Electrostatic Compensation Restores Trafficking of the Autosomal Recessive Retinitis Pigmentosa E150K Opsin Mutant to the Plasma Membrane*

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Rhodopsin is the rod photoreceptor G protein–coupled receptor responsible for capturing light. Mutations in the gene encoding this protein can lead to a blinding disease called retinitis pigmentosa, which is inherited frequently in an autosomal dominant manner. The E150K opsin mutant associated with rarely occurring autosomal recessive retinitis pigmentosa localizes to trans-Golgi network membranes rather than to plasma membranes of rod photoreceptor cells. We investigated the molecular mechanisms underlying opsin retention in the Golgi apparatus. Electrostatic calculations reveal that the E150K mutant features an overall accumulation of positive charges between helices H-IV and H-II. Human E150K and several other closely related opsin mutants were then expressed in HEK-293 cells. Spectral characteristics and functional biochemistry of each mutant were analyzed after reconstitution with the cis-retinoid chromophore. UV-visible spectra and rhodopsin/transducin activation assays revealed only minor differences between the purified wild type control and rhodopsin mutants. However, partial restoration of the surface electrostatic charge in the compensatory R69E/E150K double mutant rescues the plasma membrane localization of opsin. These findings emphasize the fundamental importance of electrostatic interactions for appropriate membrane trafficking of opsin and advance our understanding of the pathophysiology of autosomal recessive retinitis pigmentosa due to the E150K mutation.

Rod photoreceptor cells are the most abundant cell type in the mammalian retina (1–4). Mutations in rod proteins involved in a variety of cellular processes can lead to retinitis pigmentosa (RP)5 (5–9), a blinding disease inherited in either an autosomal dominant or recessive manner (6). The rod visual pigment, rhodopsin, is responsible for capturing light and triggering a series of biochemical reactions called phototransduction (10). Rhodopsin resides in rod photoreceptor outer segment membranes and is comprised of the apoprotein opsin and the chromophore 11-cis-retinylidene. This receptor is a prototypical heptatransmembrane-spanning G protein–coupled receptor that interacts with the specific heterotrimeric G protein transducin (Gt) (11, 12). More than 100 mutations in the rod opsin gene have been identified that cause autosomal dominant retinitis pigmentosa. When expressed in experimental cell lines or in transgenic rat, pig, mouse, or frog retinas (6), these mutants typically produce pathological phenotypes that include constitutive stimulation of the phototransduction pathway, dysfunctional cellular transport, or toxic accumulation of misfolded mutant proteins.

Although quite prevalent among ophthalmic diseases, autosomal dominant retinitis pigmentosa does not occur with great frequency, and the recessive form of this disease is even less common. The first report linking recessive RP to a mutation in the opsin gene involved a nonsense mutation at codon 249 within exon 4 (13), which produced a nonfunctional opsin that could not link to the chromophore. Another autosomal recessive RP mutation, the E150K mutation in the opsin gene, has been reported later (14). This point mutation does not disrupt expression of WT protein because heterozygous siblings of the individual with the E150K mutation display no signs of deteriorating rod cell function (15).

One explanation for the abnormal properties exhibited by the E150K mutant could be the disruption of electrostatic interactions resulting from substitution of a positively charged Lys for the negatively charged Glu residue. This could alter structure, stability, and folding of the opsin monomer or could interfere with formation of opsin dimers, which likely represent the physiological form of this protein (16–20). Zhu et al. (21) biochemically characterized the E150K mutant protein and found, in contrast to many autosomal dominant retinitis pigmentosa mutants, that it was retained in the Golgi. Moreover, the signaling properties of the purified mutant did not differ significantly from those of WT opsin. Interestingly, the 150 position in the primary sequence of vertebrate rhodopsin is not absolutely conserved. Skate rhodopsin has a Ser residue, whereas lamprey rhodopsin exhibits an Asn residue at this position. These residues are located in the highly conserved cytoplasmic loop III of rhodopsin, suggesting that certain substitutions in this region can be tolerated without adverse structural or signaling consequences (22). However, the size of rod outer segments is proportional to the level of opsin expression. Heterozygote opsin knock-out mice display no overt disease phenotype apart from a reduced disc diameter (19), whereas overexpression of opsin in rods increased their outer segment diameters, accommodat-

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2 The abbreviations used are: RP, retinitis pigmentosa; BTP, 1,3-bis(tris(hydroxymethyl)methylamino)propane; DDM, N-dodecyl-beta-D-maltoside; GTPyS, guanosine 5’-3-O-(thio)triphosphate; SNARE, soluble NSF attachment protein receptors.
ing the extra rhodopsins (23). Thus, available evidence supports the possibility that rhodopsin is a protein that maintains the structure of rod cells in addition to its primary function in vision. Could it be that changing from a negatively charged to a positively charged residue in the E150K mutant contributes to disruption of the previously proposed higher order organization of rhodopsin (11)? Proper formation of dimers and ordered oligomers of opsin, as illustrated by atomic force microscopy (16–20), could therefore be a prerequisite for the capability of rhodopsin to maintain disk structure. Herein, we expand on previous work searching for the basis of E150K-induced pathology by analyzing the biochemical characteristics of E150K human opsin and several closely related mutants expressed in HEK-293 cells. Our results reveal the importance of electrostatic interactions in maintaining the stability and appropriate membrane trafficking of rod opsin.

**MATERIALS AND METHODS**

**DNA Constructs**—Human opsin cloned in the pcDNA4/TO vector (Invitrogen) was obtained as described previously (21). WT opsin was mutated as indicated after subcloning into pGEM-T-Easy plasmid (Thermo Fisher Scientific) (24). Site-specific mutagenesis (E150K, R69E/E150K, R69E, K66E/E150K, K66E, Y74A, and E150A) was performed with a QuikChange site-directed mutagenesis kit purchased from Stratagene (Cedar Creek, TX), and the mutant opsin cDNAs were further cloned into the multicloning site of the pMXs-IgG3 retroviral vector (25, 26) upstream of the internal ribosome entry site and the enhanced green fluorescent protein site. Sequences of these constructs were confirmed by DNA sequencing.

**Rhodopsin/Opsin Expression in HEK-293 Stable Cell Lines**—HEK-293 cells and Phoenix Ampho cells (Orbigen, San Diego, CA) were cultured in growth media consisting of Dulbecco’s modified Eagle’s medium with 4 mM L-glutamine, 4.5 g/liter glucose, 110 mg/L-sodium pyruvate, 100 units/ml penicillin, 100 mg/ml streptomycin, and 10% fetal bovine serum (FBS). To generate stable cell lines, HEK-293 cells were transduced with a retrovirus produced with Phoenix Ampho cells carrying WT or mutant opsin genes. To generate this retrovirus, 1.6 × 10⁶ Phoenix Ampho cells in 6 ml of growth medium were seeded into a 6-cm dish. After 24 h, the medium was replaced with fresh, warm growth medium supplemented with 25 μM chloroquine (Sigma-Aldrich), and the cells were incubated for 5 min at 37 °C in 5% CO₂. Transfections were performed by using a calcium phosphate method described previously (27). Briefly, a solution of DNA (15 μg), 938 μl of water, and 62 μl of 2 M CaCl₂ was added to 1 ml of 50 mM HEPES buffer, pH 7.05, 10 mM KCl, 12 mM dextrose, 280 mM NaCl, and 1.5 mM Na₂HPO₄, and the resulting solution was mixed immediately by rapid inversion of the tube several times. After a 1-min incubation, this solution was added dropwise to Phoenix Ampho cells, and the cells were incubated at 37 °C in 5% CO₂. 10 and 24 h after transfection, the medium was replaced with fresh growth medium. To harvest the virus 48 h after transfection, the medium was transferred into 15-ml conical tubes, spun at 450 × g for 5 min to remove detached cells and then filtered through Millex 0.45-μm polyvinylidene fluoride (PVDF) syringe driven filters (purchased from Millipore, Bedford, MA). The virus-containing filtrate was then added to the HEK-293 cells.

Prior to transduction (24 h), target HEK-293 cells were seeded into a dish at 6 × 10⁵ cells/6-cm dish in 4 ml of growth medium. For transduction of target cells, the medium was replaced with a mixture of 1.5 ml of growth medium, 1.5 ml of viral supernatant, and 3.5 μl of polybrene (final concentration, 5 μg/ml). Transduction was then carried out for 24 h at 32 °C in 5% CO₂. After 24 h, the medium was replaced with fresh growth medium, and after an additional 24 h, cells were transferred to a larger culture flask. Upon recovery, cells that exhibited similar EGFP fluorescence intensity profiles were isolated with a cell sorter to ensure similar mRNA levels of WT and mutant opsins.

**Pigment Reconstitution and Purification of Isorhodopsin (Rhodopsin)—**HEK-293 cells expressing opsin were cultured (~20 plates; 150 × 20 mm) in growth medium consisting of Dulbecco’s modified Eagle’s medium with 4 mM L-glutamine, 4.5 g/liter glucose, 110 mg/liter sodium pyruvate, 100 units/ml penicillin, 100 mg/ml streptomycin, and 10% FBS. Cells were grown to 90% confluence. The following steps were performed in the dark. A fresh stock of 9-cis-retinal in dimethyl sulfoxide (from Toronto Research Chemicals, Toronto, Canada) was prepared with FBS and added to HEK-293 cells expressing opsin to provide a final concentration of 17 μM. Similarly, a fresh stock of 11-cis-retinal in dimethyl sulfoxide (a gift from Dr. R. Crouch, University of South Carolina, through a contract with the National Institutes of Health) was prepared with FBS and added to yield a final concentration of 17 μM. Isorhodopsins generated with 9-cis-retinal and rhodopsins generated with 11-cis-retinal were indistinguishable in our Gₛ activation and Meta II decay assays. The 9-cis-retinal-treated cells were incubated in the dark for 24 h at 37 °C with 5% CO₂ and then harvested with trypsin. Cells were centrifuged at 800 × g, and the pellet was resuspended in 50 mM 1,3-bis(tris(hydroxymethyl)methylamino)propane (BTP), 150 mM NaCl, and 1% n-dodecyl-β-d-maltoside (DDM), pH 7.4, and then incubated on ice for 30 min before homogenization. The homogenate was centrifuged at 90,000 × g for 30 min at 4 °C, and isorhodopsin or rhodopsin was purified from the supernatant by using an anti-rhodopsin C-terminal 1D4 antibody (28) immobilized on CNBr-activated agarose (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) (29). Briefly, the supernatant was added to 200 μl of 2 mg 1D4/ml agarose beads and incubated for 60 min. The resin was packed into a column and then washed with 50 ml of 50 mM BTP, pH 7.4, containing 100 mM NaCl and 0.1% DDM. Isorhodopsin (or rhodopsin) was eluted with 50 mM BTP, pH 7.4, containing 100 mM NaCl and 0.1% DDM supplemented with 200 μg/ml of TETSQVAPA peptide (United Biochemical Research, Seattle, WA). Isorhodopsin (or rhodopsin) concentration in eluted fractions was determined by absorption at 280 and 500 nm.

**Photosensitivity of the Visual Pigment**—UV-visible spectra were recorded with freshly purified isorhodopsin (or rhodopsin) samples. Briefly, the spectrum was recorded initially in the dark. Then, the sample was exposed to light for 2 min from a long pass wavelength filter (>490 nm) (30), and the spectrum was recorded after bleaching. To trap protonated retinylidene Lys²⁹⁶, 0.1% sul-
furic acid was added to adjust the pH to ~2.0 and the spectrum was recorded for the acidified, bleached sample.

**Meta II Decay**—The activity of isorhodopsin (or rhodopsin) was determined by using fluorescence measurements (30). The sample was prepared by using isorhodopsin/rhodopsin (final concentration, 20 nM) in 2 ml of buffer containing 20 mM BTP, 100 mM NaCl and 0.1% DDM, pH 6.0. Then, the sample was exposed to light for 15 s (λ = 490 nm). Intrinsic Trp fluorescence of the sample was measured at excitation and emission wavelengths of 295 and 330 nm, respectively, with a PerkinElmer Life Science L55 luminescence spectrophotometer (31). During all fluorescence recordings, the temperature in the cuvette chamber was maintained at 20 °C, and the sample was continuously stirred at low speed.

**Gt Activation Assay**—G protein was prepared by a method described previously (32). Gt activation was determined by fluorescence measurements (33, 34). A sample was prepared by using isorhodopsin/rhodopsin (final concentration, 30 nM) and Gt (final concentration 250 nM) in 500 μl of buffer containing 20 mM BTP, 100 mM NaCl, 5 mM MgCl₂, and 0.1% DDM, pH 6.5. The sample was exposed to light for 15 s (λ = 490 nm). Intrinsic fluorescence was measured at excitation and emission wavelengths of 300 and 345 nm, respectively. After 5 min, GTP*S was added to a final concentration of 5 μM, and the sample fluorescence was recorded. Changes in intrinsic Trp fluorescence was used as a measure of the Gt activation rate.

**Immunocytochemistry and Confocal Imaging**—HEK-293 cells expressing opsin were cultured in 24-well plates with cov-
FIGURE 2. Tuning of electrostatics on the cytoplasmic surface of rhodopsin. Electrostatic potentials calculated for WT and E150K mutant rhodopsin indicate that the E150K mutation leads to an overall increase in positive potential on the cytoplasmic surface. The electrostatic potential can be tuned by substituting charged amino acids in the vicinity of position 150 with different charged or uncharged residues (C-H). Potentials were calculated in units of $kT/e^-$ using the Adaptive Poisson-Boltzmann Solver (35). As shown by the scale under $A$, negative potentials are portrayed in red, and positive potentials are shown in blue. The top image of each panel shows the residues of interest as sticks, and the distribution of electrostatic potential on the molecular surface of each form of rhodopsin is displayed in the bottom image of each panel.
FIGURE 3. Functional characterization and subcellular localization of rhodopsin and its isorhodopsin mutants. A, a panel, WT and mutant structures of isorhodopsin showing residues in the vicinity of Glu150. Residues are rendered as sticks. Mutants were modeled with PyMOL. b panel, absorption spectra of rhodopsin. Rhodopsin was isolated from 20 plates (150 × 20 mm) of HEK-293 cells (90% confluent) and purified by 1D4 affinity chromatography. UV-visible spectra of rhodopsin in the dark were taken immediately following this purification step. Then, samples were bleached for 2 min, and the spectra were recorded immediately thereafter. Finally, samples were acidified with 0.1% concentrated sulfuric acid, and their spectra were recorded again. c panel, Meta II decay and activation of G. Intrinsic fluorescence was recorded immediately after exposure to light for 15 s. Fluorescence emission was measured at 330 nm after excitation at 295 nm. G activation was measured by monitoring the Trp fluorescence increase from the G subunit (inset). d panel, HEK-293 cells expressing WT opsin and GFP. Opsin was stained with anti-Rho B6-30 antibody (red). GFP (green) indicates the cytoplasmic space, whereas Hoechst staining (blue) displays the cell nucleus. Similarly, the E150K (B), R69E/E150K (C), R69E (D), K66E (E), K66E/E150K (F), E150A (G), and Y74A (H) mutants of rhodopsin were analyzed as described for WT rhodopsin. Purification was reproduced for WT, E150K, and R69E/E150K mutants at least 10 times and twice for the other mutants. Meta II decay and G activation assays were performed three times for each preparation. AU, absorbance units.
but an overall increase in the positive character of this protein region. We selected Ala in this position to eliminate, in addition to hydrogen bonding, possible \( \pi \) electron aromatic interactions with other residue. WT rhodopsin and all of the opsin mutants were expressed as stably transfected HEK-293 cells. Apart from the WT rhodopsin control and E150K, E150K/R69E, R69E, K66E, E150K/K66E, Y74A, and E150A variants were the focus of the current study.
Effect of E150K Mutation on Binding the Chromophore—Opsin mutants (Fig. 3, A–H, a panels) expressed in HEK-293 cells were reconstituted with 9-cis-retinal or 11-cis-retinal and then purified by 1D4-affinity chromatography. The absorbance spectra of WT isorhodopsin as well as all isorhodopsin (reconstitution with 9-cis) mutants exhibited a peak at 478 nm (Fig. 3, A–H, b panels) and of rhodopsins (reconstitution with 11-cis) at 496 nm, indicating cis-chromophore binding in the retinal binding pocket and formation of the Schiff base linkage to Lys296. The absorption spectra of the mutant proteins in the dark after light exposure, and then under acidic conditions, were similar to those of WT rhodopsin with respect to the binding and function of chromophore. These results suggest that these mutant opsinss were properly folded and inserted into the bilayer and that the chromophore binding site was not perturbed; thus, further assays were not affected by gross protein alteration.

Meta II Decay—Light-induced activation of rhodopsin results in the formation of the Meta II-activated state (11, 12). Meta II decays as a function of time, resulting in the formation of the inactive signaling states Meta III and opsin as the chromophore is released from the binding pocket, thus affecting the rate of G protein activation. This release causes structural changes reflected by an increase in Trp fluorescence. Therefore, Meta II decay can be monitored by measuring Trp fluorescence as a function of time. Meta II decay was assessed for purified preparations of WT rhodopsin and all of the mutants included in this study. Rates of Meta II decay were similar for WT rhodopsin and all tested mutants, except slightly slower rates were observed for K66E and Y74A, indicating that mutations in E150K, R69E/E150K, K66E/E150K, and E150A do not affect the activation of rhodopsin (Fig. 3, A–H, c panels). The data are summarized in Fig. 4. The duration of
active forms is comparable for all mutants, suggesting that chromophore release is not affected by the indicated mutations, which is consistent with UV-visible spectra.

Effect of Rhodopsin Mutations on Interaction with Gt—Glu150 is located at the C-terminal domain of rhodopsin involved in the binding of Gt (11, 12). Therefore, activation of Gt is an important functional parameter to measure when assessing the effect of opsin mutations at the Glu150 region. Upon activation of rhodopsin, guanylyl nucleotide exchange occurs in the α-subunit of Gt. We monitored the increase in Trp fluorescence caused by this GTPγS exchange. Interestingly, Gt activation was comparatively elevated in the mutants E150K, R69E, K66E, K66E/E150K, and E150A, with the double mutants R69E/E150K and Y74A showing similar behavior as in WT (Fig. 4). This suggests that the change in electrostatics near Glu150 in the C-terminal domain has minor effects on the activation of Gt. These data are consistent with the similar decay of Meta II, suggesting that all mutants are functional in G protein activation by maintaining the proper activation state and Gt.

Intracellular Localization of Opsin Mutants—The effect of the E150K mutation on membrane trafficking of opsin expressed in HEK-293 cells was studied by immunocytochemistry and imaged by confocal laser scanning microscopy. WT opsin was localized to the plasma membrane (Fig. 3A, d panel), suggesting proper transport. Similarly, the mutants R69E (Fig. 3D, d panel), K66E (E, d panel), E150A (G, d panel), and Y74A (H, d panel) also localized to the plasma membrane, indicating that there is no effect of these mutations on the membrane transport of opsin. In contrast, the E150K-mutated opsin failed to be appropriately transported to the cell surface and was localized instead to intracellular compartments (Fig. 3B, d panel). Notably, the compensating double mutant R69E/E150K was localized to the plasma membrane similarly to WT (Fig. 3C, d panel), whereas the other double mutant K66E/E150K behaved similarly to the E150K mutant.

To identify the localization of E150K in intracellular compartments, opsin was co-stained with markers in the endoplasmic reticulum (ER) and the Golgi network. Whereas both WT protein and the double mutant R69E/E150K localized to the plasma membrane, the E150K mutant localized to intracellular compartments of the ER (Fig. 5). However, co-staining with anti-calreticulin revealed that the E150K mutant opsin did not co-localize with calreticulin, indicating that E150K was not stuck in the ER, an observation also supported by endoglycosidase H treatment of mutant opsins showing transport to the Golgi due to the presence of endoglycosidase H-sensitive glycosylation (data not shown). E150K localization in Golgi compartments was then tested by using the medial and cis-Golgi marker, giantin, and the trans-Golgi marker, Vti1b (Fig. 6). Double staining of opsin with giantin revealed that E150K did not co-localize with giantin (Fig. 6, top panel), thereby excluding localization of the E150K mutant opsin in the medial and cis-Golgi. Double labeling of mutant opsins and Vti1b (Fig. 6) revealed that E150K closely associated with Vti1b, suggesting localization of E150K to the trans-Golgi network. Together, our data suggest that the E150K mutant is deficient in cellular transport and not in functional coupling with the chromophore or G protein.

DISCUSSION

The E150K substitution in the rod photoreceptor opsin gene leads to a disease called autosomal recessive RP (14, 15). Current studies have focused primarily on restoring the intracellular transport of this membrane protein. The change from a negatively charged glutamate to a positively charged lysine in the E150K mutant, as well as location of residue 150 on the extracellular surface of the cellular membrane, prompted our investigation to determine whether electrostatic interactions can affect the function of this visual receptor. Our results did not only indicate that the E150K mutation causes a change in local electrostatic interactions on the extracellular surface of this protein but also that reducing this electrostatic change by a compensating mutation restored proper transport of the double mutant protein to the plasma membrane. In contrast to the vast majority of other opsin mutants that cause autosomal dominant retinitis pigmentosa by acquiring constitutive activity and destabilizing the protein (9, 36–41), our present results strongly suggest that altering the overall charge in the region
encompassing Glu150 and neighboring residues can cause retention of membrane protein in the trans-Golgi.

Properties of E150K Opsin Mutant—Our basic approach was to stably express both WT rhodopsin and the E150K mutant in HEK-293 cells, along with a series of other related opsins with mutations of residues located near Glu150 in the three-dimensional structure of rhodopsin. Somewhat surprisingly, the mutant proteins displayed generally little change from WT rhodopsin in both the spectral and biochemical studies, even though their overall electrostatic charges were changed. However, the overall electrostatic charge appeared to affect proper trafficking of these receptors to cellular plasma membranes such that several mutant proteins were directed into the trans-Golgi network instead of the plasma membrane.

The similarity between the absorbance spectra of mutants and WT opsins indicates that there is no effect of the E150K mutation on binding of chromophore or formation of the Schiff base linkage with Lys296. This result is reasonable because the retinal-binding site is positioned almost 33 Å from the 150 position. This finding also indicates that the E150K mutation does not result in a major structural change that could destabilize rhodopsin. This also is supported by the similarities between WT and mutants in the function of rhodopsin in terms of Meta II decay. However, we cannot exclude that rhodopsin is more stable than the opsin owing to enhanced hydrophobic interactions that result from the binding of a hydrophobic retinal.

Localization studies reveal that the E150K mutant opsin is retained within the trans-Golgi inside the cell rather than being efficiently exported to the plasma membrane. This phenomenon can be explained by altered local electrostatic interactions. The interaction of Glu150 with Arg69 can be very important for interhelical contacts (42), which could be destabilized by the presence of a positively charged residue at position 150. The positive charge accumulation would cause repulsion between H-IV and H-II, resulting in a change in the tertiary structure of rhodopsin (16, 17, 19, 20), retention of the mutant protein in the intracellular compartments, and/or abolished interactions with intracellular transport proteins. Restoration of this electrostatic interaction by introduction of a compensatory second mutation reestablished the WT electrostatic environment and restored the altered properties of the E150K mutant.

Properties of the Electrostatic Compensatory Opsin Mutant—The positive charge introduced by mutating residue Glu150 to a Lys was compensated for through the creation of the double R69E/E150K mutant. Although this double mutant possesses a positive charge at H-IV (E150K) and a negative charge at H-II (R69E), the ionic interaction between these two residues should be preserved, thus restoring wild type properties of this rhodopsin variant. Indeed, the double mutant is transported efficiently to the plasma membrane (Fig. 3C, d panel, and Fig. 7). Arg69 in rhodopsin contributes strongly to the overall positive charge because it is located between Lys66 and Glu150. Furthermore, if the charge at position 150 was directly important for the interaction of rhodopsin with helper proteins involved in intracellular transport to the plasma membrane, it would be
logical to expect that the E150A mutant would be defective and retained in the cellular organelles. Because our compensatory experiments do not indicate this, the possibility of E150K influencing the interaction with other proteins appears to be less likely.

As shown in Fig. 7, the E150K mutant is not transported to the plasma membrane. Previous studies revealed that G protein-coupled receptor transport from the trans-Golgi network to the plasma membrane involves precise sorting in vectorial membrane transport trafficking. Vti1b is one of the proteins in the SNARE complex (43, 44) known to be involved in trafficking from the Golgi to the plasma membrane (45, 46) and in membrane fusion of vesicles. Therefore, co-localization of E150K with Vti1b suggests that this mutant opsin cannot pass through the sorting mechanisms of membrane transport and therefore is trapped in the Golgi apparatus. The compensatory double mutant restores proper transport of the opsin to the plasma membrane. We also assume that this mutant does not have significantly altered folding because the chromophore-binding properties and the ability to activate transducin are not abolished as a result of the mutation. However, the mutation could result in a local change in the tertiary protein structure that can be reversed by restoring the native interactions between helices and allowing proper membrane trafficking.

Autosomal Recessive Retinitis Pigmentosa—The first reported observation linking autosomal recessive RP to a mutation in the opsin gene involved a nonsense mutation at codon 249 within exon 4 (13). This mutation produced a shortened form of opsin that could not function because the truncation occurs before Lys286, the residue linked to the chromophore, and the mutant is missing part of the transmembrane bundle. The investigators also reported a different null mutation carried heterozygously by an unrelated and unaffected individual but did not characterize it further. It is known, however, that complementary fragments of rhodopsin expressed in experimental cell lines can assemble together and even form a functional split receptor with properties similar to rhodopsin (47). Heterozygotes with a nonsense mutation at codon 249 displayed abnormal rod responses suggesting that this fragment disrupts normal opsin/rhodopsin transport, co-localizing with WT rhodopsin and perturbing rod outer segment organization and/or WT rhodopsin function (10).

In vivo, the presence of the E150K opsin mutation does not affect the transport of wild type rhodopsin and, similar to heterozygote knock-out opsin mice (19, 48, 49), photoreceptors are functional and stable. It is likely that the mutant proteins are co-transported with WT opsin to rod outer segments. Thus, the heterozygous carriers of this mutation will not exhibit any apparent visual deficiency, as reported previously (14, 15). In contrast, photoreceptors of a homozygote carrying this mutation likely will not form rod outer segments because of the lack of structural support by opsin required to formation of rod outer segment. The retention of the mutated opsin in the Golgi apparatus would lead to rod cell death due to absence of the so-called “dark-current” similarly to knock-out rhodopsin (48, 49) and the stress imposed by overloading the inner segments of these cells with mutant opsin. Alternatively, repulsion between adjacent mutant opsin molecules will lead to formation of aberrant unstable rod outer segments prone to degeneration with time.

CONCLUSIONS

Biochemical studies of rare mutations associated with human disease can provide insights essential for the development of molecular therapeutics. Moreover, studies of many mutations leading to one type of retinal disease can lead to common approaches applicable to other retinal diseases. For example, a successful intervention that spares rods in RP caused by an opsin mutation also will permit cones to avoid “bystander” cell death and thereby preserve vision (50). Using a variety of approaches to investigate abnormalities in visual protein mutants may provide insights into how WT proteins function in the normal state. The E150K mutant opsin is a case in point. This mutant only involves a change in charge on the cytoplasmic surface of rhodopsin and does not affect its coupling or its activation of G, but still results in retinal disease. Neutralization of the aberrant charge by a second mutation appears to prevent this pathology, suggesting that a possible route to therapy for this mutation might involve the development of therapeutic compounds which specifically bind in this site and compensate for this mutation.

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