

Looking at protein misfolding neurodegenerative disease through retinitis pigmentosa

Characteristics of Retinitis Pigmentosa

Retinitis pigmentosa (RP) describes a genetically heterogeneous group of retinal dystrophies that are characterised by progressive degeneration of photoreceptor cells in the retina (see RetNet, <http://www.sph.uth.tmc.edu/RetNet/disease.htm>). RP is the most common cause of inherited blindness, and is estimated to affect one in every 4000 individuals. For recent reviews of RP and related retinal degenerations see^{1,2}.

Patients with RP lose vision because of the death of both rod and cone photoreceptors. The disease is thought to primarily compromise the function of rod photoreceptors and ultimately triggers apoptosis of these neurons. As rod cell death progresses, cone cell viability is reduced and further photoreceptor cell death occurs. Clinical examination by electroretinography reveals a reduced response from rod photoreceptors (the dim light or 'scotopic' response) early in disease and later a defect in the cone photoreceptors (the daylight or 'photopic' response), confirming rods as the primary site of pathology. As the disease progresses examination of the retina typically shows pigmentation in a 'bone-spicule' pattern, pallor of the optic disc and narrowing of retinal blood vessels.

There is a large diversity in the function of the gene products that have been implicated in RP, which reflects the complexity of the retina and photoreceptor specialisation. These include genes encoding proteins of the phototransduction cascade (for example, rhodopsin; the subunit of rod cyclic GMP phosphodiesterase; the subunit of the rod cyclic GMP-gated channel and arrestin). RP genes also encode structural proteins of the photoreceptor disc outer segment, (RDS-peripherin and Rom 1); components of the retinoid cycle; extracellular matrix proteins; proteins involved in cell adhesion; and transcription factors (CRX, NRL and TULP1). The disease also occurs as a component of syndromes such as Bardet-Biedl³ and Usher's⁴. In Usher's syndrome (types 1A and 1B) mutations in the genes for harmonin and myosin VIIa affect hair cells of the inner ear and photoreceptors. Ubiquitously expressed RP proteins, which do not cause systemic disease, have also been identified. These include RP2, which is targeted to the plasma membrane. Mutations in RP2 which prevent this localisation cause an RP phenotype^{5,6}. The role of RP2 in photoreceptors remains unclear, but it has been shown to have function-

al overlap with co-factor C a cellular chaperone involved in tubulin folding^{7,8}.

This review focuses on the most prevalent form of RP, which is caused by mutations in rhodopsin, the receptor responsible for dim light photoreception in the vertebrate retina. More than 150 distinct mutations in rhodopsin have been identified [OMIM 180380] which together account for 15% of all inherited retinal disease⁹. The majority of mutations in rhodopsin cause autosomal dominant RP.

Misfolding of rhodopsin; toxic proteins and aggregation

Rhodopsin, the prototypical seven transmembrane G-protein coupled receptor¹⁰, consists of the apoprotein opsin covalently bound to the 11-*cis*-retinal chromophore. It represents a major protein product of rod photoreceptors and accounts for over 70% of the total protein in the outer-segments where rhodopsin dimers tightly pack the disc membranes¹¹.

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-segment¹², whereas mutations in the transmembrane, intradiscal or cytoplasmic domains result in the misfolding of the protein¹³. The first misfolding mutation identified in rhodopsin (also the most frequent ADRP mutation in the US population) is a proline to histidine change at residue 23 (P23H). Studies in cultured cells have revealed that misfolded rhodopsin (P23H) undergoes retrotranslocation from the endoplasmic reticulum (ER) and degradation by the ubiquitin-proteasome system^{14,15}. Saturation of the normal proteolytic machinery causes misfolded ubiquitinated rhodopsin to accumulate in pericentriolar cytoplasmic inclusion bodies, known as aggresomes^{14,15} (Figure. 1). The formation of aggresomes has been shown to be dependent on transport of misfolded proteins by dynein dependent retrograde transport on microtubules¹⁶.

The aggregation and deposition of abnormal protein has recently been identified as a common characteristic of a broad range of neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), prion encephalopathies and polyglutamine diseases such as Huntington's disease (HD)^{17,18}. Pathogenic mutations are associated with a toxic gain of function in polyglutamine diseases¹⁹ and some other protein misfolding diseases. We have proposed that misfolded rhodopsin also acquires a gain of function that leads to cell death⁹. The role of aggregated protein deposits in disease pathogenesis is unclear and there has been considerable debate whether they are pernicious, coincidental or beneficial and this is also the case for rhodopsin aggregates and inclusions.

The cellular molecular chaperone machinery plays a vital role in the cellular



Dr Paul Chapple is a senior post-doctoral researcher at the Institute of Ophthalmology, UCL. Dr Chapple gained his PhD working on molecular chaperones in environmental biology and has been working with Dr Cheetham since 1997. He produced the first characterisation of the RP2 protein and currently researches the role of chaperones in rhodopsin folding. He is hoping to develop his own research program on the retinal cell biology of Bardet-Beid Syndrome.



Dr Mike Cheetham, Senior Lecturer, Institute of Ophthalmology, UCL, developed his interest in molecular chaperones and neurodegeneration whilst doing his PhD with Professor Brian Anderton on the molecular biology of Alzheimer's disease. He has been at the Institute of Ophthalmology since 1995 and now researches the role of chaperones in retinal degeneration.

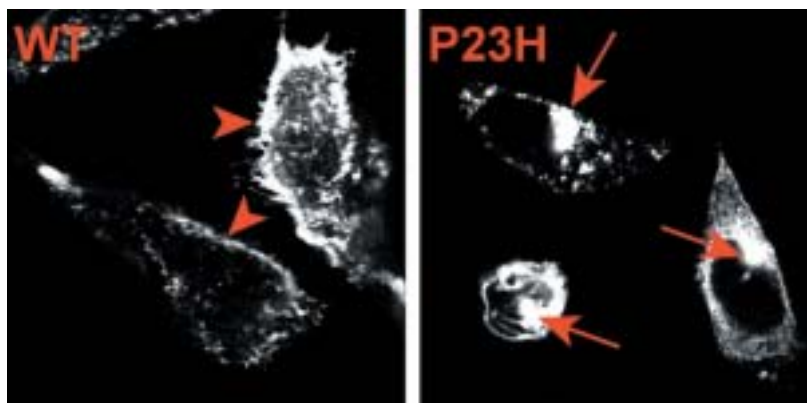


Figure 1. Comparison of the localisation of wild-type rhodopsin and rhodopsin with the misfolding mutation, P23H, in cultured neuronal cells. Wild type protein translocates to the plasma membrane (arrowheads), whereas protein with the P23H mutation forms inclusion bodies, known as aggresomes (arrows).

response to misfolded proteins²⁰ and have been shown to play a critical role in several neurodegenerative disorders²¹. Molecular chaperones and components of the ubiquitin-proteasome degradation system are present in the aggregates of misfolded protein. Molecular chaperones are known to be involved in rhodopsin folding in *Drosophila* and have been shown to be present in rhodopsin aggresomes^{9,14}.

Improving rhodopsin folding; molecular chaperones a potential therapy

The common characteristics of neurodegenerative protein misfolding diseases suggests parallel approaches to treatment based on an understanding of the normal cellular mechanisms for disposing of unwanted and potentially toxic proteins¹⁸. The role of chaperones in correct protein folding and protein degradation clearly identifies their manipulation as a potential therapy for RP. In cellular models of polyglutamine disease overexpression of members of the Hsp70 and Hsp40 families of chaperones have been shown to suppress the toxicity and aggregation of polyglutamine containing proteins. We have recently identified a member of the Hsp40 family of chaperones (HSJ1b) which has a neuronal expression pattern and is enriched in photoreceptor inner-segments, the site of rhodopsin biogenesis. HSJ1b is localised to the cytoplasmic face of the endoplasmic reticulum and will thus encounter cytoplasmic domains of rhodopsin *in vivo*. We tested whether HSJ1b could enhance the folding of mutant rhodopsin. In fact the chaperone caused wild type rhodopsin to be retained in the ER and increased the incidence of aggresome formation for both wild type and P23H rhodopsin (Chapple and Cheetham, submitted). These data provide evidence that cytoplasmic chaperones can influence the folding and processing of rhodopsin. Understanding the specialised chaperone networks within photoreceptors will be essential to exploit the potential of cellular chaperone machines to manipulate the folding of normal and mutant rhodopsin.

Several pharmacological agents have been identified that can manipulate chaperone expression/function, such that gene transfer mediated overexpression may not be required to use chaperones in RP therapies⁹. As cell death in RP, and many other neurodegenerations, is via apoptosis²², it is tempting to speculate that blocking a caspase cascade or similarly disrupting cell death pathways may be of therapeutic benefit. Chaperones play an important role in many cellular signalling pathways and could also provide a mechanism for suppressing signalling cascades which lead to apoptosis²¹.

In addition to manipulating molecular chaperones, there is the potential to manipulate protein folding by 'chemical chaperones' or stabilise protein structures using ligands. Indeed, the folding of mutant rhodopsin has been improved by the natural ligand retinoids. The addition of 11-*cis*-retinal and 9-*cis*-retinal to T17M mutant opsin expressing cells has been shown to improve folding²³.

We have also shown that addition of 9-*cis*-retinal to cultures expressing P23H mutant opsin improves the amount of opsin that reaches the plasma membrane, whilst having no effect on K296E mutant opsin¹⁴. Addition of a modified retinoid, 11-*cis*-7-ring-retinal, has also been shown to improve the folding of rhodopsin containing the P23H mutation²⁴. These data suggest that retinoids may be used as 'chemical' chaperones that can stabilise the folding of mutant opsins shifting the equilib-

rium away from aggregation and towards functional protein. High doses of vitamin A have already been shown to be of some therapeutic benefit in RP²⁵.

This clinical trial, however, was not focused on patients with misfolding mutations in rhodopsin and if it had been the clinical outcomes might have been even better. Further investigation of methods to stabilise and promote the correct folding of mutant rhodopsin, either through chemical chaperones or molecular chaperones, may lead to novel therapies for protein misfolding diseases which can be tested on the most accessible part of the CNS, the retina.

References

- Clarke G, Heon E, McInnes RR. *Recent advances in the molecular basis of inherited photoreceptor degeneration*. Clin.Genet. 2000; 57: 313-329
- Rivolta C, Sharon D, DeAngelis MM, Dryja TP. *Retinitis pigmentosa and allied diseases: numerous diseases, genes, and inheritance patterns*. Hum.Mol.Genet. 2002; 11: 1219-1227
- Katsanis N, Lupski JR, Beales PL. *Exploring the molecular basis of Bardet-Biedl syndrome*. Hum.Mol.Genet. 2001; 10: 2293-2299
- Petit C. *Usher syndrome: from genetics to pathogenesis*. Annu.Rev.Genomics Hum.Genet. 2001; 2: 271-297
- Chapple JP, Hardcastle AJ, Grayson C *et al*. *Mutations in the N-terminus of the X-linked retinitis pigmentosa protein RP2 interfere with the normal targeting of the protein to the plasma membrane*. Hum. Mol. Genet. 2000; 9: 1919-1926
- Chapple JP, Hardcastle AJ, Grayson C, Willison KR, Cheetham ME. *Delineation of the plasma membrane targeting domain of the X-linked retinitis pigmentosa protein RP2*. Invest Ophthalmol.Vis.Sci. 2002; 43: 2015-2020
- Bartolini F, Bhamidipati A, Thomas S *et al*. *Functional overlap between retinitis pigmentosa 2 protein and the tubulin-specific chaperone cofactor C*. J.Biol.Chem. 2002; 277: 14629-14634
- Grayson C, Bartolini F, Chapple JP *et al*. *Localisation in the human retina of the X-linked retinitis pigmentosa protein RP2, its homologue cofactor C and the RP2 interacting protein Arl3*. Hum.Mol.Genet. 2002; 11: 3065-3074
- Chapple JP, Grayson C, Hardcastle AJ *et al*. *Unfolding retinal dystrophies: a role for molecular chaperones?* Trends Mol.Med. 2001; 7: 414-421
- Palczewski K, Kumasaka T, Hori T *et al*. *Crystal structure of rhodopsin: A G protein-coupled receptor*. Science 2000; 289: 739-745.
- Fotiadis D, Liang Y, Filipek S *et al*. *Atomic-force microscopy: Rhodopsin dimers in native disc membranes*. Nature 2003; 421: 127-128.
- Tam BM, Moritz OL, Hurd LB, Papermaster DS. *Identification of an outer segment targeting signal in the COOH terminus of rhodopsin using transgenic Xenopus laevis*. J.Cell Biol. 2000; 151: 1369-1380.
- Sung CH, Schneider BG, Agarwal N, Papermaster DS, Nathans J. *Functional heterogeneity of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa*. Proc.Natl.Acad.Sci.U.S.A 1991; 88: 8840-8844.
- Saliba RS, Munro PM, Luthert PJ, Cheetham ME. *The cellular fate of mutant rhodopsin: quality control, degradation and aggresome formation*. J.Cell Sci. 2002; 115: 2907-2918.
- Illing ME, Rajan RS, Bence NF, Kopito RR. *A rhodopsin mutant linked to autosomal dominant retinitis pigmentosa is prone to aggregate and interacts with the ubiquitin proteasome system*. J.Biol.Chem. 2002; 277: 34150-34160.
- Kopito RR. *Aggresomes, inclusion bodies and protein aggregation*. Trends Cell Biol. 2000; 10: 524-530.
- Temussi PA, Masino L, Pastore A. *NEW EMBO MEMBER'S REVIEW: From Alzheimer to Huntington: why is a structural understanding so difficult?* EMBO J. 2003; 22: 355-361.
- Taylor JP, Hardy J, Fischbeck KH. *Toxic proteins in neurodegenerative disease*. Science 2002; 296: 1991-1995.
- Ross CA. *Polyglutamine pathogenesis: emergence of unifying mechanisms for Huntington's disease and related disorders*. Neuron 2002; 35: 819-822.
- Agashe VR, Hartl FU. *Roles of molecular chaperones in cytoplasmic protein folding*. Semin.Cell Dev.Biol. 2000; 11: 15-25.
- Muchowski PJ. *Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones?* Neuron 2002; 35: 9-12.
- Portera-Cailliau C, Sung CH, Nathans J, Adler R. *Apoptotic photoreceptor cell death in mouse models of retinitis pigmentosa*. Proc.Natl.Acad.Sci.U.S.A 1994; 91: 974-978.
- Li T, Sandberg MA, Pawlyk BS *et al*. *Effect of vitamin A supplementation on rhodopsin mutants threonine-17 --> methionine and proline-347 --> serine in transgenic mice and in cell cultures*. Proc.Natl.Acad.Sci.U.S.A 1998; 95: 11933-11938.
- Syed NM, Kuksa V, Imanishi Y *et al*. *Pharmacological chaperone-mediated in vivo folding and stabilisation of the P23H opsin mutant associated with autosomal dominant retinitis pigmentosa (ADRP)*. J.Biol.Chem. 2003;
- Berson EL, Rosner B, Sandberg MA *et al*. *Vitamin A supplementation for retinitis pigmentosa*. Arch.Ophthalmol. 1993; 111: 1456-1459.

Correspondence to:

Dr Paul Chapple
University College London
Division of Pathology
Institute of Ophthalmology
Bath Street
London EC1V 9EL
E.mail: j.chapple@ucl.ac.uk