DOI: 10.1534/genetics.107.077982

The Role of Sse1 in the *de Novo* Formation and Variant Determination of the [*PSI*$^+$] Prion

Qing Fan,* Kyung-Won Park,* Zhiqiang Du,* Kevin A. Morano† and Liming Li*,[1]

**Department of Molecular Pharmacology and Biological Chemistry, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611 and †Department of Microbiology and Molecular Genetics, University of Texas Medical School, Houston, Texas 77030*

Manuscript received June 20, 2007
Accepted for publication September 10, 2007

ABSTRACT

Yeast prions are a group of non-Mendelian genetic elements transmitted as altered and self-propagating conformations. Extensive studies in the last decade have provided valuable information on the mechanisms responsible for yeast prion propagation. How yeast prions are formed *de novo* and what cellular factors are required for determining prion "strains" or variants—a single polypeptide capable of existing in multiple conformations to result in distinct heritable phenotypes—continue to defy our understanding. We report here that Sse1, the yeast ortholog of the mammalian heat-shock protein 110 (Hsp110) and a nucleotide exchange factor for Hsp70 proteins, plays an important role in regulating [*PSI*$^+$] *de novo* formation and variant determination. Overproduction of the Sse1 chaperone dramatically enhanced [*PSI*$^+$] formation whereas deletion of *SSE1* severely inhibited it. Only an unstable weak [*PSI*$^+$] variant was formed in *SSE1* disrupted cells whereas [*PSI*$^+$] variants ranging from very strong to very weak were formed in isogenic wild-type cells under identical conditions. Thus, Sse1 is essential for the generation of multiple [*PSI*$^+$] variants. Mutational analysis further demonstrated that the physical association of Sse1 with Hsp70 but not the ATP hydrolysis activity of Sse1 is required for the formation of multiple [*PSI*$^+$] variants. Our findings establish a novel role for Sse1 in [*PSI*$^+$] *de novo* formation and variant determination, implying that the mammalian Hsp110 may likewise be involved in the etiology of protein-folding diseases.

IN the budding yeast *Saccharomyces cerevisiae*, the non-Mendelian genetic element [*PSI*$^+$] is referred to as a prion because it is epigenetically transmitted as altered and amyloid-like protein conformations (Wickner 1994; King and Diaz-Avalos 2004; Tanaka *et al.* 2004). This protein-based transmission is similar to that of a group of mammalian neurodegenerative diseases known as transmissible spongiform encephalopathies, or prion diseases (Prusiner 1998). The protein determinant of [*PSI*$^+$] is Sup35, a subunit of the translation termination factor that directs ribosomes to faithfully terminate at stop codons (Stansfield *et al.* 1995). When Sup35 enters a prion conformation, it is sequestered from its natural binding partner, Sup45, and occasionally results in translational readthrough. Therefore, [*PSI*$^+$] cells that contain a nonsense mutation in an *ADE* gene, such as *ade1-14*, are capable of growing in medium lacking adenine whereas the isogenic nonprion ([*psi*$^-$]) cells are not (Cox 1965; Firoozan *et al.* 1991). Due to the accumulation of a pigment by-product, [*psi*$^-$] cells appear red on rich growth media, *e.g.*, on YPD, but [*PSI*$^+$] cells appear white. Thus, [*PSI*$^+$] and [*psi*$^-$] cells can be easily distinguished in the laboratory due to the dramatic differences in their growth requirements and colony appearances (Cox 1965). This convenient assay, in combination with the powerful genetics available in budding yeast, has made [*PSI*$^+$] a valuable model for prion research (Liebman and Derkatch 1999; Serio and Lindquist 1999; Soto and Castilla 2004).

The formation and maintenance of [*PSI*$^+$] requires interaction of Sup35 with endogenous cellular factors. Molecular chaperones, a group of proteins that exercise protective functions inside the cell by refolding or disaggregating denatured proteins, are important components of the cellular machineries required for prion formation and propagation (Pirkkala *et al.* 2001). For example, Hsp104, a member of the Hsp100 family, Ssa and Ssb, members of the Hsp70 family, and Ydj1 and Sis1, members of the Hsp40 family, have been implicated in playing important roles in [*PSI*$^+$] propagation (Chernoff *et al.* 1995; Jung *et al.* 2000; Kushnirov *et al.* 2000; Jung and Masison 2001; Sondheimer *et al.* 2001; Allen *et al.* 2005). The Hsp90 co-chaperones Sti1 and Cpr7 are also known to influence [*PSI*$^+$] stability in the context of the mutant *ssa1-21* strain harboring an *SSA1* point mutation that destabilizes [*PSI*$^+$] (Jones *et al.* 2004). Although [*PSI*$^+$] propagation has been extensively investigated and important insights have been gained regarding the mechanisms of its transmission, the [*PSI*$^+$]

[1]*Corresponding author:* Department of Molecular Pharmacology and Biological Chemistry, The Feinberg School of Medicine, Northwestern University, Searle 5-474, MC S205, 320 E. Superior St., Chicago, IL 60611. E-mail: limingli@northwestern.edu

TABLE 1

Plasmids used in this study

Plasmid	Promoter	Marker	Copy number	Source
pCUP1-GFP	CUP1	*URA3*	CEN, low	Park *et al.* (2006)
pCUP1-NMGFP	CUP1	*URA3*	CEN, low	Park *et al.* (2006)
pRS313CUP1-GFP	CUP1	*HIS3*	CEN, low	Derkatch *et al.* (2001)
pRS313CUP1-NMGFP	CUP1	*HIS3*	CEN, low	Derkatch *et al.* (2001)
p426GPDSSE1	GPD	*URA3*	2μ high	Morano lab
p423STI1	STI1	*HIS3*	2μ high	Song *et al.* (2005)
pGAL-NMGFP	GAL	*HIS3*	CEN, low	Park *et al.* (2006)
p2UGHSP82	GPD	*URA3*	2μ, high	Lindquist lab
pKAT6	GPD	*HIS3*	2μ, high	Lindquist lab
*sse1*Δ::*LEU2*		*LEU2*	Replacement, single	Morano lab
p414TEF SSE1	TEF	*TRP1*	CEN, low	Morano lab
p414TEF SSE1K69Q	TEF	*TRP1*	CEN, low	Morano lab
p414TEF SSE1G233D	TEF	*TRP1*	CEN, low	Morano lab
p414TEF SSE1PBD	TEF	*TRP1*	CEN, low	Morano lab
pRS305-HSF1	HSF1	*LEU2*	Integrating, single	This study
*pRS305-*Δ*CTAHSF1*	HSF1	*LEU2*	Integrating, single	This study

initiation process is less well understood. We have recently reported that two truncation mutants of the heat-shock transcription factor (HSF) strongly influence [*PSI*$^+$] initiation. An *HSF* mutant lacking the carboxyl-terminal activation domain, Δ*CTA-HSF*, dramatically increases [*PSI*$^+$] *de novo* formation, whereas a mutant lacking the amino-terminal activation domain, Δ*NTA-HSF*, severely inhibits this process (Park *et al.* 2006). Interestingly, Δ*CTA-HSF* preferentially allows the formation of weak and mosaic [*PSI*$^+$] variants (Park *et al.* 2006). This finding demonstrates that the carboxyl-terminal activation domain of HSF (CTA-HSF) regulates the expression of important factors required for [*PSI*$^+$] formation and variant determination. To date, five proteins have been conclusively identified as CTA-HSF targets: Hsp90, Sse1, Sti1, Ydj1, and Cpr6 (Liu and Thiele 1999). When Hsp90 was overproduced, no significant effects were observed on preexisting [*PSI*$^+$] (Newnam *et al.* 1999). Neither overexpression nor deletion of *CPR6* had detectable effects on [*PSI*$^+$] propagation (Jones *et al.* 2004). Overexpression of *YDJ1* cured a weak [*PSI*$^+$] and a hybrid [*PSI*$^+$], [*PSI*$^+$(PS)], but had no notable influence on a strong [*PSI*$^+$] variant (Kushnirov *et al.* 2000). Overall, *STI1* overexpression weakened or destabilized whereas *sti1*Δ strengthened [*PSI*$^+$] (Jones *et al.* 2004). Recently, Sse1, an Hsp90 co-chaperone and a nucleotide exchange factor (NEF) for the cytosolic Ssa and Ssb proteins, was identified as a novel regulator of [*PSI*$^+$] propagation (Kryndushkin and Wickner 2007). Despite their importance in [*PSI*$^+$] propagation, whether these CTA-HSF targets play any roles in regulating [*PSI*$^+$] *de novo* formation and variant determination has not been investigated. We report here that manipulating the expression levels of individual CTA-HSF target genes can result in dramatic influences on [*PSI*$^+$] *de novo* formation. We show that Sse1 not only is important for [*PSI*$^+$] propagation but also is required for [*PSI*$^+$] *de novo* formation and variant determination. In addition, results from mutational analysis also demonstrate that Sse1 correlates the NEF activity of Sse1 with [*PSI*$^+$] manipulation. Our findings have therefore established Sse1 as an important regulator in [*PSI*$^+$] biology and raise the possibility that Hsp70 cofactors play critical roles in amyloidoses in higher organisms.

MATERIALS AND METHODS

Plasmids: Plasmids used in this study are listed in Table 1. To generate the plasmid *pRS305-HSF1*, *pRS314HSF1* was digested with *Xba*I and *Xho*I. The resulting fragment of 3901 bp was ligated to *pRS305-C*, which had been predigested with *Xba*I and *Xho*I. To create *pRS305-*Δ*CTA HSF1*, polymerase chain reaction (PCR) was carried out using the 5′ primer (5′-AAAGGCCT TAATGAATAGTACACAGGGCAAGGTC-3′), the 3′ primer (5′-AGGCACCCCAGGCTTTAC-3′), and *pRS305HSF1* as the DNA template. PCR with Taq polymerase (Invitrogen, San Diego) was performed with 1 cycle at 94° for 5 min; 30 cycles at 94° for 30 sec, at 55° for 30 sec, and at 72° for 2 min; and then at 72° for 10 min. The 700-bp PCR product was digested with *Stu*I and *Xho*I and the resulting 406-bp fragment was ligated to *pRS305-HSF1*, which had been predigested with *Stu*I and *Xho*I.

Yeast strains and cultures: Yeast strains used in this study are listed in Table 2. To generate the *wt-HSF* or Δ*CTA-HSF* integrated strains, the integrating constructs *pRS305-HSF1* or *pRS305-*Δ*CTAHSF1* was digested with *Cla*I and transformed into a 74D-694 ([*psi*$^-$][*RNQ*$^+$]) strain whose chromosomal *HSF1* was disrupted with a *kanR* but contained a *pRS416-HSF1* plasmid for viability (Park *et al.* 2006). Transformants selected on SC–ura–leu were streaked on SC–leu+5-FOA to eliminate the *pRS416-HSF1* and the resulting strains were termed *74D-694-I-wtHSF* and *74D-694-I-*Δ*CTAHSF*, respectively.

To create *sse1*Δ strains, the disruption construct of *sse1*Δ::*LEU2* (Shaner *et al.* 2004) was digested with *Sac*II and *Pst*I. The resulting digestion mixture was transformed into various isogenic strains with different prion backgrounds as shown in Table 2. In all cases, *SSE1* disruption was confirmed by immunoblot analysis using a polyclonal Sse1 antibody, a kind gift from J. Brodsky's laboratory.

TABLE 2

Strains of *S. cerevisiae* used in this study

Strain	Genotype description	Source
74D-694 [*psi*$^-$][*RNQ*$^+$]	*MAT***a**: *ade1-14, trp1-289, his3*Δ*-200, ura3-52, leu2-3, 112,* [*psi*$^-$][*RNQ*$^+$]	Chernoff *et al.* (1995)
74D-694 [*PSI*$^+$]^S[*RNQ*$^+$]	*MAT***a**: *ade1-14, trp1-289, his3*Δ*-200, ura3-52, leu2-3, 112,* [*PSI*$^+$]^S[*RNQ*$^+$]	Chernoff *et al.* (1995)
74D-694 [*psi*$^-$][*rnq*$^-$]	*MAT*α: *ade1-14, trp1-289, his3*Δ*-200, ura3-52, leu2-3, 112,* [*psi*$^-$][*rnq*$^-$]	Sondheimer and Lindquist (2000)
74D-694 [*PSI*$^+$]^W[*RNQ*$^+$]	*MAT***a**: *ade1-14, trp1-289, his3*Δ*-200, ura3-52, leu2-3, 112,* [*PSI*$^+$]^W[*RNQ*$^+$]	This study
74D-694 [*psi*$^-$][*RNQ*$^+$] *sse1*Δ	*MAT***a**: *ade1-14, trp1-289, his3*Δ*-200, ura3-52, leu2-3, 112,* [*psi*$^-$][*RNQ*$^+$], *sse1::LEU2*	This study
74D-694 [*PSI*$^+$]^S[*RNQ*$^+$] *sse1*Δ	*MAT***a**: *ade1-14, trp1-289, his3*Δ*-200, ura3-52, leu2-3, 112,* [*PSI*$^+$]^S[*RNQ*$^+$], *sse1::LEU2*	This study
74D-694 [*psi*$^-$][*rnq*$^-$] *sse1*Δ	*MAT***a**: *ade1-14, trp1-289, his3*Δ*-200, ura3-52, leu2-3, 112,* [*psi*$^-$][*rnq*$^-$], *sse1::LEU2*	This study
74D-694-I-wtHSF	*MAT***a**: *ade1-14, trp1-289, his3*Δ*-200, ura3-52, leu2-3, 112,* [*psi*$^-$][*RNQ*$^+$], *hsf1::kanR, pRS305HSF1* integrated at the *leu2* locus	This study
*74D-694-I-*Δ*CTAHSF*	*MAT***a**: *ade1-14, trp1-289, his3*Δ*-200, ura3-52, leu2-3, 112,* [*psi*$^-$][*RNQ*$^+$], *hsf1::kanR, pRS305-*Δ*CTAHSF1* integrated at the *leu2* locus	This study

The weak [*PSI*$^+$] variant, [*PSI*$^+$]^W, was obtained from 74D-694 cells ([*psi*$^-$][*RNQ*$^+$]) after transient overexpression of *SUP35 NMGFP* as described (Park *et al.* 2006).

Yeast cultures and other genetic manipulations were performed according to the established protocols (Sherman 1991).

[*PSI*$^+$] induction and variant determination: [*PSI*$^+$] induction in cells containing *pCUP1-NMGFP* was performed as described previously (Park *et al.* 2006). Briefly, cells were grown in selective media at 30° overnight before diluted into fresh media at a density of ~4×10^6 cell/ml. After an additional 1 hr of growth at 30°, $CuSO_4$ was added to a final concentration of 34 μM. At various induction times, cells were spotted with a fivefold serial dilution onto SC-ade and YPD plates. [*PSI*$^+$] induction in cells containing *pGAL-NMGFP* was carried out by growing cells in selective media with 2% raffinose to mid-log phase ($A_{600} = 0.5$) before adding galactose to a final concentration of 2% to induce Sup35NMGFP production. As time indicated, cultures were spread onto SC–ade and YPD plates to obtain individual colonies. In all cases, Ade$^+$ colonies were considered as [*PSI*$^+$] candidates but only GdnHCl-curable Ade$^+$ isolates were scored as [*PSI*$^+$]. Subsequent [*PSI*$^+$] confirmation and variant determination were carried out as described (Park *et al.* 2006).

For experiments described in Figure 2, isogenic strains of *74D-694-I-wtHSF* and *74D-694-I-*Δ*CTAHSF* (see Table 2 for strain descriptions) were cotransformed with either *pRS313CUP1-NMGFP* and *p2UGHSP82* or *pRS313CUP1-NMGFP* and *p426 GPDSSE1*. To examine the overexpression effect of *STI1*, the same cells were transformed with *pCUP1-NMGFP* and *pRS423 STI1* (a kind gift from D. Masison). [*PSI*$^+$] *de novo* formation was analyzed using cell patches as described (Derkatch *et al.* 2001). Briefly, individual transformants were patched onto plates selective for the containing plasmids followed by replica plating onto plates containing 70 μM $CuSO_4$. After incubation for ~20 hr at 30°, the cell patches were replica plated onto SC–ade to view potential [*PSI*$^+$] colonies. To quantify [*PSI*$^+$] *de novo* formation (Figure 2, right), three individual transformants of each transformation were grown in liquid media selective for the indicated plasmids to early log phase followed by addition of $CuSO_4$ to a final concentration of 34 μM. After 4 hr of induction, cells were counted and spotted onto SC–ade and YPD plates with a fivefold serial dilution. Ade$^+$ colonies that were cured by GdnHCl were scored as [*PSI*$^+$]. The ratio of [*PSI*$^+$] to the total number of viable cells (calculated from colonies on the YPD plates) was used to determine the frequency of [*PSI*$^+$] *de novo* formation.

To determine the effect of *sse1*Δ on [*PSI*$^+$] *de novo* formation (shown in Figure 3), an *SSE1* disruption strain of 74D-694 ([*psi*$^-$][*RNQ*$^+$]) and the isogenic wild-type strain ([*psi*$^-$][*RNQ*$^+$]) were transformed with *pCUP1-NMGFP* and the resulting transformants were assayed for [*PSI*$^+$] *de novo* formation using $CuSO_4$ as an inducer as described above. [*PSI*$^+$] determination and calculation of [*PSI*$^+$] appearance percentage were also carried out as described above. At least three transformants were used for each experiment and results from three independent transformations were summarized.

Sup35NMGFP fluorescence microscopic assay: 74D-694 cells ([*psi*$^-$][*RNQ*$^+$]) containing *pRS313CUP1-NMGFP* and *p426GPDSSE1* or *p426GPD* were grown in SC–his–ura to early log phase. After a 4-hr induction upon addition of $CuSO_4$ to 34 μM, the fluorescence patterns of Sup35NMGFP were examined under a fluorescence microscope as described previously (Park *et al.* 2006).

SDS–PAGE and immunoblot analyses: Yeast cells grown overnight in either YPD (Sse1 immunoblot analysis) or selective media to mid-log phase ([*PSI*$^+$] induction experiments) were harvested by centrifugation at 3000 rpm for 3 min followed by washing once with sterile water. The washed cells were used for preparing total protein extracts by the ethanol lysis method (Park *et al.* 2006). The resulting protein extracts were analyzed by SDS–PAGE and immunoblot analysis as described previously (Park *et al.* 2006). Antibodies used in this study were kindly provided by S. Lindquist (Hsp104, Hsp90, and Rnq1), E. Craig (Ssa, Ssb, and Sis1), J. Brodsky (Sse1), and S. Liebman (Sup35).

Semidenaturing agarose gel electrophoresis: Crude protein extracts prepared from isogenic strains of 74D-694

[*PSI*$^+$]^S[*RNQ*$^+$], [*PSI*$^+$]^W[*RNQ*$^+$], and their corresponding *sse1*Δ derivatives were subjected to SDS–PAGE analysis according to Bagriantsev *et al.* (2006) with minor modifications. Briefly, yeast cultures were grown in liquid YPD media to A_{600} of 1.5–2.0 and harvested by centrifugation at 3000 rpm for 3 min. After washing once with sterile water, the cell pellet was suspended in the extraction buffer containing 50 mm Tris–HCl (pH 7.5), 50 mm KCl, 10 mm $MgCl_2$, 5% glycerol, 8 μg/ml aprotinin, 8 μg/ml leupeptin, 10 mm phenylmethylsulfonyl fluoride (PMSF), and protease inhibitor cocktail (Roche protease inhibitor complete mini, 1/2 tablet/10 ml). After homogenization for 4 × 1 min in a bead beater with 0.5-mm glass beads, the crude lysates were centrifuged at 600 × *g* for 1 min at 4°. The supernatant was incubated for 7 min in the sample buffer containing 50 mm Tris–HCl (pH 6.8), 5% glycerol, 2% SDS, and 0.05% bromophenol blue at room temperature before being loaded onto a 1.5% horizontal agarose gel as described (Bagriantsev *et al.* 2006).

RESULTS

The role of Sse1 in [*PSI*$^+$] *de novo* formation: Sse1 is the ortholog of the mammalian Hsp110 and has been identified as a potent Hsp70 nucleotide exchange factor (Dragovic *et al.* 2006; Raviol *et al.* 2006; Shaner *et al.* 2006). Because Δ*CTA-HSF* dramatically influences the *de novo* formation of [*PSI*$^+$] and Sse1 is a CTA-HSF target, we decided to examine whether manipulating the expression levels of *SSE1* would have any effects on [*PSI*$^+$] *de novo* formation. [*PSI*$^+$] can arise spontaneously from [*psi*$^-$] cells with a very low frequency (Tuite *et al.* 1981). However, [*PSI*$^+$] formation can be greatly enhanced by Sup35 overproduction in the presence of another prion, [*RNQ*$^+$] (Chernoff *et al.* 1993; Derkatch *et al.* 1997). To examine if overexpression of *SSE1* would affect [*PSI*$^+$] *de novo* formation, we transformed 74D-694 ([*psi*$^-$][*RNQ*$^+$]) cells with *pGAL-NMGFP*, a galactose-inducible plasmid expressing a GFP fusion of the N-terminal and middle regions of Sup35 (1–265 aa), and *p426GPDSSE1*, a strong constitutive *SSE1* expression plasmid. The resulting transformants were analyzed for [*PSI*$^+$] *de novo* formation. Since the *GAL* promoter is tightly regulated, it was possible to analyze how Sse1 overproduction could kinetically affect [*PSI*$^+$] *de novo* formation. As shown in Figure 1A, Sse1 overproduction dramatically stimulated [*PSI*$^+$] formation throughout the time course of study. After 72-hr induction with galactose, the average [*PSI*$^+$] formation in cells containing the *SSE1* overexpression plasmid *p426GPDSSE1* was ~8.7% whereas cells containing the empty vector *p426GPD* had an average [*PSI*$^+$] formation rate of 0.06%, an ~145-fold difference. The stimulatory influence of Sse1 on [*PSI*$^+$] formation was also evident when Sup35NMGFP was overproduced under the regulation of a *CUP1* promoter (*pRS313CUP1-NMGFP*). Upon $CuSO_4$ addition, the number of Ade$^+$ colonies derived from cells containing *pRS313CUP1-NMGFP* and *p426GPDSSE1* was significantly greater than that derived in cells containing *pRS313CUP1-NMGFP* and *p426GPD* (Figure 1C, top). However, the stimulatory effect of Sse1 on [*PSI*$^+$] formation was less dramatic when the *CUP1* promoter was used. After a 4-hr induction, the difference in [*PSI*$^+$] formation in the presence or absence of Sse1 overproduction was ~10-fold. This smaller difference is likely due to the fact that the *CUP1* promoter is not tightly regulated (Tamai *et al.* 1994; Hahn *et al.* 2004). We also examined the pattern of Sup35NMGFP fluorescence in the presence of overproduced Sse1. As shown in Figure 1B, the observed Sup35NMGFP fluorescent foci in cells containing *p426GPDSSE1* (~20%) is significantly more than that of cells containing the empty vector *p426GPD* (~0.2%). We conclude that overproduction of Sse1 promotes both Sup35NMGFP aggregation and [*PSI*$^+$] formation.

It has been shown that overproduction of Sse1 reduces cell growth (Liu *et al.* 1999; Shaner *et al.* 2004). We also observed that the *SSE1* overexpression construct *p426GPDSSE1* reduced transformation efficiency (data not shown). To exclude the possibility that the stimulating effect of Sse1 overproduction on [*PSI*$^+$] formation is caused by chromosomal mutations in the *SSE1*-overexpressing cells, we eliminated *p426GPDSSE1* from cells containing *pRS313CUP1-NMGFP* and exhibiting stimulatory effects on [*PSI*$^+$] formation. As shown in Figure 1C (bottom), the elimination of *p426GPDSSE1* resulted in loss of the stimulatory effect on [*PSI*$^+$] *de novo* formation. Thus, the observed [*PSI*$^+$] promoting effect was not due to a chromosomal mutation(s). Rather, overproduction of Sse1 is the primary responsible factor.

To further investigate whether this [*PSI*$^+$]-promoting effect was caused indirectly by modulating other cellular factors in response to Sse1 overproduction, we examined the expression levels of several molecular chaperones known to influence [*PSI*$^+$]: Hsp104, Ssa1/2, Ssb1/2, and Sis1. Since Sse1 has been identified as an Hsp90 co-chaperone (Liu and Thiele 1999), we also examined the expression levels of Hsp90 and its co-chaperone Sti1. As shown in Figure 1D, all examined molecular chaperones displayed similar expression levels in cells with or without overproduction of Sse1, except Hsp104, which increased about twofold in cells overproducing Sse1.

Next, we examined if other CTA-HSF targets—Hsp90, Sti1, Cpr6, and Ydj1—would have effects similar to Sse1 on [*PSI*$^+$] *de novo* formation. Interestingly, as shown in Figure 2, striking differences were observed for different CTA-HSF targets. In contrast to Sse1, overexpression of *HSP82* severely inhibited [*PSI*$^+$] formation (Figure 2). Similar results were also obtained when *HSC82* was overexpressed (data not shown). As shown in Figure 2, overproduction of Sti1 also strongly inhibits [*PSI*$^+$] formation. However, elevated expression levels of Cpr6 and Ydj1 had no significant effects on [*PSI*$^+$] *de novo* formation (data not shown). The same experiments were also conducted for Δ*CTA-HSF* cells. As shown in Figure 2, in all cases, Δ*CTA-HSF* cells gave rise to significantly more Ade$^+$ colonies than *wt-HSF* cells did,

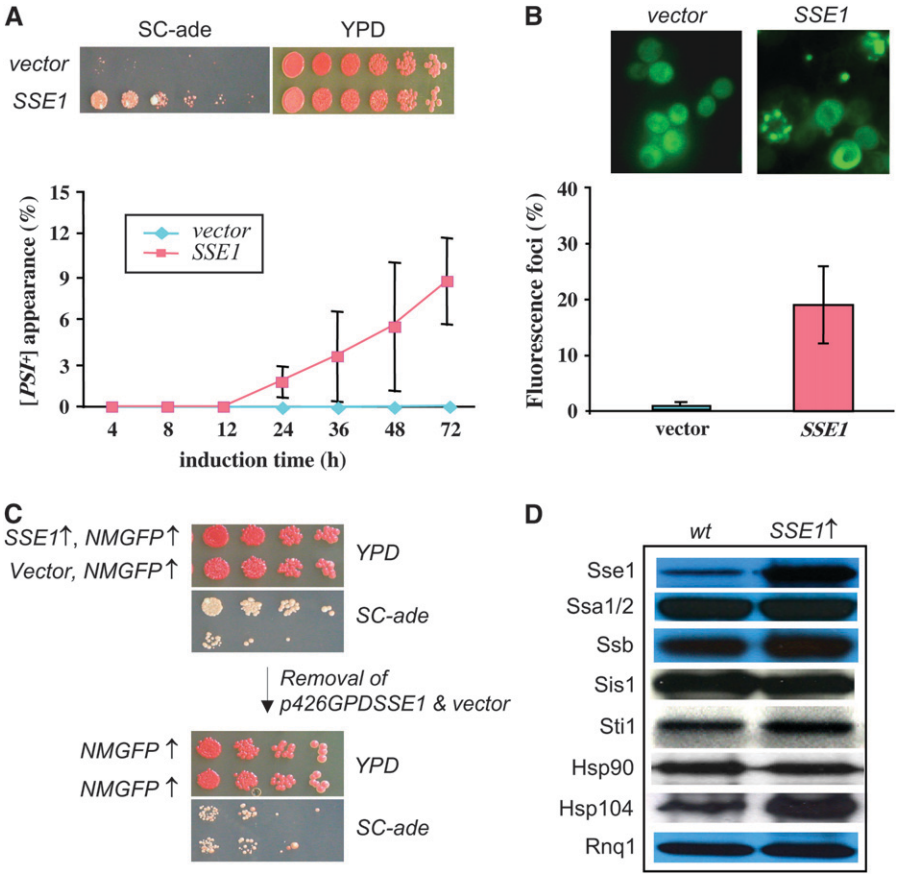

Figure 1.—The influence of Sse1 overproduction on [*PSI*$^+$] *de novo* formation. (A) Overproduction of Sse1 dramatically increases [*PSI*$^+$] *de novo* formation. Yeast cells (74D-694 [*psi*$^-$][*RNQ*$^+$]) containing *pGAL-NMGFP* and *p426GPDSSE1* (*SSE1*) or *pGAL-NMGFP* and *p426GPD* (*vector*) were assayed for [*PSI*$^+$] *de novo* formation as described in materials and methods. (Top) A representative spotting assay of [*PSI*$^+$] *de novo* formation. Pictures were taken after 7 days of growth at 30° for SC–ade and after 3 days for YPD. (Bottom) Kinetic analysis of [*PSI*$^+$] *de novo* formation in the presence or absence of Sse1 overproduction as described in materials and methods. Data shown are averages of five independent experiments. (B) Overproduction of Sse1 promotes Sup35NMGFP aggregation. Cells containing *pRS313CUP1-NMGFP, p426GPDSSE1* (*SSE1*↑, *NMGFP*↑) or *pRS313CUP1-NMGFP, p426GPD* (*vector, NMGFP*↑) were assayed for Sup35NMGFP aggregation using a fluorescence microscope as described in materials and methods. (Top) Representative images of cells expressing Sup35NMGFP in the presence (SSE1) or absence (vector) of Sse1 overproduction. (Bottom) The average percentage of cells containing Sup35NMGFP aggregates from five independent experiments. For each experiment, >2000 cells with or without *SSE1* overexpression were examined. (C) [*PSI*$^+$]-promoting effect in cells overproducing Sse1 was not caused by chromosomal mutations. The same cells used in B were also assayed for [*PSI*$^+$] *de novo* formation (top). After eliminating the *URA3* plasmids (*p426GPDSSE1* and *p426GPD*) after growing in 5-FOA, cells were reassayed for [*PSI*$^+$] *de novo* formation (bottom). (D) Immunoblot analysis of selected molecular chaperones. Cells described in B were harvested after a 4-hr induction. Protein extracts were prepared using an ethanol lysis method and examined by SDS–PAGE and immunoblot analysis as described previously (Park *et al.* 2006).

confirming our previous report that Δ*CTA-HSF* stimulates [*PSI*$^+$] *de novo* formation (Park *et al.* 2006). However, the inhibitory effects upon Hsp90 and Sti1 overproduction and the stimulatory effect upon Sse1 overproduction were also evident in Δ*CTA-HSF* cells (Figure 2). Since overproduction of each individual CTA-HSF target resulted in similar effects in both *wt-HSF* and Δ*CTA-HSF* cells, it is unlikely that the observed effects by Hsp90, Sti1, or Sse1 overproduction were caused by the deletion of the CTA of HSF. Our results thus demonstrate that different components of the Hsp90 complex play distinct roles in [*PSI*$^+$] *de novo* formation.

The effects of *SSE1* disruption on [*PSI*$^+$] formation and variant determination: To further analyze the role of Sse1 in [*PSI*$^+$] regulation, we next examined the effect of *SSE1* disruption on [*PSI*$^+$] *de novo* formation. A chromosomal *SSE1* disruption strain (*sse1*Δ) was created in 74D-694 ([*psi*$^-$][*RNQ*$^+$]). After transformation with *pCUP1-NMGFP*, isogenic *sse1*Δ and wild-type cells were assayed for [*PSI*$^+$] induction. If Sse1 is a [*PSI*$^+$]-promoting factor, *SSE1* disruption would be expected to abolish or reduce [*PSI*$^+$] formation. As shown in Figure 3A, the number of [*PSI*$^+$] colonies was indeed greatly reduced in *sse1*Δ cells when compared to that of wild-type cells. To examine if *sse1*Δ also affects [*PSI*$^+$] variant determination, Ade$^+$ colonies formed in *sse1*Δ and isogenic wild-type cells were randomly picked and streaked onto YPD plates to view their colony appearances. Interestingly, all Ade$^+$ cells formed in *sse1*Δ cells had the appearance of very weak [*PSI*$^+$] variants, which were unstable and appeared undifferentiated (Figure 3B). This appearance is similar to that of an unstable, undifferentiated [*PSI*$^+$] variant ([*PSI*$^+$]^U) formed in Δ*CTA-HSF* cells (Park *et al.* 2006). A careful, side-by-side comparison of [*PSI*$^+$]^U cells formed in Δ*CTA-HSF* and *sse1*Δ cells under identical induction conditions, however, revealed distinct colony morphologies. As shown in Figure 3C, the $_{\Delta CTA}$[*PSI*$^+$]^U colonies are more distinctly sectored but the $_{sse1\Delta}$[*PSI*$^+$]^U colonies do not have clear sectoring boundaries. Multiple independent [*PSI*$^+$] induction experiments were conducted and no uniformly colored white or pink colonies were observed in *sse1*Δ cells. Despite repeated efforts, we were not able to transfer [*PSI*$^+$]^U formed in *sse1*Δ cells to wild-type [*psi*$^-$] cells through mating/sporulation. $_{sse1\Delta}$[*PSI*$^+$]^U is easily lost during the mating process to yield [*psi*$^-$] diploids. Thus, [*PSI*$^+$]^U formed in *sse1*Δ cells is

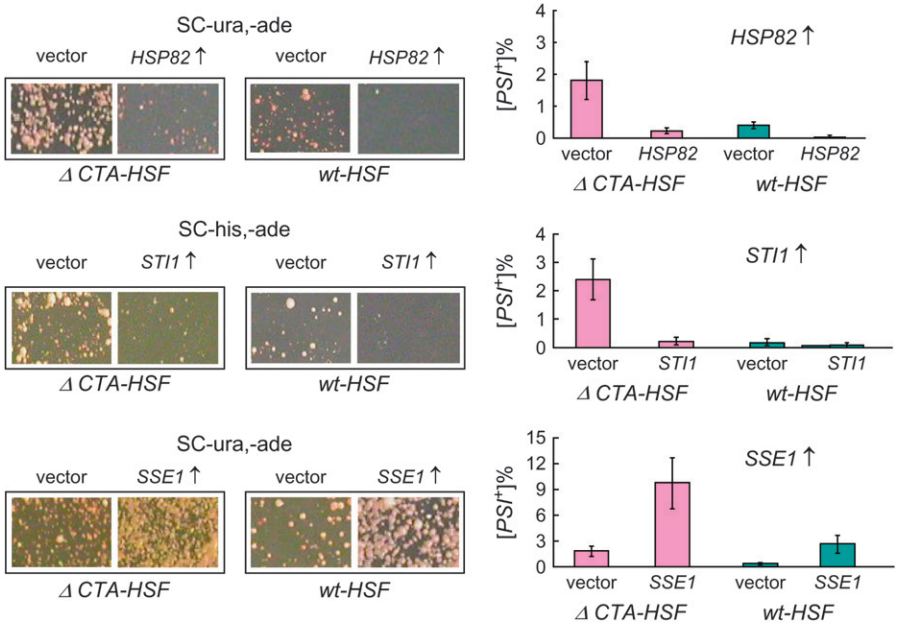

FIGURE 2.—Overproduction of Hsp90 and Sti1 severely inhibits [*PSI*$^+$] *de novo* formation. Cells of isogenic *74D-694-I-wtHSF* and *74D-694-I-ΔCTAHSF* (see Table 2 for the descriptions of the strains) containing *pRS313CUP1-NMGFP* and *p2UGHSP82* (*HSP82*), *pRS313CUP1-NMGFP p426GPDSSE1* (*SSE1*), or *pCUP1-NMGFP* and *pRS423STI1* were assayed for [*PSI*$^+$] *de novo* formation as described in MATERIALS AND METHODS. (Left) Representative cell patches showing *de novo* formation of putative [*PSI*$^+$] colonies; (Right) Quantified data of [*PSI*$^+$] *de novo* formation using a spotting assay as described in MATERIALS AND METHODS. Data shown are from three independent experiments.

extremely unstable and poorly transmitted. In comparison, $_{\Delta CTA}$[*PSI*$^+$]^U can be easily transmitted to wild-type cells as [*PSI*$^+$]^W (PARK *et al.* 2006).

We also examined the steady-state levels of several molecular chaperones in *sse1*Δ cells. As shown in Figure 3D, there were no significant changes in expression of the examined molecular chaperones, including Ssa1/2, Ssb, Hsp104, Sis1, Sti1, and Hsp90. Our data suggest that the lack of Sse1 is responsible for the observed reduction in [*PSI*$^+$] formation and deficiency in variant determination.

Mutational analysis of *SSE1* on [*PSI*$^+$] variant determination: As a nucleotide exchange factor, Sse1 stimulates ADP release from ADP-bound Hsp70 to allow it to rebind to ATP and thus promotes the Hsp70 cycle. This ADP/ATP exchange is essential for refolding thermally denatured luciferase (DRAGOVIC *et al.* 2006; RAVIOL *et al.* 2006). The nucleotide exchange function of Sse1 requires the ATP-binding domain and the substrate-binding domain (DRAGOVIC *et al.* 2006; RAVIOL *et al.* 2006; SHANER *et al.* 2006). Mutations predicted to abolish ATP hydrolysis in Sse1 do not affect its function *in vivo* or *in vitro*, while those that block nucleotide binding also prevent the binding to Hsp70 (DRAGOVIC *et al.* 2006; RAVIOL *et al.* 2006; SHANER *et al.* 2006). For example, Sse1-K69Q, a mutation that inhibits ATP hydrolysis in other Hsp70s, retains Hsp70-binding capability; Sse1-G233D, however, cannot bind to ATP or Hsp70 and Sse1-PBD, which contains only the carboxyl terminal *p*eptide (substrate)-*b*inding *d*omain, is likewise inactive *in vivo* (SHANER *et al.* 2004; SHANER *et al.* 2006). To test if the observed Sse1 effects on [*PSI*$^+$] variant determination are due to the chaperone's function as an Hsp70 NEF, we analyzed [*PSI*$^+$] variants formed in the cells whose endogenous *SSE1* was disrupted but which contain a *CEN* plasmid expressing one of the three described *sse1* mutants. Ade$^+$ colonies formed in these mutant cells were randomly picked and streaked onto YPD to view their colony appearances. As shown in Figure 3E, cells expressing wild-type *SSE1* and *SSE1-K69Q* gave rise to [*PSI*$^+$] variants from very weak to very strong. Cells expressing *SSE1-G233D* or *SSE1-PBD* gave rise only to [*PSI*$^+$]^U, an unstable [*PSI*$^+$] variant with the same colony appearance as that of [*PSI*$^+$]^U formed *de novo* in *sse1*Δ cells. To exclude the possibility that the differences that we observed among the different *sse1* mutants were due to differences in their expression levels or stabilities, the steady-state levels of individual Sse1 mutant proteins were estimated by immunoblot analysis using a polyclonal antibody recognizing Sse1. As shown in Figure 3F, there was no reactive Sse1 band in protein extracts prepared from the *sse1*Δ cells transformed with an empty vector (lane 2), confirming that the chromosomal *SSE1* was disrupted. However, immunoblot of protein extracts prepared from *sse1*Δ cells containing *p414TEFSSE1*, *p414TEFSSE1K69Q*, or *p414TEFSSE1G233D* indicated that all *SSE1* alleles were expressed at similar levels. Since ectopic expression of these *SSE1* alleles in wild-type cells gave rise to [*PSI*$^+$] variants with a full spectrum from very weak to very strong, the effects of these *sse1* mutant alleles on [*PSI*$^+$] variant establishment are not dominant (data not shown). Taken together, our data suggest that the binding of Sse1 to Hsp70, with concomitant NEF activity, is essential for establishing a full spectrum of distinct [*PSI*$^+$] variants.

The influence of Sse1 on [*PSI*$^+$] propagation: We also investigated the role of Sse1 in [*PSI*$^+$] propagation. In agreement with a recent report (KRYNDUSHKIN and WICKNER 2007), we observed that overproduction of Sse1 has no effect on preexisting [*PSI*$^+$], either [*PSI*$^+$]^S or

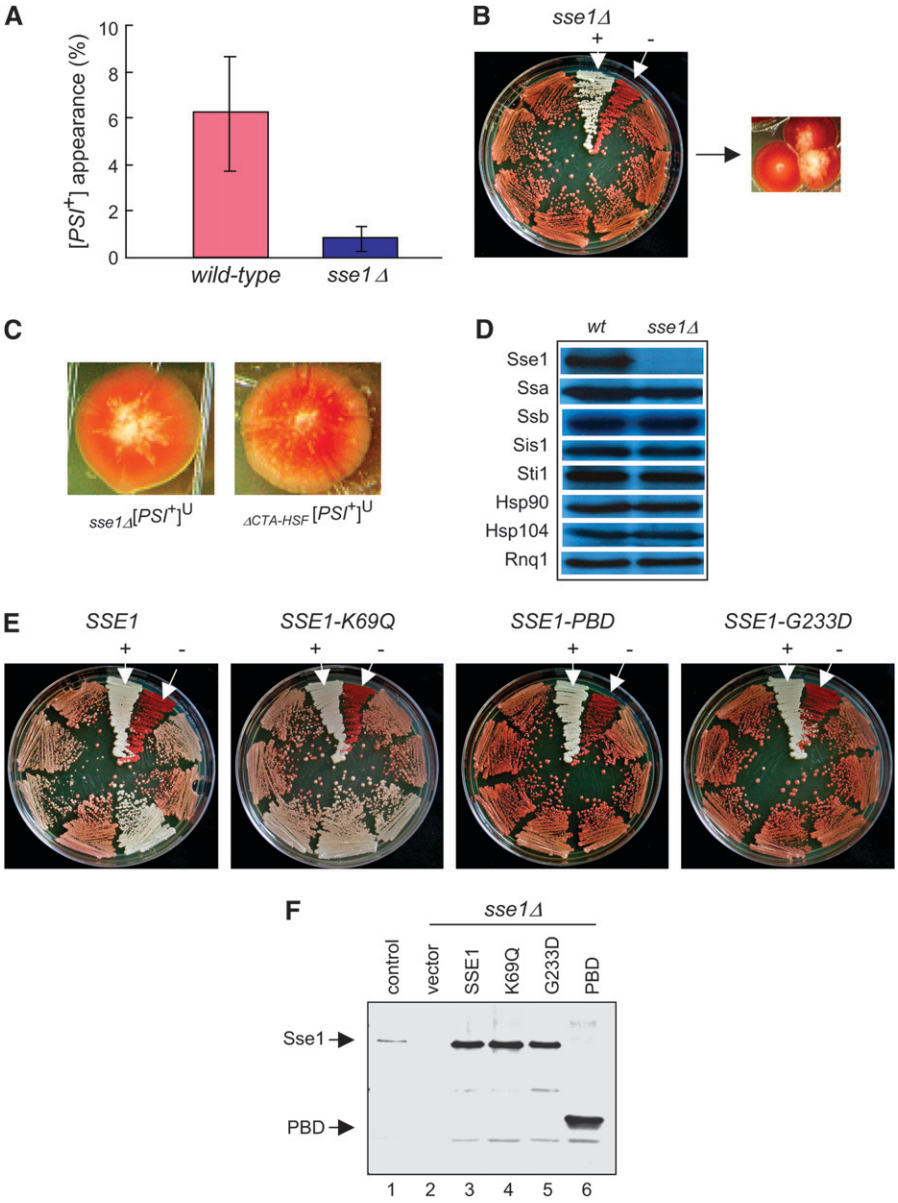

FIGURE 3.—The effects of *sse1* disruption on [*PSI*$^+$] formation, variant determination, and steady levels of selective molecular chaperones. (A) *SSE1* disruption severely inhibits [*PSI*$^+$] formation. Isogenic cells of 74D-694 ([*psi*$^-$][*RNQ*$^+$]) with (*sse1*Δ) or without *sse1* disruption (wild type) containing *pCUP1SUP35NM GFP* were assayed for [*PSI*$^+$] *de novo* formation assay as described in MATERIALS AND METHODS. The averages of three independent experiments are shown. (B) *sse1*Δ cells give rise only to an unstable and undifferentiated [*PSI*$^+$] variant, [*PSI*$^+$]^U. As shown are representative cell streaks and enlarged colonies from *sse1*Δ cells. (C) [*PSI*$^+$]^U formed in *sse1*Δ and Δ*CTA-HSF* cells is morphologically different. Enlarged images of [*PSI*$^+$]^U colonies derived from *sse1*Δ and Δ*CTA-HSF* cells under identical experimental conditions are shown. (D) *SSE1* disruption has no significant effects on the expressions of a selective group of molecular chaperones. Protein extracts were prepared from isogenic wild-type and *sse1*Δ cells using the ethanol lysis method and were examined by SDS–PAGE/immunoblot analysis as described in MATERIALS AND METHODS. Rnq1 was used as the loading control. wt, wild type. (E) Physical association of Hsp70 to Sse1 is important for [*PSI*$^+$] variant determination. 74D-694 *sse1*Δ cells ([*psi*$^-$][*RNQ*$^+$], *sse1*::LEU2) containing *pCUP1-NMGFP* and one of the following plasmids: *p414TEF* (*sse1*Δ), *p414TEF-SSE1* (*SSE1*), *p414TEF-SSE 1K69Q* (*K69Q*), *p414TEF-SSE1G233D* (*G233D*), and *p414TEF-SSE1PBD* (*PBD*) were assayed for [*PSI*$^+$] *de novo* formation as described in A. Newly formed [*PSI*$^+$] colonies were randomly picked and streaked onto YPD plates to view their colors. Plus (+) and minus (−) represent [*PSI*$^+$] and [*psi*$^-$] controls, respectively. (F) Immunoblot analysis of Sse1. Prior to the [*PSI*$^+$] induction assay, protein extracts were prepared from cultures indicated and analyzed by SDS–PAGE and immunoblot analysis using an anti-Sse1 as described in MATERIALS AND METHODS. Lane 1, 74D-694 wild-type cells (control); lanes 2–6, *sse1*Δ cells containing *p414TEF* (vector), *p414TEF-SSE1* (SSE1), *p414TEF-SSE1K69Q* (K69Q), *p414TEF-SSE1G233D* (G233D), and *p414TEF-SSE1PBD* (PBD), respectively.

[*PSI*$^+$]^W (data not shown). Disruption of *SSE1*, however, dramatically influences [*PSI*$^+$] propagation. Figure 4A (left) shows representative streaks of isogenic 74D-694 cells of [*PSI*$^+$]^S, [*PSI*$^+$]^W, and their corresponding *SSE1* disruption derivatives. *SSE1* disruption was confirmed by immunoblot analysis (Figure 4A, right). The *sse1*Δ derivatives of [*PSI*$^+$]^S had a redder colony appearance than that of the [*PSI*$^+$]^S control (Figure 4A, left), suggesting that either a new [*PSI*$^+$] variant was reestablished upon *sse1*Δ or the [*PSI*$^+$]^W-like phenotype was a different readout of [*PSI*$^+$]^S in *sse1*Δ genetic background. Upon *SSE1* disruption, [*PSI*$^+$]^W appeared as red as [*psi*$^-$] cells (Figure 4A, left). This indicates that either *sse1*Δ cured [*PSI*$^+$]^W or the [*PSI*$^+$]^W phenotype was masked in the *sse1*Δ background. For further clarification, we crossed the *sse1*Δ derivatives of both [*PSI*$^+$]^S and [*PSI*$^+$]^W with isogenic [*psi*$^-$] cells containing the wild-type *SSE1* gene. As shown in Figure 4B, the diploids derived from *sse1*Δ[*PSI*$^+$]^S × [*psi*$^-$] had the [*PSI*$^+$]^S colony appearance, indicating that disruption of *SSE1* in [*PSI*$^+$]^S did not change [*PSI*$^+$]^S into a weaker variant. The pink appearance is thus a modified readout of [*PSI*$^+$]^S in *sse1*Δ background. This conclusion was further supported by results from sporulation of the *sse1*Δ/SSE1 diploids of [*PSI*$^+$]^S, which gave rise to four spores with a ratio of 2:2 of [*PSI*$^+$]^S to [*PSI*$^+$]^W-like spores (Figure 4B). The [*PSI*$^+$]^W-like spores were verified to be *sse1*Δ cells as the colonies were smaller and they were able to grow on SC–leu media (*SSE1* was disrupted by a

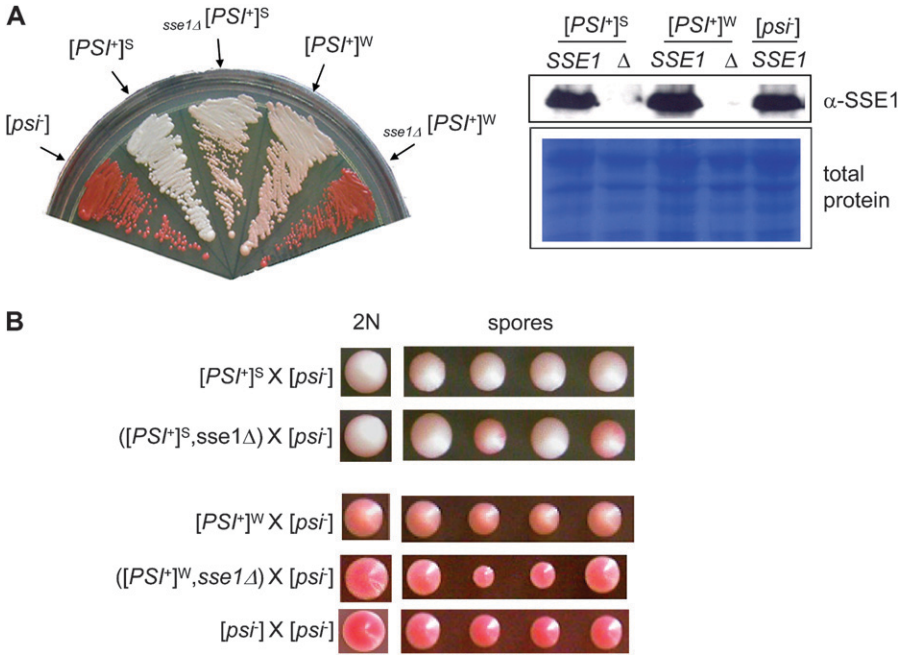

FIGURE 4.—The effects of *SSE1* disruption (*sse1*Δ) on [*PSI*$^+$] propagation. (A) The disruption of *SSE1* weakens [*PSI*$^+$]^S and cures [*PSI*$^+$]^W. (Left) Representative streaks of isogenic [*PSI*$^+$] variants and their corresponding *sse1*Δ derivatives. (Right) Immunoblot analysis to confirm that *SSE1* was disrupted in the indicated *sse1*Δ derivatives shown at the left. (B) Mating/sporulation analysis to confirm the effects of *sse1*Δ on [*PSI*$^+$] propagation. [*PSI*$^+$]^S and [*PSI*$^+$]^W cells with or without *SSE1* disruption were crossed with the α cells of 74D-694 ([*psi*$^-$][*rnq*$^-$]) and the resulting diploids and spores are shown.

LEU2 marker). In this regard, it has been reported that *sse1* null mutants grow more slowly than isogenic wild-type cells (LIU *et al.* 1999). In contrast to masking the [*PSI*$^+$]^S phenotype, results from similar crosses of [*PSI*$^+$]^W to [*psi*$^-$] indicate that *sse1*Δ had caused [*PSI*$^+$]^W loss. As shown in Figure 4B, diploids derived from *sse1*Δ[*PSI*$^+$]^W and [*psi*$^-$] had the [*psi*$^-$] colony appearance. Upon sporulation, they gave rise to four spores that were all [*psi*$^-$], indicating that [*PSI*$^+$] was eliminated in the *sse1*Δ cells. Subsequent analysis confirmed that none of the spores were able to grow in SC–ade media (data not shown).

Analyzing the effect of *sse1*Δ on the polymer sizes of Sup35: Semidenaturing agarose gel electrophoresis (SDAGE) is a useful technique that can be applied to analyze any SDS-stable amyloid protein aggregate, such as the prion polymers of Sup35 and Rnq1 in [*PSI*$^+$] and [*RNQ*$^+$] cells (KRYNDUSHKIN *et al.* 2003; BAGRIANTSEV and LIEBMAN 2004; SALNIKOVA *et al.* 2005). To examine if the disruption of *SSE1* would result in changes in Sup35 polymer sizes, we carried out the SDAGE experiment followed by immunoblot analysis using Sup35 antibody as described (BAGRIANTSEV *et al.* 2006). As shown in Figure 5, disruption of *SSE1* in [*PSI*$^+$]^S cells resulted in formation of larger-sized Sup35 polymers, which are similar to that of the [*PSI*$^+$]^W variant. However, disruption of *SSE1* in [*PSI*$^+$]^W cells resulted in a complete loss of Sup35 polymers, confirming that [*PSI*$^+$]^W was indeed eliminated upon *SSE1* disruption. The *de novo*-formed $_{sse1\Delta}$[*PSI*$^+$]^U cells have the average size of Sup35 polymers similar to that of [*PSI*$^+$]^W cells but with a much lower yield. This is likely due to the fact that some [*PSI*$^+$] elements were lost during the culturing process as a result of [*PSI*$^+$]^U instability or of the lower amount of Sup35 polymers contained in the *sse1*Δ[*PSI*$^+$]^U cells.

DISCUSSION

The yeast Sse1 protein is a member of the Hsp70 family and the ortholog of the mammalian Hsp110. It shares high sequence homology with another yeast Hsp110 protein, Sse2 (MUKAI *et al.* 1993). Both purified Sse1 and the mammalian Hsp110 can act as "holdases" that bind to unfolded proteins and maintain them in folding-competent conformations (OH *et al.* 1997; BRODSKY *et al.* 1999). The function of Sse2 is less understood and its role in [*PSI*$^+$] biology has not been investigated. Recently, Sse1 was identified as a nucleotide exchange factor for cytosolic Hsp70 members, including Ssa1 and Ssb1 (DRAGOVIC *et al.* 2006; RAVIOL *et al.* 2006; SHANER *et al.* 2006). In *S. cerevisiae*, there are four isoforms of Ssa proteins, Ssa1–4, and two isoforms of Ssb proteins, Ssb1/2, which are collectively termed Ssa and Ssb, respectively. Interestingly, Ssa and Ssb exhibit completely opposite effects with respect to the *de novo* formation and propagation of the yeast prion [*PSI*$^+$]. Ssa promotes whereas Ssb antagonizes [*PSI*$^+$]. For example, overproduction of Ssa prohibits whereas overproduction of Ssb promotes [*PSI*$^+$] curing by Hsp104 overproduction (CHERNOFF *et al.* 1999). Moreover, overproduction of Ssa but not Ssb enhances [*PSI*$^+$] *de novo* formation (ALLEN *et al.* 2005). Although Sse1 is a nucleotide exchange factor for both Ssa and Ssb proteins, we speculate that Sse1 affects [*PSI*$^+$] mainly through Ssa function. Like Ssa, Sse1 behaves as a [*PSI*$^+$]-promoting

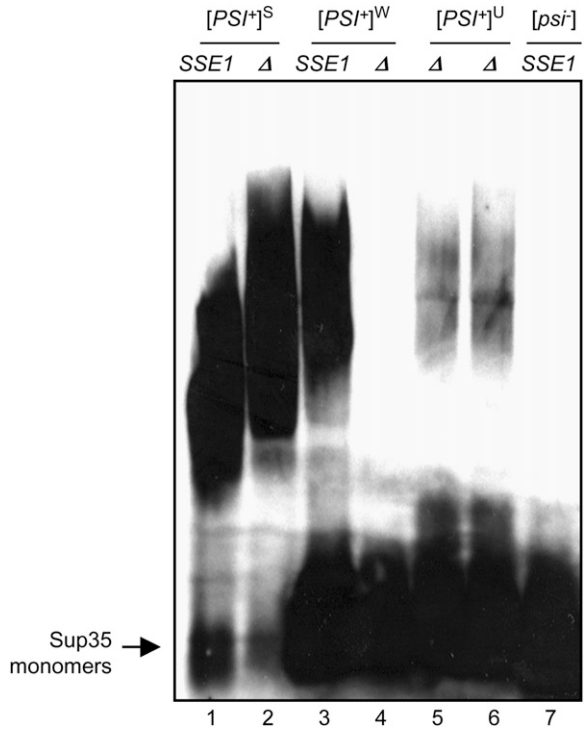

Figure 5.—Analyzing Sup35 polymer sizes using SDAGE. Crude protein extracts prepared from isogenic strains of 74D-694 ([*PSI*$^+$]^S[*RNQ*$^+$]), ([*PSI*$^+$]^W[*RNQ*$^+$]), and their corresponding *sse1*Δ derivatives were subjected to SDAGE analysis according to Bagriantsev *et al.* (2006) with minor modifications (see materials and methods). Lanes 1 and 2, [*PSI*$^+$]^S[*RNQ*$^+$] and its *sse1*Δ derivative; lanes 3 and 4, [*PSI*$^+$]^W[*RNQ*$^+$] and its *sse1*Δ derivative; lanes 5 and 6, two [*PSI*$^+$]^U isolates derived from *sse1*Δ cells ([*psi*$^-$][*RNQ*$^+$]); and lane 7, isogenic nonprion control cells ([*psi*$^-$][*rnq*$^-$]). The arrow indicates the position to which the Sup35 monomer has migrated.

factor (Allen *et al.* 2005; Figure 1A). However, the promoting effect of Sse1 on [*PSI*$^+$] is much more profound than that of Ssa proteins. Under identical conditions, we found that the amount of [*PSI*$^+$] formed under Sse1 overproduction conditions was significantly more than that formed under Ssa1 overproduction conditions (data not shown). It is possible that the excess Sse1 can more efficiently stimulate the function of Ssa than Ssb. In support of this notion, Sse1 preferentially associates with Ssa *in vivo* (Shaner *et al.* 2005). The ratio of Sse1 in association with Ssa or with Ssb is balanced by at least two factors: relative abundance and their binding affinity to Sse1. When Sse1 is overproduced, Sse/Ssa heterodimers are likely to accumulate, promoting the ADP/ATP exchange of Ssa, and thus exhibit an effect similar to that of Ssa overproduction. The fact that overproduction of either Ssa1 (Schwimmer and Masison 2002) or Sse1 (this study) has no effect on preexisting [*PSI*$^+$] but either treatment is able to cure [*URE3*] (Schwimmer and Masison 2002; Kryndushkin and Wickner 2007) further suggests that the effect of Sse1 overproduction on [*PSI*$^+$] is through Ssa1.

A fascinating phenomenon of prion biology is the existence of "strains" or variants. There are multiple PrP^{Sc} forms described, which are associated with distinct prion diseases varying in symptoms, incubation times, and brain pathologies (Prusiner 1998). All identified yeast prions are able to exist in multiple variants (Derkatch *et al.* 1996; Sondheimer and Lindquist 2000; Schlumpberger *et al.* 2001). Despite decades of effort, it remains unclear how a prion polypeptide adopts multiple heritable conformations. Identification of cellular factors responsible for establishing multiple prion conformations will likely shed light on the underlying mechanism required for prion variant determination. Although *sse1*Δ cells are capable of propagating the strong variant of [*PSI*$^+$] (Figure 4A), we have not been able to obtain uniformly colored pink or white colonies from *sse1*Δ cells upon Sup35 overproduction. Only [*PSI*$^+$]^U was permitted to form *de novo* in *sse1*Δ cells. Thus Sse1 is an important [*PSI*$^+$] variant determinant. Our finding that the physical association of Hsp70 with Sse1 is required for establishing a full spectrum of [*PSI*$^+$] variants suggests that the concerted action of Sse1–Ssa and/or Sse1–Ssb might be a key to [*PSI*$^+$] variant determination. In this regard, it has been shown that overproduced Sup35-HA is physically associated with Ssa and Ssb both *in vivo* and *in vitro* (Allen *et al.* 2005).

We have previously shown that Δ*CTA-HSF* cells preferentially give rise to a weak [*PSI*$^+$] variant that is unstable, $_{\Delta CTA}$[*PSI*$^+$]^U (Park *et al.* 2006). Although Sse1 is a CTA-HSF target (Liu and Thiele 1999) and is important for [*PSI*$^+$] variant establishment (Figure 3), the effect of Δ*CTA-HSF* on [*PSI*$^+$] variant formation is unlikely for several reasons due to the lack of Sse1. First, the [*PSI*$^+$]^U cells formed in *sse1*Δ cells have a unique colony appearance that is distinct from that of [*PSI*$^+$]^U formed in Δ*CTA-HSF* cells. As shown in Figure 3C, although both $_{\Delta CTA}$[*PSI*$^+$]^U and $_{sse1\Delta}$[*PSI*$^+$]^U have a colony appearance similar to very weak [*PSI*$^+$] variants, their detailed colony morphologies are different. Second, $_{sse1\Delta}$[*PSI*$^+$]^U is much less stable than $_{\Delta CTA}$[*PSI*$^+$]^U. Third, ectopic expression of *SSE1* driven by promoters of variable strength cannot change the preference of [*PSI*$^+$]^U formation in Δ*CTA-HSF* cells (data not shown). Thus, the preference of [*PSI*$^+$]^U formation in Δ*CTA-HSF* cells is likely caused by an unknown factor(s) other than Sse1. It is also possible that the imbalance of molecular chaperones in the Δ*CTA-HSF* cells is the responsible factor.

We have shown that overproduction of Sti1 and either isoform of Hsp90 severely inhibited [*PSI*$^+$] *de novo* formation whereas overproduction of Sse1 dramatically stimulated it (Figure 2). Overproduction of Cpr6 or Ydj1, however, exhibited no significant effects. Our finding demonstrates that different Hsp90 co-chaperones have distinct effects on [*PSI*$^+$] *de novo* formation and variant determination. This finding is intriguing as the Hsp90 complex is responsible for the maturation of a large number of client proteins and is an important

target for anticancer therapy. The effects of individual components of the Hsp90 complex on [*PSI*$^+$] *de novo* formation may be due to their dynamic association with the Hsp90 complex. It is also possible that the Hsp90 co-chaperones exercise distinct functions when they are or are not in association with Hsp90. In this regard, it has been shown that Sti1 and Cpr7 can also form stable complexes with Hsp104 both *in vivo* and *in vitro* (Abbas-Terki *et al.* 2001). How such dynamic associations of Sti1 and Cpr7 with Hsp90 and Hsp104 affect [*PSI*$^+$] formation and propagation, however, has not been investigated. Modulating the levels of one Hsp90 co-chaperone might also affect the overall structure and integrity of the Hsp90 complex and thus affect their associated cofactors and client proteins. For example, overproduction of Hsp90 might cause more Sse1–Ssa1 complex to be associated with Hsp90, thus reducing the Sup35 associated Sse1–Ssa1 complex. As a consequence, [*PSI*$^+$] *de novo* formation is reduced. However, increasing the expression levels of Sse1 would promote the nucleotide exchange of ADP to ATP of Ssa and thus enhance [*PSI*$^+$] formation. Although it is less likely, our results cannot rule out the possibility that the Hsp90 complex directly associates with Sup35 to affect [*PSI*$^+$] *de novo* formation.

A recent study by Kryndushkin and Wickner (2007) showed that overproduction of Sse1 can efficiently cure [*URE3*]. However, results from both Kryndushkin and Wickner (2007) and our independent study (data not shown) indicate that overproduction of Sse1 has no detectable effect on preexisting [*PSI*$^+$], including both [*PSI*$^+$]^S and [*PSI*$^+$]^W variants. The effect of *sse1*Δ on [*PSI*$^+$] is more complicated. Disruption of *SSE1* weakens [*PSI*$^+$]^S and completely eliminates [*PSI*$^+$]^W (Kryndushkin and Wickner 2007; Figure 4). How altered *SSE1* expression levels affect [*RNQ*$^+$] has not been systematically investigated. However, neither overexpression nor disruption of *SSE1* has detectable effects on the particular [*RNQ*$^+$] variant in the 74D-694 strains used in this study (data not shown). Taken together, the Hsp110 chaperone Sse1 is not only a prion-specific but also a variant-specific factor that plays important and distinct roles in maintaining various prion elements in yeast.

We thank J. Brodsky for the Sse1 antibody; E. Craig for the Ssa1/2, Ssb1/2, and Sis1 antibodies; J. Johnson for the Sti1 antibody; S. Liebman for the Sup35 antibody; S. Lindquist for the Rnq1, Hsp90, and Hsp104 antibodies; D. Masison for the STI1 overexpression plasmid; and C. Long for manuscript editing. This work was supported by grants from the United States Army (0650-370-R744) and Ellison Medical Foundation to L.L. and by the National Institute of General Medical Sciences of the National Institutes of Health (GM074696) to K.A.M.

LITERATURE CITED

Abbas-Terki, T., O. Donze, P. A. Briand and D. Picard, 2001 Hsp104 interacts with Hsp90 cochaperones in respiring yeast. Mol. Cell. Biol. **21:** 7569–7575.

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.

Bagriantsev, S., and S. W. Liebman, 2004 Specificity of prion assembly in vivo. [PSI+] and [PIN+] form separate structures in yeast. J. Biol. Chem. **279:** 51042–51048.

Bagriantsev, S. N., V. V. Kushnirov and S. W. Liebman, 2006 Analysis of amyloid aggregates using agarose gel electrophoresis. Methods Enzymol. **412:** 33–48.

Brodsky, J. L., E. D. Werner, M. E. Dubas, J. L. Goeckeler, K. B. Kruse *et al.*, 1999 The requirement for molecular chaperones during endoplasmic reticulum-associated protein degradation demonstrates that protein export and import are mechanistically distinct. J. Biol. Chem. **274:** 3453–3460.

Chernoff, Y. O., I. L. Derkatch and S. G. Inge-Vechtomov, 1993 Multicopy *SUP35* gene induces *de-novo* appearance of *psi*-like factors in the yeast *Saccharomyces cerevisiae*. Curr. Genet. **24:** 268–270.

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.

Chernoff, Y. O., G. P. Newnam, J. Kumar, K. Allen and A. D. Zink, 1999 Evidence for a protein mutator in yeast: role of the Hsp70-related chaperone Ssb in formation, stability, and toxicity of the [*PSI*] prion. Mol. Cell. Biol. **19:** 8103–8112.

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

Derkatch, I. L., Y. O. Chernoff, V. V. Kushnirov, S. G. Inge-Vechtomov and S. W. Liebman, 1996 Genesis and variability of [*PSI*] prion factors in *Saccharomyces cerevisiae*. Genetics **144:** 1375–1386.

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.

Firoozan, M., C. M. Grant, J. A. Duarte and M. F. Tuite, 1991 Quantitation of readthrough of termination codons in yeast using a novel gene fusion assay. Yeast **7:** 173–183.

Hahn, J. S., Z. Hu, D. J. Thiele and V. R. Iyer, 2004 Genome-wide analysis of the biology of stress responses through heat shock transcription factor. Mol. Cell. Biol. **24:** 5249–5256.

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., and D. C. Masison, 2001 Guanidine hydrochloride inhibits Hsp104 activity *in vivo*: a possible explanation for its effect in curing yeast prions. Curr. Microbiol. **43:** 7–10.

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.

King, C. Y., and R. Diaz-Avalos, 2004 Protein-only transmission of three yeast prion strains. Nature **428:** 319–323.

Kryndushkin, D., and R. B. Wickner, 2007 Nucleotide exchange factors for Hsp70s are required for [URE3] prion propagation in Saccharomyces cerevisiae. Mol. Biol. Cell **18:** 2149–2154.

Kryndushkin, D. S., I. M. Alexandrov, M. D. Ter-Avanesyan and V. V. Kushnirov, 2003 Yeast [PSI+] prion aggregates are formed by small Sup35 polymers fragmented by Hsp104. J. Biol. Chem. **278:** 49636–49643.

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.

Liebman, S. W., and I. L. Derkatch, 1999 The yeast [*PSI*$^+$] prion: making sense of nonsense. J. Biol. Chem. **274:** 1181–1184.

Liu, P. C., and D. J. Thiele, 1999 Modulation of human heat shock factor trimerization by the linker domain. J. Biol. Chem. **274:** 17219–17225.

Liu, X. D., K. A. Morano and D. J. Thiele, 1999 The yeast Hsp110 family member, Sse1, is an Hsp90 cochaperone. J. Biol. Chem. **274:** 26654–26660.

Mukai, H., T. Kuno, H. Tanaka, D. Hirata, T. Miyakawa *et al.*, 1993 Isolation and characterization of SSE1 and SSE2, new members of the yeast HSP70 multigene family. Gene **132:** 57–66.

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.

Oh, H. J., X. Chen and J. R. Subjeck, 1997 Hsp110 protects heat-denatured proteins and confers cellular thermoresistance. J. Biol. Chem. **272:** 31636–31640.

Park, K. W., J. S. Hahn, Q. Fan, D. J. Thiele and L. Li, 2006 *De novo* appearance and "strain" formation of yeast prion [PSI+] are regulated by the heat-shock transcription factor. Genetics **173:** 35–47.

Pirkkala, L., P. Nykanen and L. Sistonen, 2001 Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. FASEB J. **15:** 1118–1131.

Prusiner, S. B., 1998 Prions. Proc. Natl. Acad. Sci. USA **95:** 13363–13383.

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.

Salnikova, A. B., D. S. Kryndushkin, V. N. Smirnov, V. V. Kushnirov and M. D. Ter-Avanesyan, 2005 Nonsense suppression in yeast cells overproducing Sup35 (eRF3) is caused by its non-heritable amyloids. J. Biol. Chem. **280:** 8808–8812.

Schlumpberger, M., S. B. Prusiner and I. Herskowitz, 2001 Induction of distinct [*URE3*] yeast prion strains. Mol. Cell. Biol. **21:** 7035–7046.

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.

Serio, T. R., and S. L. Lindquist, 1999 [*PSI*$^+$]: an epigenetic modulator of translation termination efficiency. Annu. Rev. Cell Dev. Biol. **15:** 661–703.

Shaner, L., H. Wegele, J. Buchner and K. A. Morano, 2005 The yeast Hsp110 Sse1 functionally interacts with the Hsp70 chaperones Ssa and Ssb. J. Biol. Chem. **280:** 41262–41269.

Shaner, L., R. Sousa and K. A. Morano, 2006 Characterization of Hsp70 binding and nucleotide exchange by the yeast Hsp110 chaperone Sse1. Biochemistry **45:** 15075–15084.

Sherman, F., 1991 Getting started with yeast, pp. 3–21 in *Guide to Yeast Genetics and Molecular Biology*, edited by C. Guthrie and G. R. Fink. Academic Press, San Diego.

Sondheimer, N., and S. Lindquist, 2000 Rnq1: an epigenetic modifier of protein function in yeast. Mol. Cell **5:** 163–172.

Sondheimer, N., N. Lopez, E. A. Craig and S. Lindquist, 2001 The role of Sis1 in the maintenance of the [*RNQ*$^+$] prion. EMBO J. **20:** 2435–2442.

Song, Y., and D. C. Masison, 2005 Independent regulation of Hsp70 and Hsp90 chaperones by Hsp70/Hsp90-organizing protein Sti1 (Hop1). J. Biol. Chem. **280:** 34178–34185.

Soto, C., and J. Castilla, 2004 The controversial protein-only hypothesis of prion propagation. Nat. Med. **10**(Suppl.): S63–S67.

Stansfield, I., K. M. Jones, V. V. Kushnirov, A. R. Dagkesamanskaya, A. I. Poznyakovski *et al.*, 1995 The products of the *SUP45* (eRF1) and *SUP35* genes interact to mediate translation termination in *Saccharomyces cerevisiae*. EMBO J. **14:** 4365–4373.

Tamai, K. T., X. Liu, P. Silar, T. Sosinowski and D. J. Thiele, 1994 Heat shock transcription factor activates yeast metallothionein gene expression in response to heat and glucose starvation via distinct signalling pathways. Mol. Cell. Biol. **14:** 8155–8165.

Tanaka, M., P. Chien, N. Naber, R. Cooke and J. S. Weissman, 2004 Conformational variations in an infectious protein determine prion strain differences. Nature **428:** 323–328.

Tuite, M. F., C. R. Mundy and B. S. Cox, 1981 Agents that cause a high frequency of genetic change from [*PSI*$^+$] to [*psi*$^-$] in *Saccharomyces cerevisiae*. Genetics **98:** 691–711.

Wickner, R. B., 1994 [*URE3*] as an altered Ure2 protein: evidence for a prion analog in *Saccharomyces cerevisiae*. Science **264:** 566–569.

Communicating editor: F. Winston