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Wild-type opsin does not aggregate with a misfolded opsin mutant

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ABSTRACT

Rhodopsin is the light receptor in photoreceptor cells that plays a central role in phototransduction and photoreceptor cell health. Mutations in rhodopsin are the leading cause of autosomal dominant retinitis pigmentosa (adRP), a retinal degenerative disease. A majority of mutations in rhodopsin cause misfolding and aggregation of the apoprotein opsin. The pathogenesis of adRP caused by misfolded opsin is unclear. It has been proposed that physical interactions between wild-type opsin and misfolded opsin mutants may underlie the autosomal dominant phenotype. To test whether or not wild-type opsin can form a complex with misfolded opsin mutants, we examined the interactions between wild-type opsin and opsin with a G188R mutation, a clinically identified mutation causing adRP. Förster resonance energy transfer (FRET) was utilized to monitor the interactions between fluorescently tagged opsins expressed in live cells. The FRET assay employed was able to discriminate between properly folded opsin oligomers and misfolded opsin aggregates. Wild-type opsin predominantly formed oligomers and only a minor population formed aggregates. Conversely, the G188R opsin mutant predominantly formed aggregates. When wild-type opsin and G188R opsin were coexpressed in cells, properly folded wild-type opsin did not aggregate with G188R opsin and was trafficked normally to the plasma membrane. Thus, the autosomal dominant phenotype in adRP caused by misfolded opsin mutants is not predicted to arise from physical interactions between wild-type opsin and misfolded opsin mutants.

1. Introduction

Rhodopsin is the G-protein coupled receptor (GPCR) that initiates phototransduction in photoreceptor cells of the retina. Rhodopsin consists of the apoprotein opsin covalently bound to the chromophore 11-*cis* retinal. Dysfunctions in rhodopsin can impair phototransduction or lead to photoreceptor cell death and retinal degeneration. Over 100 mutations in rhodopsin have been discovered in patients with inherited retinal diseases [1]. A majority of these mutations are incapable of binding 11-*cis* retinal, cause misfolding and aggregation of the apoprotein opsin, and lead to an autosomal dominant form of retinitis pigmentosa (adRP) [2–5], the most common inherited retinal degeneration [6–9]. The complete pathogenesis of adRP caused by misfolded opsin mutants is unclear.

Multiple factors likely contribute to photoreceptor cell death in adRP caused by misfolded opsin mutants. In healthy rod photoreceptor cells, rhodopsin is synthesized in the inner segment and transported to the outer segment. Rhodopsin is predominantly found in the rod outer segment disk membranes (Fig. 1A). In adRP, photoreceptor cell death occurs, at least in part, due to toxic misfolded opsin aggregates in the endoplasmic reticulum of the inner segment [10–12]. This toxicity derives from inhibition of the proteasome and activation of the unfolded protein response [4,5,12–17]. For partially misfolded mutants, photoreceptor cell death may also result from mutants that are trafficked to the rod outer segment and disrupt normal disk membrane structure [18–20].

In rod outer segment disk membranes, rhodopsin and opsin have been shown to form oligomers arranged as nanodomains [21–24]. Quaternary structure formation has been suggested to underlie the dominant retinal degeneration phenotype in adRP, where mutant receptors physically interact with wild-type receptors [5,25]. Whether these physical interactions occur and the type of complexes formed must be determined to more fully understand the pathogenesis of adRP and rationally develop therapeutics. The possible retinal degeneration mechanisms and viable therapeutics can be different depending on whether or not mutant and wild-type opsins physically interact [26]. A loss of function mechanism is unlikely since haploinsufficiency of rhodopsin is not predicted to cause retinal degeneration, at least when the level of rhodopsin is reduced by up to half [27–29]. In fact, photoreceptor cells can adapt to reduced rhodopsin expression and maintain constant rhodopsin density in rod outer segment disk membranes, presumably and maintain a constant photon catch capability [30].

Abbreviations: A:D, acceptor to donor; CFP, cyan fluorescent protein; DM, *n*-dodecyl-β-D-maltoside; DMSO, dimethyl sulfoxide; FRET, Förster resonance energy transfer; FTIR, Fourier transform infrared; G188R-mTq, G188R opsin tagged with mTq; G188R-YFP, G188R opsin tagged with YFP; GPCR, G protein-coupled receptor; mTq, mTurquoise; PCR, polymerase chain reaction; adRP, autosomal dominant retinitis pigmentosa; WT, wild-type; WT-mTq, wild-type opsin tagged with mTq; WT-YFP, wild-type opsin tagged with YFP; YFP, yellow fluorescent protein.

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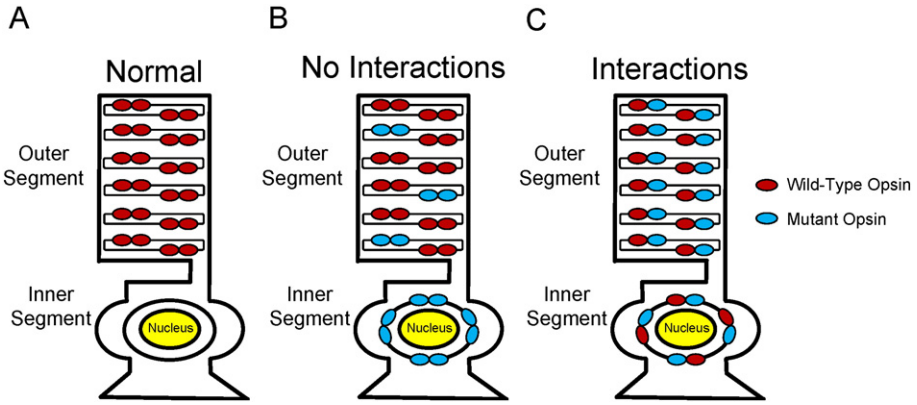

Fig. 1. Opsin localization in a rod photoreceptor cell. The localization of wild-type (red) and misfolded mutant (blue) opsins in the outer segment disk membranes or endoplasmic reticulum in the inner segment is illustrated. Localization of opsin under normal (A) and diseased (B and C) states is shown. In diseased states, scenarios where wild-type opsin cannot physically interact with mutant opsin (B) or where wild-type opsin can physically interact with mutant opsin (C) are shown.

If mutant and wild-type opsins do not interact, then the biosynthesis, trafficking and function of the wild-type receptor are predicted to be unaffected. The mechanism of adRP would then be exclusively due to a gain of function mechanism, either because mutant opsin aggregates cause toxicity in the endoplasmic reticulum or rod outer segment disk membranes are disrupted by properly trafficked mutant opsins (Fig. 1B). If mutant and wild-type opsins do interact (Fig. 1C), then the adRP mechanism could include both gain of function and dominant negative mechanisms. Dominant negative mechanisms could include the promotion of misfolding and aggregation of wild-type opsin in the endoplasmic reticulum, thereby increasing the level of toxicity and suppressing rhodopsin expression below 50%, which may be detrimental. Interactions between mutant and wild-type opsins could also disrupt normal quaternary structure and thereby alter normal packing and function of rhodopsin in rod outer segment disk membranes. To better understand the mechanism of adRP, we must discriminate between these scenarios by determining whether or not physical interactions occur between mutant and wild-type opsins and the types of complexes formed.

Studying the interactions between opsin molecules within a cellular context is a challenge. Förster resonance energy transfer (FRET) methods provide a tool to investigate protein–protein interactions within a cellular context [31]. FRET has been used to study the oligomerization of wild-type opsin and aggregation of P23H and G188R misfolding opsin mutants in COS-1 or HEK293 cells [4,32,33]. FRET has also been used to investigate the physical interactions between the P23H misfolding opsin mutant and wild-type opsin in HEK293 cells [25]. Though FRET was detected between P23H and wild-type opsins, indicating a physical interaction between the two receptors, the data was somewhat ambiguous. No negative control was conducted to ensure that the FRET signal derives from physical interactions. Since a small subset of P23H mutant opsins can fold properly [34], it was unclear whether the FRET signal originated from oligomers of properly folded opsin or from aggregates of misfolded opsin.

To overcome the ambiguities present in previous studies, more detailed FRET studies with appropriate controls were conducted on a G188R misfolding opsin mutant in the current study. The G188R opsin mutant, similarly to the more common P23H opsin mutant, misfolds, aggregates, and causes adRP [33–36]. However, the G188R mutation results in a more severe misfolding phenotype compared to the P23H mutation since all of the receptor molecules are misfolded and incapable of binding the chromophore 11-*cis* retinal [2,34,35]. Studying G188R opsin reduces ambiguity in the interpretation of results since a homogeneous population of misfolded receptor can be examined.

2. Materials and methods

2.1. DNA constructs

DNA vectors were generated for the expression of receptor tagged with either the yellow fluorescent protein (YFP) variant SYFP2 or mTurquoise (mTq). The vectors pmRho-SYFP2-1D4, pmRhoG188R-SYFP2-1D4, and pmTq-C1 were generated as described previously [33,37]. The following forward and reverse primers were used to amplify the sequence for mTq by PCR using pmTq-C1 as the template: 5′ ACGA TGGGATCCACCGGTCGCCACCATGGTGAGCAAGGGCGAGGA and 5′ CATC GTGCGGCCGCTAAGGCTGGAGCCACCTGGCTGGTCTCCGTCTTGTACAGC TCGTCCATGC. The amplified product contained a BamHI restriction endonuclease site at the 5′ end and added the sequence for a 1D4 epitope (TETSQVAPA) [38] and NotI restriction endonuclease site at the 3′ end after the sequence for mTq. This generated the product mTq-1D4. The sequences for the fluorescent proteins in pmRho-SYFP2-1D4 and pmRhoG188R-SYFP2-1D4 were replaced with the PCR product mTq-1D4 at the BamHI and NotI restriction endonuclease sites to generate the vectors pmRho-mTQ-1D4 and pmRhoG188R-mTq-1D4, respectively.

Untagged opsin constructs were generated by first PCR amplifying the opsin sequences from pmRho-SYFP2-1D4 and pmRhoG188R-SYFP2-1D4 using the following forward and reverse primers: 5′ ACGA TGAAGCTTCGAATTCGCCACCATG and 5′ CATCGTGCGGCCGCTTAGGCTG GAGCCACCTGGCTGGT. These primers added an EcoRI restriction endonuclease site at the 5′ end and a stop codon and NotI restriction endonuclease site at the 3′ end of the opsin sequences. The PCR products replaced the sequence mRho-SYPF2-1D4 in pmRho-SYFP2-1D4 at the EcoRI and NotI restriction endonuclease sites to generate the vectors pmRho and pmRhoG188R.

The vector coding for the m2 muscarinic receptor tagged with mTq containing the 1D4 epitope was constructed as follows. The template containing the sequence for the m2 muscarinic receptor was from the FLAG-m2-pBlueBac4.5 vector generated previously [39]. The sequence for the m2 muscarinic receptor was amplified by PCR from this template using the following forward and reverse primers: 5′ ACGATGAAGCTTAT GAATAACTCAACAAACTCCTCTAA and 5′ CATCGTTCTAGACCTTGTAGCG CCTATGTTC. These primers added HindIII and XbaI restriction endonuclease sites at the 5′ and 3′ end of the m2 muscarinic receptor sequence, respectively. The PCR product was inserted into the vector pFLAG-CMV-3 (Sigma Aldrich, St. Louis, MO) at the HindIII and XbaI restriction endonuclease sites to generate the vector pFLAG-m2-CMV-3, which codes for the m2 muscarinic receptor tagged at the amino terminal