Chaperoning prions: the story unfolds

by Dr David O'Connor and Dr Gary Jones

Prions are infectious proteins that are responsible for a number of mammalian degenerative diseases. The discovery of prions in yeast has allowed detailed genetic analysis to be carried out to identify cellular factors involved in prion propagation. It is now clear that a complex relationship exists between molecular chaperones and prion propagation. Prions may actually have evolved to exploit the cell's chaperone machinery to ensure their own propagation.

ROLE OF CHAPERONES IN PROTEIN FOLDING

The information necessary for a protein to fold into its native three-dimensional form is contained within the primary amino acid sequence of the polypeptide chain. As the polypeptide chain emerges from the ribosome, possibly even before its synthesis is complete, it will begin the transition through one, possibly more, partially structured states before arriving at the biologically active, pre-ordained structure. Protein folding cannot occur in a random, unbiased manner where all possible combinations are explored in order to reach the final structure; the so-called Levinthal Paradox. Rather, each protein species appears to have evolved a conserved and simplified pathway of folding [1].

Proteins are made in a sequential manner with the production of small segments of the protein at a time. As such, there are many possibilities for misfolding. Chaperone proteins (e.g. Heat Shock Protein 70, Hsp70) bind to and sequester hydrophobic regions from the surrounding environment, until the entire protein is produced and the final functional conformation can be attained. Chaperones are also important when dealing with misfolded or aggregated proteins. They may either catalyse the correct folding or assist in the degradation on these proteins. Table 1 summarises the major cytosolic chaperones and co-chaperones that exist in yeast and mammalian cells. Subsets of these chaperones are involved in the propagation of yeast and possibly mammalian prions and as such are potential drug targets.

PRIONS

The term prion was first coined by Stanley Prusiner [2] to describe the nature of the scrapie infectious agent. A prion is an infectious protein. It is a transmissible amyloid form of a cellular protein that replicates by converting the native protein into the same abnormal prion form. Prions were first described in mammals and are the causative agents of transmissible spongiform encephalopathies (TSEs). The involvement of the prion protein, PrP, in TSEs is unequivocal but solid proof that the prion form of PrP is the *sole* causative agent is still lacking. TSEs have been described in a number of species such as mink (TME), cat (FSE), elk (CWD) and of course cattle (BSE) and humans (CJD). The emergence of variant CJD due to human consumption of prion-infected beef is well documented. PrP becomes infectious when it assumes a different conformation PrP^{Sc} (its protease resistant form). PrP^{Sc} then induces the same conformational change in cellular PrP. The prion protein then becomes self-propagating and can be transmitted from cell to cell usually in the form of fibres that are rich in ß-sheets. Indeed, the presence of amyloid protein aggregates is characteristic of prion diseases.

PRIONS IN YEAST

Reed Wickner suggested that the strange

Family	Members	Human homologues	General functions
Hsp100	**Hsp104**	None	Protein disaggregation, stress tolerance.
Hsp90	Hsc82, Hsp82	Hsp90 Family	Protein folding and stress tolerance. Most substrates appear to be involved in signal transduction.
Hsp70	**Ssa1-4p** **Ssb1-2p** Sse1-2p Ssz1p	Hsp70 Family	Protein folding and stress tolerance. Bind to denatured proteins and prevent aggregation. Also involved in aspects of protein translocation and translation
Hsp40	**Ydj1p,** **Sis1p**	Hsp40	Deliver peptide substrates and stimulate ATPase activity of their relevant Hsp70 partner.
Hsp70/Hsp90 co-factors	**Sti1p,** Cpr6p, **Cpr7p,** **Cns1p**	Hop Cyclophillin 40 Cyclophillin 40	Aid in the Hsp70-Hsp90 protein folding cycle. Sti1p bridges Hsp70 to Hsp90 and regulates ATPase activity of both proteins.
Chaperones in bold have been implicated in yeast prion propagation.			

Table 1. Major chaperone and co-chaperone families in yeast cytosol.

genetic behaviour of the *Saccharomyces cerevisiae* non-mendelian elements [*PSI*] and [*URE3*] could be explained if they were prions of the Sup35 and Ure2 proteins respectively [3]. Since this suggestion, much work has accumulated to support this notion. In addition to [*PSI*] and [*URE3*] there are at least two other genetically well-characterised prion proteins and several other potential prion candidates in yeast [4]. All confirmed and potential prion proteins in yeast have no known or suspected functional relationships; the only similarity between them is that they appear to have the ability to behave in a prion-like manner. For instance, the Sup35 protein is a translation termination factor that allows efficient release of the nascent polypeptide chain from the ribosome,

Class	Technology	Principle of fabrication	Spot size (µm)	Spot density (spots/cm²)
Spotted arrays	Contact printing	Deposit biomaterial by robotic controlled pins	100-200	2000-4000
	Non-contact printing	Deposit biomaterial by robotic controlled inkjet needles	100	4000-5000
	Contact printing	Robot with cantilevers	0.1	100 million
Optical arrays	Bar coded beads (Illumina, Luminex, Qdots)	Coating beads with biomolecules	N/A	N/A
	Mask dependent fabrication	Light guided synthesis defined by masks (Affymetrix)	5	4 million
		Spotting phosphoramidites using inkjet technology (Agilent)	100	5000
In situ		Spotting phosphoramidites	100	9800

Figure 1. Regulation of Hsp70-Ssa1 reaction cycle by co-chaperones. Substrate binding is finely tuned by ATP hydrolysis and nucleotide exchange. Hsp70 ATPase stimulation occurs via Hsp40, Sti1p and Cns1p. Nucleotide exchange is facilitated by Fes1p.

whereas the Ure2 protein is involved in nitrogen metabolism. The nature of prions in yeast and fungi redefines the term prion to encompass any protein with infectious capability and suggests that prion phenomena may be widespread within biological systems and may actually provide specific functions within the cell.

Critical for establishment and propagation of yeast prion aggregates is the prion-forming domain (PrD). Amino acid regions rich in glutamines (Q) and asparagines (N) are present in all confirmed or suspected yeast prions [4]. *In vitro*, the individual PrDs are able to spontaneously undergo conformational rearrangement in the absence of any other proteins or nucleic acids, to generate highly stable amyloid fibrils that show all the biophysical characteristics of the amyloid deposits in certain neurodegenerative diseases. Therefore, in spite of the presence of the protective mechanisms provided by chaperones, yeast prion-containing cells are able to carry and efficiently propagate high molecular weight aggregates that are absent from prion-free cells. Such aggregates can be readily visualised by fusing the respective prion protein to green fluorescent protein (GFP) and observing the appearance of fluorescent foci.

CHAPERONES AND PRION PROPAGATION

The fact that prions exist in yeast provides an ideal environment for detailed genetic analysis of factors affecting amyloid formation and prion maintenance. A number of genetic analyses have implicated molecular chaperones of playing an essential role in yeast prion propagation. Table 1 highlights the chaperone proteins and families that have been implicated in propagation of yeast prions.

Currently of utmost importance in yeast prion propagation is

Hsp104. The Hsp104 protein is an ATPase that is essential for propagation of all the naturally occurring yeast prions identified so far. *Hsp104* is a member of the *E. coli* Hsp100/ClpB family and unlike other chaperones functions to disaggregate previously aggregated proteins. Deletion of *HSP104* renders yeast cells hypersensitive to heat stress due to their inability to resolubilise aggregated proteins. Both deletion and over-expression of *HSP104* were originally shown to cure the [*PSI*+] prion [5] and these results added extreme weight to the yeast prion hypothesis. It seems that Hsp104 is necessary for the solubilisation of the larger prion aggregates, allowing the formation of 'seeds' or propagons, which then induce the prion conformational change in the surrounding soluble Sup35. The production of propagons allows the efficient transmission of infectious particles to daughter cells. Inhibition of Hsp104 ATPase function, by guanidine hydro-chloride (GdnHCl) prevents the formation of new prion propagons and leads to subsequent prion-free (cured) descendants, after four of five generations.

Although Hsp104 is absent in mammalian systems, some proteins such as p97/VCP/Cdc48p share sequence features. They have a general chaperone-like function and their overexpression does prevent aggregation of non-transmissible polyglutamine aggregates but there is no evidence linking these disaggregation properties with any neurodegenerative disease propagation. It should be noted that yeast prions exist in a cell system that divides regularly whereas mammalian prions are largely associated with non-dividing cells. As such, yeast Hsp104 may have co-evolved in order to allow efficient propagation of the prion in rapidly dividing cells. The lack of a mammalian Hsp104 homologue suggests that this chaperone is not a good candidate for development of potential drug targets to treat prion or amyloid diseases.
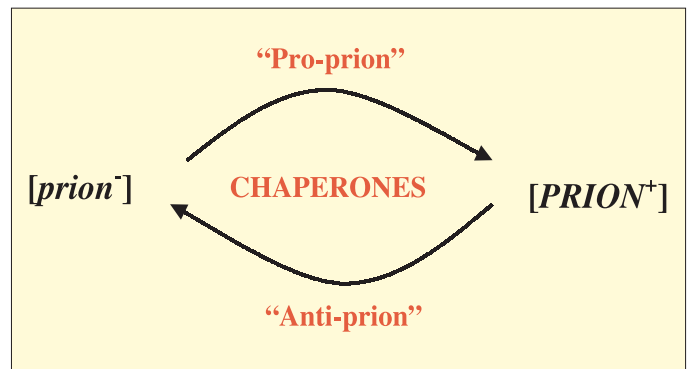
Another class of molecular chaperones implicated in yeast prion propagation is the cytosolic Hsp70s [6]. They exist in a number of different variants and in different locations within the cell. The Ssa (Stress seventy subfamily A) family comprises the main class of cytosolic Hsp70, identified as a modulator of yeast prion propagation. Collectively the four members of the Hsp70- Ssa family [Table 1] provide an essential cellular function and are also involved in various aspects of protein folding along with other chape-rones and co-chaperones. Other Hsp70 members include Ssb, Ssz and Sse proteins [Table 1].

Insight into how Hsp70-Ssa may influence prion propagation has come from the isolation of a mutation in the *SSA1* gene (*SSA1-21*) that impairs [*PSI*+] propagation [6]. Yet in spite of this clear effect on [*PSI*+] propagation, the *SSA1-21* mutation does not affect cell growth or Ssa1p function. The *SSA1-21* mutation (L483W) creates a change in the peptide-binding domain of Ssa1p, but mapping of this residue onto the crystal structure of *E. coli* Hsp70- DnaK, fails to give any clues as to what function of Hsp70 may be affected. Eight additional *SSA1* mutants with similar phenotypes to *SSA1-21* locate to the ATPase domain suggesting that an alteration of the ATPase function of Ssa1p can alter prion propagation. It is conceivable that the Ssa1-21 protein has its effects by altering the communication between the ATPase and peptide-binding domains of the chaperone.

The location of second-site suppressors of the *SSA1-21* mutation in the ATPase and C-terminal domains implicate Hsp40 and tetratricopeptide repeat (TPR) co-chaperones in aiding Ssa1-21p impairing [*PSI*+] propagation whereas those within the peptide-binding domain are located in residues involved in regulating the substrate trapping mechanism of Hsp70. That mutations that weaken the substrate binding properties of Ssa1p can suppress the

SSA1-21 mutation suggests that Ssa1-21p has enhanced substrate-binding properties. Therefore, by altering the finely tuned Hsp70 peptide binding cycle, prion propagation can be impaired. Figure 1 summarises the Hsp70- Ssa ATPase cycle and effects of perturbation of this cycle on yeast prion propagation.

In light of the fact that cells have evolved protein folding quality control systems, how prions managed to survive and replicate *in vivo* is puzzling. Not only are they able to replicate successfully but they are also able to avoid the cellular machinery for dealing with aggregates in terms of disaggregating, re-folding, and ultimately being degraded. In fact, it appears that prions may have evolved to exploit the cellular chaperone machinery to propagate their own prion proteins.

ARE CHAPERONES GOOD TARGETS FOR PRION THERAPEUTICS?

It is clear that in the yeast system the ability of a prion to propagate successfully is dependent on a fine balance between various chaperones species [7]. This gives rise to the concept that chaperones can theoretically be viewed as being pro- or anti- prion in nature [Figure 2]. The fact that chaperone mutants can be isolated that alter propagation of prions but do not affect essential cellular functions suggests that chaperones may be possible therapeutic targets. The yeast prion system has been adapted for high-throughput screening of chemical libraries to identify possible prion-curing agents [8]. The usefulness of this system has been confirmed by the ability of drugs that are known to cure mammalian prions in tissue culture, to

Figure 2. Once present in the cell [PSI+] or [PRION+] may have "hijacked" the cellular chaperone machinery to ensure propagation.

also cure yeast prions. Hence, there appears to be a conserved mechanism in the propagation of yeast and mammalian prions. A number of new drugs (eg. 6-aminophenanthridine) have been identified that cure yeast prions and whose cellular target is not Hsp104 [8]. It is likely that these new drugs target other protein chaperones and prevent prion propagation, either by inhibition of pro-prion chaperones or stimulation of anti-prion chaperones [Figure 2] [M. Blondel, G. Jones, Unpublished data]. The characterisation of how such chemicals cure prions will form the basis of future therapeutic strategies for prion diseases based on alteration of molecular chaperone function.

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