



● INVITED REVIEW

# Impacts of the retinal environment and photoreceptor type on functional regeneration

Michèle G. DuVal<sup>1</sup>, W. Ted Allison<sup>1,2,\*</sup>

<sup>1</sup> Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada

<sup>2</sup> Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada

**How to cite this article:** DuVal MG, Allison WT (2017) Impacts of the retinal environment and photoreceptor type on functional regeneration. *Neural Regen Res* 12(3):376-379.

**Open access statement:** This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

**Funding:** Grant support included MD/PhD studentships to MGD from Alberta Innovates Health Solutions and Canadian Institutes of Health Research, and operating funds to WTA were a Discovery Accelerator Grant from the Natural Sciences and Engineering Research Council of Canada.

## Abstract

Retinal regeneration is a promising central nervous system (CNS) target amongst the various stem cell therapy pursuits, due to its accessibility for manipulation and its disposition towards longitudinal monitoring of treatment safety and efficacy. We offer our perspective on current hurdles towards functional regeneration of cone photoreceptors. Cones are key: For patients suffering vision loss, cone photoreceptors are a required cellular component to restoring daytime vision, colour vision, and high acuity vision. The challenges of regenerating cones contrast with logistic challenges of regenerating rod photoreceptors, which underlines the importance of evaluating context in degeneration and regeneration studies. Foundational research is required to delineate the factors required to generate a diversity of cones in the human macula, and to coax both remaining and newly regenerating cones to rewire towards restoring daytime colour vision. A complex interplay between cell-intrinsic factors and the retinal environment determine both the specification of cone fates and the synaptic plasticity enabling their functional integration. Recent revelations that cellular materials are transferred amongst photoreceptor progenitors further emphasize the critical role of neighbouring cells in directing stem cell fates. From our vantage point, translation of stem cell therapies to restore the cone-rich human macula must be borne upon foundational research in cone-rich retinas. Research frameworks centered on patient outcomes should prioritize animal models and functional outputs that enable and report functional restoration of cone-mediated vision.

**Key Words:** zebrafish; synaptic plasticity; horizontal cells; material transfer; vision; neural development

## For Restoring Vision, the Retinal Environment and Photoreceptor Type Matter

Vision loss from retinal degenerations is increasingly prevalent and currently incurable. These degenerations are characterized by loss of photoreceptors, specialized neurons that detect light. Photoreceptors are intimately connected to the rest of the retina through intercellular communication and cell support mechanisms. The two main photoreceptor types are rods, which are highly sensitive to light and are needed for vision in low light conditions; and cones, which are less sensitive to light and are utilized in the bright light of daytime. Cones are further divided into subtypes based on maximal wavelength sensitivity; combinations of inputs from multiple cone types enable colour discrimination. For vision restoration the fovea is an important target - it is a region of human and other primate retinas containing exclusively cones, and is responsible for daytime, high acuity, and colour vision. Therefore, the ultimate goal is to restore or preserve function of the fovea and surrounding macula, which requires not just a sufficient number of cones, but also their survival, integration, and function within this unique retinal environment. Hence in modeling vision disease,

regeneration, and restoring function, more emphasis must be placed on the effects of 1) the surrounding retinal environment, and 2) the photoreceptor type, specifically cones and cone subtypes. In these endeavours, we suggest that diverse vision models would prove powerful complements to existing ones. The zebrafish retina especially can mimic the macula, because it contains a dense, even distribution of cones. Zebrafish have numerous additional advantages such as conserved eye development, genetic tools, and robust regeneration (Bibliowicz et al., 2011; Gemberling et al., 2013).

It is important to consider the retinal environment and how it affects photoreceptors. The retina is specialized to suit the unique visual environment of an organism, based on factors like circadian activity (e.g., diurnal, nocturnal) and vital visual stimuli (e.g., color sensitivity, motion detection). The composition and connectivity of photoreceptor populations are complex and variable among vertebrates, necessitating the study of multiple models. In this regard, the nocturnal rod-dominated mouse retina, a relevant system for mammalian genetics and retinal biology (but insufficient in cones), should be complemented with others such as the diurnal cone-dominated zebrafish retina, which mimics the human macula (**Figure 1**). Furthermore, the retinal environment's

**\*Correspondence to:**  
W. Ted Allison, Ph.D.,  
ted.allison@ualberta.ca.

**orcid:**  
0000-0002-8461-4864  
(W. Ted Allison)

**doi:** 10.4103/1673-5374.202930

**Accepted:** 2017-03-02

response to photoreceptor loss is an emerging question relevant to photoreceptor biology, including disease. The murine and zebrafish retinas have several intriguing contrasts, including genetic regulation of photoreceptor specification (Kim et al., 2016), responses to degeneration, and synaptic plasticity, which differentially influence rods and cones during photoreceptor loss and re-introduction. Thus we are interested in how cone subtypes regenerate, and how the retinal environment influences them. Indeed, established and emergent data indicate the retinal environment influences photoreceptor fate and survival. The diversity of retinal responses to photoreceptor loss and/or introduction can be appreciated in the contexts of disease or damage, and of an organism's life history. In many of these cases, the mechanisms are not well understood.

### Responses to Photoreceptor Loss Depend on the Retinal Environment and Photoreceptor Type

Across vertebrate species, diversity in photoreceptor development and composition (e.g., variation in rod-to-cone population ratios) underlines how photoreceptors are influenced by their environments. Surveying multiple vision models, one quickly develops an appreciation that different retinas show biases toward the development, survival, and integration of certain photoreceptor types (often comparing rods and cones, but also subtypes of cones), and have different responses to damage and disease; therefore the context of the environment should be considered when investigating photoreceptor loss and regeneration. Photoreceptor biology has been extensively studied in murine and zebrafish retinas, two complex and complementary vision models.

The mouse retina is biased to the survival and integration of primarily rods from donor precursors, suggesting mechanisms to maintain a rod-dominated milieu. Implantation of Crx<sup>+</sup> photoreceptor precursors (which have potential to become either rods or cones) into wildtype retinas results in the majority becoming rods. However, cone fate adoption is slightly higher when Crx<sup>+</sup> precursors are implanted into cone-deficient *Gucy2e*<sup>-/-</sup> retinas, though not above cone: rod population ratios seen in wild type retinas (Lakowski et al., 2010). In other words, the murine retina strongly regulates photoreceptor populations toward a rod majority and a cone minority, suggesting, essentially, that environmental context matters. The zebrafish promises to expand on this hypothesis because zebrafish retinas have both a high density of cones and a large variety, with four major cone types organized in a precise mosaic pattern (compared to two types in mice and three in humans) (Figure 1). The zebrafish retina is especially attractive for its regenerative capacity; the coordination of inflammation, neuroprotection, and latent pluripotency of Müller glia is fascinatingly effective and robust, though these topics are beyond the scope of this paper (Ahmad et al., 2011; Gorsuch and Hyde, 2014; Hippert et al., 2015; Powell et al., 2016). Adding to the wealth of literature documenting zebrafish retinal regeneration, we reported differential responses based on the photoreceptor type ablated, implicating

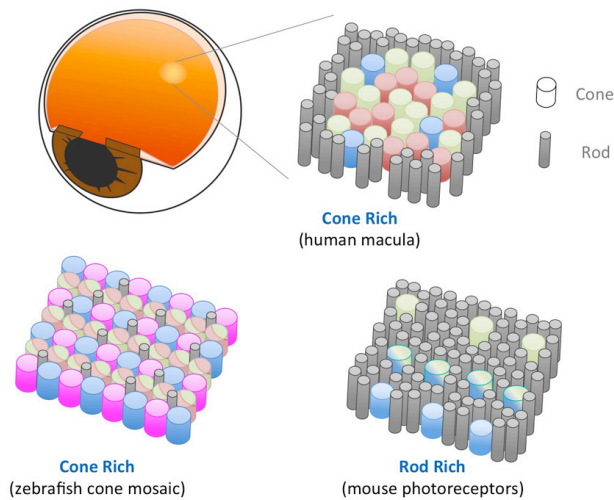
a regeneration process that is responsive to context. Following ultraviolet (UV) cone-specific ablation the regenerative response is shifted to produce proportionately more UV cones (Fraser et al., 2013), perhaps in order to restore cone subtype ratios (Figure 2). Thus in contrast to the rod-permissive/cone-restrictive murine retina, the zebrafish retina promotes a large and strictly-proportioned cone population in development and regeneration.

In zebrafish, ablation of UV cones results in regeneration with increased production of UV cones, indicating the retina (or a subset of cells therein) can, in some manner, detect the loss of specific photoreceptor types and drive pluripotent cells toward that identity (Fraser et al., 2013). In rainbow trout a similar process occurs as part of their life history; elevated thyroid hormone levels cause UV cones to disappear during preparation for migration. The subsequent return and reintegration of UV cones later in life suggests that the retinal environment retains the capacity to generate new UV cones and functionally integrate them (Allison et al., 2006a, b). Both fish systems also support the notion that the cell types lost, or the types that remain in the retinal environment, influence what photoreceptor types regenerate (Sherpa et al., 2014). Teleosts' regenerative capacity and precise response to loss of specific neural types continue to be a source of keen investigation and hope for use in the therapeutic realm.

Recent insightful work, such as material transfer between donor and host photoreceptors in mouse models of retinal degenerations, suggests unforeseen influences of the retinal environment (Ortin-Martinez et al., 2016; Pearson et al., 2016; Santos-Ferreira et al., 2016; Singh et al., 2016). Exchanging material such as RNA between cells may be a pro-survival mechanism, possibly explaining past implantations of donor cells that resulted in vision improvements, despite low integration rates. Pearson et al. (2016) suggest that donor cells may transfer vital components, helping host photoreceptors survive and thus preserving function (in contrast to restoring it). If so, implanting healthy cells may instead prove valuable for photoreceptor survival as part of overall therapeutic strategy. Recovery of visual function without photoreceptor replacement in zebrafish may be another mechanism to preserve function independently of regeneration, possibly through altering inputs from remaining cones or other forms of synaptic plasticity (Hagerman et al., 2016). Moving forward, experiments that compare different environments, rods to cones, and cone subtypes will further illuminate their influence in vision restoration.

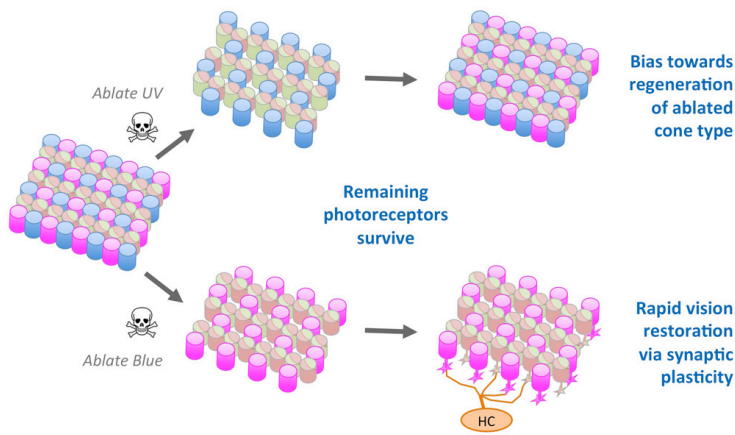
### Synaptic Plasticity in Photoreceptor Degeneration and Regeneration

Synaptic changes among photoreceptors and interneurons are prevalent during photoreceptor loss and regeneration. The establishment, patterns, and plasticity of synaptic connections are critical for visual function and are just beginning to be described. In our and others' work comparing blue and UV cones in zebrafish, we can already see that retinal interneuron synaptic development is influenced by photoreceptor identity. We also see synaptic plasticity in photoreceptor degeneration



**Figure 1 Diversity in photoreceptor composition among different retinal environments.**

The retina is a thin neural tissue that lines the inside of the eye, as shown through a cutaway in a schematized eyeball. In humans, the macula is a region of the retina that is very rich in cones, including blue-, green-, and red-sensitive cone types. The cones of the macula are responsible for daytime photopic vision, color discrimination, and visual acuity, thus the macula is an important target for vision restoration. The zebrafish and murine retinas are well-studied examples of cone rich and rod rich respectively, exemplary of a spectrum of retinal environments. Zebrafish have a high and even density of cones across the entire retina, with rods dispersed throughout. Zebrafish cones are arranged in a row mosaic where rows of ultraviolet and blue cones (colored purple and blue) alternate with rows of red and green cones (red and green, shown in double cone morphology). The mouse retina has a high density of rods and low density of cones. Mouse cones express green-sensitive opsin in the dorsal retina, blue-sensitive opsin in the ventral retina, and co-express both in the central retina. Rods are depicted as long grey bodies; cones depicted as stout bodies. In photoreceptor schematics, dorsal is towards the top.



**Figure 2 Effects of photoreceptor identity on retinal regeneration and functional vision restoration.**

Both cellular regeneration and the kinetics of synaptic plasticity are differentially affected by the identity of the photoreceptor that is lost. Loss of ultraviolet (UV) cones in zebrafish *via* pharmacogenetic ablation biases the retina to regenerate UV cones, resulting in restoration of their position and abundance. Alternatively when blue cones are ablated, a robust and rapid restoration of visual function occurs through synaptic plasticity. Meanwhile, the remaining photoreceptor types survive, likely through many pro-survival mechanisms. Thus multiple mechanisms act in response to cone loss, and the identity of the cone type lost (or perhaps the type[s] that remain) affects the mechanisms employed for regeneration and restoration of function. Based on these inferences, the need to consider photoreceptor types during regeneration strategies is apparent: regeneration, survival, and re-wiring of cones into the cone-rich human macula obliges use of appropriately cone-dominant model systems. HC: Horizontal cell.

and regeneration, and that it is subject to influences of the retinal environment within these contexts.

After blue cone ablation in zebrafish, blue cone-dependent colour discrimination is restored before regeneration, but this is not seen when UV cones are ablated (Figure 2) (Hagerman et al., 2016). H3 horizontal cells display a preference to synapse with UV more than blue cones, and do not synapse with red or green cones. This preference persists when UV cones are specifically ablated— H3 cells preferentially synapse with newly-generated UV cones (though this response can be modified under certain conditions— again, the environment matters!) (Yoshimatsu et al., 2014, 2016). Again, from these findings we infer that the zebrafish retinal environment is biased toward re-establishing a cone-rich population with specific ratios of subtypes. Next, we infer that retinal plasticity occurs to integrate new cones, and restores a connectivity pattern that discriminates among subtypes. The plasticity of the retina can restore function before regeneration depending on the cone type lost, apparently using multiple mechanisms to correct for different insults. These differential responses to cone loss underpin the need to conduct focused studies on cones and each cone subtype in turn.

Among other considerations, the retinal environment affects both surviving and new photoreceptors, including their

connections to interneurons. The teleost retina responds to photoreceptor damage using both regeneration and synaptic plasticity, based on such information as the identities of lost cells and the availability of remaining ones. These responses sharply contrast the mammalian retina, in which regeneration is not found, and photoreceptor degeneration causes diverse changes in synaptic plasticity, often with deleterious results (Jones et al., 2012; D’Orazi et al., 2014).

### Dissecting the Mechanisms behind Recovery of Visual Function

Moving forward, studying visual function and restoration will require rigorous examination of underlying cellular mechanisms. Showing, *via* electroretinogram and visual behavioral assays, the successful preservation or recovery of visual function is a powerful measure of efficacy. More often though, little or ambiguous change may be found on these assays, leaving investigators without indication where, on the road to visual function, any changes may have occurred. Assays that report on more discrete steps toward visual function would prove advantageous. Interrogating, at the cellular level, changes in photoreceptor death, survival, replacement, function, and connectivity would bridge this

gap. We showed that the optimized optomotor response can be sensitive enough to detect changes in synaptic function following loss of specific photoreceptors in zebrafish, but the mechanisms require further investigation (Hagerman et al., 2016). Characterization of H3 horizontal cells provides important starting points for interrogating connectivity and plasticity among cone subtypes (Yoshimatsu et al., 2016). A non-exhaustive list of approaches to assess photoreceptor function is provided here as food for thought.

Methods to measure neuronal activity, used successfully in other neuron types, could be utilized in the retina. Calcium-sensitive compounds and genetic tools to measure *in vivo* activity, such as CaMPARI, offer a peek into active, intact neuronal systems (Grienberger and Konnerth, 2012; Fosque et al., 2015). Functional photoreceptors respond to changes in light stimuli, which may be measurable by exploiting well-known processes such as byproducts of phototransduction. Additionally, photoreceptors must communicate with downstream neurons *via* synaptic transmission. The preservation or novel formation of synapses and the release of neurotransmitters with tools like genetically encoded transmitter indicators (GETIs) (Lin and Schnitzer, 2016) can provide context for changes on scotopic or photopic ERG, or alterations in colour sensitivity. Additionally, changes in the release of neuromodulators in response to changes in photoreceptor types or numbers would be of great interest. Horizontal cells mediate lateral inhibition and exhibit plasticity in response to changes in the environment, such as light conditions and low availability of a preferred photoreceptor type (Yoshimatsu et al., 2014).

To expand our understanding of vision biology, and to develop effective vision restoration therapies, it is becoming increasingly important to study diverse retina types. The retinal environment shapes the composition and function of the photoreceptors, and it influences regenerative capacity and synaptic plasticity. Therefore, as we learn more about photoreceptor death, survival, and regeneration, it is important to include 1) the retinal environment, and 2) the photoreceptor type as two important contexts in which to frame questions and experiments. To develop effective therapies for restoring human sight, clinical outcomes for models should especially emphasize repairing photopic vision, colour vision, and visual acuity, all of which are mediated by cones. To these ends a diversity of photoreceptor models is powerful, and to advance cone biology in particular, we need to work with cone-rich models such as the zebrafish.

**Acknowledgments:** We apologize to several colleagues whose important work could not be properly acknowledged herein as a result of space constraints.

**Author contributions:** Manuscript was written and edited by MGD, and was conceived of and edited by WTA.

**Conflicts of interest:** None declared.

## References

- Ahmad I, Del Debbio CB, Das AV, Parameswaran S (2011) Muller glia: a promising target for therapeutic regeneration. *Invest Ophthalmol Vis Sci* 52:5758-5764.
- Allison WT, Veldhoen KM, Hawryshyn CW (2006a) Proteomic analysis of opsins and thyroid hormone-induced retinal development using isotope-coded affinity tags (ICAT) and mass spectrometry. *Mol Vis* 12:655-672.
- Allison WT, Dann SG, Veldhoen KM, Hawryshyn CW (2006b) Degeneration and regeneration of ultraviolet cone photoreceptors during development in rainbow trout. *J Comp Neurol* 499:702-715.
- Bibliowicz J, Tittle RK, Gross JM (2011) Toward a better understanding of human eye disease insights from the zebrafish, *Danio rerio*. *Prog Mol Biol Transl Sci* 100:287-330.
- D'Orazi FD, Suzuki SC, Wong RO (2014) Neuronal remodeling in retinal circuit assembly, disassembly, and reassembly. *Trends Neurosci* 37:594-603.
- Fosque BF, Sun Y, Dana H, Yang CT, Ohshima T, Tadross MR, Patel R, Zlatic M, Kim DS, Ahrens MB, Jayaraman V, Looger LL, Schreier ER (2015) Neural circuits. Labeling of active neural circuits in vivo with designed calcium integrators. *Science* 347:755-760.
- Fraser B, DuVal MG, Wang H, Allison WT (2013) Regeneration of cone photoreceptors when cell ablation is primarily restricted to a particular cone subtype. *PLoS One* 8:e55410.
- Gemberling M, Bailey TJ, Hyde DR, Poss KD (2013) The zebrafish as a model for complex tissue regeneration. *Trends Genet* 29:611-620.
- Gorsuch RA, Hyde DR (2014) Regulation of Müller glial dependent neuronal regeneration in the damaged adult zebrafish retina. *Exp Eye Res* 123:131-140.
- Grienberger C, Konnerth A (2012) Imaging calcium in neurons. *Neuron* 73:862-885.
- Hagerman GF, Noel NC, Cao SY, DuVal MG, Oel AP, Allison WT (2016) Rapid recovery of visual function associated with blue cone ablation in zebrafish. *PLoS One* 11:e0166932.
- Hippert C, Graca AB, Barber AC, West EL, Smith AJ, Ali RR, Pearson RA (2015) Muller glia activation in response to inherited retinal degeneration is highly varied and disease-specific. *PLoS One* 10:e0120415.
- Jones BW, Kondo M, Terasaki H, Lin Y, McCall M, Marc RE (2012) Retinal remodeling. *Jpn J Ophthalmol* 56:289-306.
- Kim JW, Yang HJ, Oel AP, Brooks MJ, Jia L, Plachetzki DC, Li W, Allison WT, Swaroop A (2016) Recruitment of rod photoreceptors from short-wavelength-sensitive cones during the evolution of nocturnal vision in mammals. *Dev Cell* 37:520-532.
- Lakowski J, Baron M, Bainbridge J, Barber AC, Pearson RA, Ali RR, Sowden JC (2010) Cone and rod photoreceptor transplantation in models of the childhood retinopathy Leber congenital amaurosis using flow-sorted Crx-positive donor cells. *Human Mol Genet* 19:4545-4559.
- Lin MZ, Schnitzer MJ (2016) Genetically encoded indicators of neuronal activity. *Nat Neurosci* 19:1142-1153.
- Ortin-Martinez A, Tsai EL, Nickerson PE, Bergeret M, Lu Y, Smiley S, Comanita L, Wallace VA (2016) A reinterpretation of cell transplantation: GFP transfer from donor to host photoreceptors. *Stem Cells* doi:10.1002/stem.2552.
- Pearson RA, Gonzalez-Cordero A, West EL, Ribeiro JR, Aghaizu N, Goh D, Sampson RD, Georgiadis A, Waldron PV, Duran Y, Naeem A, Kloc M, Cristante E, Kruczek K, Warre-Cornish K, Sowden JC, Smith AJ, Ali RR (2016) Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors. *Nat Commun* 7:13029.
- Powell C, Cornblath E, Elsaedi F, Wan J, Goldman D (2016) Zebrafish Muller glia-derived progenitors are multipotent, exhibit proliferative biases and regenerate excess neurons. *Sci Rep* 6:24851.
- Santos-Ferreira T, Llonch S, Borsch O, Postel K, Haas J, Ader M (2016) Retinal transplantation of photoreceptors results in donor-host cytoplasmic exchange. *Nat Commun* 7:13028.
- Sherpa T, Lankford T, McGinn TE, Hunter SS, Frey RA, Sun C, Ryan M, Robison BD, Stenkamp DL (2014) Retinal regeneration is facilitated by the presence of surviving neurons. *Dev Neurobiol* 74:851-876.
- Singh MS, Balmer J, Barnard AR, Aslam SA, Moralli D, Green CM, Barnea-Cramer A, Duncan I, MacLaren RE (2016) Transplanted photoreceptor precursors transfer proteins to host photoreceptors by a mechanism of cytoplasmic fusion. *Nat Commun* 7:13537.
- Yoshimatsu T, Williams PR, D'Orazi FD, Suzuki SC, Fadool JM, Allison WT, Raymond PA, Wong RO (2014) Transmission from the dominant input shapes the stereotypic ratio of photoreceptor inputs onto horizontal cells. *Nat Commun* 5:3699.
- Yoshimatsu T, D'Orazi FD, Gamlin CR, Suzuki SC, Suli A, Kimelman D, Raible DW, Wong RO (2016) Presynaptic partner selection during retinal circuit reassembly varies with timing of neuronal regeneration in vivo. *Nat Commun* 7:10590.