

Can Fly Photoreceptors Lead to Treatments for Rho^{P23H}-Linked Retinitis Pigmentosa?

Lauren Aerni-Flessner, PhD; Mohammad Haeri, MD, PhD; Barry E. Knox, PhD
 Francesca Pignoni, PhD

SUNY Eye Institute, Department of Ophthalmology and Department of Neuroscience and Physiology, and Center for Vision Research
 SUNY Upstate Medical University, Syracuse, NY, USA

Over 1.7 million people worldwide suffer from blindness caused by retinitis pigmentosa (RP), a group of hereditary disorders that cause progressive loss of first rod and then cone photoreceptors. Mutations in rhodopsin (Rho) account for close to 30% of all autosomal dominant (ADRP) cases, and the most common mutation in North America, Rho^{P23H}, involves a single amino acid change at position 23 at the N-terminal region of the protein.^{1,2}

The molecular/cellular mechanism leading to rod cell death in Rho^{P23H}-linked RP is still subject to debate. Current models include misregulation of stress pathways, involving endoplasmic reticulum-associated degradation (ERAD) and unfolded protein response (UPR),³⁻⁷ or the disruption of membrane disks in the outer segment (see Figure 1 for current models).⁸⁻¹² Studies in transfected cells and transgenic animals show Rho^{P23H} to be a misfolded protein

that forms aberrant oligomers and aggregates, and is largely found within the cell body.^{3,13-15} Notwithstanding the many unanswered questions on the etiology of the disease, it is this accumulation of abnormal protein aggregates that is thought to eventually lead to photoreceptor cell death.^{3-6,8,10} Currently, there are no effective treatments for RP. Translational approaches focus on 1) removing the mutant protein by suppressing expression or enhancing degradation, 2) promoting cell survival by delivering neurotrophic factors, or 3) suppressing cell death.^{16,17}

Several models that recapitulate aspects of Rho^{P23H} pathology are used in these studies.^{11,15,18-21} Animal models (frog, rat, mouse, pig) offer platforms that are photoreceptor-cell-based and thus more relevant from a pathophysiological standpoint. In these systems, however, the large-scale approaches necessary

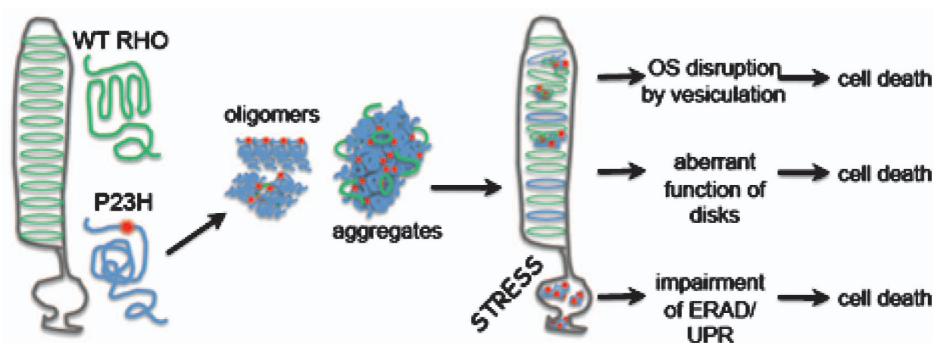


Figure 1. In healthy vertebrate photoreceptor cells (diagrammed on the left), wild type rhodopsin (WT RHO) is correctly folded and delivered to the membrane disks in the rod outer segment (OS). Very little, if any, rhodopsin is detected in the inner segment. Instead, improperly folded mutant rhodopsins (particularly Class II mutants⁴) are prone to forming oligomers and aggregates that are found largely in the cytoplasm but also in small amounts within the rod OS (diagrammed on the right). The pathogenic mechanism by which Rho^{P23H} leads to cell death is still the subject of debate³⁻¹². Experimental evidence suggests one of two possible sites of action: 1) within the cell body, by impairing the protein quality-control systems (ERAD and UPR), or 2) in the rod OS, by disrupting some critical function of the disks or the rod's structural integrity.³⁻¹²

ERAD, endoplasmic reticulum-associated degradation; UPR, unfolded protein response

for genetic or pharmacological screens are either not feasible (frog, pig) or prohibitively expensive (mouse, rat). On the contrary, cell culture is ideally suited for large-scale studies. Nevertheless, heterologous cell types lack critical features of photoreceptor cells, including specialized cellular structures and the machinery to house and process enormous quantities of membranes (protein and lipids). For these reasons and the resulting Rho-induced toxicity, the cell culture model is significantly limited.

Invertebrate models have been extensively used in the study of disease mechanisms thanks to their low cost, robust conservation of key physiological processes, and availability of sophisticated tools for detailed analyses as well as large-scale approaches (for instance, in the study of human neurodegenerative diseases).²²⁻²⁵ Among them, *Drosophila* presents an extremely well studied visual system whose developmental origin relies on many of the same molecular factors as the vertebrate eye.²⁶ Yet, the fruit fly has been rarely utilized in the analysis of mammalian proteins linked to vision loss,

largely because of the structural and functional distinctiveness of compound and camera eyes.

Is the *Drosophila* Model Relevant to the Metabolism of Mammalian Opsins?

There are striking differences between photoreceptor cells of flies and vertebrates, particularly in visual transduction and cellular structure (see comparison in Figure 2).²⁷ These differences obviously reflect a long history of independent evolution of light perception and vision in vertebrates versus invertebrates. For this reason, *Drosophila* has not been exploited as a host organism in the study of mammalian opsins. However, this should be reconsidered in light of new findings on the morphogenesis of the phototransduction compartments and the maturation, transport and degradation of opsins in flies and mouse photoreceptors.

Drosophila photoreceptor cells (aka R-cells) can produce bovine or murine Rhodopsin as stable proteins that can traffic correctly to the rhabdomere (Fig. 3A).²⁸ The bovine opsin

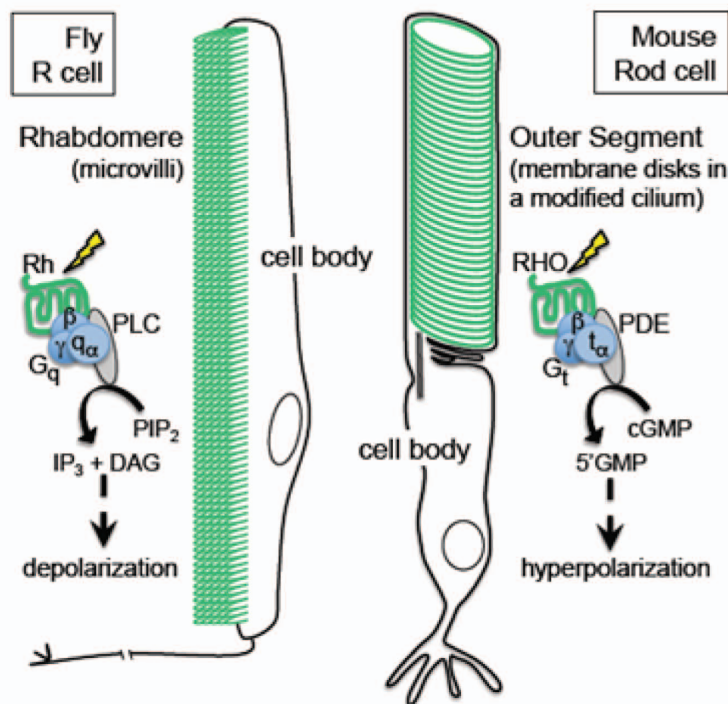


Figure 2. In *Drosophila*, rhodopsin (r-opsin) interacts with a G-protein (Gq) to activate phospholipase C (PLC) resulting in breakdown of phosphoinositide biphosphate (PIP₂) and opening of ion channels (depolarization). In vertebrates, rhodopsin (c-opsin) interacts with a G-protein transduction to activate a phosphodiesterase (PDE) resulting in hydrolysis of guanosine 3,5-cyclic monophosphate (cGMP) and closure of ion channels (hyperpolarization).²⁷

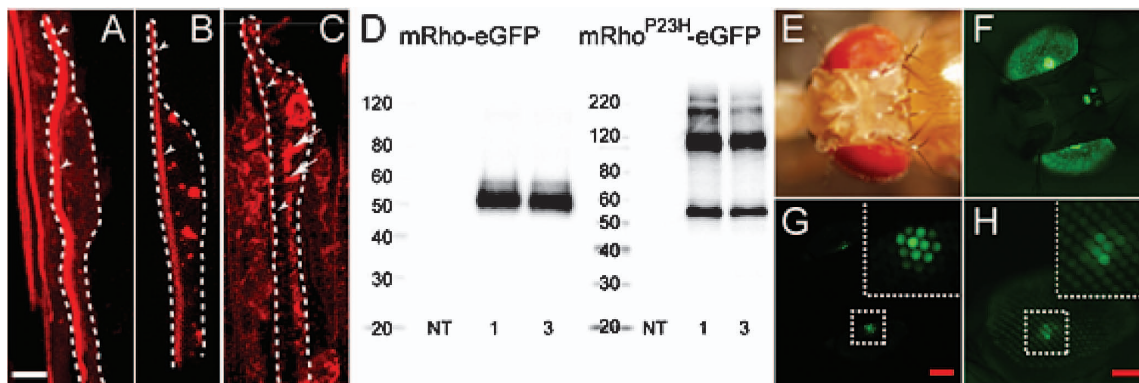


Figure 3. Analysis of wild type and P23H mouse rhodopsins-GFP fusions in fly photoreceptor cells. (A-C) Anti-GFP antibody staining of fly photoreceptors expressing (A) mRho-eGFP, (B) mRho-eGFP + Nuf^{RNAi}, or (C) mRho^{P23H}-eGFP. Cells are marked by dashed lines. White bar = 5 microns. (A) mRho-eGFP localizes to the rhabdomere (arrowheads). (B) mRho-eGFP is also found in intracellular inclusions when Nuf/FIP3 is knocked down (Nuf^{RNAi}). Thus, Nuf/FIP3, which facilitates the post-Golgi trafficking of Rho in mammals, also appears to affect mRho-eGFP localization in fly photoreceptors. (C) mRho^{P23H}-eGFP is found mostly in cytoplasmic inclusions (arrows), with very little protein reaching the rhabdomere (arrowheads). This mislocalization of mRho^{P23H}-eGFP recapitulates the phenotype observed in vertebrate models of Rho^{P23H} RP. The stability of the mutant protein is also lower. The signal in panel C has been enhanced using Adobe Photoshop for clarity. (D) Western blot analysis shows that mRho^{P23H}-eGFP (D, right side), but not mRho-eGFP (D, left side), is found in oligomers in day 1 and 3 adult fly heads. Monomers, dimers and larger oligomers can be visualized as bands of progressively higher molecular weight. NT = wild type fly, non transgenic control. (E-H) Detection of opsin-eGFP fluorescence in live flies. Red bars = 100 microns (E) Normal eye phenotype of GMR-Gal4 UAS mRho-eGFP flies shows that overexpression of mRho-eGFP does not disrupt eye morphology. (F-H) mRho-eGFP fluorescence is easily visible in the eyes of live adult flies after 1 second (F) and 50 millisecond (G) exposures. (H) Reflecting its predominantly intracellular localization and lower stability, no mRho^{P23H}-eGFP fluorescence is visible after a 50 millisecond exposure (not shown); only diffuse background fluorescence due to natural pigment is detected after 1 second (H).

has also been shown to be functional when presented with the appropriate G protein, G_t Transducin.²⁸ Moreover, a number of studies have uncovered similarities in the processing and transport of opsins. Transport of both the fly rhodopsin Rh1 and mammalian Rho involve the exocyst complex, Rab11 and myosins (V in flies and VIIA in mouse).²⁹⁻³³ In regards to Rhodopsin degradation, the ERAD effector valosin-containing protein (VCP) acts as a molecular chaperone for both fly and mammalian proteins.^{34,35} Additional evidence comes from the associations of factors required in R-cells with human eye diseases. Mutations in Crumbs homolog 1 (the vertebrate homologue of Crumbs, a critical cell polarity factor that also facilitates rhodopsin trafficking in fly photoreceptors) are associated with retinal degeneration in fly and mouse, and retinitis pigmentosa in humans.^{36,37} Multiple cases of Usher syndrome have been mapped to the region of the DENN/MADD domain containing 4A (DENND4A) locus, the human homologue of fly Crag, which plays an

integral role in the trafficking of Rh1.^{38,39} Lastly, prominin, a factor recently implicated in the elaboration of phototransduction compartments in both rhabdomeric and ciliary photoreceptors, is linked to retinitis pigmentosa and Stargardt disease.⁴⁰⁻⁴² As many genes involved in the processing of Rh1 and Rho have not been identified, additional factors in common between vertebrates and invertebrates will certainly emerge from further studies.

In addition, nearly all Rho-processing components have corresponding fly homologues that may also be active in processing of Rh1, or be recruited for production of Rho in R-cells. Interestingly, maturation of the mammalian protein does not require the Rh1-specific, endoplasmic reticulum (ER)-based chaperone NinaA (a cyclophilin),^{28,43} but does appear to depend on Nuf, a fly homologue of Rab11 family-interacting protein 3 (Fig. 3B), a critical factor for the transport of Rho from the trans-Golgi network in mouse rods.³⁰ Further analysis of evolutionarily conserved and fly-specific

factors in our mRho-GFP model will shed light on the processing of mammalian rhodopsin in *Drosophila* photoreceptors.

Is *Drosophila* Relevant to the Study of the Mutant Rho?

To explore this question, we expressed mouse mutant Rho^{P23H} (mRho^{P23H}) in R-cells. In contrast to wild type mRho, the mutant opsin does not localize to the rhabdomere, displays lower protein stability, oligomerizes and forms abnormal intracellular foci (Figures 3C and 3D). In essence, mRho^{P23H} shows a pattern of phenotypic abnormalities in R-cells strikingly similar to the aberrant behavior of this protein in vertebrate photoreceptors.

Importantly, we can detect alterations in the localization and/or stability of this mutant rhodopsin by measuring changes in GFP-fluorescence. This is achieved by using Rho-eGFP fusions and detecting fluorescence in adult eyes or dissected retinas (live or fixed tissue) (Figures 3F-I and 3A-C, respectively). Hence, modifiers that increase stability, folding, and rhabdomeric localization of Rho^{P23H}-eGFP will lead to increased fluorescence, whereas modifiers that decrease rhabdomeric localization and/or protein stability will lead to decreased fluorescence. Thus, a fly model of mammalian Rho^{P23H} may address the present lack of photoreceptor-based models suitable for large-scale applications.

In conclusion, a screening platform for genetic and pharmacological modifiers of misfolded mammalian rhodopsin is now available in the powerful genetic *Drosophila* model.⁴⁴ This provides an innovative, *in vivo* approach for the discovery of novel drugs or targets.

Conflicts of Interest

None.

REFERENCES

1. Dryja TP, McGee TL, Reichel E, Hahn LB, Cowley GS, Yandell DW, et al. A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. *Nature* 1990;343:364-366.
2. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet* 2006;368:1795-1809.
3. Frederick JM, Krasnoperova NV, Hoffmann K, Church-Kopish J, Rütther K, Howes K, et al. Mutant rhodopsin transgene expression on a null background. *Invest Ophthalmol Vis Sci* 2001;42:826-833.
4. Mendes HF, van der Spuy J, Chapple JP, Cheetham ME. Mechanisms of cell death in rhodopsin retinitis pigmentosa: implications for therapy. *Trends Mol Med* 2005;11:177-185.
5. Lin JH, Li H, Yasumura D, Cohen HR, Zhang C, Panning B, et al. IRE1 signaling affects cell fate during the unfolded protein response. *Science* 2007;318:944-949.
6. Gorbatyuk MS, Knox T, LaVail MM, Gorbatyuk OS, Noorwez SM, Hauswirth WW, et al. Restoration of visual function in P23H rhodopsin transgenic rats by gene delivery of BiP/Grp78. *Proc Natl Acad Sci USA* 2010;107:5961-5966.
7. Haeri M, Knox BE. Endoplasmic reticulum stress and unfolded protein response pathways: potential for treating age-related retinal degeneration. *J Ophthalmic Vis Res* 2012;7:45-59.
8. Liu X, Wu TH, Stowe S, Matsushita A, Arikawa K, Naash MI, et al. Defective phototransductive disk membrane morphogenesis in transgenic mice expressing opsin with a mutated N-terminal domain. *J Cell Sci* 1997;110:2589-2597.
9. Wu TH, Ting TD, Okajima TI, Pepperberg DR, Ho YK, Ripps H, et al. Opsin localization and rhodopsin photochemistry in a transgenic mouse model of retinitis pigmentosa. *Neuroscience* 1998;87:709-717.
10. Sakami S, Maeda T, Bereta G, Okano K, Golczak M, Sumaroka A, 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.
11. Haeri M, Knox BE. Rhodopsin mutant P23H destabilizes rod photoreceptor disk membranes. *PLoS One* 2012;7:e30101.
12. Price BA, Sandoval IM, Chan F, Nichols R, Roman-Sanchez R, Wensel TG, et al. Rhodopsin gene expression determines rod outer segment size and rod cell resistance to a dominant-negative neurodegeneration mutant. *PLoS One* 2012;7:e49889.
13. Saliba RS, Munro PM, Luthert PJ, Cheetham ME. The cellular fate of mutant rhodopsin: quality control, degradation and aggresome formation. *J Cell Sci* 2002;115:2907-2918.

14. Illing ME, Rajan RS, Bence NF, Kopito RR. A rhodopsin mutant linked to autosomal dominant retinitis pigmentosa is prone to aggregate and interacts with the ubiquitin proteasome system. *J Biol Chem* 2002;277:34150-34160.
15. Tam BM, Moritz OL. Characterization of rhodopsin P23H-induced retinal degeneration in a *Xenopus laevis* model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2006;47:3234-3241.
16. Jacobson SG Cideciyan AV. Treatment possibilities for retinitis pigmentosa. *New Engl J Med* 2010;363:1669-1671.
17. Rossmiller B, Mao H, Lewin AS. Gene therapy in animal models of autosomal dominant retinitis pigmentosa. *Mol Vis* 2012;18:2479-2496.
18. Olsson JE, Gordon JW, Pawlyk BS, Roof D, Hayes A, Molday RS, et al. Transgenic mice with a rhodopsin mutation (Pro23His): a mouse model of autosomal dominant retinitis pigmentosa. *Neuron* 1992;9:815-830.
19. Machida S, Kondo M, Jamison JA, Khan NW, Kononen LT, Sugawara T, et al. P23H rhodopsin transgenic rat: correlation of retinal function with histopathology. *Invest Ophthalmol Vis Sci* 2000;41:3200-3209.
20. Price BA, Sandoval IM, Chan F, Simons DL, Wu SM, Wensel TG, 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.
21. Ross JW, Fernandez de Castro JP, Zhao J, Samuel M, Walters E, Rios C, et al. Generation of an inbred miniature pig model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2012;53:501-507.
22. Lu B, Vogel H. *Drosophila* models of neurodegenerative diseases. *Annu Rev Pathol* 2009;4:315-342.
23. Hirth F. *Drosophila melanogaster* in the study of human neurodegeneration. *CNS Neurol Disord Drug Targets* 2010;9:504-523.
24. Cook T, Zelhof A, Mishra M, Nie J. 800 facets of retinal degeneration. *Prog Mol Biol Transl Sci* 2011;100:331-368.
25. Rincon-Limas DE, Jensen K, Fernandez-Funez P. *Drosophila* models of proteinopathies: the little fly that could. *Curr Pharm Des* 2012;18:1108-1122.
26. Wawersik S, Maas RL. Vertebrate eye development as modeled in *Drosophila*. *Hum Mol Genet* 2000;9:917-925.
27. Fain GL, Roger Hardie R, Laughlin SB. Phototransduction and the evolution of photoreceptors. *Curr Biol* 2010;20:R114-R124.
28. Ahmad ST, Natchin M, Barren B, Artemyev NO, O'Tousa JE. Heterologous expression of bovine rhodopsin in *Drosophila* photoreceptor cells. *Invest Ophthalmol Vis Sci* 2006;47:3722-3728.
29. Beronja S, Laprise P, Papoulas O, Pellikka M, Sisson J, Tepass U. Essential function of *Drosophila* Sec6 in apical exocytosis of epithelial photoreceptor cells. *J Cell Biol* 2005;169:635-646.
30. Mazelova J, Astuto-Gribble L, Inoue H, Tam BM, Schonteich E, Prekeris R, et al. Ciliary targeting motif VxPx directs assembly of a trafficking module through Arf4. *EMBO J* 2009;28:183-192.
31. Satoh AK, O'Tousa JE, Ozaki K, Ready DF. Rab11 mediates post-Golgi trafficking of rhodopsin to the photosensitive apical membrane of *Drosophila* photoreceptors. *Development* 2005;132:1487-1497.
32. Li BX, Satoh AK, Ready DF. Myosin V, Rab11, and dRip11 direct apical secretion and cellular morphogenesis in developing *Drosophila* photoreceptors. *J Cell Biol* 2007;77:659-669.
33. Wolfrum U, Schmitt A. Rhodopsin transport in the membrane of the connecting cilium of mammalian photoreceptor cells. *Cell Motil Cytoskeleton* 2000;46:95-107.
34. Griciuc A, Aron L, Piccoli G, Ueffing M. Clearance of Rhodopsin (P23H) aggregates requires the ERAD effector VCP. *Biochim Biophys Acta* 2010;1803:424-434.
35. Griciuc A, Aron L, Roux MJ, Klein R, Giangrande A, Ueffing M. Inactivation of VCP/ter94 suppresses retinal pathology caused by misfolded rhodopsin in *Drosophila*. *PLoS Genet* 2010;26:e1001075.
36. den Hollander AI, ten Brink JB, de Kok YJ, van Soest S, van den Born LI, van Driel MA. Mutations in a human homologue of *Drosophila* crumbs cause retinitis pigmentosa (RP12). *Nat Genet* 1999;23:217-221.
37. Pocha SM, Shevchenko A, Knust E. Crumbs regulates rhodopsin transport by interacting with and stabilizing myosin V. *J Cell Biol* 2011;195:827-838.
38. Ahmed ZM, Riazuddin S, Khan SN, Friedman PL, Riazuddin S, Friedman TB. USH1H, a novel locus for type I Usher syndrome, maps to chromosome 15q22-23. *Clin Genet* 2009;75:86-91.
39. Dad S, Østergaard E, Thykjaer T, Albrechtsen A, Ravn K, Rosenberg T, et al. Identification of a novel locus for a USH3 like syndrome combined with congenital cataract. *Clin Genet* 2010;78:388-397.
40. Yang Z, Chen Y, Lillo C, Chien J, Yu Z, Michaelides M, et al. Mutant prominin 1 found in patients with macular degeneration disrupts photoreceptor disk morphogenesis in mice. *J Clin Invest* 2008;118:2908-2916.

41. Nie J, Mahato S, Mustill W, Tipping C, Bhattacharya SS, Zelhof AC. Cross species analysis of Prominin reveals a conserved cellular role in invertebrate and vertebrate photoreceptor cells. *Dev Biol* 2012;371:312-320.
42. Gurudev N, Florek M, Corbeil D, Knust E. Prominent role of prominin in the retina. *Adv Exp Med Biol* 2013;777:55-71.
43. Baker E, Colley N, Zuker CS. The cyclophilin homolog NinaA functions as a chaperone, forming a stable complex in vivo with its protein target rhodopsin. *EMBO J* 1994;13:4886-4895.
44. Pandey UB, Nichols CD. Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. *Pharmacol Rev* 2011;63:411-436.