONL, *pax6* expression is lost and other transcription factors are expressed, most notably the basic helix-loop-helix gene *neuroD* and the cone-rod homeobox gene *crx*. Similarly, cell signaling molecules of the Notch-Delta pathway and its downstream effectors are also expressed [66]. These data suggest that progenitor cells produced in the INL in response to acute injury pass through a series of intervening, less-committed states prior to adopting a photoreceptor cell fate. Ultimately, the glia-derived cells exit the cell cycle to differentiate as photoreceptors that integrate into the existing retinal circuitry and restore vision [43].

Whereas current work in numerous species has focused on the Müller cells and the INL stem cells, understanding the regulation of rod progenitor cell activity may offer an alternative avenue to investigate the fundamental processes of photoreceptor replacement. Two previous limitations of

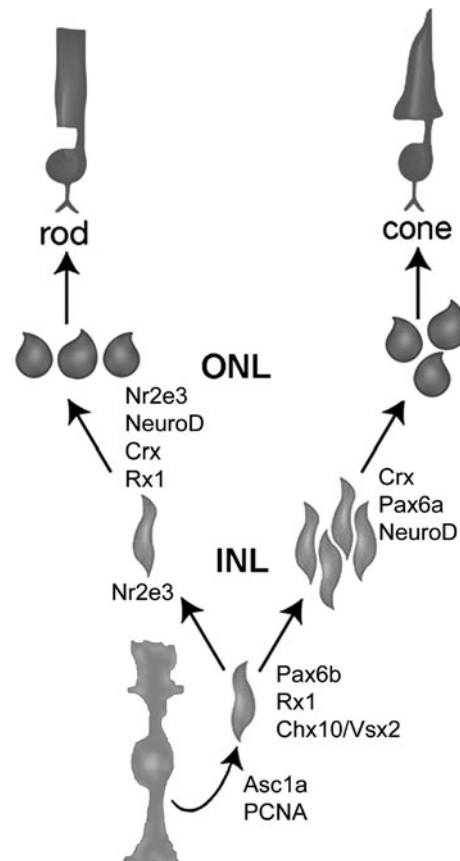


Fig. 2 A model for gene expression during persistent and injury- or degeneration-induced neurogenesis in the zebrafish central retina. Adapted from [58], see text for details. *ONL* Outer nuclear layer, *INL* inner nuclear layer

such studies have been that rod precursors are relatively few in number and no definitive marker is available. The generation of a transgenic line of zebrafish, the XOPS-mCFP line, which experiences selective degeneration of the rod photoreceptor cells due to the toxic effect of a rod-targeted fluorescent reporter gene [16], facilitates new analyses. This rod degeneration resulted in a loss of rod-mediated electrophysiological responses but did not cause any secondary cone pathology. It was, however, accompanied by a significant increase in rod progenitor mitotic activity [16]. Similar to cone regeneration, the transcription factors *NeuroD* and *Crx* are upregulated in cells in the ONL. But in contrast, expression of *nr2e3*, a rod determination gene that is expressed in mammals exclusively in postmitotic differentiating rod photoreceptors, was also upregulated, whereas expression of *pax6b* was not observed [16]. Immunolabeling of retinal cryosections with anti-BrdU showed no significant increase in INL BrdU+ cells or PCNA expression. The expression of genes with demonstrated roles in photoreceptor development, *neuroD*, *crx*, and *nr2e3*, and lack of expression of *pax6b* suggests an early commitment of the mitotic rod precursors to the rod cell

fate [16]. Supporting this conclusion, knockdown of both *pax6a* and *pax6b* resulted in increased numbers of rod precursors in the ONL and rod regeneration continued [67]. Therefore, it appears that the rod progenitors in the ONL maintain the capacity to respond to rod photoreceptor degeneration without relying on increased activity of progenitor cells in the INL or the Müller glia. These results are particularly interesting in light of a report that demonstrated that in mouse both mitotic retinal progenitors and postmitotic rod precursors could differentiate into rhodopsin-expressing cells upon transplantation into a mouse model of retinal degeneration, but only the postmitotic Nrl-positive cells could migrate to the appropriate position and integrate correctly into the host retina [69].

Recent work has begun to identify the signaling that regulates the neurogenic program in Müller glia and their derivatives *in situ*. Previous studies have implicated the growth hormone/insulin-like growth hormone I axis as a regulator of growth and mitotic activity of progenitors in the retina of goldfish, which may be more tightly associated with integrating numerous general features of overall growth and body size [70]. Phagocytosis of cellular debris stimulates mitotic activity and the regenerative potential of Müller glia in the light-damaged retina [71]. Based upon expression patterns following retinal lesion or from the perspective of a candidate gene approach, various molecules have been linked to specific aspects of the regenerative process. More recently, the zebrafish galectin, *Drgal1-L2*, has been shown to be expressed by Müller glia and neurogenic clusters [72]. Functional studies demonstrate that knockdown led to a decrease in the regeneration of rod photoreceptors but did not affect cone regeneration. Based upon studies in mammals, a neuroprotective effect of ciliary neurotrophic factor (CNTF) upon the light-lesioned zebrafish retina was observed [73]. Unexpectedly, intraocular injection of CNTF into undamaged eyes mimicked the increase in the number of proliferating Müller glia and neuronal progenitors typically observed following retinal lesions. The increase in mitotic activity was preceded by expression of Stat3, and knockdown of Stat3 expression in the CNTF-injected retinas significantly reduced the number of proliferating Müller glia [73]. Though these studies are only beginning, the results suggest an optimistic outlook for identifying compounds that stimulate resident cells to initiate a neurogenic program as an alternative strategy to stem cell replacement therapies.

In summary, investigations using the zebrafish have unequivocally identified Müller glia as a source of the regenerative cells in the neural retina and genes involved in the regenerative process. The existing genetic mutations leading to photoreceptor cell dystrophies and the isolation of new lesions as models of specific heritable disorders will be invaluable research tools to continue to unravel the

molecular and cellular pathways regulating the regenerative process. Yet, while significant progress has been made, new approaches are needed to translate these findings to specifically induce the proliferation of resident cells in the mammalian retina, engineer their fates, and direct their incorporation into an existing retinal circuit.

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