Table S1. Kinetic parameters of Sup35 fibrillization in the presence of Hsp104, Hsp104 Walker-A and sensor-1 mutants, or, Cdc48.

Condition **Sup35 (2.5μM) +**	**Lag Time (T_L)** **(h)**	**50% Assembly** **Time ($T_{A1/2}$) (h)**
-	2	2.5
Hsp104 (0.03μM)+ATP	0.5	0.9
Hsp104 (0.03μM) +AMP-PNP (5mM)	0.5	2.5
Hsp104 (0.03μM)+ADP	2	2.5
Hsp104 (0.03μM)-nucleotide	2	2.5
GdmCl (20mM)	2	2.6
Hsp104 (0.03μM) +ATP+GdmCl (20mM)	2	2.5
Hsp104 (K218T:K620T) (0.03μM)+ATP	2	2.5
Hsp104 (K620T) (0.03μM)+ATP	2	2.5
Hsp104 (K218T) (0.03μM)+ATP	2	2.7
Hsp104 (N728A) (0.03μM)+ATP	0.5	2.6
Hsp104 (T317A) (0.03μM)+ATP	0.5	2.5
Hsp104 (1μM)+ATP	*	*
Hsp104 (1μM)+AMP-PNP (5mM)	*	*
Hsp104 (1μM)+ADP	4	2.8
Hsp104 (1μM)-nucleotide	2.5	2.8
Hsp104 (1μM) +ATP+GdmCl (20mM)	4.5	3.5
Cdc48 (0.03μM)+ATP	2	2.5
Cdc48 (1μM)+ATP	2	2.5

Sup35 (2.5μM) fibrillization as in (Fig. 1B) was measured by ThT fluorescence. Lag time (T_L) refers to the time before detection of amyloid. 50% Assembly time ($T_{A1/2}$) refers to the time between the first appearance of amyloid and when 50% of conversion is complete. * indicates that no assembly was detected.

Table S2. Kinetic parameters of Ure2 fibrillization in the presence of Hsp104, Hsp104 Walker-A mutants, or, Cdc48.

Condition **Ure2 (2.5μM) +**	**Lag Time (T_L) (h)**	**50% Assembly Time ($T_{A1/2}$) (h)**
-	1	9.2
Hsp104 (0.03μM)+ATP	0	3
Hsp104 (0.03μM) +AMP-PCP (5mM)	0.5	8.1
Hsp104 (0.03μM)+ADP	1	9.2
Hsp104 (0.03μM)-nucleotide	1	8.9
GdmCl (20mM)	1	9
Hsp104 (0.03μM)+ATP+GdmCl (20mM)	1	9.5
Hsp104 (K218T:K620T) (0.03μM)+ATP	1	9.1
Hsp104 (1μM)+ATP	*	*
Hsp104 (1μM)+AMP-PCP (5mM)	*	*
Hsp104 (1μM)+ADP	4.5	10
Hsp104 (1μM)-nucleotide	4	9.2
Hsp104 (1μM)+ATP+GdmCl (20mM)	5	10
Hsp104 (K218T:K620T) (1μM)+ATP	4	9.6
Cdc48 (0.03μM)+ATP	1	9.2
Cdc48 (1μM)+ATP	1	9.2

Ure2 (2.5μM) fibrillization as in (Fig. 1C) was measured by ThT fluorescence. Lag time (T_L) refers to the time before detection of amyloid. 50% Assembly time ($T_{A1/2}$) refers to the time between the first appearance of amyloid and when 50% of conversion is complete. * indicates that no assembly was detected.

Supplemental Experimental Procedures

Proteins

Ure2 was purified as described (Baxa et al., 2003). Except that His_6-Ure2 was eluted from Ni-NTA using a linear gradient of 20-350mM imidazole. The His-tag was subsequently removed by thrombin cleavage (Baxa et al., 2003). Ure2 was then exchanged into assembly buffer (AB) (40mM Hepes-KOH pH 7.4, 150mM KCl, 20mM $MgCl_2$, 10% (w/v) glycerol, 5mM ATP, and 1mM DTT), passaged through a 0.2μm filter, and used immediately for fibrillization reactions.

Sup35 was expressed as an N-terminally His-tagged protein in *E. coli* Rosetta^{TM}2(DE3)pLacI cells (Novagen). Cells were lysed on ice by sonication in lysis buffer: 30mM TrisHCl pH 8, 300mM KCl, 10% (w/v) glycerol, 20mM imidazole, 5mM $MgCl_2$, 1mM GTP, 2mM β-mercaptoethanol, 5μM pepstatin A, and Complete protease inhibitor cocktail (1 Mini, EDTA-free tablet/50ml). Cell debris was cleared by centrifugation (40,000g, 20min, 4°C), and the supernatant applied to Ni-NTA agarose (Qiagen). The column was washed with 30 volumes of lysis buffer and His_{10}-Sup35 was eluted via a linear gradient of 20-350mM imidazole in lysis buffer. Alternatively, GTP was omitted from the lysis buffer and His_{10}-Sup35 was purified via sequential Ni-NTA and GTP-agarose (Sigma) columns. The His-tag was then removed by Factor Xa cleavage (Amersham). Uncleaved Sup35 was removed with Ni-NTA agarose and Factor Xa was removed with HiTrap Benzamidine FF (Amersham). After purification, Sup35 was exchanged into AB plus 1mM GTP, filtered through a 0.2μm filter, and used immediately for fibrillization reactions.

Fiber assembly and disassembly.

The extent of Sup35 or Ure2 fiber assembly or disassembly was monitored by EM, SDS-resistance, sedimentation analysis, CR binding or ThT fluorescence (Chernoff et al., 2002; Shorter and Lindquist, 2004).

Estimation of amount of SDS-resistant Sup35

To estimate the level of SDS-resistant (SDS-insoluble) Sup35, the amount of SDS-soluble Sup35 was first determined by quantitative densitometry of Coomassie stained gels. Values obtained from densitometry were converted to units of pmol by comparison to standard curves with known amounts of SDS-soluble Sup35. This value was subtracted from the total amount of Sup35 to obtain an estimate of the amount of SDS-resistant Sup35.

Protein transformation

Yeast cells from a W303-derived yeast strain (*MATα leu2-3, -112 his3-11 trp1-1 ura3-1 ade1-14 can1-100* [*pin*$^-$] [*psi*$^-$] [*ure-o*]) that contained an *ADE1* mutation suppressible by [*PSI*$^+$] were transformed with Sup35 proteins and a *URA3* plasmid (Tanaka et al., 2004; Krishnan and Lindquist, 2005). The final concentration of Sup35 in infection experiments was 2.5μM, and reactions were rapidly concentrated with Microcon-YM30 centrifugal filter devices (Millipore) prior to addition to spheroplasts. The proportion of Ura$^+$ transformants that acquired [*PSI*$^+$] was determined (Krishnan and Lindquist, 2005). Very similar results were obtained using another yeast strain, 74-D694.

Yeast cells from strain SL1008-3B (*MAT□ leu2-P1 ade1-14 ura2* [*pin*$^-$] [*psi*$^-$] [*ure-o*]) were grown in 25ml YPD at 30°C to OD_{600}~0.4, recovered by centrifugation at 1000g for 10min at 25°C, washed once with sterile distilled water, and once with SCE buffer (1M sorbitol, 10mM EDTA, 10mM DTT, 100mM sodium citrate, pH 5.8). Cells were resuspended in SCE buffer and incubated with lyticase (0.4mg/ml) for 10min at 30°C. Spheroplasts were recovered, and washed with STC buffer (1M sorbitol, 10mM $CaCl_2$, 10mM TrisHCl, pH 7.5). Spheroplasts were resuspended in STC buffer and mixed with Ure2 protein, a *LEU2* plasmid (YCplac111) (0.02mg/ml) and salmon sperm DNA (0.1mg/ml), and incubated for 30min at 25°C. Fusion was induced by addition of 9 volumes of PEG-buffer (20% (w/v) PEG 8000, 10mM $CaCl_2$, 10mM TrisHCl, pH 7.5), and incubation for 30min at 25°C. The final concentration of Ure2 in infection experiments was 2.5μM, and reactions were rapidly concentrated with Microcon-YM30 centrifugal filter devices (Millipore) prior to addition to spheroplasts. Spheroplasts were recovered and resuspended in SOS buffer (1M sorbitol, 7mM $CaCl_2$, 0.25% yeast extract,

0.5% bacto-peptone), incubated for 1h at 30°C, and plated on SD-Leu (with 1M sorbitol) overlaid with top agar (2.5%). Approximately 70 Leu^+ transformants were streaked onto SD-Leu medium and replica plated onto SD-Leu-Ura+Usa. To ensure Usa^+ colonies corresponded to [*URE3*] they were subsequently streaked onto SD-Leu+GdmCl (3mM), and then replica plated back onto SD-Leu-Ura+Usa to test for curing of the Usa^+ phenotype (~98% of Usa^+ transformants were cured by GdmCl).

Supplemental Discussion

While this manuscript was in preparation, another paper reported the effects of Hsp104, Hsp70, and Hsp40 on the conformational transitions of full-length, tagged Sup35 purified in the absence of GTP (Krzewska and Melki, 2006). Our results (here and in preparation) are complementary in some ways, but disagree in others. As they report, we too have found that Hsp70 and Hsp40 can inhibit the *de novo* nucleation of prions by Hsp104. However, these inhibitory activities are overcome by the prion-promoting remodeling activities of Hsp104 under *in vitro* reaction conditions that are relevant to the *in vivo* effects of environmental change on *de novo* prion formation (Shorter and Lindquist, in preparation). In fact, the ability of Hsp104 to nucleate prions is almost certainly an adaptation used by yeast cells to elicit heritable phenotypic variation in response to specific environmental cues (Shorter and Lindquist, 2005).

Furthermore, no direct interaction between Sup35 and Hsp104 could be detected, even when glutaraldehyde crosslinking was employed (Krzewska and Melki, 2006). The conditions employed varied from our own in that they were performed: (a) at 10°C; (b) at a non-physiological pH of 8; (c) with his-tagged Sup35 (ours was not tagged) purified in the absence of GTP (which makes the protein highly prone to forming non-prion aggregates); (d) with his-tagged Hsp104 purified in the absence of ATP (which can yield inactive Hsp104); (e) with higher salt concentrations based on the Na^+ ion, which is disfavored in the yeast cytosol (hence we used K^+); and (f) with lower concentrations of glycerol. It is unclear how the combination of these different conditions would alter the kinetics of conformational changes that Hsp104 undergoes during ATP binding,

hydrolysis, and ADP plus Pi release, and how this might affect substrate remodeling. Furthermore, these differences appear to have altered the physical interaction between Hsp104 and Sup35 (to undetectable levels), and this may explain why different levels of Hsp104 were required to observe any effects on Sup35 fiber assembly (Krzewska and Melki, 2006).

Supplemental References

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