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**Chaperoning prions: the cellular machinery for propagating  
an infectious protein?**

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Running title: Prions and chaperones

## Summary

Newly made polypeptide chains not only require the help of molecular chaperones to rapidly reach their final three-dimensional forms, but also to unfold and then correctly refold them back to their biologically active form should they misfold. Most prions are an unusual class of protein that can exist in one of two stable conformations, one of which leads to formation of an infectious alternatively folded form. Studies in Baker's yeast (*Saccharomyces cerevisiae*) have revealed that prions can exploit the molecular chaperone machinery in the cell in order to ensure stable propagation of the infectious, aggregation-prone form. The disaggregation of yeast prion aggregates by molecular chaperones generates forms of the prion protein that can seed the protein polymerisation that underlies the prion propagation cycle. In this article we review what we have learnt about the role of molecular chaperones in yeast prion propagation, describe a model to that can explain the role of various classes of molecular chaperones and their co-chaperones, and speculate on the possible involvement of chaperones in the propagation of mammalian prions.

## **Introduction**

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 (1). As the polypeptide chain emerges from the ribosome, possibly even before its synthesis is complete, it will begin to 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 (2; review).

In order to help a protein reach its final form and avoid going ‘off-pathway’ and misfold, many proteins call on the assistance of a number of cellular ‘chaperone’ proteins (3; review). The primary role of protein chaperones is to catalyse protein folding and they achieve this by interacting non-covalently with the nascent polypeptide chain in order to stabilize the encrypted folds and thereby prevent non-productive reactions that might lead to an incorrectly folded structure. In other words, by lowering the activation barrier between the partially folded structure and the native form, chaperones accelerate the desired folding steps thereby ensuring that the encrypted folding instructions are faithfully adhered to.

Chaperones are also required to rescue proteins that, as a consequence of having deviated from their desired folding pathway, have become misfolded and aggregated. This can

arise spontaneously or as a consequence of a cell being subject to environmental stresses such as heat shock. Specific chaperones unfold the misfolded or aggregated protein and, in conjunction with other chaperones, rescue the protein by sequential unfolding and refolding the protein back to its native and biologically active form.

Although the primary role of chaperones is to prevent protein misfolding and aggregation, an unusual class of proteins found in both mammals and fungi have apparently evolved to exploit the molecular chaperone machinery to remodel their conformation into an aggregation-prone, infectious form; the so-called prion proteins (4). Once the alternatively (mis)folded structure of a prion protein is established, it becomes self-propagating and can be transmitted from cell to cell usually in the form of protease-resistant fibres that are characteristically rich in  $\beta$ -sheets. The presence of amyloid protein aggregates is a characteristic of a number of non-transmissible neurodegenerative diseases such as Huntington's Disease and Alzheimer's Disease (5).

While the mammalian prion protein PrP (in its protease-resistant form PrP<sup>Sc</sup>) is widely recognised as the causative agent of mammalian Transmissible Spongiform Encephalopathies (TSEs) such as CJD and BSE (6), the prions found in fungi are not necessarily detrimental to the host cell. For example, the [Het-s] prion is required for heterokaryon incompatibility in *Podospora anserina* (7) and is found in a large proportion of isolates of this fungus. In some cases therefore, prions may actually be of benefit to the host cell by acting as epigenetic regulators of cell phenotype (8).

Important new insights into the role(s) played by molecular chaperones in the propagation of fungal prions are now beginning to emerge from both genetic and biochemical studies particularly with the yeast *Saccharomyces cerevisiae*. In this review we will first provide an overview of yeast's molecular chaperone machinery before considering what we have learned about the role chaperones play in prion propagation in fungi and possibly higher eukaryotes.

### **Molecular chaperones and protein folding in the yeast cell**

The unicellular eukaryote *S. cerevisiae* has proven to be an invaluable tool for elucidating protein folding pathways within the cytosol of a eukaryotic cell. All the major classes of chaperone implicated in such pathways have also been identified and extensively characterized in yeast (Table 1). For the majority of identified yeast molecular chaperones, human homologues have also been identified, but with one notable exception – no homologue of the Hsp104 chaperone has yet been described in mammals although a homologue is found in plants (9). The main classes of chaperones in the eukaryotic cytosol are generally classified as heat shock proteins (Hsps) because their synthesis is induced by conditions (such as heat shock) that trigger protein misfolding and aggregation although not all chaperones are Hsps.

The major class of chaperones belong to the Hsp70 family, a diverse collection of 70 kDa chaperones that exist in various compartments of the cell. The main Hsp70 component of the yeast cytosol is the Ssa family (Stress seventy subfamily A) comprising of the four closely related proteins Ssa1-4 and which collectively provide an essential cellular

function. The Ssa proteins are involved in various aspects of protein folding working in conjunction with other chaperones (10, a review). Other Hsp70s in yeast include the ribosome-bound Ssb and Ssz proteins and the cytosolic Sse proteins (Table 1) which collectively, carry out an array of protein folding functions that ensure correct folding of nascent polypeptides, prevent aggregation of denatured or partially unfolded proteins and, in some cases, facilitate correct sub-cellular localisation.

Yeast has two members of the Hsp90 class of chaperones; the heat-shock inducible Hsp82 and the constitutively expressed Hsc82. These two proteins are functionally redundant, but in combination provide an essential cellular function (11). The Hsp90s work in conjunction with Hsp70 and a host of other co-chaperones to facilitate the correct folding and maturation of an array of proteins (10). In mammalian cells, the Hsp90 chaperone machinery facilitates the correct folding of key regulatory proteins such as steroid hormone receptors, transcription factors and protein kinases (10,11) although the 'client list' for yeast Hsp90s is less well defined. The high degree of conservation of the Hsp70-Hsp90 chaperone machinery between yeast and mammals, including co-factor requirements, illustrates the value of using a model organism such as *S. cerevisiae* to gain real insights into protein folding in the eukaryotic cell.

The yeast chaperone that appears to have no mammalian homologue, Hsp104, is a member of the AAA+ superfamily of ATPases and is similar in both sequence and function to the *E. coli* ClpB protease (12). Although not essential for cell viability, Hsp104 plays a critical role in ensuring that cells subjected to prolonged exposure to

physical (e.g. high temperatures) or chemical (e.g. ethanol) stresses, are able to survive. Unlike Hsp70 and other chaperones such as the small heat shock proteins (sHsps, Table 1), this role is not to prevent the aggregation of denatured or partially unfolded proteins, but rather, in conjunction with both Hsp70 and the co-chaperone Hsp40, to act as a protein ‘disaggregase’ leading to the resolubilisation of protein aggregates (13). Hsp104 acts as a molecular ‘crowbar’ to shear high molecular weight aggregates into smaller aggregates that can then be more effectively dealt with by the Hsp70/Hsp40 chaperone system (Figure 1). In so doing, Hsp104 acts in a similar manner proposed for the bacterial ClpB/DnaK chaperones (14,15). As with prokaryotes, yeast therefore has evolved complimentary systems to either prevent non-productive protein aggregation or to rescue proteins that have become aggregated. It is yet to be established if such systems are present in mammalian cells although proteins such as p97/VCP/Cdc48 share both sequence and functional similarities with Hsp104 (see below). The existence of an Hsp104 homologue in plants (9) raises the possibility that a search for prions in genetically tractable plants such as *Arabidopsis* may bare fruit.

In the yeast cell, as in other eukaryotes, a number of co-chaperones and co-factors can modulate the functions of the Hsp70 and Hsp90 proteins (Table 1). The Hsp40 co-chaperones directly interact with both Hsp70 and Hsp90 to modify their function, although their main function is to bind unfolded proteins and to present these substrates to their relative Hsp70 partner. In other words, Hsp40s can act both as chaperones and as co-chaperones. Also important in the regulation of the ATPase activity of the Hsp70-Hsp90 chaperone machinery are co-chaperones that possess the tetratricopeptide repeat

(TPR) motif (16). The identity of the co-chaperones present within the Hsp70-Hsp90 complex will usually reflect the level of maturation of a particular peptide substrate (10, review). The Hsp90 co-chaperones Sti1p, Cpr7p and Cns1p also bind to Hsp104 under certain physiological conditions, although how this co-chaperone interaction modulates Hsp104 function remains to be established (17).

A specialised cytosolic chaperone machine is also present in both yeast and higher eukaryotic cells, namely CCT (cytosolic chaperonin containing T-complex polypeptide-1) or TriC (tailless complex polypeptide-1 [TCP-1] ring complex) which belongs to the same class of chaperones as the bacterial GroEL/ES chaperonin (18). CCT has 8 different sub-units which form a heteromeric complex of two stacked rings and acts primarily to fold actins and tubulins (18). The CCT rings undergo an ATPase-dependent conformational change when bound to their relatively limited repertoire of substrates and provide an enclosed environment for polypeptides to fold correctly in. In terms of both form and function, the CCT complex is highly conserved between yeast and humans (18,19).

Hsp26 and Hsp42 are two low molecular weight chaperones within the yeast cytosol (Table 1) that function independently to prevent the non-specific aggregation of about 30% of the cytosolic protein species (20,21). As with the CCT complex, both Hsp26 and Hsp42 form oligomeric structures, but these are homopolymeric rather than heteropolymeric in nature (20,24). Only the synthesis of Hsp26 is induced by thermal stress while Hsp42 represents the major cellular chaperone in non-stressed cells (21).



What happens when a cell finds itself hosting and propagating prion aggregates? Chaperones represent a first line of defense against the generation and accumulation of misfolded, potentially toxic, protein aggregates. Yet, the very fact that prions are remarkably effective at self-propagation, would suggest that the prion aggregates have not only evolved features to avoid the disaggregation and refolding activities of the chaperone machinery, but can also avoid the back-up defense of degradation by the polyubiquitin/proteasome system (22). In fact, studies with yeast suggest that prions may have actually evolved to exploit the properties of the cellular chaperone machinery in order to propagate their infectious altered conformer.

### **Yeast prions and their propagation**

*S. cerevisiae* has at least three proteins that meet the necessary genetic (23) and biochemical criteria (24) to be defined as a prion: Sup35p/[*PSI*<sup>+</sup>], Ure2p/[*URE3*<sup>+</sup>] and Rnq1p/[*RNQ/PIN*<sup>+</sup>]. Of these perhaps the best understood in terms of its chaperone requirement is the translation termination factor Sup35p. In its prion state, Sup35p aggregates giving rise to [*PSI*<sup>+</sup>] cells that show a defect in translation termination (25). In [*PSI*<sup>+</sup>] cells one usually finds greater than 90% of the Sup35p present in the form of a high molecular weight aggregate (26,27) although there are some natural ‘variants’ of [*PSI*<sup>+</sup>] where this figure is nearer 50% (28) and concomitantly, show a weaker termination phenotype. In contrast, in prion-free [*psi*<sup>-</sup>] cells >95% of the Sup35p is in a soluble functional form.

Critical for establishment and propagation of the  $[PSI^+]$  prion aggregate is the prion-forming domain (PrD) located at the N-terminus of Sup35p and separated from the essential release factor domain by a highly charged region called 'M' (29, review). Both Ure2p and Rnq1p also contain distinct regions that act as self-contained prion-forming domains (30,31). *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 (32, review). Therefore, in spite of the presence of the protective mechanisms provided by the chaperones and the ubiquitin/proteasome systems, 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 in both *S.cerevisiae* (26, 31,33) and *Podospora* (34) by fusing the respective prion protein to green fluorescent protein (GFP) and observing the appearance of fluorescent foci (Figure 2). Speransky *et al* (35) have also directly demonstrated that the presence of protein filaments in  $[URE3]$  strains that contain Ure2p although no direct evidence that any of the fungal prion proteins form amyloids *in vivo* has been forthcoming.

The spontaneous conversion of Sup35p to its prion form occurs is less than one in  $10^6$  cells suggesting that although the cell is not able to eliminate the prion once it is formed, nevertheless it has an effective mechanism to keep spontaneous prion protein conversion in check. The rate of such *de novo* conversion can be dramatically elevated by overexpressing the corresponding full-length prion protein or its isolated PrD (36)

provided the cells already have at least one other prion already resident in the same cell. Originally referred to as the [*PIN*] determinant - for **P**si **I**nducibility (37) – we now know that [*PIN*] is commonly (but not exclusively) the prion form of Rnq1p (38,39). The requirement of a second prion for efficient prion conversion suggests that either the prion form of one protein can template the formation of a second, sequence-unrelated protein, but with amyloid-forming potential (38), or the [*PIN*] prion titrates out an endogenous inhibitor of prion conversion which may be one of the cytosolic chaperones (39). It is only the *de novo* conversion that requires [*PIN*] since once the [*PSI*<sup>+</sup>] prion is established, it can be stably propagated in the absence of [*PIN*] (37).

Stable propagation of yeast prion aggregates requires not only the generation of new propagons (i.e. oligomeric prion ‘seeds’, 40) to drive soluble forms of the protein into prion aggregates, but also the propagons must be efficiently distributed during mitosis and meiosis. In yeast, propagon generation and transmission requires the molecular chaperone Hsp104 and thus plays an essential role in the propagation of all three native yeast prions (31,41,42). However, the structural features of the prion aggregates, or the propagons that seed their formation, remain to be established. The high molecular weight aggregates, as visualized by GFP fusions (Figure 2), may be the dead-end by-products of Sup35p aggregation. Exactly what constitutes a propagon at the molecular level, how its structure relates to the high molecular weight (amyloid-like) prion aggregates found in cells, and how the various chaperone proteins functionally interact to generate and transmit these unusual heritable units so effectively, remain important questions that,

through *in vivo* and *in vitro* studies (discussed below) we are at last beginning to understand.

### **Do protein disaggregation and prion propagation go hand-in-hand?**

Yeast strains carrying a deletion of the *HSP104* gene, although viable, are unable to propagate any of the three native yeast prions so far described (31,41,42). The requirement for Hsp104 is not however absolute because Sup35p chimaeras containing heterologous sequences in place of either the highly charged M region (43) or the PrD (44) can form transmissible prion aggregates that are propagated in the absence of Hsp104.

Hsp104 is an ATPase and, like other members of the AAA+ protein superfamily mediates ATP-dependent unfolding/disassembly of protein-protein complexes and contains two AAA modules with motifs for ATP binding and hydrolysis (12, review). Inhibition of the ATPase activity of Hsp104 *in vivo* by 3 mM guanidine hydrochloride (GdnHCl) (45-47) leads to a rapid block in the ability of the cell to generate new propagons resulting in the appearance, after 4 or 5 generations, of prion-free i.e. [*prion*<sup>-</sup>] cells (46,48).

The model that is most consistent with these data is that Hsp104 generates new [*PSI*<sup>+</sup>] propagons by disassembly of the high molecular weight prion aggregates, into smaller seeding competent oligomers (49,50) (see Figure 1). Inhibition of Hsp104 by GdnHCl does not block prion protein aggregation *per se* (51) but does lead to a block in the

fragmentation of Sup35p aggregates *in vivo* (52). This has led to the proposal for a two-cycle model for prionisation of Sup35p (and most likely, other yeast prions proteins): an Hsp104-dependent process that generates new propagons and an Hsp104-independent process that converts propagons into a high molecular weight, possibly non-seeding dead-end product (51) (Figure 1). Consistent with this model, over expression of Hsp104 in exponentially growing [*PSI*<sup>+</sup>] cells also leads to a rapid loss of the [*PSI*<sup>+</sup>] prion (41) presumably by accelerating the disaggregation of Sup35p polymers that can then be efficiently dealt with by the Hsp40/70 chaperone system.

The mechanism(s) by which either the absence of, or high levels of, Hsp104 can eliminate the [*PSI*<sup>+</sup>] prion has recently emerged from studies using a reconstituted *in vitro* fibrillisation assay based on recombinant Sup35p<sup>NM</sup> (53). In this assay low levels of Hsp104 catalyse the formation of relatively small oligomeric intermediates of Sup35p<sup>NM</sup> that are required for the nucleation of new fibrils *in vitro*. Critically, at higher concentrations, Hsp104 blocks the maturation of these oligomers into fibrils and promotes disassembly of the pre-existing fibrils into non-seeding species. To fully understand the consequences of manipulating Hsp104 levels on prion propagation in the cell it will be important to establish the nature and relationship of the various Sup35p oligomers present in the [*PSI*<sup>+</sup>] cell and the mechanism by which they are generated. Nevertheless, the *in vitro* studies provide us with strong clues to why the cellular levels of Hsp104 are critical for ensuring efficient prion propagation in non-stressed cells. It is conceivable that Hsp104 may in fact have a different function or mode of action in non-

stressed cells that effectively propagate prions such as [*PSI*<sup>+</sup>], compared with stressed cells (54).

What about mammalian prions? Higher eukaryotes, including mammals, encode various AAA+ proteins that share sequence features with the Hsp104/ClpB proteins. These include p97/VCP/Cdc48p which has a general chaperone-like function and participates in a number of different cellular processes (55), torsin A, an ER-luminal protein implicated in torsion dystonia, a movement disorder, and SKD3, a 76kDa ATPase first identified in mouse macrophages (56). Although overexpression of both p97/VCP/Cdc48p (57) and torsin A (58) suppress the aggregation of non-transmissible polyglutamine aggregates, there are no data that conclusively link the disaggregation properties of these proteins with the propagation of any neurodegenerative disease (12,59). Furthermore, the ability of p97/VCP/Cdc48p to bind to denatured proteins and maintain them in a soluble state, does not require p97/VCP/Cdc48p-associated ATPase activity (55) and is unable to promote Sup35pNM fibre disassembly in a reconstituted *in vitro* system (53). These findings may simply reflect a failure to recognise the heterologous substrate or, more likely, through a fundamental difference in the mode of action of p97/VCP/Cdc48p and Hsp104.

It is important to remember that mammalian prions are largely associated with non-dividing cells in the brain, whereas a yeast cell undergoes division every 2-3 hours without loss of the prion. Perhaps Hsp104 has co-evolved with prion-based epigenetic regulators of phenotype and has acquired specific functions that are lacking in other

related AAA+ ATPases, but which ensure rapid generation of new prion seeds to ensure efficient propagation of the prion in rapidly dividing cells?

The interactions between a AAA+ protein and its substrate(s) are mediated largely through their N or C termini which either recognise the substrate directly or may bind 'adaptor' proteins which in turn mediate substrate binding (60). Three yeast Hsp90-binding co-chaperones, Sti1p, Cpr7p and Cns1p, interact with the C-terminus of Hsp104 in respiring cells (17), but although both Sti1p and Cpr7p play a minor, non-essential role in  $[PSI^+]$  propagation, this appears to be mediated via their regulation of the substrate binding properties of Ssa1p (Hsp70) rather than Hsp104 (61).

Genetic studies using both native and artificially constructed yeast prions have demonstrated that, in addition to Hsp104, both Hsp70 (Ssa1/2p, Ssb1/2p) and Hsp40 (Ydj1p, Sis1p) chaperones (see Table 1) are also components of the prion propagation machinery and work in conjunction with Hsp104 to generate new propagons (40,62, reviews) although no direct *in vitro* interaction, using purified proteins, has yet been reported.

### **Hsp70: a key modulator of prion propagation?**

The Hsp70s are a highly conserved family of chaperones that form the central part of a ubiquitous protein folding system (10) and are also involved in aiding protein translocation across membranes, translation and regulation of the heat shock response (10, 63,64). The yeast genome encodes at least fourteen distinct Hsp70-related proteins

that are located in various cellular compartments, but which all share the property of binding to short hydrophobic segments of partially folded or unfolded polypeptides thereby preventing their aggregation and hence aid correct folding.

The Hsp70s consist of an amino-terminal ATPase domain that regulates the function of an adjacent peptide-binding domain, and a carboxyl-terminal variable domain. The finely tuned ATPase cycle of Hsp70 regulates the ability of the chaperone to bind non-covalently to its substrate (Figure 3). When ATP is bound, the peptide-binding domain is in the “open” conformation and can rapidly bind and release the peptide substrate. ATP hydrolysis causes a conformational change to the “closed” form that establishes a tight association between Hsp70 and its substrate, while nucleotide exchange restores the ATP bound form and returns it to the “open” conformation. Various co-chaperones fine-tune the Hsp70 ATPase cycle (Figure 3) (65, review).

Amino acid sequence divergence has allowed varying functions of Hsp70s to evolve. In addition, functional differences are achieved through interactions with different co-chaperones and also through interaction with other non-Hsp70 chaperones. For instance, the ATPase activity of Ssa1p can be stimulated by both Sis1p and Ydj1p whereas Ssb1p cannot (66). For each cellular compartment in which Hsp70 resides, specific Hsp40 partner(s) can be identified.

Two sub-families of cytosolic Hsp70 have been implicated in yeast prion propagation; the Ssa (61, 67-72) and the Ssb sub-families (69,73,74). In addition to effects on prion



propagation, overexpression of Hsp70 can suppress the polyglutamine-associated pathogenicity and reduce the formation of the associated amyloid fibrils (reviewed in 75). Recently, a more tractable yeast model for the study of polyglutamine expansion diseases has been developed and has also implicated the Ssa family in regulating amyloid formation and toxicity *in vivo* (76). The Hsp70 family may therefore have a universal role in suppressing the formation of both transmissible and non-transmissible pathogenic aggregates.

Over-expression of Ssa1p reduces the efficiency of curing of [*PSI*<sup>+</sup>] by Hsp104 over-expression (67) and the complexity of the chaperone-prion interaction is highlighted by the differing abilities of combinations of over-expressed chaperones and co-chaperones to cure different strains and variants of [*PSI*<sup>+</sup>] (69). This most likely reflects underlying differences in structure of the prion oligomers that in turn affects the ability of the various chaperones to interact efficiently with the prion substrate. The complexity of the chaperone-prion interaction is further emphasised by the differing effects of over-expression of Hsp104, Ssa1p and Ssa2p on [*PSI*<sup>+</sup>] and [*URE3*]; over-expression of Hsp104 can cure [*PSI*<sup>+</sup>] but does not cure [*URE3*] while over-expression of Ssa1p but not Ssa2p can cure [*URE3*]. Yet neither Ssa1p nor Ssa2p have any effect on [*PSI*<sup>+</sup>] when over-expressed in unstressed cells (41,42,70).

If the non-essential Hsp70-Ssb family of chaperones is deleted in a [*PIN*<sup>+</sup>] background, the spontaneous level of [*PSI*<sup>+</sup>] appearance increases ten-fold, and the ability of Hsp104 over-expression to eliminate [*PSI*<sup>+</sup>] is severely reduced in these cells (73). Over-

expression of Ssb1p can also eliminate artificially constructed variants of  $[PSI^+]$  (69,74). A possible explanation for these findings is that lack of Ssb leads to an increase in the *de novo* appearance of  $[PSI^+]$  as a direct consequence of the loss of the Ssb-associated chaperone function needed to act on the ribosome-associated nascent polypeptide. Conversely, over-expression of Ssb may reduce the amount of newly synthesised Sup35 that can be recruited into the prion form.

An insight into how Hsp70-Ssa may influence prion propagation has come from the isolation of a novel *SSA1* mutant (*SSA1-21*), that impairs  $[PSI^+]$  propagation, reduces propagation number and, in cells where the encoded protein is the sole Ssa,  $[PSI^+]$  cannot be maintained (68,71). 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 DnaK fails to give any clues as to what function of Hsp70 may be affected (68). Eight additional *SSA1* mutants with similar phenotypes to *SSA1-21* locate to the ATPase domain (71) 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 (68,71).

The location of second-site suppressors of the *SSA1-21* mutation in the ATPase and C-terminal domains implicate Hsp40 and 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 (71). 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.

### **Hsp70 co-chaperones and yeast prion propagation**

The Ssa1p co-chaperones Ydj1p and Sis1p also play a role in yeast prion propagation as judged by monitoring the consequences of overexpression of the co-chaperone or by studying genetic interactions with the *SSA1-21* allele. For example, over-expression of Ydj1p eliminates [*URE3*] (42) and certain variants of [*PSI*<sup>+</sup>] (69), while the over-expression of Sis1p or Ydj1p, efficiently eliminate an “artificial” variant of [*PSI*<sup>+</sup>] (77). Since deletion of the non-essential *YDJI* gene enhances the effects of *SSA1-21* on [*PSI*<sup>+</sup>] propagation, this indicates that the Sis1p:Ssa1p interactions might be crucial in [*PSI*<sup>+</sup>] propagation (71). Sis1p also plays a role in the propagation of the [*RNQ*<sup>+</sup>] prion through its interaction with Ssa1p (78,79). The ability of both Sis1p and Ydj1p to bind various peptide substrates and deliver them to Ssa1p is controlled via a common chaperone module within these proteins and by a region (the G/F region), adjacent to their putative peptide binding domains (78-82).

Over-expression of Sti1p, the yeast homologue of the mammalian TPR co-chaperone Hop, also leads to loss of an “artificial” [*PSI*<sup>+</sup>] variant (77) and impairment of the propagation of a natural [*PSI*<sup>+</sup>] variant (61). Second-site intragenic suppressors of *SSA1-*

21 that map to the conserved GPTVEEVD C-terminal motif of Ssa1p, suggest that a productive interaction between Ssa1p and TPR co-chaperones is required for the Hsp70-mediated effects on  $[PSI^+]$  (71). Although deletion of the non-essential genes encoding the TPR co-chaperones Sti1p and Cpr7p negates the effects of Ssa1-21p, deletion of the genes encoding two other TPR co-chaperones (Cpr6p or Sgt2p) does not. Furthermore, suppression of *SSA1-21* by deletion of the *CPR7* gene is reversed if the *CNS1* gene is over-expressed in these cells. Therefore, for Ssa1-21p to alter prion propagation, a productive interaction with a sub-set of TPR co-chaperones is required (61). The effects on yeast prion propagation of over-expression of either TPR co-chaperones or Hsp40s suggests an Hsp70-dependent mechanism that involves the stimulation of Hsp70 ATPase activity and promotion of substrate binding.

The finding that Ssa1-21p requires a productive interaction with TPR co-chaperones raises the possibility that chaperone complexes containing Hsp90 may be involved in prion propagation since Hsp90s also contain the highly conserved EEVD C-terminal motif in common with Hsp70s (83). However, over-expression of Hsp90 has no effect on prion propagation or curing by Hsp104, and the defects in prion propagation seen in the *SSA1-21* mutant are not altered in strains expressing Hsp90 deleted for the C-terminal MEEVD domain (61,67). Perhaps it is the functional interaction between Hsp70 and the TPR co-chaperones that is independent of physical interactions between the TPR motif and the C-terminus of Hsp70 that is important for prion propagation?

Fes1p, a recently described nucleotide exchange factor for Ssa1p in both yeast (84) and humans (85), accelerates the release of ADP from substrate-bound Ssa1p (Figure 3) while Sti1p and Cns1p are activators of Ssa1p ATPase (86,87). In keeping with this antagonistic action to Hsp70 TPR co-chaperones, if *FES1* is deleted in *SSA1-21* cells then the  $[PSI^+]$  prion is no longer propagated (61). Conversely, over-expression of *FES1* in *SSA1-21* cells produces a “stronger”  $[PSI^+]$  phenotype (61). These data suggest that promotion of substrate binding by Hsp70 impairs  $[PSI^+]$  propagation.

The genetic data emerging suggest that in conjunction with a sub-set of co-chaperones, Hsp70 plays a central role in the propagation of yeast prions (Figure 4). This role involves the binding and release of a prion or prion-related substrate that is essential for the maintenance of the prion. In the same manner as Hsp70 interacts with denatured or partially folded polypeptides, the Hsp70-prion substrate interaction is finely balanced and linked to the Hsp70 ATPase cycle.

### **Chaperones and mammalian prions?**

Given that the Hsp70 chaperones have been highly conserved through evolution, it is conceivable that the mammalian Hsp70 homologues may play a role in the propagation of the prion form of PrP. However, although it has been reported that BiP (a member of the Hsp70 family) is able to bind to mutant forms of PrP (88), the only chaperone that appears to facilitate the *in vitro* conversion of PrP<sup>C</sup> to a protease resistant form is the bacterial chaperonin GroEL (89,90), but importantly this form has not been shown to be infectious. Other chaperones must also play a role in the correct folding of PrP<sup>C</sup>, for

example inhibition of Hsp90 by geldanamycin leads to a change in PrP<sup>C</sup> conformation and/or glycosylation (91). How prion seeds are generated in non-dividing cells is not known but their generation via a molecular chaperone-mediated process of aggregate dissociation is an attractive model and one that is made all the more attractive by the discoveries made in yeast..

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## ABBREVIATIONS USED

AAA+, ATPase associated with a variety of cellular activities; BSE, Bovine Spongiform Encephalopathy; CCT, cytosolic chaperonin containing T-complex polypeptide-1; CJD, Creutzfeldt-Jakob disease; GdnHCl, guanidine hydrochloride; GFP, green fluorescent protein; Hsp, heat shock protein; PrD, prion-forming domain; PrP<sup>C</sup>, soluble, non-infectious form of the mammalian prion protein PrP; PrP<sup>Sc</sup>, insoluble, infectious form of PrP; TPR, tetratricopeptide repeat motif; TSE, Transmissible Spongiform Encephalopathy



## FIGURE LEGENDS

### Figure 1. **Cooperative functions of Hsp104, Hsp70 and Hsp40**

When exposed to stress, such as heat-shock, proteins can become denatured or misfolded and form into amorphous aggregates. The action of Hsp104, aided by Hsp70 and Hsp40, results in a disaggregation of amorphous aggregates into substrates that Hsp70 and Hsp40 can act upon and aid in their correct refolding. It is still unclear in what order these chaperones interact with the initial larger aggregate, and this order may well vary depending on size or other physical nature of the aggregate.

### Figure 2. **Aggregation of GFP when fused to a yeast prion domain**

When the prion-forming domain (PrD) of a yeast prion protein is fused in-frame with Green Fluorescent Protein (GFP), the resulting GFP fusion protein will to coalesce into large aggregates if the cell already contains the prion form of the wild-type prion protein. In the example shown, the Sup35p-PrD –GFP fusion protein has been expressed in a [*PSI*<sup>+</sup>] strain and the resulting aggregates indicated by arrows..

### Figure 3. **Regulation of Ssa1p reaction cycle by co-chaperones.**

Substrate binding is finely tuned by hydrolysis of ATP and nucleotide exchange. Stimulation of Hsp70 ATPase has been demonstrated for Ydj1p, Sis1p, Sti1p and Cns1p. Genetic data suggest Cpr7p may also stimulate Hsp70 ATPase. Nucleotide exchange is facilitated by the action of Fes1p.

**Figure 4. A model for the role of the Hsp40 and Hsp70 chaperones in yeast prion propagation.**

Once a prion has formed in *S. cerevisiae*, the chaperone functions of Hsp104 and Hsp70 maintain the propagation of the infectious protein. Hsp104 is the key component in generating infectious prion seeds from pre-existing amyloid aggregates (see Figure 1). Hsp70 also affects the seed generation process, and appears to require interaction with Hsp40 and TPR co-chaperones to function in this capacity. The complex genetic behaviour of the various Hsp70s and their co-chaperones in prion propagation suggest that subtle differences in Hsp70 substrate recognition may be achieved by altering the composition of an Hsp70-Hsp40-Tpr complex. The nature of the Hsp40 and Tpr co-chaperones interacting with a particular Hsp70 may also affect Hsp70 substrate preference in its function in the stress response. It is also conceivable that a preference exists between highly homologous cytosolic Hsp70s and the choice of Hsp40 and Tpr partners. The conserved nature of the majority of chaperones involved in prion propagation in yeast and their mammalian counterparts, suggests that a similar mechanism for PrP<sup>Sc</sup> maintenance may exist.

**Table 1: Major chaperone and co-chaperone families in the yeast cytosol**

<b>Family</b>	<b>Members</b>	<b>General functions</b>
Hsp100 <sup>a</sup>	<b>Hsp104</b>	Protein disaggregation, stress tolerance.
Hsp90 <sup>b</sup>	Hsc82, Hsp82	Protein folding and stress tolerance. Most substrates appear to be involved in signal transduction.
Hsp70 <sup>b</sup>	<b>Ssa1-4p</b> <b>Ssb1-2p</b> Sse1-2p Ssz1p	Protein folding and stress tolerance. Bind to denatured proteins and prevent aggregation. Also involved in aspects of protein translocation and translation.
Hsp40 <sup>c</sup>	<b>Ydj1p,</b> <b>Sis1p</b>	Deliver peptide substrates and stimulate ATPase activity of their relevant Hsp70 partner. Sis1p is involved in translation initiation.
Hsp70/Hsp90 <sup>b</sup> co-factors	<b>Sti1p,</b> Cpr6p, <b>Cpr7p,</b> <b>Cns1p</b>	Aid in the Hsp70-Hsp90 protein folding cycle. Sti1p bridges Hsp70 to Hsp90 and regulates ATPase activity of both proteins.
Small Hsps <sup>d</sup>	Hsp26, Hsp42	Form oligomeric complexes that bind to unfolded proteins and prevent aggregation.

**FOOTNOTE**

<sup>a</sup> Detailed review in Weibezahn et al. (12).

<sup>b</sup> Detailed review in Wegele et al. (10).

<sup>c</sup> Detailed review in Fan et al. (82).

<sup>d</sup> Detailed review in Walter and Buchner (2)

Chaperones and co-chaperones in **bold** have been implicated in the propagation of yeast prions (see text).

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