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# Misfolded Proteins and Retinal Dystrophies

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## Abstract

Many mutations associated with retinal degeneration lead to the production of misfolded proteins by cells of the retina. Emerging evidence suggests that these abnormal proteins cause cell death by activating the Unfolded Protein Response, a set of conserved intracellular signaling pathways that detect protein misfolding within the endoplasmic reticulum and control protective and proapoptotic signal transduction pathways. Here, we review the misfolded proteins associated with select types of retinitis pigmentosa, Stargadt-like macular degeneration, and Doyne Honeycomb Retinal Dystrophy and discuss the role that endoplasmic reticulum stress and UPR signaling play in their pathogenesis. Last, we review new therapies for these diseases based on preventing protein misfolding in the retina.

## 14.1 Endoplasmic Reticulum Stress and Retinal Degeneration

The retina collects and transmits light information through an intricate network of highly specialized neural cells. To accomplish this unique sensory function, cells of the retina produce a specialized array of proteins such as rhodopsin. Transmission of accurate visual information depends on the continuous production of high-quality, functional proteins by cells of the retina. Cells have evolved elaborate mechanisms to ensure that membrane and secreted proteins are accurately folded and assembled before export or delivery to the cell surface. Stringent quality control is imposed by the endoplasmic reticulum (ER), a membrane-bound organelle, where virtually all plasma and secreted proteins begin their journey to the surface. Only properly folded proteins are allowed to exit the ER. Misfolded proteins are retained by the ER and degraded to prevent the generation of proteins that may be dysfunctional or potentially toxic. If ER protein quality control fails and chronic protein misfolding ensues, cell death occurs via apoptosis. Cells have evolved a set of intracellular signaling pathways termed the Unfolded Protein Response (UPR) that detect protein misfolding within the ER and direct protective and proapoptotic responses (Lin et al. 2008). The UPR provides an attractive molecular framework to investigate the molecular pathogenesis of retinal dystrophies arising from protein misfolding. Over 100 different heritable mutations have been identified that lead to death of retinal cells and loss of vision (www.sph.uth.tmc.edu/retnet/). Many of these genetic defects lead to the production of abnormal proteins by retinal cell types. Here we review some of the protein mutations linked to retinal degeneration and evidence that implicates UPR signaling in the cell death elicited by these mutations.

## 14.2 Misfolded Proteins in Photoreceptors

Rhodopsin is by far the predominant protein within photoreceptors, where it comprises ~30% of the entire proteome of photoreceptors and over 90% of all proteins in the outer segment region of photoreceptors (Hargrave 2001). Rhodopsin plays acriticalrolein phototransduction


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andisexpressed solelybyrodphotoreceptors. Rhodopsin is the archetypal serpentine G-protein coupled receptor and consists of a348 amino acid polypeptide organized into7transmembrane helices and abinding pocket for light-sensitive 11-cis-retinal (Hargrave 2001; Palczewski et al. 2000). Like virtually all membrane proteins, rhodopsin synthesis occurs at the ER, where the nascent rhodopsin polypeptide is co-translationally inserted into the membrane and undergoes multiple post-translational modifications including disulfide bond formation and glycosylation at asparagine residues (Fukuda et al. 1979; Kaushal et al. 1994; Krebs et al. 2004). Once properly folded, rhodopsin exits the ER and enters the Golgi apparatus where it undergoes additional sugar modifications and is eventually delivered to the rod photoreceptor outer segment (Liang et al. 1979). In the rod outer segment, the 11-cis-retinal vitamin A derivative is covalently linked to opsin (the apoprotein of rhodopsin) by a protonated Schiff base at a lysine residue to create the final rhodopsin chromophore. When light strikes rhodopsin, a photon is absorbed that causes the retinal to isomerize to the all-trans form which drives the rhodopsin protein through a series of transient photo-intermediates that bind and activate the G protein, transducin. A cascade of biochemical events ensues that result in a drop in cGMP concentration, the closing of the calcium conductance channels in the plasma membrane, and hyperpolarization of the cell, thereby generating an electrical signal and activating the neural circuitry underlying vision.

Mutations in the visual pigment, rhodopsin, are the most common cause of hereditary of RP and account for 25–30% of autosomal dominant RP (adRP) (Berson et al. 2001; Sohocki et al. 2001). Over 100 distinct missense mutations in rhodopsin have been identified that lead to retinal degeneration, including recessive and autosomal dominant forms of retinitis pigmentosa, and congenital stationary night blindness (Retnet http://www.sph.uth.tmc.edu/RetNet). These mutations are found throughout the rhodopsin molecule. Seminal studies performed by the labs of Jeremy Nathans and Gobind Khorana in the 1990s demonstrated that the vast majority of adRP-linked rhodopsin mutations lead to misfolding of the rhodopsin protein.

Many of these studies have focused on the most common rhodopsin mutation leading to adRP in the United States, a missense mutation at amino acid position 23 of rhodopsin that replaces a proline with a histidine residue (P23H RHO) (Retnet http://www.sph.uth.tmc.edu/RetNet). P23H RHO fails to bind 11-cis-retinal (Kaushal and Khorana 1994; Kaushal et al. 1994; Liu et al. 1996). The crystal structure of rhodopsin indicates that the proline$^{23}$ residue is located in the N-terminal intradiscal tail within one of the β-strands that comprise the N-terminal plug of the molecule (Palczewski et al. 2000). The N-terminal plug normally positions and binds 11-cis-retinal to form fully functional rhodopsin. Mutations in this region, such as the P23H substitution, could lead to misfolding of the N-terminal plug and hence impair binding of the chromophore. Biochemical data support that the P23H mutation induces rhodopsin misfolding: mutant rhodopsins form oligomeric aggregates; P23H rhodopsin displays abnormal sensitivity to trypsin compared to wild-type rhodopsin when expressed in cell culture; P23H rhodopsin is not properly glycosylated compared to wild-type rhodopsin, and instead, is complexed with ER-resident chaperones such as BiP or Grp94 (Anukanth and Khorana 1994; Liu et al. 1996; Noorwez et al. 2004). Immunocytochemical and ultrastructural studies in cultured cells and retinas from transgenic mice and frogs also revealed that P23H rhodopsin is localized to the ER/Golgi, whereas wild-type rhodopsin translocates to the surface membrane (Frederick et al. 2001; Kaushal and Khorana 1994; Saliba et al. 2002; Sung et al. 1991; Tam and Moritz 2006). In sum, these findings provide biochemical, cellular, and genetic evidence that P23H rhodopsin is misfolded in the ER and set the foundation for investigating the molecular events downstream of rhodopsin misfolding and retention in the ER leading to photoreceptor cell death. Moreover, data established for P23H rhodopsin are likely to hold true for additional adRP-linked mutant rhodopsins; indeed, in Liu, Garriga, and Khorana PNAS 93:4554–4559, 1996, they state that: “We suggest that most, if not all, of the point mutations in the intradiscal

domain identified in adRP cause partial or complete misfolding of rhodopsin (Liu et al. 1996)."

Recent studies indicate that the Unfolded Protein Response (UPR) signaling pathways link rhodopsin misfolding in the ER and cell fate. In a Drosophila model of retinal degeneration arising from rhodopsin misfolding, robust activation of the IRE1 signaling pathway of the UPR was observed in the fly and intriguingly, genetic down-regulation of this pathway accelerated retinal degeneration (Ryoo et al. 2007). In multiple rodent models of adRP that express different levels of P23H rhodopsin and undergo different rates of retinal degeneration, multiple distinct UPR signaling pathways were selectively activated in P23H animals compared to wild-type siblings. In these animals, proapoptotic UPR signaling molecules such as CHOP were markedly elevated in animals expressing misfolded rhodopsin at time points that preceded frank loss of photoreceptors, raising the possibility that activation of UPR signaling by misfolded rhodopsin directly drives photoreceptor cell death (Lin et al. 2007). Future studies will focus on determining causality between UPR activation and photoreceptor cell survival after rhodopsin misfolding.

Mutations in *ElovL4* have been identified in Stargadt-like macular degeneration, an autosomal dominant form of juvenile retinal degeneration (Zhang et al. 2001). *ElovL4* encodes an enzyme thought to be involved in the generation of long-chain fatty acids (Karan et al. 2004; Oh et al. 1997; Vasireddy et al. 2008). Consistent with its role in lipid biosynthesis, ELOVL4 is a membrane protein targeted to the ER (Karan et al. 2004; Vasireddy et al. 2005). Photoreceptors express high levels of ELOVL4, but in contrast to rhodopsin, its expression has also been reported in other cell types in the eye (Zhang et al. 2003). At the molecular level, mutations in *ElovL4* that trigger macular degeneration lead to premature truncations of the protein that all result in loss of an ER retention motif. In biochemical studies, mutant ELOVL4 is isolated as a higher-order complex, and in transfected cells, immunofluorescence reveals that mutant ELOVL4 is no longer distributed in a reticular manner but instead found as perinuclear aggregates (Karan et al. 2005; Vasireddy et al. 2005). These findings are consistent with misfolding of mutant ELOVL4. Intriguingly, in these in vitro studies, mutant ELOVL4 binds and sequesters wild-type ELOVL4 into higher order aggregates, perhaps accounting for its dominant phenotype (Karan et al. 2005; Vasireddy et al. 2005). Mutant ELOVL4 also activates the UPR in transfected cells, raising the possibility that these signaling pathways link ELOVL4 misfolding and aggregation in the ER and photoreceptor cell fate (Karan et al. 2005).

The *rd1* mutation results in the profound reduction of PDE6-β protein presumably through destabilization of the mutated mRNA or nascent protein. PDE6-β is acatalytic subunit ofaphosphodiesterase that regulates cGMPlevels in photoreceptors in response to light activation. Absence of the PDE6-β disrupts phosphodiesterase activity and accumulation of cGMP, which in turn, enhances cGMP-gated ion channels, leading to significant rises in intracellular calcium and ultimately photoreceptor cell death. Recent work demonstrates that multiple UPR signaling pathways are also activated in the *rd1* mouse at ages that precede frank photoreceptor cell death (Yang et al. 2007). These findings suggest that UPR signaling may also play a role in the pathogenesis of this type of retinal degeneration. However, PDE6-β is a cytosolic enzyme, whereas UPR signaling pathways are activated by perturbations in the ER. How would the *rd1* mutation then trigger the UPR? Besides protein folding, the ER also performs crucial cellular functions that include the synthesis of lipids and sterols and the storage of calcium. Defects in lipid/sterol metabolism and calcium homeostasis can also elicit endoplasmic reticulum stress (Lin et al. 2008). Calcium dysregulation in *rd1* and other retinal diseases could activate the UPR, though this remains to be investigated.

## 14.3 Misfolded Proteins in Retinal Pigment Epithelial Cells

A missense mutation in *fibulin-3* leading to an arginine-to-tryptophan substitution at amino acid 345 (R345W) has been found in an autosomal dominant maculopathy, malattia leventinese and Doyne Honeycomb Retinal Dystrophy (ML/DHRD) (Stone et al. 1999). *Fibulin-3* encodes an extracellular protein that is expressed and secreted by retinal pigment epithelial (RPE) cells (Marmorstein et al. 2002). In cell culture studies, mutant R345W Fibulin-3 is inefficiently secreted, and the majority is retained in the ER (Roybal et al. 2005). UPR signaling pathways are activated leading to increased BiP/grp78 and VEGF production (Blais et al. 2006; Roybal et al. 2005). These findings support a model whereby mutant fibulin-3 leads to macular degeneration through its misfolding in the ER and activation of UPR signaling pathways in RPE cells, followed by enhanced VEGF production and choroidal neovascularization. Enhanced VEGF levels and choroidal neovascularization are also key features of AMD (Campochiaro 2007), raising the possibility that abnormal ER stress and UPR activity in RPE may also be at play in sporadic types of macular dystrophy.

## 14.4 Pharmacologic Targeting of Protein Misfolding to Prevent Retinal Degeneration

Given the link between protein misfolding and retinal degeneration, most clearly established in the case of rhodopsin, pharmacologic prevention of protein misfolding has emerged as an exciting new strategy to treat these diseases. Chaperones are ubiquitous proteins dedicated to folding and stabilizing proteins, and recent studies by Noorwez and colleagues have demonstrated that retinoids and other related diffusible artificial chaperones can promote mutant rhodopsin binding of 11-cis-retinal such that it can function as a light chromophore (Noorwez et al. 2003,2004,2008). Mendes and colleagues have also recently demonstrated, in vitro, that retinoids and other agents that prevent protein aggregation can prevent cell death elicited by rhodopsin misfolding (Mendes and Cheetham 2008). These compounds suggest that preventing rhodopsin misfolding may be a new strategy to prevent retinal degeneration. This approach may also be efficacious in other retinal diseases arising from protein misfolding.

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