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Nucleotide Exchange Factors for Hsp70s Are Required for [URE3] Prion Propagation in *Saccharomyces cerevisiae*

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The [URE3] and [*PSI*$^+$] prions are infectious amyloid forms of Ure2p and Sup35p. Several chaperones influence prion propagation: Hsp104p overproduction destabilizes [*PSI*$^+$], whereas [URE3] is sensitive to excess of Ssa1p or Ydj1p. Here, we show that overproduction of the chaperone, Sse1p, can efficiently cure [URE3]. Sse1p and Fes1p are nucleotide exchange factors for Ssa1p. Interestingly, deletion of either *SSE1* or *FES1* completely blocked [URE3] propagation. In addition, deletion of *SSE1* also interfered with [*PSI*$^+$] propagation.

INTRODUCTION

The yeast nonchromosomal genes, [URE3], [*PSI*$^+$], and [*PIN*$^+$], are infectious proteins (prions) of Ure2p, Sup35p, and Rnq1p, respectively (Wickner, 1994; Derkatch *et al.*, 2001). Each is a self-propagating amyloid form of the corresponding protein (Paushkin *et al.*, 1996; Glover *et al.*, 1997; King *et al.*, 1997; Taylor *et al.*, 1999; Sondheimer and Lindquist, 2000; Derkatch *et al.*, 2001), and thus these systems bring the facile yeast molecular genetic tools to bear on the broader problem of amyloidoses.

Chaperones of the Hsp104, Hsp70, and Hsp40 groups are critical for propagation of [*PSI*$^+$], [URE3], and [*PIN*$^+$] (Chernoff *et al.*, 1995; Newnam *et al.*, 1999; Jung *et al.*, 2000; Moriyama *et al.*, 2000; Jones *et al.*, 2004). Hsp104 can disaggregate denatured proteins aided by Hsp70 and Hsp40 (Glover and Lindquist, 1998), and Hsp104 probably breaks up large filaments to make smaller seeds (Eaglestone *et al.*, 2000; Kryndushkin *et al.*, 2003).

Mutation of the cytoplasmic Hsp70, Ssa1p, destabilizes the [*PSI*$^+$] prion (Jung *et al.*, 2000), and Ssa1p overproduction inhibits curing of [*PSI*$^+$] by overproduced Hsp104 (Newnam *et al.*, 1999). Overproduction of Ssa1p (but not the nearly identical Ssa2p) cures [URE3] (but not [*PSI*$^+$]; Schwimmer and Masison, 2002), whereas mutation or deletion of *SSA2* (but not *SSA1*) destabilizes [URE3] (Roberts *et al.*, 2004). Overproduction of the Hsp40, Ydj1p, also cures [URE3] (Moriyama *et al.*, 2000), whereas deletion of the G/F-rich domain of Sis1p cures [*PIN*+] (Sondheimer *et al.*, 2001).

Hsp70s (including the cytoplasmic Ssa1 to Ssa4, Ssb1 and Ssb2) bind hydrophobic regions of proteins ("holdase" activity), preventing their aggregation and promoting their refolding (reviewed by Mayer and Bukau, 2005). Various cochaperones, such as Hsp40s (including Ydj1p, Sis1p), bind to both specific substrates and to Hsp70s bringing the substrates to be acted on by Hsp70s. Hsp70 action is mediated by ATP binding, hydrolysis and release: the Hsp·ATP complex is in an open conformation that rapidly binds and releases substrate, whereas Hsp·ADP is closed and binds tightly to substrate. Nucleotide exchange factors, such as Fes1p and Sse1p, promote the release of ADP by Hsp70, allowing ATP to bind, and thus affect the balance of binding, release, and refolding activities of Hsp70s. Sti1p, which interacts with both Hsp90s and Hsp70s, also stimulates the Ssa1p ATPase.

The key role of Hsp104 in prion propagation was discovered by screening for proteins whose overproduction cured [*PSI*$^+$] (Chernoff *et al.*, 1995). A similar screen using a hybrid of the *Pichia methanolica* Sup35 prion domain and the *Saccharomyces cerevisiae* Sup35 functional domain (Sup35PS) identified curing activity of several chaperones (Ydj1, Sis1, Sti1) as well as several factors that regulate chaperone activity at the transcriptional level (Kryndushkin *et al.*, 2002). Here, we performed a similar screen on [URE3].

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MATERIALS AND METHODS

Strains, Genetic Methods, and Plasmids

Strains used are listed in Table 1. Standard rich (YPD) or synthetic (SC) yeast media were used (Sherman, 1991). Adenine-poor medium (1/2 YPD) contains half the normal amount of yeast extract and was used for the red-white assay of *ADE2* expression (Cox, 1965). Strains BY241 (used in the screening) and BY251 contain the *ADE2* gene under control of the *DAL5* promoter (Brachmann *et al.*, 2005), allowing detection of the prion state of Ure2p and even different [URE3] prion variants by colony color. Three different [URE3] variants were characterized in those strains (Brachmann *et al.*, 2005): v1, v2, and v3; BY241 v1, the same as [URE3-1] (Lacroute, 1971), was used in the screening. To measure [URE3] loss under chaperone overproduction, ~30 yeast colonies from a transformation plate were inoculated in liquid YPD media and grown overnight to allow plasmid loss (which was ~90% complete), and ~10^4 cells were spread on a YPD plate. The ratio of red to white colonies was scored.

For [*PSI*$^+$] experiments, strain 5V-H19 with strong or weak prion variants (Kryndushkin *et al.*, 2003) or 74D-694 [*PSI*$^+$] (Chernoff *et al.*, 1995) was used. To detect the [*PSI*$^+$] phenotype, strain 5V-H19 includes the *ade2-1* UAA nonsense mutation and the *SUQ5* tRNA suppressor, which suppresses *ade2-1* only in combination with [*PSI*$^+$]. [*psi*$^-$] cells are Ade− and accumulate a red pigment related to impaired adenine biosynthesis. [*PSI*$^+$] cells are Ade+ and form white ("strong" [*PSI*$^+$] variants) or pink ("weak" variants) colonies. To enhance colony color development, only one third (6 mg/l) of normal adenine concentration was used in transformation plates. 5V-H19-PS, obtained from 5V-H19 by replacing *SUP35* with the chimeric *SUP35-PS* allele encoding amino acids 1-186 of Sup35 of the yeast *P. methanolica* instead of amino acids

Table 1. Yeast strains

Strain name	Genotype	Reference
BY241	MAT**a**, *leu2, trp1, ura3,* P_{DAL5}*:ADE2,* P_{DAL5}*:CAN1, kar1,* [URE3-1]	Brachmann *et al.* (2005)
BY241-HA	MAT**a**, *leu2, trp1, ura3,* P_{DAL5}*:ADE2,* P_{DAL5}*:CAN1, kar1, URE2::URE2-HA*	This study
BY241 ΔSSE1	MAT**a**, *leu2, trp1, ura3,* P_{DAL5}*:ADE2,* P_{DAL5}*:CAN1, kar1, sse1*Δ::kanMX	This study
BY241 ΔFES1	MAT**a**, *leu2, trp1, ura3,* P_{DAL5}*:ADE2,* P_{DAL5}*:CAN1, kar1, fes1*Δ::kanMX	This study
BY241 ΔSTI1	MAT**a**, *leu2, trp1, ura3,* P_{DAL5}*:ADE2,* P_{DAL5}*:CAN1, kar1, sti1*Δ::kanMX	This study
BY251	MATα, *leu2, trp1, ura3,* P_{DAL5}*:ADE2,* P_{DAL5}*:CAN1, kar1,* [URE3-1]	Brachmann *et al.* (2005)
5V-H19	MAT**a**, *ade2, SUQ5, leu2, ura3,* [PSI^+]	TerAvanesyan *et al.* (1994)
5V-H19 ΔSSE1	MAT**a**, *ade2, SUQ5, leu2, ura3, sse1*Δ::kanMX	This study
5V-H19 ΔFES1	MAT**a**, *ade2, SUQ5, leu2, ura3, fes1*Δ::kanMX	This study
5V-H19-PS	MAT**a**, *ade2, SUQ5, leu2, ura3, SUP35::SUP35-PS,* [PSI^+PS]#1	Kushnirov *et al.* (2000)
74D-694	MAT**a**, *ade1, leu2, trp1, ura3, his3,* [PSI^+]	Chernoff *et al.* (1995)
74D-694 ΔSSE1	MAT**a**, *ade1, leu2, trp1, ura3, his3, sse1*Δ::kanMX	This study
74D-694 ΔFES1	MAT**a**, *ade1, leu2, trp1, ura3, his3, fes1*Δ::kanMX	This study
62δ-1DC	MATα, *ade2, SUQ5, trp1, lys2, can1, kar1,* [*psi-*]	D. Masison

1-123 of Sup35 of *S. cerevisiae*, was used with the strong prion variant [PSI^+ PS]#1. Four millimolar GuHCl was used to cure either [URE3] or [PSI^+].

Plasmids and DNA Manipulations

Plasmids used are listed in Table 2. The *S. cerevisiae* genomic library (catalogue no. 37323) was purchased from American Type Culture Collection (Manassas, VA). Individual genes were tested for [URE3] curing by cloning into pRS425-based plasmids, using the Gateway Technology system (Invitrogen, Carlsbad, CA). The AttR1+CmR+ccdB+AttR2 block of pYES-Dest52 (Invitrogen) was amplified by PCR using primers p11 and p12 and transferred into pRS425 using XhoI and HindIII sites to produce a new destination vector (pRS425-Dest). Corresponding genes (*SSE1*, *FES1*, and *YDJ1*) were amplified from plasmids (genomic inserts in the case of *SSE1* and *YDJ1*) or yeast genomic DNA (for *FES1*) using proofreading polymerase Pfx (Invitrogen) and primers p13 and p8 for *SSE1*, p14 and p9 for *FES1*, and p15 and p16 for *YDJ1*. Then these genes were inserted first by TOPO cloning into pENTR/D-TOPO vector (Invitrogen) containing AttL1,2 sites and then by recombination between AttL and AttR sites into pRS425-Dest vector using Clonase II Enzyme Mix (Invitrogen). Insertions were verified by sequencing. Sse1 K69M and G233D mutations were inserted via quick-change PCR, using p17 and p18 (for K69M) and p19 and p20 (for G233D) primers. Then mutated genes were inserted into pRS425-Dest similar to wild-type *SSE1*.

URA3-based plasmids Yeplac195-SIS1, Yeplac195-STI1, Yeplac195-SSB1, and pFL44-HSP104 were kindly provided by M. Ter-Avanesyan (Kryndushkin *et al.*, 2002). pH125 and pH400 were a kind gift of H. Edskes (Edskes and Wickner, 2000). pH125 is a pRS425-based episomal vector with inserted *ADH1* cassette, containing *ADH1* promoter and terminator, divided by multiple cloning sites. pH400 is the same as pH125 but contains *TRP1* instead of *LEU2*. *SSA1* was amplified from yeast genomic DNA using primers p21 and p22, containing BamHI and HindIII restriction sites for cloning. After overnight digestion BamHI-HindIII fragment was cloned into BamHI and HindIII linearized pH125 and pH400, resulted in pH125-SSA1 and pH400-SSA1 plasmids.

SSE1, *FES1*, and *STI1* disruption cassettes were obtained by amplifying yeast genomic DNA of corresponding strains from the *S. cerevisiae* knockout collection (Winzeler *et al.*, 1999) using primers: p1 and p2 for *SSE1*, p3 and p4 for *FES1*, and p5 and p6 for *STI1* (see Table 3). Disrupted mutants were then obtained by transforming the resulting PCR fragment into yeast and selecting for G418-resistant colonies at a final concentration of 0.5 g/l. In each case disruption was confirmed with additional primers, one primer outside the cassette (p8 for *SSE1*, p9 for *FES1*, and p10 for *STI1*) and another inside the *KanMX* gene (p7 for all). The phenotypes (see *Results*) of each disruption were analyzed in three independent clones.

For biochemical experiments the BY241-HA strain had *URE2* with three C-terminal tandem hemagglutinin (HA) tags made by homologous recombination (Longtine *et al.*, 1998), and the resulting Ure2-3HA was functional. [URE3] was selected on−ade medium and checked by curing with 4 mM GuHCl. This [URE3] was affected by overexpression of SSE1 or YDJ1 with efficiency similar to that of [URE3-1] in wild-type BY241.

Analysis of Yeast Cell Lysates and Electrophoresis. Strain BY241-HA was grown in liquid media to an OD_{600} of 1.5. The cells were harvested, washed in buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM dithiothreitol, and Complete protease inhibitor mixture; Roche Applied Science, Indianapolis, IN) and lysed by glass beads in the same buffer. Cell debris was removed by centrifugation at 10,000 × *g* for 10 min. Protein was measured with BCA reagent (Pierce, Rockford, IL). To determine the size distribution of Ure2p, the cell lysates (0.4 ml of 5 mg/ml) were fractionated by centrifugation through a 30% sucrose pad (0.5 ml) in the SW55 rotor (Beckman, Fullerton, CA) for 40

Table 2. Plasmids

Plasmid name	Markers	Reference
pRS425-SSE1	*LEU2*, episomal	This study
pRS425-SSE1 K69M	*LEU2*, episomal	This study
pRS425-SSE1 G233D	*LEU2*, episomal	This study
pRS425-FES1	*LEU2*, episomal	This study
pRS425-YDJ1	*LEU2*, episomal	This study
Yeplac195-SIS1	*URA3*, episomal	Kryndushkin *et al.* (2002)
Yeplac195-STI1	*URA3*, episomal	Kryndushkin *et al.* (2002)
Yeplac195-SSB1	*URA3*, episomal	Kryndushkin *et al.* (2002)
pFL44-HSP104	*URA3*, episomal	Kryndushkin *et al.* (2002)
pH400-SSA1	*TRP1*, episomal	This study
pH125-SSA1	*LEU2*, episomal	This study

Table 3. Primers

Primer name	Primer sequence, 5′–nn–3′
p1	gcataatcatttgttacgtg
p2	ATACGCGCTGGCATGTCCC
p3	cagtgtactaacctatttcc
p4	CTATTGAATGATGATCGAACG
p5	caaactattgaactaaacgc
p6	GTAAAGTTGTGCCAATTACG
p7	tgattttgatgacgagcgtaat
p8	GGTAGACTTTATGGATGGAG
p9	CTGTTTACTTGTACTGATCC
p10	ATGGAGCTTAGATATTCTCG
p11	gttCTCGAGgctatcaaacaagtttgtac
p12	CACaagcttATCGAACCACTTTGTACAAG
p13	CACCaactagtataactcccatc
p14	CACCaatgatgaaatgttatccc
p15	CACCtgagttgtgtgtattcaac
p16	ACAAAAAATGGTTTTCTGTGG
p17	gccaacttgaTGagaattattggtttggattacc
p18	GGTAATCCAAACCAATAATTCTCATCAAGTTGGC
p19	ctgcgacaagcattttgAtggtagggacttcgatttgg
p20	CCAAATCGAAGTCCCTACCAtCAAAATGCTTGTC GCAG
p21	CAG GGATCC atgtcaaaagctgtcgg
p22	GCA AAGCTT TTAATCAACTTCTTCAACGG

min at 43,000 rpm. Ure2-HA protein was detected by the anti-HA antibody (Roche Applied Science).

Agarose Gel Electrophoresis. Agarose gel electrophoresis of prion aggregates of Ure2p was adopted from previously published procedures for Sup35p (Kryndushkin *et al.*, 2003) or Rnq1p (Bagriantsev *et al.*, 2006) prion polymers. Briefly, cell lysates of BY241-HA [URE3] were obtained as described above. Ure2p prion aggregates were sedimented through a 30% sucrose pad (0.5 ml) in the SW55 rotor (Beckman) for 40 min at 43,000 rpm. Resulted pellets were treated with 2% of SDS for 10 min at 37°C (prion polymers are resistant to such treatment in contrast to most cellular protein–protein interactions as shown in Kryndushkin *et al.*, 2003) and were separated by horizontal 1.5% agarose gel electrophoresis in LB buffer with 0.1% SDS. As molecular weight markers, a preparation of chicken pectoralis extract containing abundant giant proteins titin (3 MDa) and nebulin (0.8 MDa) was used. After the electrophoresis, proteins were transferred from gels to Immobilon-P PVDF sheets (Millipore, Bedford, MA) by electric transfer unit (Invitrogen) for 1 h, followed by immunostaining. Because the polyclonal antibody obtained previously against recombinant Ure2p (Wickner, 1994) reacts poorly with aggregated Ure2p (Speransky *et al.*, 2001), strain BY241-HA [URE3] was used. Ure2-HA protein was detected by the anti-HA antibody (Roche Applied Science).

RESULTS

A Screen for Prion-eliminating Factors

Strain BY241 carries *ADE2* under control of the *DAL5* promoter to monitor activity of Ure2p (Brachmann *et al.*, 2005). Active Ure2p makes such a strain Ade− and red on adenine-limiting medium, but prion inactivation of Ure2p allows *ADE2* transcription, resulting in pink or white colonies according to the level of transcription. We used the mitotically stable [URE3-1] prion variant (Lacroute, 1971) that results in a white colony phenotype. Strain BY241 [URE3-1] was transformed with a *S. cerevisiae* genomic library based on the multicopy plasmid YEp13. Red, pink, or red-sectored transformant colonies have lost [URE3], most spontaneously (~1 per 1000 transformants). But in some cases the red colony color was due to the plasmid, either curing [URE3-1] or slowing prion conversion, causing increased levels of soluble Ure2p without eliminating the prion.

Among ~150,000 transformants, ~200 red "positives" were tested for spontaneous prion loss by crossing with strain BY251 carrying the same [URE3-1]. Plasmids isolated from transformants that were red or pink both as haploids and after mating with BY251 were transformed again into BY241 [URE3-1] to confirm the prion-curing effect. Confirmed plasmids were sequenced, and individual genes were tested again. Two proteins that cured [URE3-1] very efficiently were Ydj1p and Sse1p (Figure 1A, Table 4). Also, the lack of prion phenotype after the spontaneous loss of the plasmids, confirmed that [URE3-1] was indeed cured, ruling out mere suppression of the phenotype.

Overproduction of either Ydj1p or Ssa1p is known to impair [URE3] propagation (Moriyama *et al.*, 2000; Schwimmer and Masison, 2002), but the effect of Ssa1p is much smaller at least in our genetic background (Figure 1A, Table 1), so we did not identify it in the screening.

Sse1p is an Hsp110 family chaperone (Mukai *et al.*, 1993), with 35% identity to Ssa1p and overall structure similarity, including an ATPase domain. The exact function of Sse1p in protein folding in vivo is not yet clear. Besides its "holdase" activity, Sse1p was recently found to be a nucleotide exchange factor (NEF) for yeast Hsp70 members Ssa1p and Ssb1p (Dragovic *et al.*, 2006; Raviol *et al.*, 2006), facilitating the release of a substrate from Hsp70.

We further tested overproduction of Ydj1p and Sse1p on [*PSI*$^+$] and on [*PSI*$+_{PS}$], based on a *Pichia-Saccharomyces* (Sup35-PS) hybrid. [*PSI*$^+$PS] variant 1, in contrast to [*PSI*+], is cured by Ydj1p overexpression (Kryndushkin *et al.*, 2002; Table 4). Overexpression of Sse1p slightly destabilized [*PSI*$^+$PS]#1, but had no effect on [*PSI*$^+$] (Table 4).

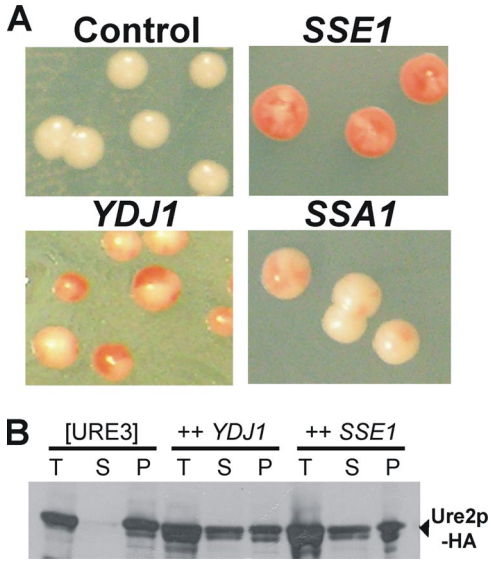

Figure 1. Overproduced chaperones cure [URE3]. (A) Transformants of BY241 [URE3-1] with multicopy plasmids carrying the indicated genes. Control, pRS425 vector. (B) Centrifugation analysis of BY241-HA [URE3]. T, total lysate; S, supernatant fraction; P, pellet fraction. Fractions were analyzed by Western blot with anti-HA antibody.

Table 4. Relative prion loss after chaperone overproduction

Overproduced proteins	None	Ydj1p	Sse1p	Ssa1p
Relative [URE3] v1 loss (%)	1	55	85	20
Relative [*PSI*$^+_{PS}$]#1 loss (%)	1	40	12	20

Data shown are the average of three experiments, with a variation of about ±10%.

Ydj1p and Sse1p also showed high curing potency for two other [URE3] variants (BY241 v2 and v3; Brachmann *et al.*, 2005) and in different host strains (BY251, BY241-HA [URE3] de novo induced). Supporting the genetic results, overproduction of either Ydj1 or Sse1 caused increased solubility of Ure2p (Figure 1B) and increased the length of Ure2p prion polymers (Figure 2B). Further, overexpression of Sse1p does not significantly change levels of Hsp104 or Ssa proteins (Figure 3).

Sse1p ATPase Activity Is Needed To Cure [URE3]

Because Sse1p is homologous to Ssa1p, it might act on [URE3] as a chaperone by direct interaction with soluble or filamentous Ure2p or some intermediate. Alternatively, Sse1p might act as a NEF for Ssa1p, affecting [URE3] indirectly. To distinguish between these two possibilities, we generated two point mutations within the ATPase domain of Sse1p. Sse1-G233D is known to reduce both ATP binding and hydrolysis and results in the loss of interaction and NEF function with Ssa1p (Shaner *et al.*, 2004, 2005; Dragovic *et al.*, 2006). In contrast, the Sse1p-K69M mutant can bind ATP (hydrolysis is defective) and retains both Ssa1p binding and NEF activity (Raviol *et al.*, 2006). We found that overproduction of Sse1p-K69M efficiently cured [URE3-1], but Sse1p-

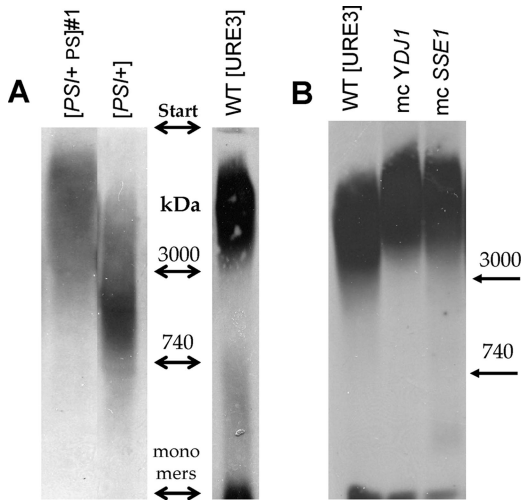

Figure 2. Agarose gel electrophoresis of prion polymers. Particulate fractions from sucrose pad centrifugation analyzed on 1.5% agarose gels and blotted to nitrocellulose. Immunostaining was with anti-Sup35 or anti-HA antibody. (A) Size comparison of [*PSI*$^+$PS], [*PSI*$^+$], and [URE3]. (B) Size differences of [URE3] prion polymers for wild-type cells and cells overproducing (mc) either Ydj1p or Sse1p.

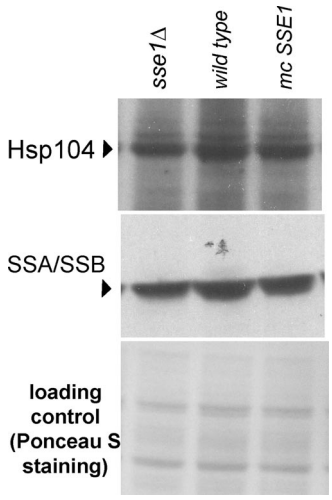

Figure 3. Levels of Hsp104p or Ssa proteins in BY241 [URE3-1] wild type, *sse1*Δ, and with overproduced (mc) Sse1p. Immunodetection was with anti-Hsp104 antibody (StressGen, San Diego, CA) or anti-Hsp70 (StressGen).

G233D did not (Figure 4A). Because ATPase activity (not merely ATP binding) is generally essential for chaperone activity of Hsp70s, this suggests that Sse1p is not affecting [URE3] by a direct chaperone activity.

Deletions of Either SSE1 or FES1 Disrupt [URE3] Propagation

Because Sse1p apparently affects [URE3] stability via regulation of Ssa1p, we examined whether Fes1p, the other known NEF for Ssa1p (Kabani *et al.*, 2002), affects [URE3]. Fes1p promotes ADP release upon binding to Ssa1p and may inhibit the ATPase activity of Ssa1p (Kabani *et al.*, 2002). Several other Ssa1p cofactors (Ydj1p, Sis1p, Sti1p, Cpr7p, and others) stimulate ATPase activity of Ssa1p, pushing the reaction in the opposite direction (see Figure 5A). Unlike

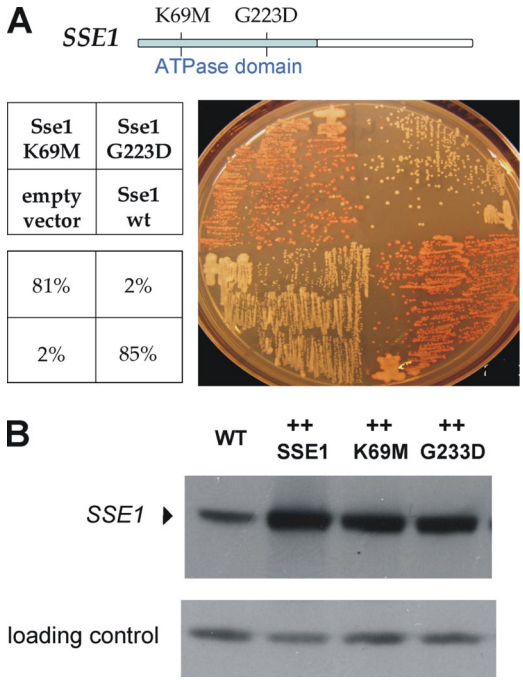

Figure 4. Effect of Sse1 ATPase domain point mutations. (A) Strain BY241 [URE3-1] with overproducted wild-type Sse1p, Sse1-K69Q, Sse1-G233D, or vector streaked on ½ YPD (figures indicate relative prion loss). (B) Levels of normal and mutant Sse1p overproduction, measured with anti-Sse1 antibody and compared with vector alone (WT). The loading control is a nonspecific band recognized by anti-Sse1 antiserum, and the results were confirmed by Ponceau S staining.

Ydj1p and Sse1p, overexpression of Sis1p, Sti1p, or Fes1p does not produce significant loss of [URE3-1] (data not shown). However, we found complete loss of prion propagation after deletion of *SSE1* or *FES1*, and partial destabilization of prion propagation after deletion of *STI1* (Figure 6A). The loss of [URE3] by *sse1*Δ was confirmed by mating BY241 *sse1*Δ with BY251 [URE3-1] and tetrad analysis: all *sse1*Δ spores had lost [URE3], but many *SSE1* spores remained [URE3]. Similar data were obtained for BY241 *fes1*Δ× BY251.

Deletion of SSE1 Can Also Affect [PSI$^+$] Propagation

Using two independent strains (5V-H19 and 74D-694) and three different prion variants, deletion of *SSE1* weakened the [*PSI*$^+$] phenotype in all cases, but deletion of *FES1* did not (although Jones *et al.*, 2004 did observe both weakening and instability of [*PSI*$^+$] in *fes1*Δ). Prion variant 335 (strong) in strain 5V-H19 *sse1*Δ showed white diploids after crossing with a [*psi*$^-$] *SSE1* partner, showing that the *sse1*Δ mutation only masked the [*PSI*$^+$] phenotype, but variant 1111 (weak) in host strain 5V-H19 *sse1*Δ produced all red diploids, indicating complete prion loss (Figure 6B). Thus, the requirement for Sse1p is both prion and prion variant specific.

DISCUSSION

Sse1p Effects: Direct or Indirect?

Either the overexpression or depletion of Sse1p disrupts propagation of [URE3]. Our mutant results indicate that ATP binding, but not the ATPase of Sse1p are important for its prion-curing effects. Because the ATPase activity should

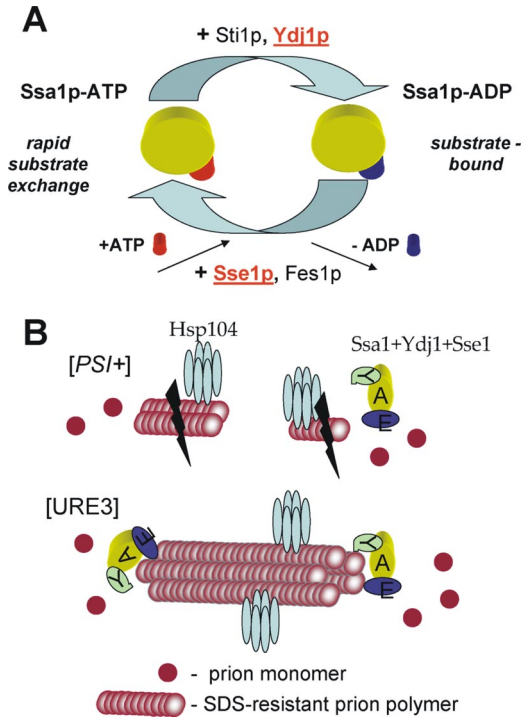

Figure 5. Schematic models. (A) Functional cycle of Ssa1p ATPase. Different cofactors either stimulate ATPase activity of Ssa1p or stimulate ADP exchange. (B) Possible difference between [*PSI*$^+$] and [URE3] prion propagation: because of the relatively shorter polymers, [*PSI*$^+$] is more sensitive to Hsp104 action; instead, [URE3] is more sensitive to the Ssa1/Ydj1/Sse1 complex.

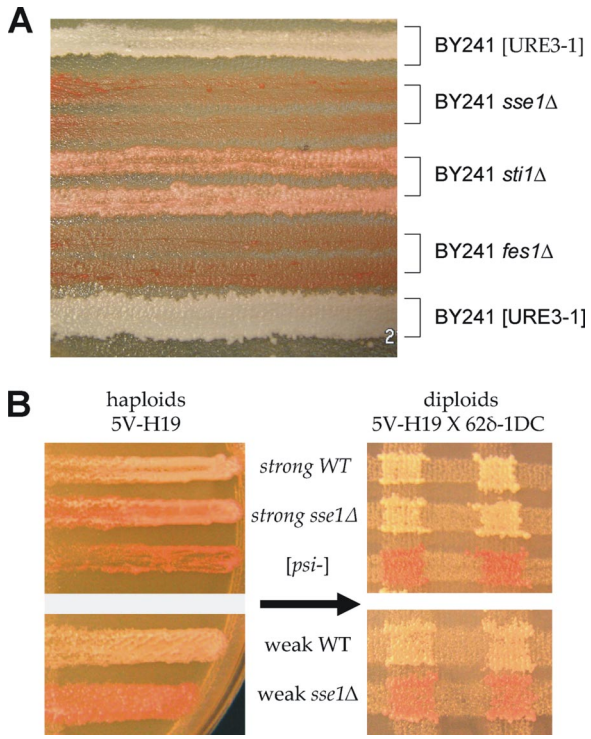

Figure 6. Deletion of either *SSE1* or *FES1* disrupt [URE3] propagation. (A) BY241 [URE3-1] deleted for the indicated genes streaked on ½ YPD. Red color indicates loss of [URE3]. (B) Strain 5V-H19 with [*PSI*$^+$]$_{strong}$ or [*PSI*$^+$]$_{weak}$, *sse1*Δ derivatives and a guanidine-cured derivative and diploids with the wild-type [*psi*-] strain 62δ-1DC were streaked on ½ YPD. White color indicates the presence of [*PSI*+].

be necessary for any Hsp70-like chaperone activity, this result suggests that prion curing is mediated by its association with and action on the Ssa proteins. Surprisingly, the ATPase-dead Sse1-K69Q mutant fully complements all phenotypes of an *sse1*Δ strain (Shaner *et al.*, 2004), suggesting that many Sse1p functions are independent of its ATPase activity.

Optimal Ssa activity is required for stable propagation of [URE3], with overexpression of Ssa1p (Schwimmer and Masison, 2002) or deletion of *SSA2* (Roberts *et al.*, 2004) effectively curing [URE3]. We find that alteration of different cofactors can also efficiently disrupt [URE3] propagation. Sse1p also cooperates with Ssa1p in renaturing luciferase in vivo and in vitro (Dragovic *et al.*, 2006), with either excess or deficient Sse1p impairing activity.

Although mutations of *SSE1* suggest an indirect action through Ssa1p, differences in effects of NEFs (overproduction of Fes1p did not cure) might indicate an additional action for Sse1p. Masison's group showed that overexpression of Fes1p (Jones *et al.*, 2004) and Sse1p (D. Masison, personal communication improved [*PSI*$^+$] stability in an *SSA1-21* background, whereas deletion of *FES1* further destabilized it; overexpression and deletion of *STI1* did the respective opposites. Their results imply that increasing the substrate-bound ADP form of Ssa1p impaired [*PSI*$^+$] propagation, whereas the ATP form promotes [*PSI*$^+$] (Jones *et al.*, 2004). [URE3] appears to be more complex in that alteration of Ssa1 activity in either direction can damage prion propagation.

Interestingly, both NEFs for Ssa1p are required for [URE3]: independent deletions of either *SSE1* or *FES1* completely disrupted [URE3] propagation. Fes1p binds preferentially to Ssa1p·ADP, stimulating ADP release and inhibiting the ATPase activity of Ssa1p (Kabani *et al.*, 2002). In contrast Ydj1p stimulates the Ssa1p ATPase. *fes1*Δ and *ydj1-151ts* suppress each other, suggesting antagonism (Kabani *et al.*, 2002). Yjd1p overproduction cures [URE3] as does *fes1*Δ, consistent with this idea. Although *fes1*Δ restores growth at 37°C to *ydj1-151* strains, *sse1*Δ makes the thermosensitive phenotype more severe (Goeckeler *et al.*, 2002). These results argue against functional redundancy of Fes1p and Sse1p, though their functions may overlap. Sse1p forms specific complexes with Ssa1p and Ydj1p, as observed in vitro (Dragovic *et al.*, 2006); Fes1p may form distinct complexes also involved in [URE3] propagation.

Chaperone-Typing of Prions

Either overproduction or depletion of Sse1p cured [URE3] mimicking the effects of Hsp104 on [*PSI*$^+$] (Chernoff *et al.*, 1995). Only [*PSI*$^+$] was destabilized by Hsp104 overexpression, whereas [URE3] and hybrid [*PSI*$^+$PS] showed sensitivity to overexpression of Hsp70 (Ssa1p) and its cofactors (Sse1, Ydj1). Such "chaperone-typing" indicates structural similarity between [URE3] and hybrid [*PSI*$^+$PS] and distinction from [*PSI*$^+$]. Although the structural differences between [URE3] and [*PSI*$^+$] amyloids are unclear, SDS-resistant polymers on agarose gels are apparently smaller in many [*PSI*$^+$] variants compared with the [URE3] and hybrid [*PSI*$^+$PS] #1 (Figure 2A). Longer [URE3] polymers better adhere to each other to form large insoluble aggregates (Kryndushkin *et al.*, 2003), some resistant to boiling in SDS. Perhaps this makes them more resistant to Hsp104 action than [*PSI*$^+$] polymers.

The Ssa1p/Ydj1p/Sse1p complex must act on polymers by a different mechanism, possibly by binding to the ends of

prion filaments. Depletion of Sse1p (or Fes1p) might shift Ssa's toward the ADP form, which binds substrates tightly. Such binding might block addition of new monomers to the fibril. The larger [URE3] aggregates have fewer ends compared with [*PSI*$^+$] filaments and thus are more sensitive to Sse1p or Fes1p depletion. Overexpressed Sse1p might activate a disaggregating activity of Hsp70s on Ure2p polymers or stabilize Ure2p monomers, preventing their joining the amyloid filaments (Figure 5B).

In summary, Sse1p and Fes1p affect Ssa1p activity as NEFs, and, in addition, Sse1p cooperates with Ssa1p and Ydj1p in a stoichiometric complex in folding of certain proteins. Differences between Sse1p and Fes1p may be explained by their forming different complexes with Ssa1p, or by the fact that Sse1p is a more potent NEF.

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