DOI: 10.1534/genetics.106.066019

Importance of the Hsp70 ATPase Domain in Yeast Prion Propagation

Harriët M. Loovers, Emma Guinan and Gary W. Jones[1]

Department of Biology, National University of Ireland, Maynooth, County Kildare, Ireland

Manuscript received October 11, 2006
Accepted for publication November 21, 2006

ABSTRACT

The *Saccharomyces cerevisiae* non-Mendelian genetic element [*PSI*$^+$] is the prion form of the translation termination factor Sup35p. The ability of [*PSI*$^+$] to propagate efficiently has been shown previously to depend upon the action of protein chaperones. In this article we describe a genetic screen that identifies an array of mutants within the two major cytosolic Hsp70 chaperones of yeast, Ssa1p and Ssa2p, which impair the propagation of [*PSI*$^+$]. All but one of the mutants was located within the ATPase domain of Hsp70, which highlights the important role of regulation of Hsp70–Ssa ATP hydrolysis in prion propagation. A subset of mutants is shown to alter Hsp70 function in a way that is distinct from that of previously characterized Hsp70 mutants that alter [*PSI*$^+$] propagation and supports the importance of interdomain communication and Hsp70 interaction with nucleotide exchange factors in prion propagation. Analysis of the effects of Hsp70 mutants upon propagation of a second yeast prion [*URE3*] further classifies these mutants as having general or prion-specific inhibitory properties.

PRIONS are infectious proteins. They are a transmissible amyloid form of a cellular protein that replicates by converting the native protein into the same abnormal prion form. The term prion was first used to describe the nature of the scrapie infectious agent (Prusiner 1982). The conversion of the normal form of the mammalian prion protein PrP^c to the infectious prion form PrP^{Sc} is associated with a number of transmissible spongiform encephalopathies (TSEs) in mammals (Collinge 2001). The discovery of proteins that can behave in a prion-like manner in yeast and filamentous fungi (Wickner 1994; Coustou *et al.* 1997) has expanded the prion theory from solely disease-causing agents into novel epigenetic phenomena that may carry out a functional biological role within the cell and raises the possibility that prions may be found in other species as well.

Saccharomyces cerevisiae has at least three proteins that meet the genetic criteria to be defined as a prion (Wickner 1994). Confirmed yeast prions include [*PSI*$^+$], the prion form of Sup35p (Cox 1965), [*URE3*], the prion form of Ure2p (Aigle and Lacroute 1975), and [*PIN*$^+$], the prion form of Rnq1p (Derkatch *et al.* 1997) but also possibly the prion form of a number of other prion-like proteins (Derkatch *et al.* 2001). The most widely studied yeast prion is [*PSI*$^+$], which is the prion form of the translation termination factor Sup35p.

Sup35p can be subdivided into three distinct domains, an N-terminal domain of ~130 amino acids that is required for prion formation and propagation, a highly charged middle domain, and a large C-terminal region that carries out essential functions in translation termination (reviewed in Tuite 2000). In [*PSI*$^+$] cells a high proportion of Sup35p is present in the form of non-functional high molecular weight aggregates (Patino *et al.* 1996; Paushkin *et al.* 1997), which reduces the amount of translation termination release factor and therefore reduces the efficiency of translation termination. Thus, [*PSI*$^+$] cells have high levels of nonsense suppression (Cox 1965). Natural variants of [*PSI*$^+$] exist where a reduced amount of Sup35p can be found within these aggregates (Uptain *et al.* 2001) and such variants show a corresponding reduction in nonsense suppression. In contrast, the vast majority of Sup35p in [*psi*$^-$] cells is present in a soluble functional form.

Another well-characterized yeast prion is [*URE3*]. Conversion of Ure2p into the infectious prion form causes a proportion or Ure2p to form high molecular weight amyloid aggregates that prevent the protein from carrying out its normal function in regulating nitrogen metabolism. Hence, [*URE3*] cells are capable of utilizing poor nitrogen sources while [*ure-0*] cells are not (Taylor and Wickner 2001; Lian *et al.* 2006).

Genetic screens have identified a number of protein chaperones and cochaperones as modulators of prion propagation in yeast (reviewed in Jones and Tuite 2005). The nonessential chaperone Hsp104p plays a critical role in propagation of all naturally occurring yeast prions (reviewed in True 2006). Hsp104p is a protein disaggregase that is required for recovery from exposure to stresses such as heat shock and works in concert with Hsp70 and Hsp40 in the refolding of

[1]*Corresponding author:* Department of Biology, National University of Ireland, Maynooth, County Kildare, Ireland.
E-mail: gary.jones@nuim.ie

aggregated proteins (Glover and Lindquist 1998). Deletion of Hsp104p prevents propagation of naturally occurring yeast prions while overexpression has been shown to efficiently cure [*PSI*$^+$] but not [*URE3*] or [*PIN*$^+$] (Chernoff *et al.* 1995; Derkatch *et al.* 1997; Moriyama *et al.* 2000), presumably due to differences in aggregated prion substrates.

Another major factor in efficient prion propagation is the cytosolic Hsp70 family. Hsp70's are a highly conserved family of chaperones that form the central part of a ubiquitous protein-folding system (Wegele *et al.* 2004). The yeast genome encodes at least 14 distinct Hsp70-related proteins that are located in various cellular compartments. Members of the Hsp70 family share the property of binding to short hydrophobic segments of partially folded or unfolded polypeptides, thereby preventing their aggregation. One subfamily of cytosolic Hsp70 that has been implicated in yeast prion propagation is the Hsp70–Ssa family. The Hsp70–Ssa family consists of four genes, *SSA1–4*, and the presence of at least one is required for vegetative growth (Werner-Washburne *et al.* 1989). Each Ssa protein is composed of an ATPase domain, a peptide-binding domain (PBD), and a C-terminal variable domain (James *et al.* 1997). *SSA1* and *SSA2* are constitutively expressed and under normal growth conditions ~80% of Ssa protein is Ssa2p; expression of *SSA3* and *SSA4* is induced by stress conditions (Werner-Washburne *et al.* 1989).

The binding and hydrolysis of ATP dictates the interaction between Hsp70 and substrate. In the ATP-bound state the peptide substrate is rapidly exchanged between the peptide-binding pocket and the surrounding environment. Upon ATP hydrolysis the peptide-binding domain traps hydrophobic substrate, which is released upon nucleotide exchange from an ADP- to an ATP-bound state (reviewed in Mayer *et al.* 2001). The ATPase cycle of Hsp70 is influenced by cochaperones, including Hsp40's (*e.g.*, Ydj1p in yeast) and tetratricopeptide repeat (TPR)-containing proteins (*e.g.*, Sti1p in yeast) (reviewed in Jones and Tuite 2005). Efficient nucleotide exchange requires interactions with nucleotide exchange factors such as Fes1p (Kabani *et al.* 2002a,b) or Sse1p (Dragovic *et al.* 2006; Raviol *et al.* 2006).

Genetic and biochemical studies on Hsp70–Ssa proteins have provided insight into how cytosolic Hsp70 functions in yeast prion propagation. Overexpression of Hsp70–Ssa reduces the efficiency of curing of [*PSI*$^+$] by Hsp104 overexpression (Newnam *et al.* 1999; Allen *et al.* 2005). Overexpression of Ssa1p also increases the *de novo* appearance of [*PSI*$^+$] when Sup35p is overexpressed and Ssa1p appears to interact physically with Sup35p *in vivo* (Allen *et al.* 2005). Other studies have shown that overexpression of Ssa1p or the Hsp40 family members Ydj1p or Sis1p can efficiently cure natural and "artificial" variants of [*PSI*$^+$] (Kushnirov *et al.* 2000; Krynduskin *et al.* 2002). Despite the high sequence identity between Ssa1p and Ssa2p (98% identity) they have been shown to have differing effects on propagation of [*URE3*] (Schwimmer and Masison 2002) and the presence of Ssa2p is required to maintain [*URE3*] (Roberts *et al.* 2004). Furthermore, different Hsp70–Ssa species have different effects upon the toxicity of amyloid formed by polyglutamine (Gokhale *et al.* 2005) and mutant α-synuclein (Flower *et al.* 2005).

Further insight into how Hsp70–Ssa may influence prion propagation has come from characterization of the *SSA1-21* mutant. *SSA1-21* (L483W) is a dominant mutant of *SSA1* that impairs propagation of [*PSI*$^+$] but does not have any effect upon vegetative cell growth (Jung *et al.* 2000). Genetic analysis of *SSA1-21* cells suggests that Ssa1-21p binds more avidly to prion aggregates and interferes with the production of propagons (Jung *et al.* 2000; Jones and Masison 2003). Analysis of prion aggregates in *SSA1-21* cells suggests that the average size of aggregates is larger when compared to wild-type cells (Song *et al.* 2005). The ability of *SSA1-21* to impair prion propagation is dependent on interaction with TPR cochaperones Sti1p and Cpr7p, and these effects appear independent of Hsp90 (Jones *et al.* 2004).

A previous study identified a number of *SSA1* mutations located within the ATPase domain that impair [*PSI*$^+$] propagation by a mechanism probably similar to that of the well-characterized *SSA1-21* allele (Jones and Masison 2003). To further our understanding of how Hsp70–Ssa species are involved in yeast prion propagation we have carried out an extensive genetic screen to identify mutants in both the major cytosolic Ssa proteins, Ssa1p and Ssa2p, that impair [*PSI*$^+$] propagation. All but one of these new Ssa mutants are located within the ATPase domain, which highlights the importance of the Ssa ATPase cycle in prion propagation. Location of mutated residues on the Hsp70 ATPase domain crystal structure supports a role for regulated interdomain communication in prion maintenance. Clustering of mutants within the ATPase domain in a region known to interact with the nucleotide exchange factor Fes1p indicates the importance of coordination of Hsp70 and its exchange factor(s) for efficient prion propagation. We also analyzed the effects of our new *SSA* mutants upon propagation of a second yeast prion [*URE3*], which has allowed the classification of *SSA* mutants into those having general effects or prion-specific effects. Furthermore, we demonstrate that a subset of these new *SSA* mutants appears to impair prion propagation by a mechanism that is distinct from that of previously well-characterized *SSA1* mutants.

MATERIALS AND METHODS

Strains, plasmids, and genetic methods: Strains used in this study were G400-1C (*MAT***a** *ade2-1 SUQ5 kar1-1 his3 leu2 lys2 trp1 ura3 ssa1::KanMX, ssa2::HIS3, ssa3::TRP1, ssa4::URA3-1f/*pRDW10 [*PSI*$^+$]) (Jones and Masison 2003), G632 *MAT***a**

ade2.1 SUQ5 kar1-1 his3 leu2 trp1 ura3 ssa1::KanMX ssa2::HIS3, which is equivalent to strain 1012 (Jones *et al.* 2004), and SB34 (Bach *et al.* 2003), which is a derivative of CC34 *MAT***a**, *trp1-1, ade2-1, leu2-3,112, his3-11,15, URA2::HIS3*, [*URE3*] (Fernandez-Bellot *et al.* 2000), which has the *ERG6* gene deleted with *TRP1* and the *DAL5* coding sequence replaced by *ADE2*. Plasmid pRDW10 and pJ120 have been described previously (Jones and Masison 2003) and are single-copy plasmids containing the *SSA1* gene. Plasmid pGJ100 is the *SSA2* coding sequence plus 500 bp of upstream and downstream sequence cloned into pRS315 (Sikorski and Hieter 1989). The *SSA2* gene was amplified by PCR from strain 1001 (Jones *et al.* 2004) with oligonucleotide primers containing *Bam*HI sites and subsequently cloned into the *Bam*HI site of pRS315. All *SSA1* mutants are derivatives of pJ120 and all *SSA2* mutants are derivatives of pGJ100.

Monitoring of [*PSI*$^+$] was carried out as described (Jones and Masison 2003). Briefly, the presence of [*PSI*$^+$] (the nonfunctional aggregated form of Sup35p) and *SUQ5* in our cells causes efficient translation readthrough of the *ochre* mutation in the *ade2-1* allele. Nonsuppressed *ade2-1* mutants are Ade$^-$ and are red when grown on medium containing limiting amounts of adenine due to the accumulation of a pigmented substrate of Ade2p. Partial suppression of *ade2-1* by [*PSI*$^+$] allows growth without adenine and eliminates the pigmentation (Cox 1965).

Monitoring of [*URE3*] again made use of the red/white selection based on the *ADE2* gene. The strain SB34 has *ADE2* under control of the *DAL5* promoter. In [*URE3*] cells expression of the *DAL5* promoter is high due to the action of Gln3p. In [ure-0] cells soluble Ure2p can interact with Gln3p and prevent transcription from the *DAL5* promoter. Hence, when [*URE3*] is present the SB34 strain will grow on medium lacking adenine and is white on medium with limiting adenine. When [ure-0] this strain will not grow on medium lacking adenine and is red on medium with limiting adenine.

Generation of mutant *SSA* plasmid libraries: Plasmids pJ120 and pGJ100 were subjected to treatment with hydroxylamine for 60 min (Schatz *et al.* 1988). For these plasmids this treatment results in mutation frequencies of ~6%.

Isolation of *SSA1* and *SSA2* mutants that impair [*PSI*$^+$] propagation: *SSA* mutants were isolated using the plasmid shuffle technique. Strain G400-1C was transformed with either the *SSA1* or the *SSA2* mutagenized plasmid library. Transformed cells were selected on medium lacking leucine. Any red or dark-pink colonies were scored at this point as potential *SSA* mutants that could weaken [*PSI*$^+$]. Transformation plates were replica plated onto medium containing limiting amounts of adenine and also 5-fluoroorotic acid (5-FOA), a chemical that selects against *URA*$^+$ cells and hence against the presence of the pRDW10 plasmid. Colonies appearing red or dark pink at this stage were scored as potentially harboring a mutant *SSA* allele that cannot maintain [*PSI*$^+$]. All potential *SSA* mutant-containing plasmids were isolated and retransformed back into G400-1C and analyzed for their effects upon [*PSI*$^+$]. After retransformation the color phenotype of colonies was scored subjectively from 0 to 9, with 0 being white and 9 being red.

Assaying *SSA* mutant effects upon [*URE3*] propagation: SB34 was grown to log phase growth under conditions that maintain [*URE3*] (medium lacking adenine). Cells were transformed with mutant *SSA* alleles and transformants were selected on medium lacking leucine. At this stage all cells (at least 100) were scored for color phenotype on the basis of being white, red, or sectored. Three white colonies were then selected and spread onto medium lacking both leucine and adenine, which will select for the *SSA* mutant plasmid and also for [*URE3*]. Cells were then restreaked onto medium lacking only leucine and scored for color phenotype on a subjective scale of 0–9, as described earlier.

Site-directed mutagenesis: Mutant alleles were introduced into *SSA* coding sequence by using appropriate primers containing the required DNA base mismatch and using the Quickchange kit (Stratagene, La Jolla, CA) with PCR conditions as recommended by the manufacturer. All *SSA* alleles subjected to site-directed mutagenesis were fully sequenced to verify that only the desired mutation had been introduced.

Western analysis: Western analysis was performed essentially as described in Jung *et al.* (2000) and Jones and Masison (2003). Hsp70 monoclonal antibody was purchased from Stressgen (Victoria, BC, Canada) (SPA 822) and Hsp104 polyclonal antibody was a gift from John Glover (University of Toronto).

RESULTS

Mutants of *SSA1* and *SSA2* that impair [*PSI*$^+$] propagation: Using the plasmid shuffle technique described in materials and methods we have isolated 25 new alleles of *SSA1* and *SSA2* that impair [*PSI*$^+$] propagation (Table 1, Figure 1). Five mutants (A53V, T64I, R74K, G287D, and T295I) were isolated in both *SSA1* and *SSA2* (Table 1, columns 1 and 2). This strongly suggests that *SSA1* and *SSA2* are carrying out the same function in [*PSI*$^+$] prion propagation and supports the suggestion that the *SSA* gene family provides a redundant activity in [*PSI*$^+$] prion propagation (Allen *et al.* 2005). While all mutants greatly impair [*PSI*$^+$] propagation when they are the sole Ssa source in the cell, only one (*SSA2*^{A176T}) has any significant effect upon growth rate (Table 1, last column). This trend has been observed for virtually all previously characterized *SSA1* mutants that alter [*PSI*$^+$] propagation and supports the suggestion that either Ssa function in essential cellular pathways can be separated from Ssa function in prion propagation (Jung *et al.* 2000; Jones and Masison 2003) or [*PSI*$^+$] is much more sensitive to perturbation in Ssa function compared to other cellular substrates.

The most striking observation about the mutants listed in Table 1 is that 24 of the 25 are located within the ATPase domain of Ssa (Table 1, columns 1 and 2). The one mutant located in the PBD is G481D, which is in very close proximity to the *SSA1-21* allele (L483W) (Jung *et al.* 2000). Hence we suspect that G481D is behaving in a similar manner to L483W. The screen performed by Jones and Masison (2003) has previously alluded to the importance of the ATPase domain in prion propagation. In this more exhaustive screen of two Ssa species our findings support the importance of regulation of the Ssa ATPase cycle for propagation of [*PSI*$^+$].

Pre-FOA treatment suggests that most *SSA* mutants show a degree of dominance with respect to [*PSI*$^+$] propagation (Table 1, column 5). Only when plated onto FOA medium, thereby selecting for loss of the wild-type *SSA1* plasmid, did all mutants develop colonies with a uniform color (Table 1, column 6). However, when

TABLE 1

Relative effects of *SSA1* and *SSA2* mutants on prion propagation and cell growth

Mutations[a]				Color[c] in G400-1C		
In Ssa1p	In Ssa2p	*E. coli* DnaK[b]	Times isolated	Pre-FOA	Post-FOA	Generation time (% of wt)[d]
WT	WT		—	0	0	100
	A4T	I4	1	6	9	81 ± 8
	D30N	A30	1	3	7	95 ± 1
	G41D	A41	5	0–6	9	118 ± 2
	G50D	G51	1	7	9	90 ± 9[e]
A53V		A54	1	4	7	95 ± 4
	A53V		2	6	8	100 ± 5
	A53T		4	7	8	105 ± 3
T64I		T65	3	0–8	8	101 ± 2
	T64I		4	0–8	8	101 ± 4
G73D		G74	3	5	9	101 ± 5
R74K		R75	1	5	7	94 ± 4
	R74K		1	6	7	77 ± 30
S119F		A117	2	3	7	96 ± 4
	A146T	A144	2	3	8	101 ± 3
A155V		A153	2	5	8	113 ± 10
A155T			1	4	7	94 ± 4
	A176T	A174	1	4	8	131 ± 7
T223I		T225	8	2–7	8	94 ± 2
R259K		R261	2	5	9	100 ± 6
G287D		D289	4	7	9	102 ± 5
	G287D		5	7	9	101 ± 5
T295I		T301	1	6	8	95 ± 3
	T295I		1	2	8	105 ± 5
A297T		A303	1	4	8	107 ± 3
G481D		G482	1	2–6	9	98 ± 5

[a] Mutations isolated in a previous screen (Jones and Masison 2003) were also isolated in our screen: A17V, five times; R23H, one time; G32D, three times; and R34K, six times.
[b] Corresponding amino acid in *E. coli* DnaK.
[c] Color: 0, white, [*PSI*$^+$]; 9, red, [*psi*$^-$]; FOA, selection against presence of WT *SSA1 URA3* plasmid.
[d] Growth rates measured with *SSA* mutants as sole cytosolic Ssa species and in [*psi*$^-$] variants.
[e] G50D was the only allele to show a severe growth defect at 37°.

placed in an environment containing all *SSA* genes the mutants have no or little effect on [*PSI*$^+$] impairment (data not shown). The reason for this phenotypic difference is unknown but does not involve altered expression levels of Ssa3p or Ssa4p as these proteins are undetectable in these strains.

Given the high degree of homology between the ATPase domains of Hsp70 species across genera, it is reasonable to assume that the location of our new *SSA* mutants on the structure of the DnaK (*Escherichia coli* Hsp70) ATPase domain will provide insight into possible functional alterations. Figure 2 and Table 2 summarize

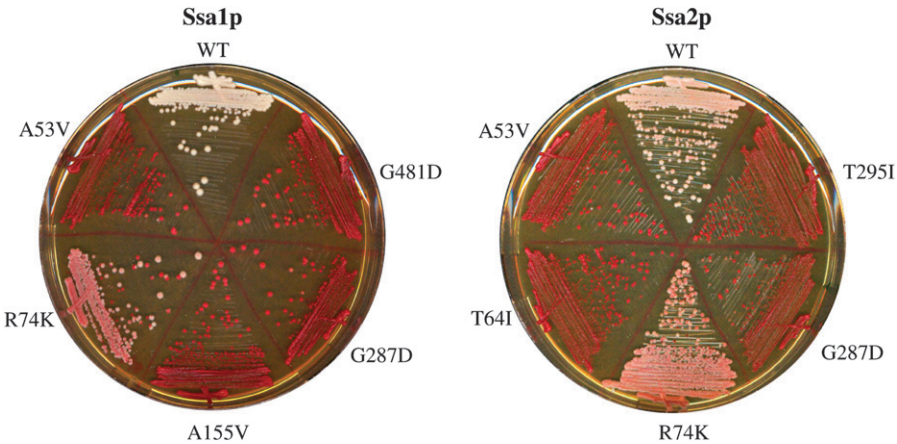

Figure 1.—[*PSI*$^+$] phenotypes of yeast cells expressing *SSA1* or *SSA2* mutant alleles as sole source of Ssa protein within the cell. Cells were streaked onto YPD and incubated at 30° for 2 days followed by a further 2 days at room temperature. Mutated residues are indicated. White color is indicative of the presence of [*PSI*$^+$] prion, and pink and red colonies reflect a reduction in readthrough of the *ade2-1* reporter gene and [*psi*$^-$] cells, respectively.

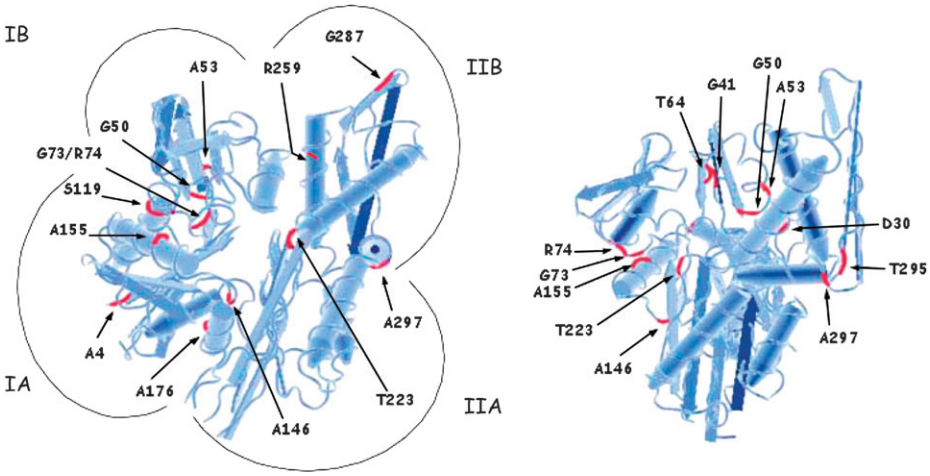

FIGURE 2.—Location of *SSA* mutant alleles on the ATPase domain of DnaK. A tubular diagram of the Protein Data Bank structure for 1DKG is shown from two different angles. Some mutants are shown on both structures to illustrate location more clearly.

the locations of the 18 ATPase domain *SSA* mutations within the ATPase domain of DnaK and also provide the corresponding residues for bovine Hsc70 to show further conservation.

A striking observation from comparing the location of the ATPase domain mutants (Figure 2) is the clustering of residues both in primary and in tertiary structures. In addition to the clustering of specific residues in relation to each other, there is also a generalized clustering of residues to the broadly categorized IA, IB, and IIB region of the Hsp70 ATPase domain. There is only one mutated residue, T223, which is located within the IIA region (Table 2). We can see the clustering in three-dimensional space of G41 with T64 and also of G73 and R74 with A155.

TABLE 2

Ssa ATPase domain mutations and location within Hsp70 ATPase crystal structure

Mutation in Ssa	*E. coli* DnaK	bHsc70	Location
A4T	I4	A6	IA: start β-strand
D30N	A30	D32	IA: loop
G41D	A41	A43	IB: middle β-strand
G50D	G51	G52	IB: end β-strand
A53V	A54	A55	IB: loop
T64I	T65	T66	IB: small β-strand
G73D	G74	G75	IB: loop
R74K	R75	R76	IB: loop
S119F	A117	S121	IA: start α-helix
A146T	A144	A148	IA′: loop
A155V	A153	A157	IA: middle α-helix
A176T	A174	A178	IA: middle α-helix
T223I	T225	T226	IIA: end β-strand
R259K	R261	R262	IIB: middle α-helix
G287D	D289	G290	IIB: top β-strand
T295I	T301	T298	IIB: end β-strand
A297T	A303	A300	IIB: start α-helix
G481D	G482	G484	Peptide-binding domain

Comparison of new *SSA1* mutants to well-characterized *SSA1* alleles that weaken [*PSI*$^+$] propagation: An array of *SSA1* mutations have been reported previously that can suppress the prion-weakening effects of the *SSA1-21* allele (JONES and MASISON 2003). To assess whether our new *SSA* mutants may function in a similar manner to *SSA1-21*, we introduced three known *SSA1-21* second-site suppressor mutations (A519T, E540K, and P636S) into five of our new *SSA1* mutant alleles. We observed the effects upon [*PSI*$^+$] propagation when these double *SSA* mutant alleles were the only *SSA* being expressed in the cell. As a control we used the A17V mutation of *SSA1*; as shown before, its prion-weakening effect is fully suppressed by the chosen suppressor mutations (JONES and MASISON 2003).

The G73D mutation appears to function in a similar manner to the A17V allele in that G73D is fully suppressed by all three second-site suppressor mutations (Table 3). On the other hand the effect of G287D on [*PSI*$^+$] propagation is unaltered by the presence of the second-site suppressors. This suggests that G287D is functioning differently than previously identified *SSA1* mutants that alter [*PSI*$^+$] propagation.

The presence of E540K causes a loss of essential function for *SSA1* and this loss of function can be overcome by A17V or L483W mutations (JONES and MASISON 2003). All new *SSA1* mutations tested in combination with E540K could suppress the growth defect, but not to the same extent as A17V or L483W (Table 3).

Hsp70 and Hsp104 abundance: The propagation of [*PSI*$^+$] can be influenced by the expression levels of Hsp70 and Hsp104 chaperones (CHERNOFF *et al.* 1995; KUSHNIROV *et al.* 2000). We therefore measured abundance of these chaperones in G400-1C expressing our new *SSA* mutants by Western blotting. In all of our mutants we found no difference in the abundance of Ssa or Hsp104 protein levels (Figure 3). This suggests that the effects upon [*PSI*$^+$] propagation are not due to alterations in Ssa or Hsp104 protein abundance and suggest a more direct effect on prion propagation.

TABLE 3

Effects of *SSA1-21* known second-site suppressor mutations upon new prion-weakening *SSA1* mutants

	SSA1 mutation					
Second mutation	A17V	A53V	G73D	R259K	G287D	T295I
None	9	7	9	9	9	8
A519T	0	ND	0	ND	9	ND
E540K	0[a]	7[b]	0[a]	9[b]	9[a]	0[a]
P636S	0	ND	0	ND	9	ND

Effects on colony color are scored subjectively as described in the Table 1 legend. ND, not determined.
[a] Normal growth.
[b] Poor growth.

Different effects of *SSA1* and *SSA2* mutants upon propagation of [*URE3*]: Overexpressed Ssa1p is capable of curing [*URE3*], whereas Ssa2p is not (Schwimmer and Masison 2002). The only known *SSA* allele to negatively affect [*URE3*] is *ssa2-10* (Roberts *et al.* 2004). No *SSA1* allele has been identified. It has therefore been suggested that Ssa1p and Ssa2p have different roles in propagation of the [*URE3*] prion. To investigate if our *SSA* mutants could alter the propagation of [*URE3*] we transformed yeast strain SB34 (Bach *et al.* 2003) with our mutants. This strain contains wild-type copies of all *SSA* genes and therefore any change in [*URE3*] propagation relies on a dominant effect from the mutant Ssa protein. In addition, we constructed plasmids carrying *SSA1* and *SSA2* alleles that harbored a mutation equivalent to *ssa2-10* (P395L) to compare the effects of the *ssa2-10* mutation with our new *SSA* alleles under the same experimental conditions.

In contrast to weak effects on [*PSI*$^+$] when wild-type *SSA*s are present, the *SSA* mutants had major effects on [*URE3*] stability. As shown in Table 4, introduction of an extra copy of *SSA1* or *SSA2* did not have a dramatic effect upon [*URE3*] stability, although there was a reproducible tendency for the appearance of a small percentage of [*ure-0*] cells by an extra copy of *SSA1*. We also observed an effect on stability of [*URE3*] for both *SSA1* and *SSA2* carrying the P395L mutation. The *SSA2* allele showed slightly more impact upon [*URE3*] strength than the *SSA1* allele.

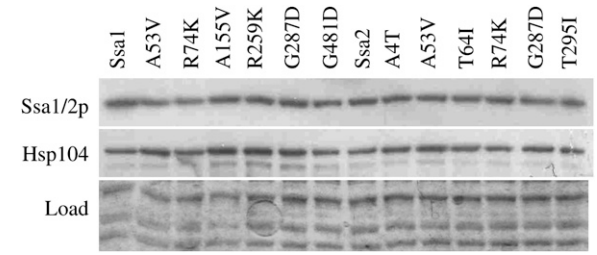

Figure 3.—Abundance of Ssa1p or Ssa2p and Hsp104: Western blot to show chaperone levels in yeast cells harboring mutant alleles of *SSA1* or *SSA2* as sole Ssa protein in the cell. A representative sample of mutants is shown. Mutant alleles are as indicated. Identical aliquots were loaded onto two gels, one of which was probed with antibody to Hsp70, stripped, and probed with antibody to Hsp104. The second gel was stained with Coomassie to show equal loading of samples.

The most informative way to view the data in Table 4 is to combine the effect upon [*URE3*] strength after restreaking (Table 4, last column, *i.e.*, reflected by colony color) with the initial effect upon [*URE3*] stability after transformation (Table 4, columns 3–5, *i.e.*, reflected appearance of [*ure-0*] cells). It is clear that a number of mutants in *SSA2* (A53V, A53T, A146T, and A176T) and *SSA1* (S119F, A155V, and A155T) have a dramatic effect upon [*URE3*] propagation. The effect of these alleles on [*URE3*] propagation is greater than that of the previously identified P395L mutant in these experimental conditions. There are also a number of mutants in both *SSA1* and *SSA2*, such as A4T and R74K, which behave similar to wild-type *SSA* under these experimental conditions.

The *SSA* mutants that showed the strongest effect upon [*URE3*] propagation (A146T and A176T) are *SSA2* alleles. In the case of the A53V mutation, a greater effect upon [*URE3*] propagation is seen for the *SSA2* version compared to the *SSA1* version. However, other mutations found in both *SSA1* and *SSA2* (T64I, R74K, and T295I) behave in virtually the same manner with regard to [*URE3*] propagation.

Western blot analyses for Hsp70–Ssa and Hsp104p expression levels in SB34 strains carrying a copy of *SSA* mutants indicate that there is no correlation between expression levels of these chaperones and the ability to impair [*URE3*], thereby suggesting a more direct effect on prion propagation (data not shown).

Interestingly, we also found that cells harboring *SSA1* or *SSA2* carrying the P395L mutation as the sole *SSA* source show no effect whatsoever on propagation of [*PSI*$^+$] (data not shown). This supports the view that there are differences in the way Ssa proteins influence the propagation of different prions.

DISCUSSION

We have identified 25 new mutations within the two major cytosolic Hsp70–Ssa molecular chaperones of *S. cerevisiae* that impair the propagation of the [*PSI*$^+$] prion. Five of these mutants were identified in identical residues of both Ssa1p and Ssa2p. This provides clear evidence that the Hsp70–Ssa family is providing a redundant function with respect to the [*PSI*$^+$] prion. All but one of the mutants were located within the ATPase domain, which further highlights the importance of the ATPase regulation of Hsp70–Ssa in its role in prion propagation. Only one mutant, *SSA2*^{A176T}, had a major effect on growth rate. This supports previous observations that essential and prion-related functions of Hsp70–Ssa can be separated (Jung *et al.* 2000; Jones and Masison 2003) or that prion substrates are more

TABLE 4

Relative effects of *SSA1* and *SSA2* mutants on [*URE3*] propagation

Mutations		% white colonies	% red colonies	% sectored colonies	Color after restreak
In Ssa1p	In Ssa2p				
WT		92	7	1	0
	WT	100	0	0	0
	A4T	98	1	1	1
	D30N	31	54	15	1
	G41D	96	1	3	2
	G50D	99	1	0	1
A53V		14	14	72	1
	A53V	2	76	22	5
	A53T	48	22	20	5
T64I		97	0	3	1
	T64I	97	1	2	2
G73D		76	7	17	2
R74K		99	1	0	1
	R74K	96	3	1	1
S119F		20	30	50	5
	A146T	9	33	58	6
A155V		7	15	78	5
A155T		12	30	58	4
	A176T	45	5	50	8
T223I		90	5	5	1
R259K		85	10	5	1
G287D		99	1	1	3
	G287D	40	40	20	3
T295I		90	5	5	2
	T295I	98	1	1	1
A297T		33	34	33	1
P395L[a]		14	16	70	2
	P395L[a]	17	29	54	4
G481D		40	30	30	2

Colony color was scored subjectively as for Table 1. Colony percentage is given after transformation of the *SSA* mutant into SB34 and color after restreak is for a colony from [*URE3*] selective medium, as described in MATERIALS AND METHODS.

[a] P395L is a *SSA2* mutation isolated as weakening [*URE3*] propagation (ROBERTS *et al.* 2004) and was used to provide comparison with our new *SSA* mutants.

sensitive to perturbation of Ssa function than other substrates.

We further classified our Ssa mutants by whether or not they impaired propagation of a second yeast prion [*URE3*]. Previous genetic studies have uncovered clear differences in how chaperones influence the propagation of different yeast prions. Hsp104p overexpression has been shown to efficiently cure [*PSI*$^+$] but has no effect on [*URE3*] or [*RNQ*$^+$] (CHERNOFF *et al.* 1995; DERKATCH *et al.* 1997; MORIYAMA *et al.* 2000) and overexpression of Ssa1p but not Ssa2p can efficiently cure [*URE3*] (SCHWIMMER and MASISON 2002). Only the *ssa2-10* mutant allele has previously been shown to affect [*URE3*] propagation (ROBERTS *et al.* 2004). We identified some mutants in both Ssa1p and Ssa2p that are capable of impairing [*URE3*] propagation. The data clearly show that specific mutations in Hsp70–Ssa that impair one prion are not guaranteed to impair a different prion. These results are not too surprising as there is clearly a complex relationship between chaperones and amyloid propagation both *in vivo* and *in vitro* (NEWNAM *et al.* 1999; KUSHNIROV *et al.* 2000; INOUE *et al.* 2004; SHORTER and LINDQUIST 2004; FLOWER *et al.* 2005; GOKHALE *et al.* 2005; KRZEWSKA and MELKI 2006; SHORTER and LINDQUIST 2006). Differences in how different chaperones alter amyloid propagation and how the same chaperone may alter propagation of different amyloid species probably arise from *in vivo* interactions of the chaperone with cochaperones and from the physical structure of the amyloid (reviewed in JONES and TUITE 2005).

Previous genetic studies have suggested that promoting the substrate-bound ADP form of Hsp70–Ssa can impair prion propagation (Figure 4) (JONES and MASISON 2003; JONES *et al.* 2004). While direct biochemical evidence is lacking to confirm this hypothesis, strong indirect evidence exists in the form of biochemical analysis of equivalent DnaK mutations (MAYER *et al.* 2001) and also the effect of Hsp40 and TPR cochaperones on ATPase stimulation of Ssa1p (CYR *et al.* 1992; WEGELE *et al.* 2003; HAINZL *et al.* 2004). Promoting the interaction of Hsp70–Ssa with the prion substrate may disrupt the finely tuned ATPase hydrolysis cycle and thus prevent prion propagation by not releasing substrate for prion conversion. Deregulation of the Hsp70–Ssa ATPase cycle can be achieved by interfering with interdomain communication or by altering interaction with cochaperones that influence ATP hydrolysis.

NMR studies (REVINGTON *et al.* 2005) have recently identified a number of residues in bacterial Hsp70 that are important in communication between the ATPase domain and the PBD. Comparing our new *SSA* mutants to the data of REVINGTON *et al.* (2005), we can infer that A53, G73, R74, A146, A176, and T223 may all play an important role in interdomain communication in Ssa proteins. Mutations at these residues may therefore disrupt the communication between the ATPase and the PBD of Ssa1p and Ssa2p and in turn alter [*PSI*$^+$] propagation.

VOGEL *et al.* (2006) have proposed an allosteric regulation of Hsp70 conformation by a proline switch. In DnaK, residues K70, P143, R151, and E171 play a role in controlling conformational changes in the substrate-binding domain and ATP hydrolysis. A significant number of our new Ssa mutants (G73D, R74K, A146T, A155V, and A176T) are located in regions around the corresponding residues in Ssa1p and Ssa2p, which further suggests that they may alter the interdomain communication control mechanism. We did not isolate mutants at these specific residues. However, these are essential residues for this interdomain communication mechanism and alteration would inactivate Hsp70 function. The nature of our genetic screen in identifying Hsp70

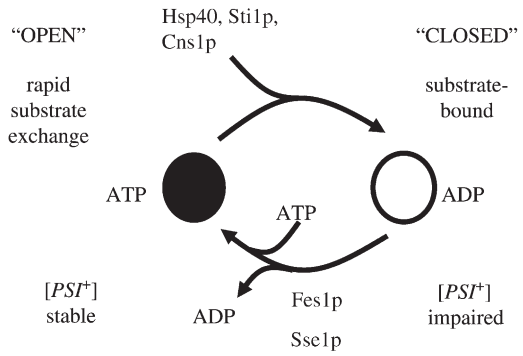

Figure 4.—ATPase cycle of Ssa1p. The highly coordinated hydrolysis of ATP is used to switch between the open and closed forms of Hsp70 chaperone. The ATPase cycle is influenced by the action of Hsp40, TPR cochaperones, and nucleotide exchange factors. Genetic and biochemical data suggest that promotion of the ADP closed form of Hsp70 promotes impairment of prion propagation.

mutants requires maintenance of essential functions, ensuring that we would never succeed in isolating mutations at these essential sites.

Recently a crystal structure of bovine Hsc70 lacking the C terminus of the protein was solved (Jiang *et al.* 2005). This structure has allowed the identification of residues that form molecular interactions at the interface between the ATPase and substrate binding domains of Hsc70. A number of our new Hsp70 mutants (A146T, A155V, A176T, and T223I) are located in regions close to residues that are predicted to be important in forming productive interactions at the interface of ATPase and the PBD of Ssa proteins. This further adds weight to the prediction that a significant number of our Ssa mutants are affecting interdomain communication.

Altering the interaction between Hsp70–Ssa and its nucleotide exchange factors Fes1p or Sse1p will affect the finely tuned ATP hydrolysis cycle. A number of mutants (R259K, G287D, T295I, and A297T) are located within the IIB region that has been implicated as the interaction site between mammalian Hsp70 and the mammalian Fes1p homolog HspBP1 (Shomura *et al.* 2005). The IIB mutants may therefore be altered in their interaction with the *S. cerevisiae* nucleotide exchange factor Fes1p in a way that alters [*PSI*$^+$] propagation. Fes1p has already been implicated in playing a role in [*PSI*$^+$] propagation (Jones *et al.* 2004). When these mutant alleles are the sole *SSA* source in the cell [*PSI*$^+$] propagation is severely impaired and this impairment is much stronger than is observed for a *fes1Δ* strain (Jones *et al.* 2004). This clear difference suggests that the *SSA* mutants do not cause a simple reduction or loss of interaction with this nucleotide exchange factor but probably affect the interaction in a more complex manner. Recently the yeast Hsp70/Hsp110 family member Sse1p has been identified as a second nucleotide exchange factor for Hsp70–Ssa (Dragovic *et al.* 2006; Raviol *et al.* 2006). It is conceivable that both exchange factors interact with Hsp70–Ssa through the IIB region and that our mutants are altering interaction with both or either of the nucleotide exchange factors in a complex manner. Similar to *fes1Δ* a deletion of *SSE1* in our genetic backgrounds has a minor effect upon [*PSI*$^+$] propagation (H. M. Loovers and G. W. Jones, unpublished data). If our Ssa mutants within the IIB region are promoting or increasing the time period Ssa stays bound to the prion-like substrate this may explain why these mutants impair prion propagation (Figure 4).

Hsp70–Ssa is an abundant protein chaperone that has the potential to interact with a wide variety of substrates and influence protein folding. It was recently reported that mature functional Sup35p is readily converted into the prion form (Satpute-Krishnan and Serio 2005). Sup35p is often found in complex with many other cellular proteins. It is possible that Hsp70–Ssa may play a role in [*PSI*$^+$] propagation by participating in the process of remodeling protein complexes that contain Sup35p and during this process provide potential Sup35p prion substrate. Ssa1p and Ssa2p are involved in remodeling of the Rad9–Rad53 complex in response to DNA damage (Gilbert *et al.* 2003) and this remodeling function could extend to many as yet unidentified cellular complexes within yeast.

Further genetic and biochemical analysis of these new Ssa mutants will aid in understanding the precise role of Hsp70–Ssa in amyloid prevention, formation, and propagation within the yeast cell. Given the extent of conservation between yeast and mammalian cytosolic Hsp70's, what we discover in the yeast system may well be directly applicable in the mammalian cell.

We are indebted to Dan Masison for strains and plasmids. We thank Marc Blondel for strain SB34 and John Glover for Hsp104p antibody. We thank Sarah Perrett and Cathy Jones for critical reading of the manuscript. We thank David O'Connor for general help and advice and Peter Mowlds for initial experiments to identify some mutant alleles. This work was supported by a grant from the Irish Health Research Board (RP/04/227) and by a Marie Curie International Reintegration grant (516449) awarded to G.W.J.

LITERATURE CITED

Aigle, M., and F. Lacroute, 1975 Genetical aspects of [URE3], a non-mitochondrial, cytoplasmically inherited mutation in yeast. Mol. Gen. Genet. **136:** 327–335.

Allen, K. D., R. D. Wegrzyn, T. A. Chernova, S. Muller, G. P. Newnam *et al.*, 2005 Hsp70 chaperones as modulators of prion life cycle: novel effects of Ssa and Ssb on the *Saccharomyces cerevisiae* prion [PSI+]. Genetics **169:** 1227–1242.

Bach, S., N. Talarek, T. Andrieu, J. M. Vierfond, Y. Mettey *et al.*, 2003 Isolation of drugs active against mammalian prions using a yeast-based screening assay. Nat. Biotechnol. **21:** 1075–1081.

Chernoff, Y. O., S. L. Lindquist, B. Ono, S. G. Inge-Vechtomov and S. W. Liebman, 1995 Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi+]. Science **268:** 880–884.

Collinge, J., 2001 Prion diseases of humans and animals: their causes and molecular basis. Annu. Rev. Neurosci. **24:** 519–550.

Coustou, V., C. Deleu, S. Saupe and J. Begueret, 1997 The protein product of the het-s heterokaryon incompatibility gene of the fungus Podospora anserina behaves as a prion analog. Proc. Natl. Acad. Sci. USA **94:** 9773–9778.

Cox, B. S., 1965 [PSI+], a cytoplasmic suppressor of super-suppressors in yeast. Heredity **20:** 505–521.

Cyr, D. M., X. Lu and M. G. Douglas, 1992 Regulation of Hsp70 function by a eukaryotic DnaJ homolog. J. Biol. Chem. **267:** 20927–20931.

Derkatch, I. L., M. E. Bradley, P. Zhou, Y. O. Chernoff and S. W. Liebman, 1997 Genetic and environmental factors affecting the de novo appearance of the [PSI+] prion in *Saccharomyces cerevisiae*. Genetics **147:** 507–519.

Derkatch, I. L., M. E. Bradley, J. Y. Hong and S. W. Liebman, 2001 Prions affect the appearance of other prions: the story of [PIN(+)]. Cell **106:** 171–182.

Dragovic, Z., S. A. Broadley, Y. Shomura, A. Bracher and F. U. Hartl, 2006 Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. EMBO J. **25:** 2519–2528.

Fernandez-Bellot, E., E. Guillemet and C. Cullin, 2000 The yeast prion [URE3] can be greatly induced by a functional mutated URE2 allele. EMBO J. **19:** 3215–3222.

Flower, T. R., L. S. Chesnokova, C. A. Froelich, C. Dixon and S. N. Witt, 2005 Heat shock prevents alpha-synuclein-induced apoptosis in a yeast model of Parkinson's disease. J. Mol. Biol. **351:** 1081–1100.

Gilbert, C. S., M. van den Bosch, C. M. Green, J. E. Vialard, M. Grenon *et al.*, 2003 The budding yeast Rad9 checkpoint complex: chaperone proteins are required for its function. EMBO Rep. **4:** 953–958.

Glover, J. R., and S. Lindquist, 1998 Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. Cell **94:** 73–82.

Gokhale, K. C., G. P. Newnam, M. Y. Sherman and Y. O. Chernoff, 2005 Modulation of prion-dependent polyglutamine aggregation and toxicity by chaperone proteins in the yeast model. J. Biol. Chem. **280:** 22809–22818.

Hainzl, O., H. Wegele, K. Richter and J. Buchner, 2004 Cns1 is an activator of the Ssa1 ATPase activity. J. Biol. Chem. **279:** 23267–23273.

Inoue, Y., H. Taguchi, A. Kishimoto and M. Yoshida, 2004 Hsp104 binds to yeast Sup35 prion fiber but needs other factor(s) to sever it. J. Biol. Chem. **279:** 52319–52323.

James, P., C. Pfund and E. A. Craig, 1997 Functional specificity among Hsp70 molecular chaperones. Science **275:** 387–389.

Jiang, J., K. Prasad, E. M. Lafer and R. Sousa, 2005 Structural basis of interdomain communication in the Hsc70 chaperone. Mol. Cell **20:** 513–524.

Jones, G. W., and D. C. Masison, 2003 *Saccharomyces cerevisiae* Hsp70 mutations affect [*PSI*$^+$] prion propagation and cell growth differently and implicate Hsp40 and tetratricopeptide repeat cochaperones in impairment of [*PSI*$^+$]. Genetics **163:** 495–506.

Jones, G. W., and M. F. Tuite, 2005 Chaperonin prions: The cellular machinery for propagating an infectious protein? BioEssays **27:** 823–832.

Jones, G., Y. Song, S. Chung and D. C. Masison, 2004 Propagation of Saccharomyces cerevisiae [PSI+] prion is impaired by factors that regulate Hsp70 substrate binding. Mol. Cell. Biol. **24:** 3928–3937.

Jung, G., G. Jones, R. D. Wegrzyn and D. C. Masison, 2000 A role for cytosolic hsp70 in yeast [*PSI*$^+$] prion propagation and [*PSI*$^+$] as a cellular stress. Genetics **156:** 559–570.

Kabani, M., J. M. Beckerich and J. L. Brodsky, 2002a Nucleotide exchange factor for the yeast Hsp70 molecular chaperone Ssa1p. Mol. Cell. Biol. **22:** 4677–4689.

Kabani, M., C. McLellan, D. A. Raynes, V. Guerriero and J. L. Brodsky, 2002b HspBP1, a homologue of the yeast Fes1 and Sls1 proteins, is an Hsc70 nucleotide exchange factor. FEBS Lett. **531:** 339–342.

Kryndushkin, D. S., V. N. Smirnov, M. D. Ter-Avanesyan and V. V. Kushnirov, 2002 Increased expression of Hsp40 chaperones, transcripional factors, and ribosomal protein Rpp0 can cure yeast prions. J. Biol. Chem. **277:** 23702–23708.

Krzewska, J., and R. Melki, 2006 Molecular chaperones and the assembly of the prion Sup35p, an in vitro study. EMBO J. **25:** 822–833.

Kushnirov, V. V., D. S. Kryndushkin, M. Boguta, V. N. Smirnov and M. D. Ter-Avanesyan, 2000 Chaperones that cure yeast artificial [PSI+] and their prion-specific effects. Curr. Biol. **10:** 1443–1446.

Lian, H. Y., Y. Jiang, H. Zhang, G. W. Jones and S. Perrett, 2006 The yeast prion protein Ure2: structure, function and folding. Biochim. Biophys. Acta **1764:** 535–545.

Mayer, M. P., D. Brehmer, C. S. Gassler and B. Bukau, 2001 Hsp70 chaperone machines. Adv. Protein Chem. **59:** 1–44.

Moriyama, H., H. K. Edskes and R. B. Wickner, 2000 [URE3] prion propagation in Saccharomyces cerevisiae: requirement for chaperone Hsp104 and curing by overexpressed chaperone Ydj1p. Mol. Cell. Biol. **20:** 8916–8922.

Newnam, G. P., R. D. Wegrzyn, S. L. Lindquist and Y. O. Chernoff, 1999 Antagonistic interactions between yeast chaperones Hsp104 and Hsp70 in prion curing. Mol. Cell. Biol. **19:** 1325–1333.

Patino, M. M., J. J. Liu, J. R. Glover and S. Lindquist, 1996 Support for the prion hypothesis for inheritance of a phenotypic trait in yeast. Science **273:** 622–626.

Paushkin, S. V., V. V. Kushnirov, V. N. Smirnov and M. D. Ter-Avanesyan, 1997 Interaction between yeast Sup45p (eRF1) and Sup35p (eRF3) polypeptide chain release factors: implications for prion-dependent regulation. Mol. Cell. Biol. **17:** 2798–2805.

Prusiner, S. B., 1982 Novel proteinaceous infectious particles cause scrapie. Science **216:** 136–144.

Raviol, H., H. Sadlish, F. Rodriguez, M. P. Mayer and B. Bukau, 2006 Chaperone network in the yeast cytosol: Hsp110 is revealed as an Hsp70 nucleotide exchange factor. EMBO J. **25:** 2510–2518.

Revington, M., Y. Zhang, G. N. Yip, A. V. Kurochkin and E. R. Zuiderweg, 2005 NMR investigations of allosteric processes in a two-domain Thermus thermophilus Hsp70 molecular chaperone. J. Mol. Biol. **349:** 163–183.

Roberts, B. T., H. Moriyama and R. B. Wickner, 2004 [URE3] prion propagation is abolished by a mutation of the primary cytosolic Hsp70 of budding yeast. Yeast **21:** 107–117.

Satpute-Krishnan, P., and T. R. Serio, 2005 Prion protein remodelling confers an immediate phenotypic switch. Nature **437:** 262–265.

Schatz, P. J., F. Solomon and D. Botstein, 1988 Isolation and characterization of conditional-lethal mutations in the TUB1 alpha-tubulin gene of the yeast *Saccharomyces cerevisiae*. Genetics **120:** 681–695.

Schwimmer, C., and D. C. Masison, 2002 Antagonistic interactions between yeast [PSI(+)] and [URE3] prions and curing of [URE3] by Hsp70 protein chaperone Ssa1p but not by Ssa2p. Mol. Cell. Biol. **22:** 3590–3598.

Shomura, Y., Z. Dragovic, H. C. Chang, N. Tzvetkov, J. C. Young *et al.*, 2005 Regulation of Hsp70 function by HspBP1: structural analysis reveals an alternate mechanism for Hsp70 nucleotide exchange. Mol. Cell **17:** 367–379.

Shorter, J., and S. Lindquist, 2004 Hsp104 catalyzes formation and elimination of self-replicating Sup35 prion conformers. Science **304:** 1793–1797.

Shorter, J., and S. Lindquist, 2006 Destruction or potentiation of different prions catalyzed by similar Hsp104 remodeling activities. Mol. Cell **23:** 425–438.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

Song, Y., Y. X. Wu, G. Jung, Y. Tutar, E. Eisenberg *et al.*, 2005 Role for Hsp70 chaperone in Saccharomyces cerevisiae prion seed replication. Eukaryot. Cell **4:** 289–297.

Taylor, K. L., and R. B. Wickner, 2001 [URE3] and [PSI]: prions of Saccharomyces cerevisiae. Contrib. Microbiol. **7:** 21–31.

True, H. L., 2006 The battle of the fold: chaperones take on prions. Trends Genet. **22:** 110–117.

Tuite, M. F., 2000 Yeast prions and their prion-folding domain. Cell **100:** 289–292.

Uptain, S. M., G. J. Sawicki, B. Caughey and S. Lindquist, 2001 Strains of [PSI(+)] are distinguished by their efficiencies of prion-mediated conformational conversion. EMBO J. **20:** 6236–6245.

Vogel, M., B. Bukau and M. P. Mayer, 2006 Allosteric regulation of Hsp70 chaperones by a proline switch. Mol. Cell **21:** 359–367.

Wegele, H., M. Haslbeck, J. Reinstein and J. Buchner, 2003 Sti1 is a novel activator of the Ssa proteins. J. Biol. Chem. **278:** 25970–25976.

Wegele, H., L. Muller and J. Buchner, 2004 Hsp70 and Hsp90—a relay team for protein folding. Rev. Physiol. Biochem. Pharmacol. **151:** 1–44.

Werner-Washburne, M., J. Becker, J. Kosic-Smithers and E. A. Craig, 1989 Yeast Hsp70 RNA levels vary in response to the physiological status of the cell. J. Bacteriol. **171:** 2680–2688.

Wickner, R. B., 1994 [URE3] as an altered URE2 protein: evidence for a prion analog in Saccharomyces cerevisiae. Science **264:** 566–569.

Communicating editor: A. P. Mitchell