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Chapter 74
Endoplasmic Reticulum Stress in Vertebrate Mutant Rhodopsin Models of Retinal Degeneration

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Abstract *Rhodopsin* mutations cause many types of heritable retinitis pigmentosa (RP). Biochemical and in vitro studies have demonstrated that many RP-linked mutant *rhodopsins* produce misfolded rhodopsin proteins, which are prone to aggregation and retention within the endoplasmic reticulum, where they cause endoplasmic reticulum stress and activate the Unfolded Protein Response signaling pathways. Many vertebrate models of retinal degeneration have been created through expression of RP-linked *rhodopsins* in photoreceptors including, but not limited to, *VPP/GHL* mice, *P23H Rhodopsin* frogs, *P23H rhodopsin* rats, *S334ter rhodopsin* rats, *C185R rhodopsin* mice, *T17M rhodopsin* mice, and *P23H rhodopsin* mice. These models have provided many opportunities to test therapeutic strategies to prevent retinal degeneration and also enabled in vivo investigation of cellular and molecular mechanisms responsible for photoreceptor cell death. Here, we examine and compare the contribution of endoplasmic reticulum stress to retinal degeneration in several vertebrate models of RP generated through expression of mutant *rhodopsins*.

Keywords Rhodopsin · Retinitis pigmentosa · P23H rhodopsin · Endoplasmic reticulum · Unfolded Protein Response · Photoreceptors

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Abbreviations

RP	Retinitis pigmentosa
ER	Endoplasmic reticulum
UPR	Unfolded Protein Response
VPP	**V**20G, **P**23H, and **P**27L mutations
GHL	V20**G**, P23**H**, and P27**L** mutations

74.1 Introduction

Rhodopsin encodes a G protein-coupled multipass transmembrane protein that is expressed solely in rod photoreceptors and is essential for phototransduction [1]. Many heritable types of RP are caused by mutations in *rhodopsin* (www.sph.uth.tmc.edu/retnet). Biochemical studies in heterologous cell culture expression systems have found that many RP-linked *rhodopsin* mutations generate mutant rhodopsin proteins that are misfolded, abnormally aggregated, and are retained within the endoplasmic reticulum (ER) by ER protein quality control mechanisms such as the Unfolded Protein Response (UPR) [2–7]. Many animal models of retinal degeneration have also been developed through expression of mutant rhodopsins in photoreceptors. Here, we compare roles for ER stress in several vertebrate models of retinal degeneration expressing mutant rhodopsins.

74.2 VPP and GHL Transgenic Mice

"*VPP*" and "*GHL*" transgenic mice both express genetically modified mouse *opsin* bearing V20G, P23H, and P27L mutations under mouse opsin promoter control and have been widely studied as models of human RP [8, 9]. In these mice, in the absence of any wild-type rhodopsin (in rhodopsin knockout background), the triple mutant rhodopsin aggregates as abnormal dimers and is found mostly within the rod inner segment co-localizing with ER markers [9]. By contrast, in the presence of wild-type rhodopsin, fewer abnormal rhodopsin dimers are formed, and mutant rhodopsin can be detected in the rod outer segment [10]. These findings indicate that the photoreceptor recognizes the triple mutant rhodopsin as a misfolded protein and retains it in the ER, where it likely causes ER stress and activates UPR signaling. These findings also suggest that wild-type rhodopsin somehow reduces the levels of abnormal rhodopsin dimers and enables mutant rhodopsin protein to exit from ER to the outer segment, when both wild-type and mutant rhodopsin are co-expressed in photoreceptors. This alleviation may be incompletely sustained since these animals still ultimately develop photoreceptor cell loss.

74.3 P23H Rhodopsin Transgenic *Xenopus Laevis*

Transgenic *Xenopus laevis* expressing *X. laevis* rhodopsin bearing a P23H mutation under the control of the *X. laevis* opsin promoter develop progressive retinal degeneration in a transgene dose-dependent manner [11]. Mutant *X. laevis* P23H rhodopsin predominantly localizes within the rod inner segment in transgenic *X. laevis*, co-localizing with the ER-resident calnexin protein [11]. Mutant *X. laevis* P23H rhodopsin protein also forms abnormal dimers and other higher order protein aggregates in solubilized retina lysates from these animals [11]. These findings in transgenic *X. laevis* indicate that *X. laevis* mutant P23H rhodopsin is misfolded and retained in the ER. Interestingly, endogenous wild-type *X. laevis* rhodopsin is still expressed in these animals, but amelioration of the abnormal aggregation and ER retention has not been reported for P23H rhodopsin protein despite co-expression of the wild-type protein.

74.4 P23H Rhodopsin Transgenic Rat

Transgenic rats expressing mouse *opsin* bearing P23H mutation under mouse opsin promoter control develop retinal degeneration in a transgene dose-dependent manner and are widely used to study retinal degeneration mechanisms and therapeutics [12–14]. Molecular studies have found increased levels of ER stress-induced and UPR signaling pathway-activated mRNAs and proteins, such as the ER-resident chaperone *Grp78/BiP* and the transcription factor *Chop*, at ages that roughly correspond with the onset and early progression of retinal degeneration [6, 15]. These findings mirror cell culture studies that found activation of UPR signaling pathways and ER stress-induced genes in response to P23H rhodopsin expression [6, 16]. UPR signaling promotes selective degradation of misfolded P23H rhodopsin in vitro and could also operate in these animals to remove P23H rhodopsin protein from photoreceptors [7, 17]. P23H rhodopsin protein aggregation and subcellular localization has been difficult to determine precisely in these transgenic rats, in part because of nearly identical homology between the transgenic mouse P23H rhodopsin protein and endogenous rat rhodopsin protein.

74.5 S334ter Rhodopsin Transgenic Rat

Transgenic rats expressing mouse *opsin* bearing a premature termination codon at residue S334 also develop retinal degeneration in transgene dose-dependent manners [14, 18]. S334ter rhodopsin lacks carboxy-terminal residues required for accurate rhodopsin protein intracellular localization and accurate phototransduction signaling by rhodopsin [19–22]. In vitro studies have reported that many carboxy-

tail mutant rhodopsin proteins fold with sufficient fidelity that they do not form abnormal aggregates and can journey out of the ER to the outer segment [3, 23]. Surprisingly, recent reports found increased levels of ER stress-induced proteins, BiP/Grp78 and Chop, in retinas of transgenic S334ter rats compared to wild-type animals [24, 25]. It is unclear why and how S334ter rhodopsin causes ER stress, but ER stress could arise through the disruption of photoreceptor calcium homeostasis due to abnormal rhodopsin phototransduction. Recent biochemical studies have also found that some S334ter rhodopsin is retained within the ER to a greater degree compared to wild-type rhodopsin (albeit less than the ER retention seen with P23H rhodopsin) [17]. Increased ER latency of S334ter rhodopsin could also contribute to elevated ER stress levels seen in transgenic S334ter rhodopsin rats.

74.6 *R3* (C185R Rhodopsin) Mouse

The *R3* mouse line was identified in an N-ethyl-N-nitrosourea mutagenesis mouse screen [26]. These animals develop an autosomal dominant retinal degeneration that mapped to a C185R mutation in the native mouse *opsin* gene [26]. Coincidentally, orthologous human C185R mutations have also been identified in RP patients [27]. Structural modeling of the C185R mutant rhodopsin predicted that the long side chain of the abnormal arginine residue would interfere with rhodopsin folding and thereby lead to generation of misfolded rhodopsin protein [26]. C185R rhodopsin protein entirely localized to the inner segment in R3/R3 homozygous mice [26]. Ultrastructural studies further found that R3/R3 homozygous animals produced virtually no outer segment, while R3/+ heterozygous animals showed short outer segments with extensive disc disorganization [26]. These findings suggest that photoreceptors recognize C185R rhodopsin as a misfolded protein to be retained in the ER and targeted for degradation.

74.7 T17M Transgenic Mouse

Transgenic mice expressing the human *rhodopsin* gene and flanking sequences bearing the T17M mutation develop a progressive retinal degeneration and have been used to study therapeutic effects of vitamin A [28]. In vitro studies previously demonstrated that T17M rhodopsin was misfolded and retained within the ER similar to P23H rhodopsin [2, 3, 7]. Up-regulation of several ER stress-induced and UPR activated target genes were observed in the retinas of transgenic T17M mice [29]. Furthermore, increased GFP fluorescence was seen in photoreceptors when T17M mice were crossed with ER stress-sensitive GFP reporter mice [29, 30]. Multiple additional intracellular signaling pathways and cellular processes, including the Akt signaling pathway, autophagy, and mitochondrial intrinsic apop-

tosis regulators, were also dysregulated in T17M animals in addition to activation of UPR signaling pathways [29]. These findings suggest that many intracellular mechanisms are disrupted during the course of T17M rhodopsin-induced retinal degeneration.

74.8 P23H Rhodopsin Knock-In Mouse

P23H rhodopsin knock-in mice have recently been generated through targeted replacement of the *P23H* codon in endogenous mouse *opsin* gene [31]. By contrast to prior vertebrate P23H rhodopsin models, the P23H rhodopsin knock-in mice express no exogenous transgenic copies of rhodopsin. P23H rhodopsin knock-in heterozygous mice develop a progressive retinal degeneration that is significantly more rapid and severe in mice homozygous for the P23H rhodopsin knock-in allele. In P23H rhodopsin knock-in mice, P23H rhodopsin was incompletely glycosylated, retained within the ER, and found at very low levels compared to wild-type rhodopsin, presumably because ER-retained P23H rhodopsin was quickly targeted for degradation [31]. In heterozygous animals, 1D4-immunoreactivity to visualize rhodopsin protein localization showed rhodopsin labeling in rod outer segments with minimal rhodopsin labeling elsewhere in the photoreceptor [31]. Moreover, the outer segments were significantly shorter and contained abnormal, perpendicularly polarized discs [31]. Interestingly, the retinal degeneration seen in the P23H knock-in mice was worsened by genetic depletion of 11-cis-retinal, a molecular chaperone of P23H opsin in vitro [31].

74.9 P23H Rhodopsin-GFP Knock-In Mouse

P23H rhodopsin-GFP knock-in mice have also recently been generated through targeted replacement of an endogenous mouse *opsin* allele with homologous human *opsin* genomic region carrying a mutated *P23H* codon and *GFP* fused to the carboxy terminus of rhodopsin [32]. Heterozygous mice develop a mild retinal degeneration that is severely worsened in homozygous animals. In these animals, the P23H rhodopsin-GFP knock-in allele was transcribed as efficiently as endogenous *opsin* [32]. However, P23H rhodopsin-GFP protein levels were significantly lower than that of the wild-type rhodopsin protein, presumably through decreased stability and enhanced degradation of the P23H rhodopsin-GFP protein [32]. The fusion of GFP to P23H rhodopsin in these animals provided an opportunity to specifically track the subcellular localization of P23H rhodopsin-GFP independent of wild-type rhodopsin in photoreceptors. P23H rhodopsin-GFP was found to be predominantly mislocalized to the rod inner segment and outer nuclear layer, where the ER and nuclear membranes reside, with smaller amounts of GFP signal found in the outer segment and inner plexiform layer [32].

74.10 Discussion

Misfolded membrane proteins commonly aggregate, are retained within the ER, elicit ER stress, and activate the UPR [33, 34]. UPR signaling then enhances degradation of irreparably damaged proteins. Many mutant rhodopsins linked to RP display all of these features in heterologous cell culture expression studies. In vertebrate models of retinal degeneration generated through mutant rhodopsin expression in photoreceptors, abnormal rhodopsin protein aggregation, ER retention, and UPR activation/ER stress are also seen to varying degrees suggesting that ER stress is also involved in retinal degeneration in vivo. Vertebrate models have also revealed additional intriguing properties and effects of mutant rhodopsins in retina including: (1) photoreceptors can rapidly identify and clear mutant rhodopsins from the ER via unclear mechanisms, (2) co-expression of wild-type rhodopsin can enable mutant rhodopsin to escape ER and/or promote its degradation via unclear mechanisms in photoreceptors, and (3) disorganization of discs and outer segments are frequently in photoreceptors expressing mutant rhodopsins. Investigating functions and properties of ER unique to photoreceptors may provide further insight into the role of ER stress in retinal degeneration.

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