Unfolding retinal dystrophies: a role for molecular chaperones?

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Inherited retinal dystrophy is a major cause of blindness worldwide. Recent molecular studies have suggested that protein folding and molecular chaperones might play a major role in the pathogenesis of these degenerations. Incorrect protein folding could be a common consequence of causative mutations in retinal degeneration disease genes, particularly mutations in the visual pigment rhodopsin. Furthermore, several retinal degeneration disease genes have recently been identified as putative facilitators of correct protein folding, molecular chaperones, on the basis of sequence homology. We also consider whether manipulation of chaperone levels or chaperone function might offer potential novel therapies for retinal degeneration.

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Diseases affecting the function of the retina leading to visual impairment and blindness display remarkable genetic and clinical heterogeneity[1]. Indeed, Online Mendelian Inheritance in Man (OMIM, www3.ncbi.nlm.nih.gov/Omim/) and the online retinal information network RetNet (www.sph.uth.tmc.edu/RetNet/) list over 120 distinct disorders that include some form of retinal degeneration, which can be inherited as autosomal dominant, autosomal recessive, X-linked, mitochondrial, digenic, polygenic and syndromal diseases affecting over 1 in 2000 people (Fig. i, Box 1). Here, we will review recent developments in the molecular and cellular understanding of these diseases to examine the potential role of protein folding and molecular chaperones, and whether molecular chaperones might represent novel targets for therapeutic intervention.

Protein misfolding and retinal disease

Correct protein folding is an essential biological process. To ensure this happens in the intracellular milieu many proteins interact with molecular chaperones (Box 2). The importance of correct protein folding and the potential involvement of molecular chaperones in retinal degeneration are emphasized by the most common and studied form of inherited retinal degeneration, retinitis pigmentosa (RP) caused by mutations in rhodopsin – the molecular sensor for light.

Rhodopsin and misfolding in RP

Mutations in the gene encoding rhodopsin were the first identified in 1990[2], and over 100 mutations have now been described that account for approximately 15% of all inherited human retinal degenerations (see OMIM). Many studies have suggested that the vast majority of these mutations cause disease through the misfolding or mislocalisation of the protein[3]. Wild-type rhodopsin is almost entirely restricted to the specialized rod photo-sensing organelle, the outer segment. In contrast, mutant rhodopsin is localized within the cell body of photoreceptors in animal models[4,5]. In transfected cells, rhodopsin with mutations in the intradiscal, transmembrane and cytoplasmic domains fails to translocate to the plasma membrane, and accumulates in the endoplasmic reticulum (ER) and Golgi (Fig. 1). These mutant proteins trapped within the cell cannot form the visual pigment with 11-*cis*-retinal and are found in complex with the molecular chaperones BiP and Grp94 (members of the Hsp70 and Hsp90 families, respectively), supporting the notion that they are incorrectly folded[6] (Fig. 2b). It appears, therefore, that these mutant proteins fail to translocate because of protein misfolding.

The failure of rhodopsin to translocate to the outer segment, *per se*, does not appear to be enough to cause RP, as heterozygous rhodopsin knockout mice display little photoreceptor cell death[7]. Rather, it would appear that misfolded rhodopsin acquires a 'gain of function' that leads to cell death. The nature of this gain of function is unclear at present, but could be related to a saturation of the normal protein processing, transport and degradation machinery in such a highly-specialized cell. Defining the cellular consequences of incorrect rhodopsin folding and targeting might help bring to light the pathogenetic mechanisms that link rhodopsin misfolding with photoreceptor apoptosis. Given the importance of correct rhodopsin folding for photoreceptor survival, it is perhaps not surprising that specialized chaperones of opsin biogenesis have been identified, such as the *Drosophila* protein NinaA (Ref. 8).

Specialized opsin chaperones

NinaA is a photoreceptor-specific integral membrane glycoprotein with a central cyclophilin (CyP) homology domain (Box 2) that extends into the ER lumen[9]. The major visual pigment in *Drosophila*, rhodopsin 1 (Rh1), is expressed in photoreceptor cells R1-6 and is synthesized in the ER, and then transported through the secretory pathway to the rhabdomere (a light transducing organelle similar to the mammalian rod outer segment). NinaA and Rh1 colocalize in the ER and transport vesicles within photoreceptor cells (Fig. 2e). Mutations in NinaA lead to the accumulation of immature, misfolded Rh1 in the ER that is prevented from reaching the rhabdomere. The misfolded Rh1 is degraded, resulting in reduced levels

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Box 1. Retinal dystrophies

Retinitis pigmentosa (RP)
RP (OMIM 180380 for rhodopsin RP and 312600 for RP2 RP) is the most common cause of inherited blindness with over 25 genetic loci identified (see RetNet). Clinically, the disorder is characterized by night-blindness, loss of peripheral vision, followed by loss of central vision. Examination typically shows pigmentation in a 'bone-spicule' pattern in the retina, pallor of the optic nerve and narrowing of retinal blood vessels. There is much variability in the severity of symptoms and signs between different families as well as between different individuals of the same family, and although total blindness can occur, this is rare. Electroretinography, which involves the detection and characterization of the microvoltage potentials that occur on light stimulation of the retina, reveal a reduced response from rod photoreceptors and later cone photoreceptors, and confirm that the primary pathology involves the maintenance of rod photoreceptor function. Fig. I shows a fundus photograph of normal retina alongside a patient fundus with RP. Genetically, there is considerable overlap between RP and other retinal dystrophies such as those that affect the macula (macular dystrophies) and those that affect primarily cone photoreceptors (cone and cone–rod dystrophies).

Leber congenital amaurosis (LCA)
LCA (OMIM 604393 for AIPL1 LCA) accounts for approximately 10% of inherited retinal disease and is the most common cause of congenital visual impairment in infants and children. Five distinct genetic loci have been reported. This severe form of inherited retinal dystrophy presents within a few months of birth when a non-seeing infant has a normal appearing fundus but a flat electroretinogram. Later, the retina can appear to resemble that seen in RP, and a subset of the disease can present with an area of deficient retinal tissue at the macula (macular coloboma).

Bardet–Biedl syndrome (BBS)
BBS [OMIM 605231 for McKusick–Kaufman syndrome (MKKS)–BBS] has an ocular component, which is characterized by pigmentary retinopathy similar to that seen in RP as a result of rod–cone disease. Associated symptoms of BBS include obesity, polydactyly, hypogonadism, learning difficulties and renal failure. Six loci have been described to date, with MKKS representing the first cloned gene for this disorder.

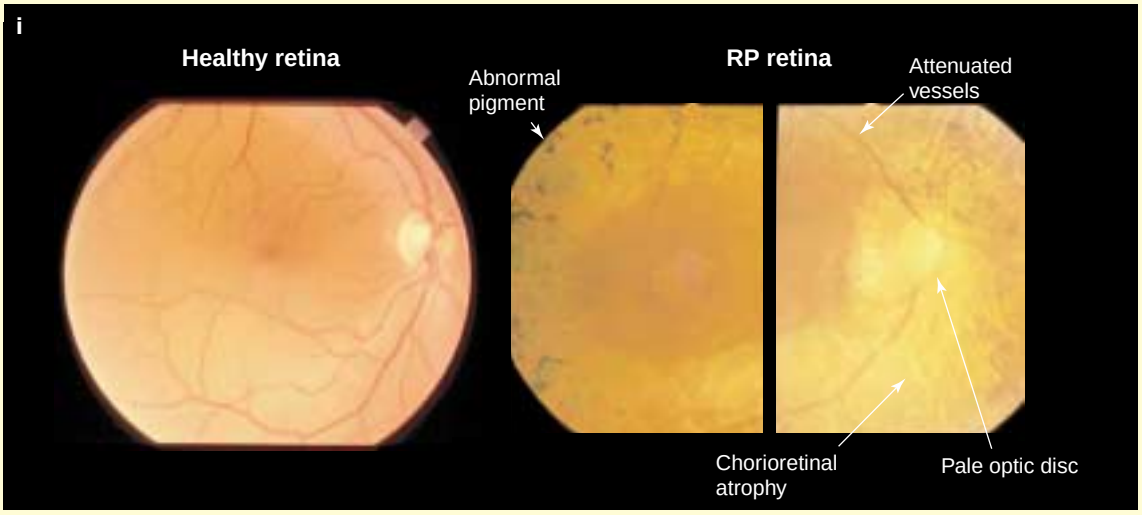


Fig. I. A comparison of normal and RP fundus. Retinal dystrophies are classified by (i) ophthalmoscopic findings (fundus examination), (ii) psychophysics and electrophysiology, (iii) age of onset, and (iv) genetics or family history.

of Rh1 found in NinaA mutant flies[10]. Furthermore, NinaA forms a stable and highly specific complex with Rh1 and the amount of functional NinaA directly reflects the production of mature rhodopsin[8]. Mutations in the CyP homology domain are thought to compromise the peptidyl-prolyl *cis–trans* isomerase (PPIase) activity of NinaA, preventing proline isomerization within Rh1 that might be required for its proper folding[8]. Collectively, these observations suggest that processing and transport of Rh1 are dependent on NinaA, and that NinaA acts as a specific molecular chaperone for Rh1.

A mammalian orthologue of NinaA has not yet been identified. Ferreira *et al.*[11] identified a putative retina-specific cyclophilin from a bovine retina cDNA library with two splice variants encoding isoform types I and II, which were predominately expressed in cone photoreceptors. The type II isoform is identical to RanBP2 (Ran-binding protein 2), a large protein that was identified as a component of the nuclear transport machinery[12]. RanBP2 might also play a role in opsin processing. Two adjacent domains in RanBP2, the Ran-binding domain 4 (RBD4) and the cyclophilin domain, form a complex with the bovine

Box 2. What are molecular chaperones?

By the simplest definition, molecular chaperones are facilitators of protein conformational change that, in themselves, provide no information as to the final protein structure. Several more precise definitions exist but these seldom cover the breadth of molecular chaperone action. Molecular chaperones are a group of functionally related but otherwise diverse protein families. They bind to and stabilize conformers of other proteins and, through cycles of regulated binding and release, are able to facilitate the correct fate of their client. Via this mechanism, molecular chaperones, in conjunction with their cochaperones, play an essential role in many cellular processes (Fig. 2a–f). They have a principal role in protein folding, where they are involved in the *de novo* synthesis of polypeptides (Fig. 2a), transport across membranes (Fig. 2b and e) and the refolding of proteins denatured by adverse environmental conditions (Fig. 2f). Chaperones also function in oligomeric assembly and disassembly of protein complexes, controlled switching between active and inactive conformations of client proteins, intracellular transport and proteolytic degradation (Fig. 2c,d and e)[40,58].

More than 20 different families of proteins have been shown to have chaperone activity. Principal chaperone families include the Hsp90, Hsp70, chaperonins (e.g. Hsp60), DnaJ (e.g. Hsp40) and small heat-shock proteins (e.g. Hsp27, α-crystallin). The best-studied and mechanistically understood chaperone machines are Hsp70 and the chaperonins. These chaperones recognize and bind to unfolded or partially folded polypeptides by binding to exposed hydrophobic regions preventing them from aggregating and maintaining them in a folding competent state until release. This is particularly important as the nascent polypeptide emerges from the ribosome, where efficient folding of the newly synthesized chain is achieved by a transient interaction with the chaperone that prevents aggregation due to unwanted interactions with hydrophobic regions of other proteins or within the extending polypeptide (Fig. 2a).

The different families of chaperone proteins recognise various intermediates of non-native polypeptides and interact through different modes of binding. Hsp70 proteins bind short regions of peptides with a certain position and pattern of hydrophobic residues in a substrate-binding pocket[40]. By contrast, chaperonins can facilitate folding by enclosing non-native polypeptides in the central cavity of a double ring structure formed from identical or closely related rotationally symmetrical subunits[39,40]. Another example of chaperone activity is the peptidyl-prolyl *cis–trans* isomerase (PPIase) activity found in the cyclophilins (e.g. NinaA) that overcomes a rate-limiting step in protein folding, the correct orientation of proline residues[59].

For many chaperones, cycles of client protein binding and release are coupled to conformational changes in the chaperone protein, which are dependent on the hydrolysis and exchange of ATP that is regulated by cochaperones[40]. Cochaperones function synergistically with the major chaperones in protein folding and often have independent chaperone activity, but their major role might be to provide these folding machines with specificity. For example, the Hsp70 protein machinery achieves its multiple cellular functions because of various cochaperone proteins, such as the DnaJ family, which stimulate Hsp70 ATP hydrolysis through their conserved J domain[60]. Several other cochaperones (e.g. Hip, Hop, Chip and some immunophilins including AIPL1) utilise a degenerate 34-amino-acid repeat motif, the tetratricopeptide repeat (TPR), in tandem arrays to promote chaperone–cochaperone interactions[61] and modify chaperone function. In addition, there is functional cooperation between the individual chaperone machines. For example, Hsp90 and Hsc70 cooperate in the assembly of steroid receptor and transcription factor complexes (Fig. 2c), and the Hsp70 chaperone machine might pass nascent chains onto chaperonins to complete folding (Fig. 2a). Through these complementary but distinct roles in protein folding, molecular chaperones can facilitate changes in protein conformation from initial folding through function and ultimately to degradation.

long- and medium-wavelength (red/green) sensitive opsin[13]. Similar to NinaA, the cyclophilin domain might function to induce a *cis*→*trans* isomerization of one or more peptidyl-prolyl bonds in the long-wavelength (red) opsin[14].

A specific molecular chaperone for mammalian rhodopsin biogenesis and transport has yet to be identified, but other, less specialized, molecular chaperones might participate in rhodopsin biogenesis. For example, BiP and Grp94 might interact transiently with wild-type opsin as part of normal protein folding and quality control in the ER. In addition, the small heat shock protein family member α-crystallin has been found to be enriched in post-golgi membranes in the inner segment of frog rod cells along with newly synthesized rhodopsin[15], suggesting that α-crystallin might associate with rhodopsin and be involved in its processing (Fig. 2e).

Rhodopsin is not the only example of a retinal disease protein that can cause disease through misfolding. Point mutations in other essential photoreceptor proteins might destabilize the protein structure and lead to misfolding. It is also possible that retinal degeneration can result from a dysfunction in the specialized chaperone machinery of the retina, and in the following sections we will examine some recently identified retinal disease genes as putative chaperones.

Putative chaperones as causes of retinal degeneration

RP2 and RP

Mutations in RP2 account for 15–20% of X-linked retinitis pigmentosa (XLRP)[16]. RP2 was identified as a putative chaperone on the basis of its similarity (30.4% identity over 151 amino acids) to cofactor C, a component of the tubulin folding pathway[17,18]. The significance of this similarity is supported by pathogenic amino-acid substitutions in RP2 at conserved residues[16,19,20], suggesting that RP2 has functional homology with cofactor C.

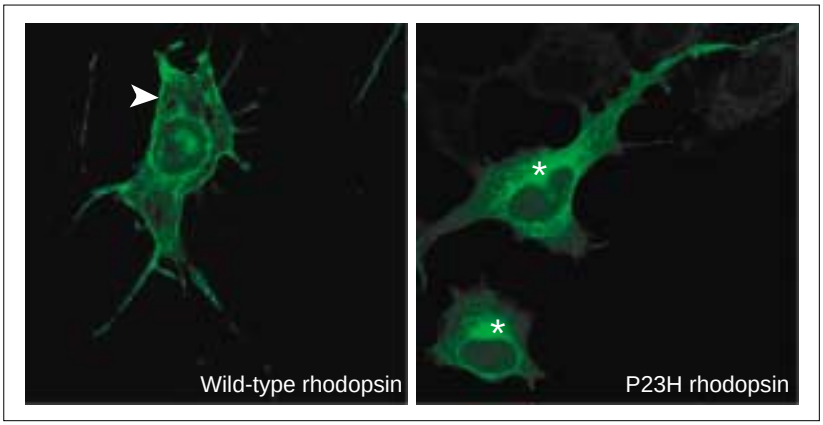


Fig. 1. Comparison of the cellular localization of wild-type and mutant rhodopsin in transfected COS-7 cells. Wild-type protein translocates to the plasma membrane (arrowhead), whereas protein with a P23H mutation accumulates within the ER/Golgi (star) and fails to reach the plasma membrane.

Molecular chaperones, in particular the cytosolic chaperonin containing TCP1 (CCT), play an essential role in the biogenesis of several components of the cytoskeleton, including actin (microfilaments) and tubulin (microtubules)[21]. The production of native α–β tubulin heterodimers before their assembly into microtubules depends on the action of several cofactors (A–E), including cofactor C, following the release of near native folded subunits from CCT (Fig. 2d)[17,18]. These cofactors might also play a role in sequestering tubulin or modifying microtubule function and dynamics, for example, by acting as a GTPase activating protein (GAP) for the tubulin heterodimer[22–24]. However, the precise role of cofactor C in tubulin and microtubule dynamics remains unclear.

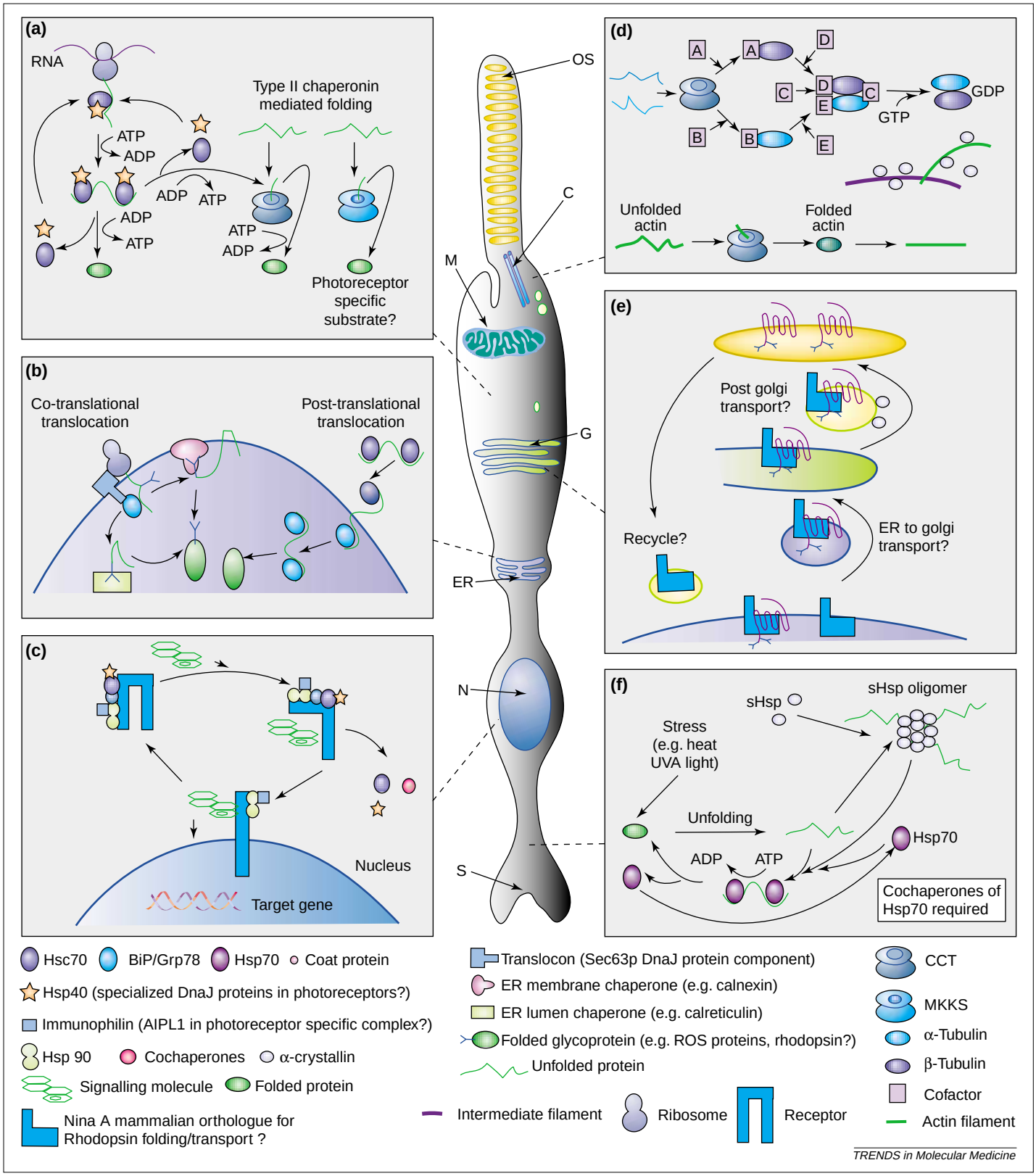
RP2 is ubiquitously expressed (i.e. it is not retina specific) and is post-translationally modified at its N-terminus by the addition of two acyl moieties that target the protein to the plasma membrane[25]. This acyl-mediated membrane targeting is disrupted by a pathogenic mutation in RP2 (ΔS6), suggesting that the membrane localization is essential for protein function in the retina (Fig. 3)[25]. Therefore, it seems unlikely that RP2 functions exclusively in tubulin folding. It is possible that RP2 does still interact with tubulin and/or microtubules and might provide a link between membranes and the cytoskeleton, potentially as part of the cellular protein traffic machinery or a signalling cascade. This hypothesis is supported by the identification of ADP ribosylation factor (ARF)-like proteins and src as interacting partners of RP2 (Ref. 26), a function that appears to be conserved with cofactors C and D (Refs 23,24). As more functional information on RP2 and cofactor C is ascertained, their putative chaperone function can be evaluated. Two other proteins homologous to known chaperones have more recently been identified as causes of non-syndromic and syndromic retinal degenerations.

AIPL1 and Leber congenital amaurosis

Mutations in the gene encoding a novel photoreceptor–pineal-specific protein, AIPL1, cause approximately 10% of recessive Leber congenital amauroses (LCA)[27]. This protein shares 49% amino acid identity with the human aryl hydrocarbon receptor-interacting protein (AIP), and was subsequently designated the aryl hydrocarbon receptor-interacting protein-like (AIPL) 1. AIP facilitates the signalling of a transcription factor, aryl hydrocarbon receptor (AhR) (Fig. 2c)[28,29]. In the absence of ligands, inactivated AhR exists in a cytosolic multiprotein complex, which includes AIP and the molecular chaperone Hsp90. In the presence of ligands, AhR translocates to the nucleus where it dissociates from Hsp90 and AIP, and subsequently heterodimerizes with a structurally related protein termed the aryl hydrocarbon receptor nuclear translocator (Arnt). Importantly, both the transactivation activity and subcellular compartmentalization of AhR are modulated by AIP (Refs 30–32). AIP is able to protect the inactivated AhR from ubiquitination, thereby stabilizing the cytosolic AhR from degradation and enhancing the levels of total and functional AhR in the cytosol[32–34]. Furthermore, an AIP-dependent delay in the nuclear accumulation of the AhR is observed in the presence of ligand. AhR-mediated transactivation is thus significantly enhanced in the presence of AIP through an increased availability of AhR ligand-binding sites. AIP can also help prevent AhR aggregation by thermal denaturation. These data, in addition to sequence similarities between AIP and the immunophilin FKBP52 (see below), support a function for AIP as a molecular chaperone and suggests that AIPL1 also acts as a chaperone or cochaperone.

FKBP52 is a high-molecular-weight member of the family of immunophilins. Analogous to the AhR signalling system, a direct interaction of the high-molecular-weight immunophilins with the molecular chaperone Hsp90 mediates their association with cytosolic steroid hormone receptor complexes. These immunophilins are thought to assist the targeted translocation of the associated activated steroid receptor to the nucleus. Furthermore, these immunophilins might function as molecular chaperones in their own right, as they can both inhibit the aggregation of thermally denatured substrates and maintain them in a folding-competent conformation[35].

Although there are no obvious structural similarities, AhR and members of the steroid receptor superfamily exhibit similarities in their signalling mechanism. Both the AIP and immunophilin containing complexes represent the penultimate stage of a procedure that involves the maturation of the receptor to a high-affinity ligand-binding conformation. This procedure is tightly regulated by the molecular chaperones Hsp90 and Hsp70 and their associated cochaperones, which collectively comprise a cytosolic heterocomplex chaperoning machine[36]. Similarly, a retina-specific system of interacting chaperones and cochaperones might exist and fulfil an essential chaperone function in the retina. Within this system, AIPL1 could fulfil a molecular chaperone function in retinal protein folding, or assist receptor nuclear translocation in a manner analogous to that of AIP and FKBP52 in their respective signalling systems.



MKKS and Bardet–Biedl

Mutations in *MKKS* have recently been identified as the cause of the developmental diseases, McKusick–Kaufman syndrome (MKKS)[37], and a rare form of the more severe disease Bardet–Biedl syndrome (BBS6), which also has a characteristic retinal dystrophy (Box 1)[38]. Amino acid sequence homology strongly suggests that MKKS is a member of the family of type II chaperonins (Fig. 4)[37]. Chaperonins can been classified into two families; type I (or GroEL subclass) chaperonins found in eubacteria and in organelles of eubacterial descent, and; type II (or TCP-1 subclass) chaperonins found in *archaea* and the eukaryotic cytosol[39]. Chaperonins are large homo- or

Fig. 2. Schematic of chaperone-facilitated events in a photoreceptor cell, including putative specialized pathways in the outer segment (OS), the cilium (C), mitochondria (M), the golgi (G), the endoplasmic reticulum (ER), the nucleus (N) and the synapse (S). (a) Folding in the cytosol: nascent chains emerging from the ribosome are bound by the Hsp70 chaperone machine, in particular the constitutive Hsp70, Hsc70, and members of the DnaJ family including Hsp40. The nascent polypeptides can then be passed onto the cytosolic chaperonin CCT, or putatively MKKS, to complete folding. (b) Folding in the ER: proteins can be cotranslationally inserted into the ER with the help of the translocon (including Sec63) or post-translationally with the assistance of Hsc70. Once within the ER, the unfolded proteins are bound by the ER resident Hsp70, BiP, and the glycan-binding chaperones calnexin and calreticulin that promote and monitor correct folding. (c) Signalling: nuclear receptors, such as the glucocorticoid receptor, form a complex with Hsp90 and Hsc70 and several other cochaperones (Hop, DnaJ proteins). This complex 'matures', through the exchange of several components (e.g. Hop and Hsc70 exchange for immunophilins and p23), to a complex that facilitates the binding of ligand. Upon ligand binding the complex changes conformation again and the activated receptor is transported to the nucleus where the chaperones are released. (d) Cytoskeleton: actin and tubulin require CCT for correct folding. Near-native α and β tubulin subunits are released from CCT and bound by cofactors that facilitate formation of the heterodimer and stimulate GTP hydrolysis. Small heat shock proteins (sHsps) also interact with components of the cytoskeleton. (e) Vesicular transport: immature, unfolded rhodopsin is bound by NinaA in the ER. The NinaA remains associated with the rhodopsin through the secretory pathway and is recycled to the ER upon release of the mature folded opsin. α crystallin associates with post-golgi rhodopsin vesicles. (f) Stress response: proteotoxic stress (e.g. heat) leads to the partial unfolding of proteins. The unfolded proteins are bound by the stress-induced Hsp70 (Hsp70) or sHsp oligomers. sHsps can hold the protein until Hsp70 is ready to 'refold' them with the assistance of other chaperones and cochaperones (e.g. Hsp90 and Hsp40).

hetero-oligomeric complexes that promote the folding of proteins to native states in an ATP-dependent cycle of binding and release[39,40] (Fig. 2a and d). The subunits are arranged in two rings stacked together back-to-back such that they form a toroidal structure, with a central cavity in each ring where it is believed protein folding can occur in isolation from the rest of the cell.

In type II chaperonins, the rings consist of eight or nine subunits and, although some thermosomes (chaperonins from high temperature living *archaea*) are homo-oligomeric, the majority are hetero-oligomeric consisting of at least two different homologous subunits (Fig. 4). The eukaryotic cytosolic chaperonin (CCT or TriC) has eight unique subunits per ring, with each subunit occupying a well-defined position[41]. Currently, it is not known whether the MKKS protein is a subunit in a homo- or hetero-oligomeric ring. The thermosomes from *Thermoplasma acidophilum*, whose fold best resembles the MKKS protein (Fig. 4)[37], are hexadecamers composed of alternating α and β subunits[42]. If the MKKS protein is one subunit of a hetero-oligomeric type II chaperonin, then other as yet unidentified subunits could represent candidate genes for other BBS loci.

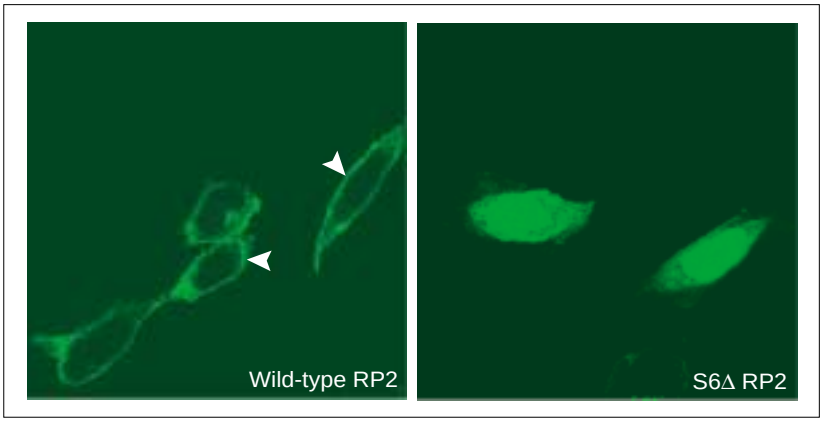


Fig. 3. Targeting of the N-terminus of RP2-GFP in CHO cells. Wild-type protein localizes to the plasma membrane (arrowhead), whereas RP2, which harbours the Ser6deletion mutation, is localized to the cytoplasm and nucleus, as a result of the disruption of N-terminal acylation of the protein[25].

Two of the mutations found in MKKS patients (Y37C and H84Y) are predicted to lie in the highly conserved equatorial domain of the ring structure. The structure of this domain of the MKKS protein can be efficiently modelled on the thermosome structure (Fig. 4). The Y37C mutation is in a region of the protein involved in making intra-ring contacts in the thermosome, whereas the H84Y mutation lies in a region that is responsible for ATP hydrolysis in type II chaperonins. It seems likely, therefore, that the correct assembly of the toroidal structure and the hydrolysis of ATP are essential for the function of MKKS. The putative identity of MKKS as a type II chaperonin strongly suggests a function in the cellular protein folding machinery in the retina and other tissues (Fig. 2a).

Chaperonins are thought to assist the folding of their 'client proteins' in the central cavity of their toroidal structure (Fig. 2a and d, Fig. 4 and Box 2). If MKKS is indeed a component of a novel chaperonin it will be important to identify its client proteins, as they could also represent BBS causative genes. It has been estimated that ~15% of newly synthesised eukaryotic proteins interact with CCT (Fig. 2a)[43], although other studies suggest that CCT predominantly has the cytoskeletal proteins actin and tubulin as client proteins (Fig. 2d)[44]. Therefore, it is difficult to predict whether MKKS will facilitate the folding of a wide range of proteins or only a few specialised clients. Recently, two other BBS genes (*BBS2* and *BBS4*) have been identified[45,46]. Neither protein has homology to MKKS, suggesting that they are not other subunits of a hetero-olgomeric complex, however, BBS4 contains a putative tetratricopeptide repeat (TPR) domain (Box 2). BBS4, therefore, could also be a chaperone or cochaperone involved in the same folding pathway, whereas BBS2 could represent a putative client.

The diseases MKKS and BBS both show variable penetrance and expressivity[37]. The underlying genetic fault might result in compromised cellular chaperone machinery, and this in turn could compromise the stress handling capacity (Fig. 2f). The variation in disease penetrance and expression could, therefore, reflect differences in stress during embryonic development that expose the inability of the developing embryo to compensate for protein damaging insults, such as elevated temperatures caused by maternal illness.

The therapeutic potential of manipulating molecular chaperones in retinal degeneration

The upregulation of many molecular chaperones as part of the cellular response to stress is one of their characteristic features, and led to their initial identification as heat shock proteins (Fig. 2f). This is not only a reactive but also an adaptive response and can protect the cell against future stress, a phenomenon known as thermotolerance. Even before the identification of the first rhodopsin mutations, it had been demonstrated that thermotolerance could be exploited to protect the retina from damage. Barbe *et al.*[47]

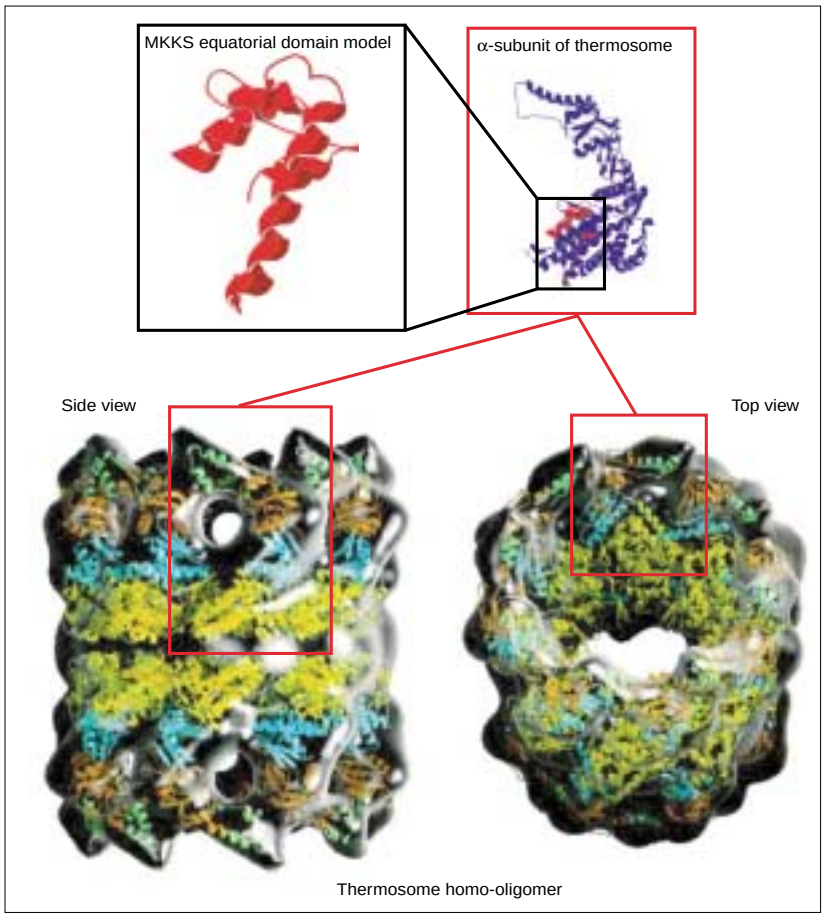


Fig. 4. The McKusick–Kaufman syndrome-causing protein (MKKS) shares structural homology with type II chaperonins. A three-dimensional ribbon model of the equatorial domain of the MKKS protein (red), which includes residues mutated in MKKS and BBS, was based on the crystal structure of the α-subunit of the thermosome from *Thermoplasma acidophilum* (blue) and modelled using SwissModel (http://www.expasy.ch/swiss-mod/SWISS-MODEL.html). Note the importance of this domain for inter-subunit and inter-ring contacts. These subunits are then arranged into a hexadecamer. The arrangement of the α-subunits in the thermosome homo-oligomer in its 'open conformation' is shown in side and top view (reproduced, with permission, from Ref. 43). Client proteins are bound in the central cavity.

showed that a prior mild heat shock could protect the retina from light-induced damage. Although the mechanisms of this protection are not known, the overexpression of individual or combinations of molecular chaperones can mimic many of the protective properties engendered by heat shock. Next, we will discuss the potential of manipulating the chaperone machinery for treatment of retinal degeneration.

The fate of misfolded protein in retinal degenerations is not known, and the accumulation of misfolded protein could be a slow process – RP sometimes does not develop until midlife. Granular aggregates, immunoreactive for rhodopsin, form in human[48] and animal models of RP (Ref. 49), a phenomenon that begs parallels with other neurodegenerations in which intracellular protein aggregates form and have been linked to cell death. The mechanism by which misfolded proteins lead to apoptosis is not known and could differ between proteins. Photoreceptors in rhodopsin RP, however, are more sensitive to stress such as light damage[50]. Manipulating molecular chaperones, therefore, could have two modes of action, preventing protein aggregate formation and protection from environmental stress.

The balance and potential synergy between these two modes of action are perhaps best exemplified by the effect that molecular chaperones have on neurodegenerations mediated by polyglutamine expansions[51]. In mammals, only SCA7 polyglutamine expansions cause retinal degeneration, in addition to central nervous system or peripheral neuropathy[52], but in *Drosophila*, polyglutamine expansions result primarily in retinal degeneration. This model organism has been used in several elegant studies to show the protective effects of chaperones. Overexpression of Hsp70 in transgenic flies resulted in a reduction of polyglutamine induced retinal degeneration[53]. Furthermore, complementary studies using P-element insertional mutagenesis in *Drosophila* identified two cochaperones, Hsp40/Hdj1 and TPR2/Hcp, as suppressors of polyglutamine mediated degeneration in the eye[54]. In these studies, the incidence of the hallmark intranuclear inclusions, which are thought to be a response to misfolded protein, was not reduced. Many other studies, however, have shown that the formation of these inclusions can be modulated by chaperone overexpression[51] and it is unclear which chaperone mechanisms are important for protection from polyglutamine mediated retinal degeneration.

There are now several pharmacological agents that can manipulate chaperone expression and/or function, so that gene transfer mediated overexpression might not be necessary to develop therapeutic strategies involving chaperones[55]. In particular, bimoclomol can potentiate the endogenous stress response and increase chaperone levels[56], and has been shown to protect photoreceptor cells in an experimental model of diabetic retinopathy[57]. The potential of chaperone-modulating compounds in treating acquired and inherited retinal degenerations needs to be further explored, but the developing basic science suggests that manipulating protein folding through molecular chaperones should be widely applicable in the treatment of retinal degenerations.

Outstanding questions

- How does the misfolding of rhodopsin lead to photoreceptor cell death by apoptosis?
- Does the retina contain specialized molecular chaperone machines and what are their client proteins?
- Can molecular chaperone manipulation be used as a general treatment for misfolded proteins in retinal degeneration; in particular, can the folding of rhodopsin be manipulated to improve photoreceptor survival (by molecular chaperones or ligand, such as vitamin A)?
- Can the anti-apoptotic effects of molecular chaperones be used to promote photoreceptor viability?

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