Review

Restoration of Cone Photoreceptor Function in Retinitis Pigmentosa

Henry J. Kaplan[1], Wei Wang[1], and Douglas C. Dean[1,2]

[1] University of Louisville, Department of Ophthalmology and Visual Sciences, Louisville, KY, USA
[2] University of Louisville, Molecular Targets Program, James Graham Brown Cancer Center, Louisville, KY, USA

Correspondence: Henry J. Kaplan, Evans Professor of Ophthalmology, Chair, Ophthalmology & Visual Sciences, University of Louisville, Louisville, KY, USA. e-mail: hjkapl01@louisville.edu

Copyright 2017 The Authors

The major cause of hereditary blindness in North America is retinitis pigmentosa (RP), which is a group of inherited diseases of the retina characterized by the onset of night blindness, the early loss of the peripheral visual field, and the late loss of central vision. In the late stage of the disease when retinal degeneration approaches the macula and cone degeneration ensues, most patients find themselves with a profoundly incapacitating visual handicap because of the loss of central vision which precedes this stage of the disease, is also debilitating (e.g., for driving motor vehicles and reading). If therapy can prevent or reverse the onset of cone degeneration within the macula most patients would be immeasurably helped and able to live a normal life despite the loss of peripheral vision and decreased dark adaptation.

Over 67 mutant genes (www.sph.uth.tmc.edu/RetNet/home.htm) have been identified in monogenic forms of RP, all leading to generally similar photoreceptor pathology. Some of these mutations arise in genes, such as *RHO* that play a direct role in rod visual transduction, and *ROM1* that contribute to visual pigment-rich outer segment (OS) structure. But, other mutations arise in seemingly unrelated pathways including metabolism and RNA processing. And, even rod-autonomous mutations in retinal pigment epithelial (RPE)-specific genes can give rise to RP. How these disparate target genes expressed in both rods and RPE are linked into a seemingly common RP-sensitive pathway remains a crucial unanswered question including the loss of a high-resolution central cone vision in end-stage RP. It is still unclear why cones lose function as rods began to die during RP progression.

Mutant *RHO* becomes trapped in the endoplasmic reticulum (ER) and the rods undergo apoptosis.[1–3] With rod degeneration, cone photoreceptors begin to lose function. Diminished cone function is highlighted by loss of functional structures, including visual pigment-rich OS and mitochondrial–rich inner segments (IS).[4–6] Importantly, in contrast to rod cell bodies that die during rod photoreceptor degeneration there is long-term persistence of cone cell bodies after cone photoreceptor degeneration with little more than residual cone nuclei in RP patients, referred to as cone dormancy[6–10] (Fig. 1).

Photoreceptors are among the most metabolically active cells, and like other neurons, depend on glucose,[11] which is thought to be critical not only for energy production but for OS synthesis.[12] The importance of glycolysis in both rod and cone photoreceptors can be illustrated by inhibition of the essential glycolytic pathway enzyme glyceraldehyde 3-phosphate dehydrogenase with iodoacetic acid[13] and enhancing photoreceptor glycolysis by mutation of *Sirt6*[14] or overexpression of rod-derived cone viability factor (RdCVF), which promotes glucose uptake into cones to enhance glycolysis.[15] Following a glycolytic block, both rods and cones rapidly lost OS. As the glycolytic block diminished, dormant cones resumed OS synthesis and function, but rods failed to do so and died.[13] By contrast,

A.

B.
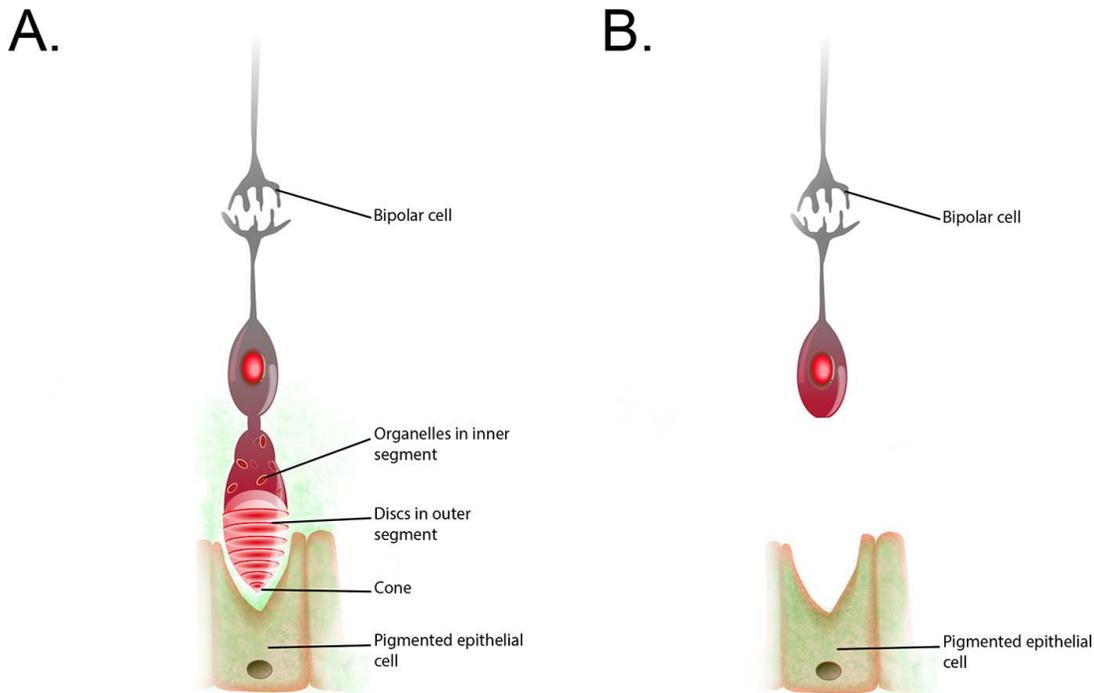
Figure 1. Cone dormancy is the degenerative stage cone photoreceptors (A) enter following rod photoreceptor death and results in loss of central vision. Cone dormancy (B) results following degeneration of COS, followed by the subsequent disassembly of CIS so that only a viable cone nucleus remains. COS, cone outer segment; CIS, cone inner segment.

enhancing glycolysis in both rods and cones by *Sirt6* mutation delayed rod death and loss of cone function in a mouse RP model, and enhancing glycolysis selectively in cones by early viral overexpression of RdCVF delayed loss of cone function in mouse RP.[14,15] Taken together, these studies demonstrate both rods and cones depend upon glycolysis for OS synthesis. Rod viability is dependent upon glycolysis, but cones survive a block in glycolysis and persist in a functionless state lacking OS.[16] Early enhancement of photoreceptor glycolysis can both delay mutant rod death and loss of cone function in RP mice, but neither *Sirt6* mutation nor early RdCVF overexpression ultimately prevented loss of photoreceptor function in RP, and cone function was not restored by such photoreceptor glycolysis-promoting therapies after it was lost.

Taken together, these findings suggest that cone dormancy in end-stage RP might be driven by starvation for glucose. In support of this possibility, we found that glucose-responsive genes were downregulated in cones as they began to lose their OS during RP progression.[16,17] And, injection of glucose into the subretinal space transiently restored cone expression of these glucose-responsive genes, OS

synthesis and the photopic electroretinogram (ERG). Taken together, these in vivo results provided evidence that cone loss of OS synthesis and function during RP progression is indeed somehow linked to glucose starvation.

We previously compared uptake of circulating glucose into photoreceptors in RP mice and wild-type (WT) littermates. Glucose from the choroid circulation is transported to the RPE and then into the subretinal space, where it is taken up by photoreceptors in the outer retina via the glucose transporter Glut1 on their IS.[12] By contrast, glucose is transported directly to the inner retina through branches of the central retinal artery. Fluorescently labeled 2-deoxyglucose was injected into the tail vein of RP and WT littermates, and uptake into eye muscles, the inner and outer retina, and RPE was examined in tissue sections. WT mice showed glucose uptake into photoreceptor inner segments (IS) and cell bodies as well as the inner retina and eye muscles, but little glucose was evident in the RPE.[16] Like WT mice, RP mice also showed glucose uptake into the inner retina and eye muscles, but glucose became high in the RPE and diminished in photoreceptors as RP mice began to show rod OS shortening.[16] We concluded glucose
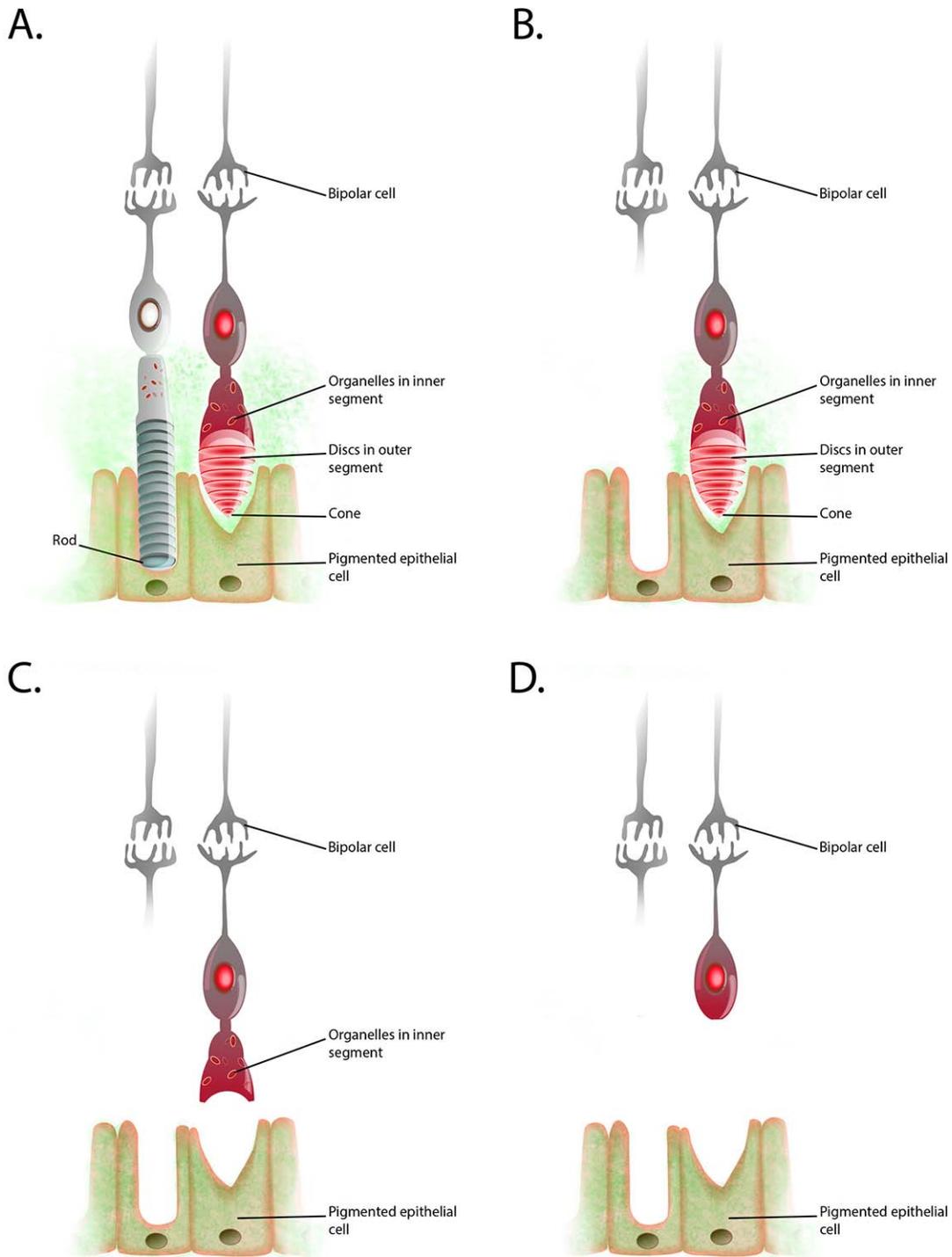
Figure 2. Cone dormancy in RP results in viable cone nuclei for many years after OS/IS degeneration. (A) Glucose (*green dots*) transport to the subretinal extracellular space and cone IS is essential to provide energy for regeneration of cone OS. (B) Degeneration of ROS and loss of contact with apical microvilli results in entrapment of glucose in RPE. Ensuing glucose starvation of cone photoreceptors results in OS degeneration (C) and IS disassembly but with maintenance of viable cone nuclei (D). ROS, rod outer segment.

delivery to eye muscles and the inner retina is unaffected in RP; however, with the onset of rod OS shortening, glucose becomes sequestered in the RPE and is no longer transported to photoreceptors leading to their starvation (Fig. 2).

To experimentally examine the effect of rods on cone function in RP, WT rod precursors were transplanted into RP pigs after mutant rods were lost. We found that these WT rods were able to restore induction of glucose-responsive genes in cones, OS synthesis and the photopic ERG. This effect of the transplanted rods on cone function correlated with their ability to generate OS following transplant. But, interestingly, it did not require integration of the transplanted rods into the outer nuclear layer (ONL).[16] Because the effect of the transplanted rods was linked to their OS generation, but not to cell integration into the ONL, we asked whether injection of WT rod OS alone might mimic transplanted rods in restoration of glucose transport from the RPE to photoreceptors in RP. Indeed, injection of WT rod OS into the subretinal space triggered glucose transport from the RPE to photoreceptors in RP mice (Wang W, et al. *IOVS* 2017;58:ARVO E-Abstract 3028). We then concluded that contact of abundant WT rod OS with the RPE is somehow triggering glucose release from the RPE for uptake by photoreceptors. Because glucose from the RPE must be released into the subretinal space for uptake by Glut1 on photoreceptor IS, which are distal to the RPE, it is reasonable that the process required for new OS tip synthesis would be tightly coupled to onset of the daily OS tip phagocytosis cycle, initiated by OS contact with the RPE. We reason that therapies such as *Sirt6* mutation or RdCVF overexpression aimed at enhancing glycolysis in photoreceptors ultimately fail to maintain photoreceptor function as RP progresses because the underlying defect in photoreceptor glycolysis is progressive retention of glucose in the RPE. The signaling pathway(s) potentially linking OS phagocytosis to glucose transport to photoreceptors is then of keen interest, and might provide new therapeutic targets in RP.

Acknowledgments

References

1. Roof DJ, Adamian M, Hayes A. Rhodopsin accumulation at abnormal sites in retinas of mice with a human P23H rhodopsin transgene. *Invest Ophthalmol Vis Sci*. 1994;35:4049–4062.

2. Price BA, Sandoval IM, Chan F, et al. Mislocalization and degradation of human P23H-rhodopsin-GFP in a knockin mouse model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2011;52:9728–9736.

3. Sakami S, Maeda T, Bereta G, et al. Probing mechanisms of photoreceptor degeneration in a new mouse model of the common form of autosomal dominant retinitis pigmentosa due to P23H opsin mutations. *J Biol Chem*. 2011;286: 10551–10567.

4. Cai CX, Locke KG, Ramachandran R, Birch DG, Hood DC. A comparison of progressive loss of the ellipsoid zone (EZ) band in autosomal dominant and x-linked retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2014;55:7417–7422.

5. Litts KM, Messinger JD, Freund KB, Zhang Y, Curcio CA. Inner segment remodeling and mitochondrial translocation in cone photoreceptors in age-related macular degeneration with outer retinal tabulation. *Invest Ophthalmol Vis Sci*. 2015;56:2243–2253.

6. Wong F, Kwok SY. The survival of cone photoreceptors in retinitis pigmentosa. *JAMA*. 2016;134:249–250.

7. Lin B, Masland RH, Strettoi E. Remodeling of cone photoreceptor cells after rod degeneration in rd mice. *Exp Eye Res*. 2009;88:589–599.

8. Busskamp V, Picaud S, Sahel JA, Roska B. Optogenetic therapy for retinitis pigmentosa. *Gene Ther*. 2012;19:169–175.

9. Busskamp V, Krol J, Nelidova D, et al. miRNAs 182 and 183 are necessary to maintain adult cone photoreceptor outer segments and visual function. *Neuron*. 2014;83:586–600.

10. Sahel JA, Leveillard T, Picaud S, et al. Functional rescue of cone photoreceptors in retinitis pigmentosa. *Graefes Arch Clin Exp Ophthalmol*. 2013; 251:1669–1677.

11. Hurley JB, Lindsay KJ, Du J. Glucose, lactate, and shuttling of metabolites in vertebrate retinas. *J Neurosci Res*. 2015;93:1079–1092.

12. Gospe SM, Baker SA, Arshavsky VY. Facilitative glucose transporter Glut1 is actively excluded from rod outer segments. *J Cell Sci*. 2010;123:3639–3644.

13. Wang W, Fernandez de Castro J, Vukmanic E, Zhou L, Emery D, Demarco PJ, Kaplan HJ, Dean DC. Selective rod degeneration and partial cone inactivation characterize an iodoacetic acid model of swine retinal degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:7917–7923.

14. Zhang L, Du J, Justus S, et al. Reprogramming metabolism by targeting sirtuin 6 attenuates retinal degeneration. *J Clin Invest*. 2016;126:4659–4673.

15. Ait-Ali N, Fridlich R, Millet-Puel G, et al. Rod-derived cone viability factor promotes cone survival by stimulating aerobic glycolysis. *Cell*. 2015;161:817–832.

16. Wang W, Lee SJ, Scott PA, et al. Two-step reactivation of dormant cones in retinitis pigmentosa. *Cell*. 2016;15:372–385.

17. Ross JW, de Castro JP, Zhao J, et al. Generation of an inbred miniature pig model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2012;53:501–507.

translational vision science & technology