The Chaperone Environment at the Cytoplasmic Face of the Endoplasmic Reticulum Can Modulate Rhodopsin Processing and Inclusion Formation

J. Paul Chapple and Michael E. Cheetham
From the Division of Pathology, Institute of Ophthalmology, University College of London, London EC1V 9EL, United Kingdom

The human DnaJ (Hsp40) proteins HSJ1a and HSJ1b are type II DnaJ proteins with different C termini generated by alternate splicing. Both protein isoforms can regulate the ATPase activity and substrate binding of Hsp70. In this study, we have confirmed the neuronal expression of HSJ1a and HSJ1b proteins and localized their expression in human neural retina using isoform-specific antisera. HSJ1a and HSJ1b were enriched in photoreceptors, particularly the inner segments, but had different intracellular localization due to the pre-nylation of HSJ1b by a geranylgeranyl moiety. Because of their enrichment at the site of rhodopsin production, we investigated the effect of HSJ1 isoforms on the cellular processing of wild-type and mutant rhodopsin apoprotein in SK-N-SH cells. The expression of HSJ1b, but not HSJ1a, inhibited the normal cellular processing of wild-type rhodopsin-GFP, which co-localized with HSJ1b at the ER. HSJ1b expression also increased the incidence of inclusion formation by the wild-type protein. Both isoforms were recruited to mutant P23H rhodopsin inclusions, but only HSJ1b enhanced inclusion formation. Investigation of a prenylation-null mutant showed that the modulation of rhodopsin processing and inclusion formation was dependent on the correct subcellular targeting of HSJ1b to the cytosolic face of the ER. An Hsp70 interaction-null mutant of HSJ1b had the same effect as HSJ1b, suggesting that these phenomena were independent of Hsp70 and, furthermore, over-expression of Hsp70 with HSJ1b did not modulate the HSJ1b effect on inclusion formation, showing that Hsp70 was not limiting. The data provide evidence that cytoplasmic chaperones when targeted to the ER can influence the folding and processing of a GPCR and show that DnaJ protein function can be further specialized by alternative splicing and post-translational modification.

Rhodopsin, the visual pigment of rod photoreceptors, is the prototypical G-protein-coupled receptor. Mutations in rhodopsin are the major cause of the neurodegenerative disease retinitis pigmentosa with more than 100 distinct mutations identified (OMIM 180380). Rhodopsin mutations can be divided into two categories on the basis of the mechanism of pathogenesis. Mutations at the C terminus of the protein interfere with its normal targeting to the photoreceptor outer segment (1–3), whereas mutations in the transmembrane, intradiscal, or cytoplasmic domains result in the misfolding of the protein (4–8).

When wild-type rhodopsin is heterologously expressed in cultured mammalian cells it translocates to the plasma membrane, whereas rhodopsin with misfolding mutations accumulates within the cell. Misfolded rhodopsin does not acquire mature oligosaccharides, indicating it does not transit through the Golgi apparatus, and also fails to produce functional visual pigment with 11-cis-retinal (4, 6, 8). Instead, misfolded rhodopsin undergoes retrotranslocation from the endoplasmic reticulum (ER) and degradation by the ubiquitin-proteasome system, (9, 10). Saturation of the normal proteolytic machinery causes misfolded ubiquitinated rhodopsin to accumulate in pericentriolar cytoplasmic inclusion bodies, known as aggresomes (9–11).

Recently, there has been much interest in the role of molecular chaperones in diseases of protein misfolding where the formation of cellular inclusions of aggregated protein is a common feature (12). Molecular chaperones are recruited to mutant rhodopsin aggresomes (9); however, the roles of chaperones in the biogenesis and aggregation of rhodopsin are relatively unexplored. A specialized molecular chaperone, NinaA, has been described for Drosophila rhodopsin, but a mammalian orthologue of NinaA has not yet been identified (13). ER resident chaperones appear to play a role in the quality control of mutant rhodopsin folding and processing (9, 14), but the effect of manipulating chaperones on mammalian rhodopsin processing and mutant opsin aggregation and/or degradation has not yet been investigated.

The diverse members of the DnaJ family of cochaperone proteins are characterized by a highly conserved 70-amino acid domain called the J-domain. The J-domain interacts with Hsp70 proteins, stimulating ATP hydrolysis and altering substrate binding, such that DnaJ proteins play an essential role in the regulation of the Hsp70 cochaperone machine. In addition to this cochaperone function, several DnaJ proteins, including E. coli DnaJ, Ydj1 and Csp, have been shown to have independent cochaperone activity in vitro (15, 16). Different DnaJ proteins are found in subcellular compartments including the cytosol.
nucleus, mitochondria, and ER. DnaJ proteins can also be targeted to membranes by transmembrane domains or post-translational lipid modifications, such as prenylation. Prenylated proteins have either a farnesyl (C15) or a geranylgeranyl (C20) isoprenoid group attached to a C-terminal cysteine residue by a thioether linkage (17, 18). Prenylation requires the recognition of a C-terminal CAAX tetrapeptide motif by a prenyl transferase.

HSJ1a and HSJ1b are human type II DnaJ proteins (16) that have different C termini generated by alternate splicing from a single copy gene (Fig. 1) and the mRNAs are preferentially expressed in neurons (19). Both protein isoforms can regulate the ATPase activity and substrate binding of Hsp70 (20, 21). In this study we show that the larger isoform is targeted to the cytoplasmic face of the ER by prenylation and that this localization can facilitate an interaction with rhodopsin, modulating rhodopsin processing and influencing inclusion formation.

EXPERIMENTAL PROCEDURES
Antibody Generation and Construction of Plasmids—Recombinant HSJ1a was purified as described previously (21) and used to produce, and affinity purify, sheep polyclonal anti-HSJ1 serum, sHSJ11–277. Rabbit polyclonal anti-peptide sera specific for HSJ1a (rHSJ1a267–320) and HSJ1b (rHSJ1b307–320) were raised against C-terminal peptides CEAAAGKKPADVF (HSJ1a) and CATKRSPSPEEKASR (HSJ1b) as previously described (22). Cloning and mutagenesis of HSJ1 isoform open reading frames (21) into mammalian expression and fusion vectors pPBSTR-1 (23), pEGFP-C1 (Clontech, Palo Alto, CA) and pCMV-Tag3 (Stratagene Ltd, Cambridge, UK) were facilitated by PCR-directed changes (24). The untagged bovine rhodopsin constructs, rhodopsin-GFP fusions and use of anti-rhodopsin antibody 1D4 have been described previously (9). pCMV70 encoding human-inducible Hsp70 was a gift from Dr. H. Kampinga (Groningen).

Immunofluorescence Labeling and Confocal Microscopy of Human Retina—Immunofluorescence was performed as previously described (25). rHSJ1a267–320 and rHSJ1b307–320 antisera were used at a titer of 1:100, C3′-conjugated donkey anti-rabbit (Jackson Immunoresearch) secondary antibody, 1:50; peptide competition was at 250 μM. GFP fluorescence was monitored, and cell fixation/permeabilization for immunocytochemistry were as described (24) with the following antibody titers: sHSJ11–277 1:100; Hsp70 (SPA-810, which specific for the inducible form) 1:250; BIP (SPA-826) 1:200 and calnexin (SPA-860; Stressgen, Victoria, Canada) 1:200; C3′-conjugated donkey secondary antibody (Jackson Immunoresearch) 1:50; Alexa Fluor 647-conjugated chicken anti-mouse (Molecular Probes) 1:500.

Subcellular Fractionation of Cells Expressing GFP-HSJ1 Chimeras—Subcellular fraction and Western blotting to determine the membrane association of HSJ1 chimeric proteins were performed as previously described (24, 26).

RESULTS
HSJ1 Proteins Are Expressed in Neural Tissues and Are Enriched in Photoreceptors—Previously it had been shown that neuronal layers of the brain contained higher levels of HSJ1 mRNAs than other tissues (19). Human tissues were screened for HSJ1 protein expression by Western analysis (Fig. 2A) using an affinity-purified sheep polyclonal antiserum (sHSJ11–277) raised against purified recombinant HSJ1a protein. HSJ1a and HSJ1b proteins were most abundant in neocortex, cerebellum, spinal cord, and retina. Both HSJ1 isoforms could also be detected at very low levels (100-fold lower than in cerebellum) in non-neuronal tissues; kidney, spleen, lung, heart, and testis (data not shown). HSJ1b appeared to be more abundant in neocortex and cerebellum than HSJ1a.

To analyze the localization of the HSJ1a and HSJ1b isoforms within tissues we generated isoform-specific polyclonal anti-peptide sera to the C termini of HSJ1a (rHSJ1a267–320) and HSJ1b (rHSJ1b307–320). These antisera were isoform-specific as demonstrated by immunoreactivity of cells transfected with GFP-HSJ1a1 and GFP-HSJ1b chimeric proteins. sHSJ11–277 did not react with either HSJ1a or HSJ1b by Western blotting. sHSJ1a267–320 detected a single band corresponding to HSJ1a in cerebellum (Fig. 2B), confirming that this antiserum (which was raised to a peptide with nine residues common to HSJ1b), was HSJ1a-specific and that the shorter alternative transcript is translated into protein. All specific immunoreactivities could be competed with the immunizing peptide.

To investigate whether both isoforms are expressed in the same cell or if the alternative splicing of HSJ1a was cell-type specific, we used these antisera for immunohistochemistry of paraformaldehyde-fixed agarose-embedded adult human retina optically sectioned by confocal microscopy (Fig. 2C). Both HSJ1a and HSJ1b were detected in all layers of the neural retina; however, the staining patterns for the two proteins were distinct. HSJ1a labeling was most intense in neuronal processes (e.g. the axons of the ganglion cell layer) and photoreceptor inner and outer segments. The intracellular staining pattern for HSJ1a appeared diffuse and cytoplasmic with no nuclear staining. In contrast, HSJ1b staining was more intense in rod and cone photoreceptor inner segments than outer segments and the rest of the retina. Within the inner segments, the HSJ1b staining appeared punctate corresponding to the protein being associated with intracellular membranes. These staining patterns were not observed with either preimmune
Differences in the Intracellular Localization of HSJ1 Protein Isoforms Are Dependent on Geranylgeranylation—We have not identified an established cell line that endogenously expresses HSJ1 proteins. Therefore, to further investigate the subcellular localization of HSJ1a and HSJ1b we used immunofluorescent staining of CHO-1 cells heterologously expressing HSJ1 proteins (Fig. 3A). HSJ1a was localized throughout cells with diffuse nuclear and cytoplasmic staining. In contrast, HSJ1b was excluded from the nucleus, as with the localization observed in photoreceptors the staining pattern was indicative of the protein being association with internal membranes, in particular the ER. To further analyze this we performed immunofluorescent staining of ER marker proteins in cells expressing GFP-HSJ1b (Fig. 3B). HSJ1b localization overlapped with the staining pattern of the ER lumen protein BiP and the ER transmembrane protein calnexin, as would be expected if HSJ1b were anchored to the ER.

We identified a putative prenylation motif at the C terminus of HSJ1b (Fig. 1; accession number GenBankTM X63368). To test if a prenyl modification was responsible for the differences in localization between HSJ1 proteins we mutated HSJ1b’s putative CAAX box at the cysteine residue where a prenyl group would be attached (C321S). The localization of GFP-HSJ1b(C321S) was identical to the localization of HSJ1a (Fig. 3C). GFP-HSJ1b(C321S) was not excluded from the nucleus and did not appear to be associated with internal membranes (Fig. 3C).

Whether substrate proteins are prenylated with a farnesyl or geranylgeranyl group is dependent on the residue at position X of the CAAX box (18). The CAAX box in HSJ1b has a leucine at...
fractions by Western analysis with sHSJ1. HSJ1b was diffusely localized throughout the nucleus and the membrane component of the staining was cytoplasmic, while HSJ1b staining was perinuclear-associated. Cell lysates were fractionated by centrifugation and subsequent association. Treatment of cells expressing GFP-HSJ1b(L324M) with GGTI did not affect the localization of the protein (Fig. 3D).

The membrane association of wild-type and mutant HSJ1 proteins was also analyzed using a well-characterized subcellular fractionation (24, 26). This fractionation generates a supernatant that is principally cytosolic and a pellet of membranes, which also contains some nuclear and cytoskeletal material. CHO-1 cells were transfected with HSJ1 constructs, following cell breakage and fractionation by centrifugation, the presence of HSJ1 proteins in supernatant and pellet fractions was determined by Western analysis with sHSJ1 (Fig. 3E). HSJ1b partitioned principally to the pellet fraction, whereas HSJ1a and HSJ1b(C321S) were more abundant in supernatant than pellet fractions. Collectively, these data are consistent with HSJ1b being attached to the cytosolic face of intracellular membranes dependent upon geranylgeranylation of the cysteine residue at position 321.

**HSJ1b and Rhodopsin Co-localize at the ER and in Rhodopsin Inclusions**—As HSJ1 proteins are enriched in photoreceptor inner segments, we investigated a potential interaction between them and the major protein product of the rod inner segment, rhodopsin, in its apoprotein state (i.e. without 11-cis-retinal) and whether expression of HSJ1 proteins could modulate rhodopsin aggregation. SK-N-SH neuroblastoma cells were co-transfected with HSJ1 proteins and either wild-type rhodopsin-GFP or rhodopsin-GFP with the retinitis pigmentosa-causing mutation P23H (9). Wild-type rhodopsin-GFP localized predominantly to the plasma membrane (Fig. 4A). P23H rhodopsin-GFP was not processed to the plasma membrane, but accumulated in the ER and in pericentriolar inclusion bodies (Fig. 4A) that have previously been characterized as aggresomes (9–11). Co-transfection with HSJ1a had no effect on wild-type rhodopsin-GFP processing and no co-localization was observed between HSJ1a and rhodopsin-GFP (Fig. 4B). In contrast, HSJ1b had a dramatic effect on the localization of wild-type rhodopsin, greatly reducing plasma membrane targeting and causing rhodopsin-GFP to accumulate in the ER and in a proportion of cells in intracellular inclusions (Fig. 4B). HSJ1b co-localized with rhodopsin-GFP at the ER and in intracellular inclusions.

We investigated if HSJ1b’s effect on rhodopsin processing was dependent on the sequestration of Hsp70, or the disruption of another Hsp70/DnaJ protein interaction, using a point mutation in HSJ1b that is defective for binding to Hsp70. The interaction between the J-domain and Hsp70 requires a 3-amino acid (HPD) motif in the J-domain. Mutation of the histidine residue in this motif eliminates the regulation of Hsp70 by DnaJ proteins (15, 16). In cells expressing rhodopsin-GFP and HSJ1b(H31Q), rhodopsin was not processed to the plasma membrane and accumulated in the ER and intracellular inclusions (Fig. 4B). The effect of HSJ1b prenylation on rhodopsin processing was tested (Fig. 4B). In cells expressing rhodopsin-GFP with HSJ1b(C321S), rhodopsin localized predominantly to the plasma membrane and did not co-localize with HSJ1b(C321S). HSJ1a and HSJ1b(C321S) had no effect on the localization of rhodopsin-GFP with the P23H mutation; however, the HSJ1 staining was increased in some intracellular inclusions (Fig. 4C). HSJ1b and HSJ1b(H31Q) co-localized with mutant rhodopsin at the ER and the staining was particularly intense in intracellular inclusions (Fig. 4C). The same
phenomena were observed with untagged rhodopsin (available as supplementary data).

**HSJ1b Is Present in a Complex with Wild-type and Mutant Rhodopsin**—To investigate if HSJ1b was in a complex with rhodopsin we performed immunoprecipitation of rhodopsin-GFP and HSJ1 proteins. Immunoprecipitation of rhodopsin-GFP from a DM soluble cell lysate with monoclonal antibody 1D4 showed that HSJ1b and HSJ1b(H31Q) were present in a complex with wild-type and mutant P23H rhodopsin-GFP (Fig. 5A). Only small amounts of HSJ1a could be recovered in a complex with P23H rhodopsin-GFP, and no HSJ1b(C321S) could be detected co-purifying with rhodopsin-GFP. The reciprocal immunoprecipitation of HSJ1 proteins showed that wild-type and P23H rhodopsin-GFP could be co-purified with HSJ1b from a DM-soluble cell lysate as measured by GFP fluorescence. More P23H rhodopsin-GFP co-immunoprecipitated with HSJ1b than wild-type, suggesting that there may be a stronger interaction with the mutant protein (Fig. 5B). The mutant P23H rhodopsin-GFP also co-purified with HSJ1a. No interaction was observed between HSJ1 proteins and GFP alone (data not shown).

**HSJ1b Increases Rhodopsin Inclusions Dependent on Prenylation**—The effect of HSJ1 co-expression on the proportion of rhodopsin-GFP expressing cells with inclusions was quantified (Fig. 6). Approximately 5% of cells expressing wild-type rhodopsin-GFP had inclusions 24 h after transfection. In cells that had been co-transfected with rhodopsin-GFP and HSJ1a or HSJ1b(C321S) the proportion of cells containing intracellular inclusions did not increase. In contrast, a significantly greater proportion (~25%) of cells expressing HSJ1b or HSJ1b(H31Q) contained inclusions of rhodopsin-GFP, compared with control cells (p < 0.0001) (Fig. 6A). Approximately 55% of cells expressing mutant P23H rhodopsin-GFP contained intracellular inclusions 24 h after transfection. Expression of HSJ1a or HSJ1b(C321S) had no significant effect on the proportion of cells containing inclusions of P23H-rhodopsin. Expression of HSJ1b and HSJ1b(H31Q) again significantly increased the proportion of cells with inclusions (~75%; p < 0.0001) (Fig. 6B).

**The Effect of Hsp70 on HSJ1b Mediated Rhodopsin ER Retention and Inclusion Formation**—The HSJ1b(H31Q) mutant showed that the effects of HSJ1b on wild-type and mutant rhodopsin processing were not dependent on the sequestration of Hsp70 or the disruption of another DnaJ protein/Hsp70 interaction. However, this did not exclude a role for Hsp70 proteins in rhodopsin processing and inclusion formation or the possibility that levels of Hsp70 could be limiting in a functional cycle of rhodopsin folding mediated by HSJ1b. Indeed, Hsp70 has previously been shown to be present in mutant rhodopsin aggresomes (9). We investigated if Hsp70 co-immunoprecipitated with wild-type and mutant rhodopsin using an antibody that detects both the cognate Hsc70 and inducible Hsp70 (Fig. 7A). No Hsp70 could be detected with wild-type rhodopsin alone, but a small amount of Hsp70 proteins could be detected with rhodopsin in the presence of HSJ1b and HSJ1b(H31Q). More Hsp70 was detected in complex with the P23H rhodopsin and this was further increased in the HSJ1b and HSJ1b(H31Q) co-transfections. The presence of Hsp70 in complex with rhodopsin was increased when HSJ1b was present, but appeared to be unaffected by the HSJ1b(H31Q) mutation, suggesting...
that the Hsp70 may not be binding to HSJ1b but may be binding directly to the retained and/or aggregated rhodopsin. As Hsp70 proteins are present in a complex with rhodopsin in the presence of HSJ1b, we overexpressed the inducible form of Hsp70 with HSJ1b and monitored the localization of the proteins by confocal microscopy. In the absence of HSJ1b, Hsp70 overexpression did not affect wild-type rhodopsin-GFP processing, and Hsp70 did not co-localize with the rhodopsin-GFP, although Hsp70 co-localized with rhodopsin in inclusions of P23H rhodopsin-GFP (Fig. 7B). When Hsp70 was overexpressed in the presence of HSJ1b and wild-type or P23H rhodopsin-GFP, all three proteins could be observed to co-localize within the cell, particularly in rhodopsin inclusions (Fig. 7B). The overexpression of Hsp70 with HSJ1b did not dramatically alter the processing of the wild-type protein, as the HSJ1b mediated intracellular retention of wild-type rhodopsin did not appear to be affected in cells expressing high levels of Hsp70 compared with cells expressing low levels of Hsp70. The co-localization of HSJ1b and Hsp70 with rhodopsin-GFP, coupled to the immunoprecipitation data, suggests that the proteins may be present in a ternary complex.

To test if increased levels of Hsp70 modulated the increase in the incidence of inclusion formation mediated by HSJ1b the proportion of rhodopsin-GFP expressing cells with inclusions was quantified (Fig. 8). If Hsp70 was the limiting factor in completing a functional cycle of rhodopsin folding then co-expression with HSJ1b should reduce the incidence of inclusions, whereas the Hsp70 interaction null HSJ1b(H31Q) should be unaffected. Comparison of the incidence of inclusions revealed that the expression of Hsp70 had no effect on wild-type rhodopsin-GFP or P23H rhodopsin-GFP either by itself or when co-expressed with HSJ1b or HSJ1b(H31Q) (Fig. 8). These data suggest that Hsp70 was not a limiting factor in these reactions and that it has no effect on rhodopsin inclusion formation independent of HSJ1b.

**DISCUSSION**

In the adult human retina HSJ1a and HSJ1b proteins were enriched in rods and cones. This suggested they may have photoreceptor-specific functions and/or interact with photoreceptor specific proteins. HSJ1a and HSJ1b had different intracellular localizations, which are likely to reflect differences in chaperone functions within cells. HSJ1a was cytoplasmic and excluded from nuclei in retina, but was cytoplasmic and nuclear in cultured cells. The reason for these differences between tissue and cultured cells are unclear, but may be due to HSJ1a being excluded from the nucleus in vivo due to specific interactions with other proteins. In both retina and cultured cells, HSJ1b was excluded from the nucleus and had a predominantly perinuclear localization consistent with the protein being on intracellular membranes. This membrane association was dependent on prenylation. The farnesylation of DnaJ family proteins is relatively common. Ydj1, Hdj-2, and ANJ1 are all farnesylated (28–30), and examination of the amino acid sequence of other DnaJ proteins with putative CAA X motifs predicts farnesylation. HSJ1b is the first DnaJ family protein identified as being modified with a geranylgeranyl moiety. HSJ1b was targeted to endomembranes and co-localized with the ER marker proteins BiP and calnexin. Whether HSJ1b contains any other signals that mediate its
rhodopsin acting as a chaperone as opposed to a cochaperone. The difference between our cell culture model and the in vivo situation, where the presence of HSJ1b does not appear to arrest the processing of rhodopsin in photoreceptors, could be the result of several factors. The amount of HSJ1b at the face of ER would not appear to be the major cause, as rhodopsin retention and enhanced inclusion formation were observed in cells that expressed low levels of HSJ1b. In photoreceptors, HSJ1b may have another client protein that it binds with greater affinity than rhodopsin. Alternatively, there may be other members of the photoreceptor cell chaperone machinery that are not present at sufficient levels in the SK-N-SH cells to complete a functional cycle of rhodopsin folding in which HSJ1b takes part.

Rhodopsin is continuously synthesized in huge amounts (about $10^{-7}$ molecules per day per photoreceptor) within the inner segments and then vectorially transported to the base of the outer segments. Therefore, the chaperone machinery that facilitates rhodopsin folding is expected to be highly efficient in photoreceptor cells. It is interesting to note that wild-type rhodopsin spontaneously forms aggresomes in transfected cells (in around 10% of cells after 24 h in SK-N-SH), even in the absence of HSJ1b. These cells express much less rhodopsin than a photoreceptor, illustrating that although rhodopsin can fold normally it appears to do so far less efficiently in these cells than in photoreceptors, possibly because a specialized chaperone machinery for rhodopsin biogenesis is not fully present. In Drosophila there is a specialized chaperone, NinaA, for Rh1 rhodopsin, and homologues of NinaA have been suggested to act as chaperones for red opsin (13). To date, however, no specific chaperone has been identified for mammalian rhodopsin.

Given the enrichment of HSJ1b at the site of rhodopsin production and its ability to modulate rhodopsin folding in SK-N-SH cells, HSJ1b would appear to be an excellent candidate to participate in rhodopsin folding. Nonetheless, our data show that it cannot stimulate rhodopsin folding by itself and appears to cause a stalling in rhodopsin processing instead. This would suggest that if HSJ1b does participate in rhodopsin folding in vivo, other components of the photoreceptor chaperone machinery are required to complete a functional cycle and stimulate efficient rhodopsin folding. We examined whether Hsp70 levels were a limiting factor in this chaperone cycle by overexpressing the inducible form of the protein in conjunction with HSJ1b. Although Hsp70 did co-localize with HSJ1b and was present in a complex with rhodopsin, overexpression did not alter the fate of the wild-type and mutant rhodopsin proteins, suggesting that Hsp70 was not the missing factor. Elucidation of the factors involved in rhodopsin processing is a major challenge, but heterologous systems may be of use in reconstituting the photoreceptor chaperone machinery.

The cellular processing of the cystic fibrosis transmembrane-conductance regulator (CFTR) has parallels with rhodopsin processing, and several cytosolic chaperones and cochaperones have been demonstrated to be involved in folding and degradation of CFTR. Like mutant rhodopsin, mutant CFTR is retained in the ER and degraded by the ubiquitin proteasome pathway (31). Furthermore, when the proteasomal degradation of misfolded CFTR is inhibited the protein accumulates in aggresomes (32). In conjunction with a cochaperone, CHIP, Hsp70 senses the folded state of CFTR and targets aberrant forms for proteasomal degradation by promoting their ubiquitination (33, 34). Interestingly, the farnesylated DnaJ protein Hdj-2 has also been localized to the cytosolic face of the ER, where in concert with Hsp70 it has been shown to facilitate CFTR biogenesis (35). Whether similar chaperone assisted pathways of folding and
DnaJ Proteins and Rhodopsin Folding

degradation exist for rhodopsin and other G-protein-coupled receptors still remains to be determined. However, the data presented in this study provide proof-of-principle that the chaperone environment at the cytoplasmic face of the ER plays an important role in the biogenesis of rhodopsin and other G-protein-coupled receptors. The manipulation of chaperones in particular DnaJ proteins, has been shown to be effective in reducing inclusions and alleviating cell death associated with polyglutamine expansions (12). The potential for modulating rhodopsin folding and degradation through manipulation of the cell chaperone machinery could have important implications for developing therapies for retinitis pigmentosa caused by misfolding mutations in rhodopsin. It will be important to understand the specialized chaperone networks within photo-receptors and their localizations to fully exploit this potential.

Acknowledgments—We thank the MRC Brain Bank, Institute of Psychiatry for providing human tissue samples and P. Luthert for retinal tissue. We would also like to thank P. Adamson for the gift of pCMV70. We are grateful to C. Grayson and J. van der Spuy for help with retinal immunohistochemistry, to S. Wilkins for assistance with cell culture, and A. Hardcastle for critical reading of the article.

REFERENCES
1. Tam, B. M., Moritz, O. L., Hurd, L. B., and Papermaster, D. S. (2000) J. Cell Biol. 151, 1269–1280
2. Tai, A. W., Chuang, J. Z., Bode, C., Wolfrum, U., and Sung, C. H. (1999) Cell 97, 877–887
3. Sung, C. H., Makino, C., Baylar, D., and Nathans, J. (1994) J. Neurosci. 14, 5818–5833
4. Kaushal, S., and Khorana, H. G. (1994) Biochemistry 33, 6121–6128
5. Roof, D. J., Adamian, M., and Hayes, A. (1994) Invest Ophthalmol. Vis. Sci. 35, 4949–4962
6. Sung, C. H., Davenport, C. M., and Nathans, J. (1993) J. Biol. Chem. 268, 26645–26649
7. Olsson, J. E., Gordon, J. W., Pawlyk, B. S., Roof, D., Hayes, A., Molday, R. S., Mukai, S., Cowley, G. S., Berson, E. L., and Dryja, T. P. (1992) Neuron 9, 815–830
8. Sung, C. H., Schneider, B. G., Agarwal, N., Papermaster, D. S., and Nathans, J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8840–8844
9. Saliba, R. S., Munro, P. M., Luther, P. J., and Cheetham, M. E. (2002) J. Cell Biol. 155, 2907–2918
10. Ilting, M. E., Rajaan, R. S., Bence, N. F., and Kopito, R. R. (2002) J. Biol. Chem. 277, 34150–34160
11. Rajaan, R. S., Ilting, M. E., Bence, N. F., and Kopito, R. R. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 13066–13065
12. Muchowski, P. J. (2002) Neuron 35, 9–12
13. Chapple, J. P., Grayson, C., Hardesty, A. J., Saliba, R. S., van der Spuy, J. P., and Cheetham, M. E. (2001) Trends Mol. Med. 7, 414–421
14. Anzawa, A., and Khorana, H. G. (1994) J. Biol. Chem. 269, 19738–19744
15. Kelley, W. L. (1998) Trends Biochem. Sci. 23, 222–227
16. Cheetham, M. E., and Caplan, A. J. (1998) Cell Stress. Chaperones. 3, 28–36
17. Zhang, P. L., and Casey, P. J. (1996) Annu. Rev. Biochem. 65, 241–269
18. Casey, P. J., and Seabra, M. C. (1990) J. Biol. Chem. 271, 5298–5292
19. Cheetham, M. E., Brion, J. P., and Anderton, B. H. (1992) Biochem. J. 284, 469–476
20. Cheetham, M. E., Anderson, B. H., and Jackson, A. P. (1996) Biochem. J. 319, 103–108
21. Cheetham, M. E., Jackson, A. P., and Anderton, B. H. (1994) Eur. J. Biochem. 226, 89–97
22. van der, S. J., Chapple, J. P., Clark, B. J., Luthert, P. J., Sethi, C. S., and Cheetham, M. E. (2002) Hum. Mol. Genet. 11, 823–831
23. Paulus, W., Baur, I., Boyce, F. M., Breakfield, X. O., and Reeves, S. A. (1996) J. Virol. 70, 62–67
24. Chapple, J. P., Hardcastle, A. J., Grayson, C., Caplan, A. J., Luthert, P. J., and Cheetham, M. E. (2000) Hum. Mol. Genet. 9, 1919–1926
25. Grayson, C., Bartolini, F., Chapple, J. P., Willison, K. R., Bhamidipati, A., Lewis, S. A., Luthert, P. J., Hardcastle, A. J., Cowan, N. J., and Cheetham, M. E. (2002) Hum. Mol. Genet. 11, 3065–3074
26. Chapple, J. P., Hardesty, A. J., Grayson, C., Willison, K. R., and Cheetham, M. E. (2002) Invest Ophthalmol. Vis. Sci. 43, 2015–2020
27. Vogt, A., Sun, J., Qian, Y., Hamilton, A. D., and Sekit, S. M. (1997) J. Biol. Chem. 272, 27224–27229
28. Caplan, A. J., Tsai, J., Casey, P. J., and Douglas, M. G. (1992) J. Biol. Chem. 267, 18890–18895
29. Kanazawa, M., Terada, K., Kato, S., and Mori, M. (1997) J. Biol. Chem. 272, 27224–27229
30. Zhu, J. K., Bressan, R. A., and Hasegawa, P. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8557–8561
31. Ward, C. L., Omura, S., and Kopito, R. R. (1995) Cell 83, 121–127
32. Nagy, K. R. (1999) Physiol. Rev. 79, S167–S173
33. Meacham, G. C., Patterson, C., Zhang, W., Younger, J. M., and Bry, D. M. (2001) Nat. Cell Biol. 3, 100–105
34. Zhang, Y., Nijbroek, G., Sullivan, M. L., McCracken, A. A., Watkins, S. S., Michaelis, B., and Brodsky, J. L. (2001) Mol. Biol. Cell 12, 1303–1314
35. Meacham, G. C., Lu, Z., King, S., Sursch, E., Tousson, A., and Bry, D. M. (1999) EMBO J. 18, 1492–1505

Downloaded from http://www.jbc.org/ by guest on July 23, 2018
The Chaperone Environment at the Cytoplasmic Face of the Endoplasmic Reticulum Can Modulate Rhodopsin Processing and Inclusion Formation

J. Paul Chapple and Michael E. Cheetham

J. Biol. Chem. 2003, 278:19087-19094.
doi: 10.1074/jbc.M212349200 originally published online March 6, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M212349200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2003/04/04/M212349200.DC2

This article cites 35 references, 17 of which can be accessed free at
http://www.jbc.org/content/278/21/19087.full.html#ref-list-1