Hsp104 disaggregase at normal levels cures many [*PSI*$^+$] prion variants in a process promoted by Sti1p, Hsp90, and Sis1p

Anton Gorkovskiy[a], Michael Reidy[a], Daniel C. Masison[a], and Reed B. Wickner[a,1]

[a]Laboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0830

Contributed by Reed B. Wickner, April 15, 2017 (sent for review March 12, 2017; reviewed by Vitaly V. Kushnirov and Susan Liebman)

Overproduction or deficiency of many chaperones and other cellular components cure the yeast prions [*PSI*$^+$] (formed by Sup35p) or [*URE3*] (based on Ure2p). However, at normal expression levels, Btn2p and Cur1p eliminate most newly arising [*URE3*] variants but do not cure [*PSI*$^+$], even after overexpression. Deficiency or overproduction of Hsp104 cures the [*PSI*$^+$] prion. Hsp104 deficiency curing is a result of failure to cleave the Sup35p amyloid filaments to make new seeds, whereas Hsp104 overproduction curing occurs by a different mechanism. Hsp104(T160M) can propagate [*PSI*$^+$], but cannot cure it by overproduction, thus separating filament cleavage from curing activities. Here we show that most [*PSI*$^+$] variants arising spontaneously in an *hsp104(T160M)* strain are cured by restoration of just normal levels of the WT Hsp104. Both strong and weak [*PSI*$^+$] variants are among those cured by this process. This normal-level Hsp104 curing is promoted by Sti1p, Hsp90, and Sis1p, proteins previously implicated in the Hsp104 overproduction curing of [*PSI*$^+$]. The [*PSI*$^+$] prion arises in *hsp104(T160M)* cells at more than 10-fold the frequency in WT cells. The curing activity of Hsp104 thus constitutes an antiprion system, culling many variants of the [*PSI*$^+$] prion at normal Hsp104 levels.

prion | antiprion system | [*PSI*$^+$] | Sup35p

Most organisms devote a substantial part of their genome to opposing viral propagation and takeover of cellular processes. Humoral and cellular immune systems, RNAi-based systems, innate immunity, physical barriers to penetration, interferons, the *SKI*-based systems, and many others have been selected to keep virus propagation in check. Infectious proteins, prions, have not been studied as widely, but already antiprion systems have been recognized.

The translation termination subunit, Sup35p, can form an infectious protein, [*PSI*$^+$] by conversion to a self-propagating amyloid form (1–6). Similarly, Ure2p, a mediator of nitrogen catabolite repression, can form the amyloid-based [*URE3*] prion (1, 7–9) and Rnq1p forms the [*PIN*$^+$] prion (10, 11). Amyloid is a filamentous β-sheet–rich protein polymer, and the yeast prion amyloids have a folded, in-register, parallel β-sheet architecture (12–15). This architecture provides a mechanism by which proteins can template their conformation, much as DNA templates its sequence, and explains the rather stable propagation of many different prion variants (called "prion strains" in mammals) based on different conformations of a single prion protein (16, 17).

Chernoff's seminal discovery that Hsp104 overproduction or deficiency could cure the [*PSI*$^+$] prion (18, 19) led to detailed dissection of the mechanisms of these effects, and discovery of the involvement of many other chaperones and cochaperones. Hsp104 (20) is a disaggregating chaperone, which acts with Hsp70s and Hsp40s to solubilize proteins (21). Monomers are removed from the aggregate and fed through the central cavity of the Hsp104 hexamer, thereby denaturing them and allowing them a chance to properly refold (22–24). Millimolar guanidine HCl is a surprisingly specific inhibitor of Hsp104 (25–29), and has been used to show that the effect of Hsp104 inactivation on prion propagation is to block the generation of new seeds (also called propagons) (30–32). Hsp104's prion-propagating activity, like its general disaggregating activity, also involves Hsp70s and nucleotide-exchange factors, as well as Hsp40s. Hsp70s, the cytoplasmic Ssas of *Saccharomyces cerevisiae*, are necessary for stable prion propagation (33–37), and can antagonize the curing of [*PSI*$^+$] by overproduction of Hsp104 (38), an effect requiring Sgt2 (39). The Hsp40 role in prion propagation includes considerable prion-specificity of the various Hsp40s (40, 41). The need for collaboration between Hsp104, Hsp70s, Hsp40s, and nucleotide-exchange factors in prion propagation was shown by the ability of *Escherichia coli* homologs, ClpB, DnaK, and GrpE, to substitute for their yeast relatives only if they could interact with their *E. coli* partners (42).

The mechanism of Hsp104 overproduction curing of [*PSI*$^+$] differs from that of its prion–propagation action. Mutation or complete deletion of the Hsp104 N-terminal domain has no effect on [*PSI*$^+$] propagation, but eliminates the ability of the overproduced protein to cure [*PSI*$^+$] (43). Sti1p and Cpr7 are cochaperones containing tetratricopeptide repeat sequences that determine their binding to conserved EEVD/DDLD sites at the C termini of Hsp70, Hsp90, and Hsp104 (44, 45). The *sti1Δ* mutation also prevents curing of [*PSI*$^+$] by overproduced Hsp104 (46, 47), although this deletion does not affect propagation of the same [*PSI*$^+$] variant in an otherwise WT strain (48). However, deletion of the Hsp104 C-terminal DDLD prevents its binding to Sti1p (45), but does not prevent overproduced Hsp104 from curing [*PSI*$^+$] (47), suggesting Sti1p is needed for the Hsp104 overproduction curing via another interaction. The part of Sti1p most important for Hsp104 curing of [*PSI*$^+$] is the tetratricopeptide repeat 2 domain involved in interaction with Hsp90s (47). Indeed, inhibition of Hsp90s with radicicol blocks the curing activity of overproduced Hsp104 (47). These results suggest a role of Hsp90s in the Hsp104 prion curing process.

The mechanism of Hsp104 overproduction curing of [*PSI*$^+$] remains controversial. One proposal is that overproduced Hsp104 binds to a special site in the middle (M) domain of Sup35p (49) and so prevents Hsp70s from having access to the filaments, which access is believed necessary for the Hsp104-Hsp70-Hsp40 machine to extract a monomer from the filament and thereby cleave it (37).

Significance

Prions (infectious proteins) pose a substantial risk to yeast, as they do for humans. Overproduction of the disaggregase, Hsp104, has long been known capable of curing [*PSI*$^+$], a prion based on amyloid formation by the Sup35 protein. We find that most [*PSI*$^+$] variants arising spontaneously in the absence of this Hsp104 overproduction curing activity are cured when that activity is restored at normal levels. This activity is thus an antiprion system, largely protecting the cells from prion formation by this protein.

Author contributions: A.G., M.R., D.C.M., and R.B.W. designed research, performed research, contributed new reagents/analytic tools, analyzed data, and wrote the paper.

Reviewers: V.V.K., Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences; and S.L., University of Nevada.

The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. Email: wickner@helix.nih.gov.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1704016114/-/DCSupplemental.

Table 1. Restoration of the WT Hsp104 (at normal levels) cures most of the spontaneously arising [PSI$^+$] variants

Donor *HSP104* genotype and [*PSI*$^+$] variant (phenotype on 1/2 YPD)	Recipient	Cytoductants	Ade$^+$ cytoductants	% Ade$^+$	*P* value
WT [*PSI*$^+$1] (ss)	WT	12	11	92	
WT [*PSI*$^+$1] (ss)	*hsp104*^{T160M}	9	9	100	
WT [*PSI*$^+$2] (ss)	WT	12	2	17	6×10^{-5}
WT [*PSI*$^+$2] (ss)	*hsp104*^{T160M}	16	16	100	
WT [*PSI*$^+$3] (ss)	WT	13	12	92	
WT [*PSI*$^+$3] (ss)	*hsp104*^{T160M}	14	14	100	
hsp104^{T160M} [*PSI*$^+$4] (vwu)	WT	32	13	41	4×10^{-4}
hsp104^{T160M} [*PSI*$^+$4] (vwu)	*hsp104*^{T160M}	22	21	95	
hsp104^{T160M} [*PSI*$^+$5] (vwvu)	WT	13	0	0	
hsp104^{T160M} [*PSI*$^+$5] (vwvu)	*hsp104*^{T160M}	12	0	0	
hsp104^{T160M} [*PSI*$^+$6] (vwu)	WT	21	17	81	
hsp104^{T160M} [*PSI*$^+$6] (vwu)	*hsp104*^{T160M}	15	15	100	
hsp104^{T160M} [*PSI*$^+$7] (vwu)	WT	23	6	26	1×10^{-4}
hsp104^{T160M} [*PSI*$^+$7] (vwu)	*hsp104*^{T160M}	15	12	80	
hsp104^{T160M} [*PSI*$^+$8] (vwu)	WT	3	0	0	2×10^{-3}
hsp104^{T160M} [*PSI*$^+$8] (vwu)	*hsp104*^{T160M}	19	19	100	
hsp104^{T160M} [*PSI*$^+$9] (vwvu)	WT	37	6	16	1×10^{-5}
hsp104^{T160M} [*PSI*$^+$9] (vwvu)	*hsp104*^{T160M}	18	15	83	
hsp104^{T160M} [*PSI*$^+$10] (vwu)	WT	28	18	64	1×10^{-5}
hsp104^{T160M} [*PSI*$^+$10] (vwu)	*hsp104*^{T160M}	21	20	95	
hsp104^{T160M} [*PSI*$^+$11] (vwvu)	WT	26	7	27	1×10^{-5}
hsp104^{T160M} [*PSI*$^+$11] (vwvu)	*hsp104*^{T160M}	14	14	100	

The spontaneously arising prion variants isolated in either WT (AG666) or *hsp104*^{T160M} (AG667) background were used as cytoduction donors to isogenic WT (AG686) or *hsp104*^{T160M} (AG687) recipients. The cytoduction efficiency of the [*PSI*$^+$2], [*PSI*$^+$4], [*PSI*$^+$7], [*PSI*$^+$8], [*PSI*$^+$9], [*PSI*$^+$10], and [*PSI*$^+$11] prion variants into *hsp104*^{T160M} recipients was significantly higher than into WT recipients. ss, strong stable; vwu, very weak unstable; vwvu, very weak very unstable (see Fig. S1*B*). Statistical tests were carried out as described in *Methods*.

Another model proposes that overproduction curing represents removal of Sup35p monomers from the ends of filaments, thereby eventually solubilizing the filaments (50). A third group posits assymetric segregation of prion seeds that have been collected by the overproduced Hsp104, with some cells emerging from the division with no seeds (51). There is substantial evidence for each of these models.

Although extensive studies have probed the mechanism of Hsp104 overproduction curing, less is known about its biological role. During the usual heat-shock regime, little curing of [*PSI*$^+$] occurs, perhaps because the higher levels of Hsp104 are largely occupied renaturing the wide array of denatured cellular proteins. Transient heat shock produces some curing of a weak [*PSI*$^+$], a phenomenon attributed to a demonstrated temporary excess of Hsp104 over Hsp70s of the Ssa group (52). However, it is likely that other heat-shock proteins and other factors are also varying in amount or activity during this treatment.

The rare occurrence of the [*PSI*$^+$] and [*URE3*] prions in wild yeast (53) is a function of their low frequency of generation, their spontaneous loss, their spread by mating, and their effects on their host, the latter varying from lethal to mild (54–57; reviewed in ref. 58). As organisms have an array of systems to deal with viral, bacterial, and parasite infections, it is not surprising that yeast has antiprion systems. The ribosome-associated Hsp70s, Ssb1 and Ssb2, at their normal levels, repress the formation of the [*PSI*$^+$] prion and, if overproduced, can cure [*PSI*$^+$] (59, 60). The Ssb proteins are involved in assuring correct folding of nascent proteins (61, 62). Most variants of [*URE3*] are quickly eliminated by the normal levels of Btn2p and Cur1p and overproduction of either protein cures all known [*URE3*] variants (63, 64). Btn2p acts by collecting Ure2p amyloid aggregates to one place in the cell so that following division, one of the daughter cells is cured (63, 64). Btn2p and Cur1p are each also able to collect nonprion protein aggregates (63, 65, 66), but neither cures [*PSI*$^+$] (63).

The fact that Hsp104 overproduction cures [*PSI*$^+$], and that this prion-curing activity appears to be distinct from Hsp104's prion-propagation activity, led us to suspect that, as in the Btn2p/Cur1p effects on [*URE3*], the prion-curing activity of Hsp104 might be working without overexpression of the protein to eliminate many [*PSI*$^+$] variants as they arise.

Results

Hsp104 at Its Normal Level Cures Many [*PSI*$^+$] Variants. Overproduction of Hsp104^{T160M} fails to cure [*PSI*$^+$], but this mutant is fully able to propagate [*PSI*$^+$] (43). The *hsp104*^{T160M} mutation does not simply lower amyloid fiber cutting activity, as such a change would make a strong [*PSI*$^+$] appear to be weaker. In fact, the mutation makes a weak [*PSI*$^+$] appear stronger (43). We isolated spontaneous [*PSI*$^+$] variants in a [*PIN*$^+$] *hsp104*^{T160M} strain (Fig. S1*A*). We restored the WT Hsp104 (with the curing activity, but at normal levels) by cytoduction (cytoplasmic transfer) (*Methods*) of these prions into an isogenic WT host, or into another *hsp104*^{T160M} strain as a control (Table 1). We also performed cytoductions of [*PSI*$^+$]s generated in a WT strain as a control.

Most [*PSI*$^+$]s arising spontaneously in the *hsp104*^{T160M} strain propagated poorly when cytoduced to WT, but not when cytoduced to *hsp104*^{T160M} recipients. This finding supports the hypothesis that Hsp104, at its normal level, cures a significant fraction of the [*PSI*$^+$]s appearing spontaneously in a yeast cell. We call such prions [*PSI*$^+$hhs] for Hsp104 hypersensitive. To make reading the tables of data easier, we use "wt" for variants isolated in a WT host and "hsp" for those isolated in an *hsp104*^{T160M} host. Variant numbers are "Ax" or "Bx" (isolated in two different experiments). In most of the tables, [*PSI*$^+$hhs] variants are shown in orange, and those that are not hypersensitive are shown in green, and will be referred to by those colors herein.

We also performed the same experiment with [*PSI*$^+$] cytoduction donors generated by Sup35NM overexpression in either WT or *hsp104*^{T160M} background (Table 2). All of the donors were guanidine-curable (Fig. 1*A*). As expected, we found [*PSI*$^+$] variants generated in the *hsp104*^{T160M} background that cytoduced significantly better to *hsp104*^{T160M} than to WT recipients (orange variants

Table 2. [*PSI*$^+$] isolates induced by overproduction of Sup35NM

[*PSI*$^+$] donor	Recipient		[*PSI*$^+$] donor	Recipient		[*PSI*$^+$] donor	Recipient		[*PSI*$^+$] donor	Recipient	
WT	WT	*hsp104 T160M*	WT	WT	*hsp104 T160M*	*hsp104 T160M*	WT	*hsp104 T160M*	*hsp104 T160M*	WT	*hsp104 T160M*
	%Ade$^+$ cytoductants (total cytoductants) *P* value										
wtB1 ss	100 (48)	100 (89)	wtA1 su	97 (30)	100 (20)	hspB1 vwvu	97 (63)	44 (59)*	hspA1 ws	100 (48)	100 (20)
wtB2 ss	99 (167)	100 (123)	wtA2 ss	95 (41)	100 (14)	hspB2 ws	100(137)	100 (161)	hspA2 vwvu	65 (32)	41 (37)
wtB3 ss	95 (112)	100 (51)	wtA3 svu	10 (20)*	59 (17)	hspB3 wvu	97 (35)	31 (45)*	hspA3 wvu	23 (48)*	100 (36)
wtB4 ss	96 (52)	86 (73)	wtA4 svu	33 (42)	16 (62)	hspB4 wvu	65 (98)	78 (72)	hspA4 ss	30 (27)*	100 (33)
wtB5 ss	100 (63)	100 (90)	wtA5 ss	100 (16)	100 (16)	hspB5 vwvu	33 (180)*	87 (230)	hspA5 wvu	14 (65)*	100 (31)
wtB6 ss	100 (71)	100 (86)	wtA6 ss	100 (22)	100 (36)	hspB6 wu	88 (77)	97 (34)	hspA6 wvu	0 (25)	20 (15)
wtB7 vu	19 (48)	25 (52)	wtA7 svu	0 (22)	18 (50)	hspB7 ss	32 (185)*	98 (95)	hspA7 wvu	24 (17)*	100 (18)
wtB8 ss	100 (70)	100 (93)	wtA8 ss	100 (16)	100 (21)	hspB8 ws	100 (58)	100 (75)	hspA8 ws	100 (21)	100 (23)
wtB9 ss	100 (62)	100 (53)	wtA9 svu	17 (47)	9 (45)	hspB9 wu	100 (130)	100 (78)	hspA9 ws	41 (32)*	94 (18)
wtB10 ss	100 (79)	100 (68)	wtA10 svu	20 (30)	17 (46)	hspB10 vwvu	98 (81)	40 (30)*	hspA10 wu	100 (16)	96 (23)
wtB11 ss	100 (42)	100 (84)	wtA11 ss	95 (19)	92 (39)	hspB11 wu	99 (71)	100 (61)	hspA11 wu	96 (50)	100 (30)
wtB12 svu	22 (54)	38 (49)	wtA12 ss	98 (40)	100 (29)	hspB12 vwvu	89 (96)	90 (49)	hspA12 ws	100 (24)	100 (32)
wtB13 ss	100 (41)	100 (98)	wtA13 ss	81 (73)*	100 (19)	hspB13 vwvu	96 (74)	54 (48)*	hspA13 wvu	64 (28)	78 (32)
wtB14 ss	100 (72)	100 (83)	wtA14 ss	100 (18)	98 (45)	hspB14 wvu	81 (91)	1/2	hspA14 wvu	13 (32)	17 (23)
wtB15 ss	100(70)	100 (52)	wtA15 su	52 (38)	30 (37)	hspB15 wu	100 (128)	100 (112)	hspA15 wvu	49 (39)*	100 (17)
wtB16 svu	14 (49)	20 (64)	wtA16 ss	91 (21)	100 (15)	hspB16 wu	100 (93)	100 (60)	hspA16 wvu	96 (46)	86 (21)
wtB17 ss	100 (38)	100 (41)	wtA17 su	85 (20)	100 (17)	hspB17 wu	100 (50)	90 (82)	hspA17 ss	41 (44)*	100 (17)
wtB18 svu	33 (48)	38 (76)	wtA18 su	94 (36)	100 (18)	hspB18 ws	100 (45)	100 (76)	hspA18vwvu	48 (31)	24 (46)
wtB19 svu	26 (65)*	73 (71)	wtA19 ss	100 (28)	100 (25)	hspB19 ws	100 (102)	100 (99)	hspA19vwvu	25 (53)*	82 (22)
wtB20 ss	100 (50)	100 (56)	wtA20 ss	100 (14)	100 (18)	hspB20 vwvu	81 (47) 0.05	100 (38)	hspA20 wu	70 (30) 0.06	100 (11)

WT (wt, AG666) and *hsp104*^{T160M} (hsp, AG667) [*psi*$^-$] [*PIN*$^+$] strains were induced to [*PSI*$^+$] by transient overproduction of Sup35NM. Guanidine curable clones were each used as cytoduction donors to WT (wt, AG686) and *hsp104*^{T160M} (hsp, AG687) [*psi*$^-$] ρ^o recipients. The color-coding in this table is as follows: "green" variants cytoduce well to both WT or *hsp104*^{T160M} recipients; "orange" variants cytoduce well to *hsp104*^{T160M} but not to WT recipients; "blue" variants cytoduce well to WT but not to *hsp104*^{T160M} recipients; and "white" variants cytoduce poorly, but with approximately equal efficiency to both recipients. *$P < 10^{-5}$.

hspB5, hspB7, hspB20; hspA3, hspA4, hspA5, hspA6, hspA7, hspA9, hspA15, hspA19, hspA20). We also found a few such variants among those generated in the WT background (wtB19; wtA3, wtA13). Two of these were very unstable in the WT parent and were apparently stabilized by the *hsp104*^{T160M} mutation. Most variants generated by Sup35NM overexpression in the WT background cytoduced well to both WT and *hsp104*^{T160M} recipients (28 of 40, green variants). The fraction of orange variants among those generated in the *hsp104*^{T160M} background was higher than in variants generated in the WT background [12 of 40 in *hsp104*^{T160M} vs. 3 of 40 isolated in the WT host ($P < 10^{-4}$)]. We also found variants that cytoduced to both recipients poorly, but with near equal efficiency [white variants (shown as white in the tables)]. Surprisingly, we found several variants generated in the *hsp104*^{T160M} background that cytoduced significantly better to WT recipients than to *hsp104*^{T160M} ones (variants hspB1, hspB3, hspB10, hspB13). Apparently, normal Hsp104 curing activity aids their propagation. These variants are reminiscent of a [*PSI*$^+$] described by Borchsenius et al., which requires elevated Hsp104 for its propagation (67).

Ade$^-$ *HSP104* Cytoductants Are [*psi*$^-$]. To confirm that Ade$^-$ cytoductants from an *hsp104*^{T160M} [*PSI*$^+$hhs] strain to a WT recipient were really [*psi*$^-$], we performed back-cytoductions from Ade$^-$ cytoductants (resulting from cytoduction from hspB7 to the WT) (Table 2) to WT and *hsp104*^{T160M} [*psi*$^-$] recipients (Fig. 2). All of the cytoductants and diploids formed were Ade$^-$, including the *hsp104*^{T160M} cytoductants, confirming that [*PSI*$^+$] was really lost upon cytoduction from hspB7 to the WT recipient. The phenotypic assay for [*PSI*$^+$] measures translational read-through of the *ade2-1* mutation that results from the deficiency of translation termination factor Sup35p, produced by its being largely tied up in the amyloid filaments. A phenotypic masking effect would be a direct effect of the *hsp104*^{T160M} mutation on translational read-through. It has been previously shown that *hsp104*^{T160M} has no such [*PSI*+]-independent effect (43).

We also did back-cytoductions from Ade$^+$ cytoductants (Table 3). The orange variant converted to a green variant when transferred to a WT. This result is consistent with the "cloud of variants" model according to which a [*PSI*$^+$] cell contains a mixture of prion variants, with one or another becoming stochastically dominant (68–70). By cytoducing this mixture from the curing-defective *hsp104*^{T160M} host to one with a fully functional Hsp104, we applied selective pressure that eliminated the susceptible (orange) [*PSI*$^+$hhs] variants leaving resistant (green) variants uncured. As expected, passing hspB7 through the *hsp104*^{T160M} recipient maintained the orange character of the [*PSI*$^+$] variant as no new selective pressure was applied by this process. Note that the green character of a [*PSI*$^+$] is not changed in an *hsp104*^{T160M} host.

Loss of [*PSI*$^+$hhs] Was Indeed Because of Hsp104. To make sure that the T160M mutation in *HSP104* and not some other accompanying mutation underlies the inability of our *hsp104*^{T160M} recipient strain to cure a significant fraction of spontaneously appearing [*PSI*$^+$] variants, we used the CRISPR-Cas technique to restore the WT allele of *HSP104* in this particular strain, and performed the cytoductions as previously. The results confirm the primary role of the T160M mutation in making Hsp104 unable to cure some [*PSI*$^+$] variants because transmission of orange prion variants into both WT-CRISPR strains was significantly less efficient compared with cytoduction into *hsp104*^{T160M} recipients, whereas green variants were well propagated by the CRISPR-restored hosts (Table 4).

Strong/Weak, Seed Number, and Hsp104 Hypersensitivity. We do not find a correlation between the strong/weak or stable/unstable nature of a variant and its ability/inability to propagate in the presence of normal levels of WT Hsp104. For example, hspA4 is a strong very stable [*PSI*$^+$] (Fig. 1*A*), but is highly sensitive to curing

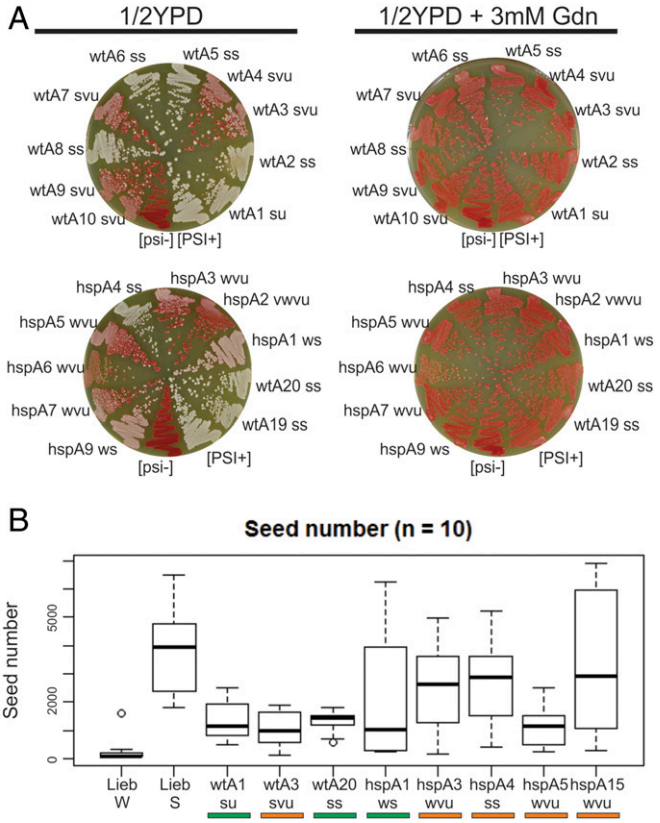

Fig. 1. Characterization of the [*PSI*$^+$] cytoduction donors generated by Sup35NM overexpression in either wt or *hsp104*^{T160M} background (Table 2). [*PSI*$^+$], positive control. [*psi*$^-$], negative control. (*A*) [*PSI*$^+$] prion variants were grown on 1/2 YPD medium with or without 3 mM guanidine HCl (Gdn). Strong [*PSI*$^+$] variants are white and weak variants are pink reflecting the lower or higher amount of the soluble Sup35 in the cytoplasm. Red colonies are prion-free. Only guanidine-curable donors were used in further experiments. Phenotypes: ss, strong stable; su, strong unstable; svu, strong very unstable; ws, weak stable; wu, weak unstable; wvu, weak very unstable; vwvu, very weak very unstable. (*B*) The [*PSI*$^+$] prion seed number determined for the indicated strains (*Methods*). LiebW and LiebS are weak and strong strains from S. Liebman, University of Nevada, Reno, NV.

by normal levels of Hsp104 (Table 2). Similarly, the weak very unstable variant hspA5 (Fig. 1) shows a similar sensitivity to normal levels of WT Hsp104 (Table 2). Furthermore, we did not observe any correlation between seeds/cell and the green vs. orange property of variants (Fig. 1*C*).

Mutability of Hsp104 Hypersensitive [*PSI*$^+$]. We noted the gradual change of orange variants toward green on repeated passage in an *hsp104*^{T160M} host on –Ade medium (Table S1). The difference in cytoduction efficiencies of these variants into WT and *hsp104*^{T160M} recipients gradually decreased with growth. In contrast, we did not observe such changes in the case of green variants. This finding suggests that initially orange variants include a small minority of green variants (the prion cloud), and that the green variants have an advantage over orange variants, even in an *hsp104*^{T160M} host. Note that even the strong, stable orange variant hspA4 gradually becomes green on repeated culturing (Table S1).

Proteins Associated with Hsp104 and Hsp104(T160M). We used coimmunoprecipitation (co-IP) to compare the binding partners of WT Hsp104 and Hsp104^{T160M}. The binding of most interactors with the mutant Hsp104 was approximately as effective as with WT Hsp104 (Table S2). The only protein that consistently co-IP with Hsp104^{T160M} less effectively was Ura2 (aspartate transcarbamylase), a known Hsp90 client (71). Both Hsp90 and its cochaperone, Cpr6, were shown to be able to independently bind Ura2 (71), and the binding of Cpr6 to the mutant Hsp104 was slightly reduced (Table S2), thus leaving the possibility that the decrease in Ura2 binding is a result of somewhat weaker interaction of Cpr6 with Hsp104^{T160M}. As for three other Hsp90 cochaperones, the T160M mutation slightly impairs interaction with Sti1 (Table S2). Hsp104 is a hexamer and, as expected, we detected the binding of Hsp104 to itself that was not changed in the mutant strain (Table S2).

Increased Frequency of Spontaneous [*PSI*$^+$] in *hsp104*^{T160M} Strains. Finding that many variants generated in an *hsp104*^{T160M} strain are curable by normal levels of Hsp104 predicts that the frequency of spontaneous [*PSI*$^+$] generation may be increased in this mutant. We crossed a WT (779-6A [*psi*$^-$] [*PIN*$^+$]) strain with isogenic WT or *hsp104*^{T160M}::*hisG URA3 hisG* [*psi*$^-$] [*pin*–] strains and the spontaneous [*PSI*$^+$] generation frequency was measured for each spore clone (Table 5). As anticipated, the average frequency of [*PSI*$^+$] generation in *hsp104*^{T160M} mutant strains was approximately 10 times higher than in WT (130×10^{-6} vs. 10×10^{-6}, respectively) (Table 5 and Fig. S2). These differences are probably not a result of different variants of [*PIN*$^+$] because each spore clone of a tetrad should get the same [*PIN*$^+$] variant. The cosegregation of elevated [*PSI*$^+$] frequency with *hsp104*^{T160M} again indicates that it is this mutation that allows the orange variants to propagate, and not some adventitious change at another locus. The *hsp104*^{T160M} meiotic segregants were [*PIN*$^+$] (Fig. S1*A*), and [*PSI*$^+$] generation in these strains was still [*PIN*$^+$]-dependent, as no [*PSI*$^+$] colonies were found when their [*pin*$^-$] derivatives were tested (Table 5). Most [*PSI*$^+$]s generated spontaneously in *hsp104*^{T160M} strains were weak (dark pink) and unstable on 1/2 YPD, whereas most [*PSI*$^+$]s generated spontaneously in WT strains were strong (white or slightly pink) and stable (Fig. S1*B*). However, in most cases [*PSI*$^+$] colonies generated in a *hsp104*^{T160M} mutant grew on –Ade medium significantly faster than those generated in WT strains, as previously shown for a standard [*PSI*$^+$] variant (43).

Chaperone Levels in *HSP104* vs. *hsp104*^{T160M}. Potentially, the difference in curing ability of WT and *hsp104*^{T160M} recipient strains could be a result of differences in protein levels of Hsp104 or other chaperones/cochaperones and related proteins between these strains. Western blot analysis (Fig. 3) showed that the levels of Hsp104, Ssa1-4, Sti1, Cpr7, and Ydj1 were not changed in the *hsp104*^{T160M} mutant compared with the isogenic WT, whereas the level of Sis1 was 1.4 times increased, and the level of Sse1/2 was 1.3 times decreased. It is known that having two copies of the *SIS1* gene in the cell does not influence [*PSI*$^+$] propagation (72), so it is unlikely that this slight protein level increase is the cause of the phenotype. Similarly, deletion of *SSE1* has generally detrimental effects on [*PSI*$^+$] generation and propagation (73, 74), so it is unlikely that the slight decrease of Sse1 level leads to better propagation of orange [*PSI*$^+$] variants in *hsp104*^{T160M} than in WT. The antibody used in our work recognizes both Sse1 and Sse2, but without heat shock, *SSE2* transcripts are nearly undetectable and Sse2 is known not to support [*PSI*$^+$] propagation (74). We also find the levels of Sup35 and Hsp104 are approximately the same in the mutant as in the CRISPR-corrected WT (Fig. 3).

Proteins Modulating the Curing of [*PSI*$^+$]s by Normal Levels of Hsp104. To determine whether any of the factors affecting [*PSI*$^+$] curing by overexpressed Hsp104 can modulate [*PSI*$^+$] curing by normal levels of Hsp104, we generated a set of mutant recipients and performed cytoductions into them from green and orange [*PSI*$^+$] donors, as well as from standard [*PSI*$^+$] and [*psi*$^-$] strains (Table 6). Depletion of Ssa1p (in favor of Ssa2p) is known to be detrimental to [*PSI*$^+$] propagation (75). Moreover, overproduction of Ssa1p counteracts curing of [*PSI*$^+$] by overproduction of Hsp104 (38). Consistent with these findings, we also observed that the cytoduction efficiency of some variants (e.g., orange hspB5, hspB7, hspA9; white wtA4, wtA10, hspA2) into *ssa1Δ* was impaired compared with that into a WT recipient. Sgt2p is known to help overproduced Ssa1p antagonize Hsp104 overproduction curing of [*PSI*$^+$], indicating a

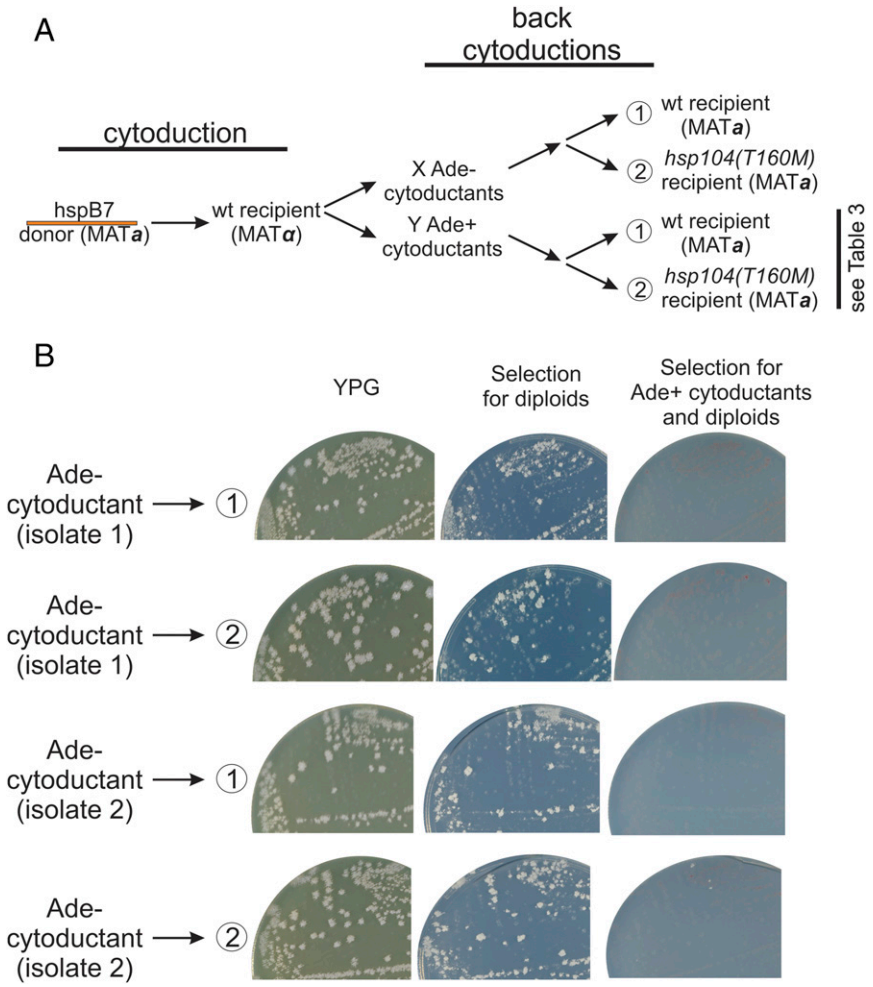

Fig. 2. Ade$^-$ phenotype of cytoductants is because of loss of [*PSI*$^+$hhs]. Cytoduction of Hsp104 hypersensitive [*PSI*$^+$] from *hsp104*^{T160M} to a WT recipient produced mostly Ade$^-$ clones, but also a minority of Ade$^+$ cytoductants (Tables 1 and 2). Back-cytoductions from the Ade$^-$ cytoductants into either WT or *hsp104*^{T160M} recipients produced only Ade$^-$ cytoductants and diploids. (*A*) The scheme of the back-cytoduction experiment. The hspB7 orange prion variant was cytoduced into WT recipient resulting in 126 Ade$^-$ cytoductants and 59 Ade$^+$ cytoductants. Two Ade$^-$ (see *B*) and several Ade$^+$ cytoductants (Table 3) were used as donors and were back-cytoduced into WT and *hsp104*^{T160M} recipients. (*B*) The results of the back-cytoduction of Ade$^-$ cytoductants. Cytoductant colonies grow on YPG medium (ρ^+) but not on medium selective for diploids (*Methods*). The back-cytoduction of the Ade$^-$ cytoductants into their original *hsp104*^{T160M} background did not result in any Ade$^+$ cytoductants. This proves that their Ade$^-$ phenotype is a result of the loss of [*PSI*$^+$] and is not because of phenotype masking.

pro-[*PSI*$^+$] activity for Sgt2 (39). We found that most orange variants (e.g., wtA3, hspA4, hspA5, hspA15, hspA19) cytoduced into *sgt2Δ* nearly as efficiently as into the *hsp104*^{T160M} recipient, and generally better than into the WT. Several variants (e.g., white wtA10 and hspA14; orange hspA3, hspA4, hspA5, hspA6 and hspA7) cytoduced into *sti1Δ* significantly better than into the WT recipient. These results suggest that Sgt2 and Sti1 are helping, whereas Ssa1 is counteracting the curing of some [*PSI*$^+$] variants by the normal level of Hsp104. Most orange variants (hspB5, wtA3, wtA7, hspA3, hspA4, hspA5, hspA6, hspA7, hspA9, hspA17, hspA19) cytoduced into *sis1*$^{\Delta 338–352}$ recipients nearly as efficiently as into *hsp104*^{T160M} (Table 6), consistent with this deletion's known total abrogation of Hsp104 overproduction curing (72). Thus, Sis1 helps the normal level of Hsp104 to cure many orange [*PSI*$^+$] variants.

We could not find evidence for the involvement of Btn2 and Cur1 in curing of [*PSI*$^+$] by the normal level of Hsp104 because both green and orange variants that we've tested cytoduced with similar efficiency into 74-D694 WT and 74-D694 *btn2Δ cur1Δ* recipients (Table S3). Deletion of *HSP42* does not substantially affect the propagation of any variants tested (Table 7), consistent with our earlier findings (64).

Yeast cells need at least one of the highly similar isoforms of Hsp90 (*HSP82* and *HSC82*) to be viable (76), so we used the strains in which the *hsp82Δ hsc82Δ* double deletion was compensated by either a WT or mutant copy of *HSP82* expressed under the control of its native promoter from a centromere plasmid. We used either *hsp82ΔMEEVD* or *hsp82*^{W585T} mutants in our experiments. The C-terminal MEEVD motif of Hsp82 is critical for interaction with tetratricopeptide repeat domain-containing cochaperones such as Cpr7, Sti1, Cpr6, and Cns1, so its deletion disturbs these interactions (45). Hsp82^{W585T} protein is defective in client binding and chaperone activity (77). Mutant Hsp82 results in increased cytoduction efficiency of orange [*PSI*$^+$] variants, suggesting that Hsp82 is involved in the Hsp104 normal-level curing (Table 7), as it has previously been shown to be involved in Hsp104 overexpression curing of [*PSI*$^+$] (47).

Discussion

The lethal potential of yeast prion infections (54) suggested that cellular defense mechanisms should have evolved. Because overproduction of Hsp104 cures [*PSI*$^+$] (18, 19), and the mechanism of this curing appears to be distinct from that of its prion propagation-promoting activity (43) (see *Introduction*), we suspected that normal levels of Hsp104 might be curing some [*PSI*$^+$] variants as they arise.

We isolated [*PSI*$^+$] variants in an *hsp104*^{T160M} mutant shown by Hung and Masison (43) to lack the [*PSI*$^+$]-curing activity, and

Table 3. Back-cytoductions from Ade$^+$ guanidine-curable cytoductants

[*PSI*$^+$] donor of original cytoduction	Recipient of first and Ade$^+$ donor of second cytoduction	Final recipient: wt	Final recipient: hsp104^{T160M}
		%Ade$^+$ (total cytoductants)	
(hspB7 →	wt) 1 →	92 (37)	89 (27)
(hspB7 →	wt) 3 →	89 (35)	73 (55)
(hspB7 →	hsp) 1 →	47 (32)*	90 (41)
(hspB7 →	hsp) 2 →	31 (32)*	89 (27)
(hspB7 →	hsp) 3 →	40 (25)*	100 (16)
(wtB2 →	wt) 1 →	94 (34)	92 (36)
(wtB2 →	wt) 2 →	69 (48)	94 (35)
(wtB2 →	wt) 3 →	100 (21)	81 (21)
(wtB2 →	wt) 4 →	83 (42)	90 (31)
(wtB2 →	hsp) 1 →	97 (34)	100 (27)
(wtB2 →	hsp) 2 →	91 (64)	100 (32)
(wtB2 →	hsp) 3 →	91 (21)	100 (18)

Some cytoductants of Hsp104 hypersensitive [*PSI*+]s (e.g., hspB7) to WT recipients remained Ade$^+$. These cytoductants were used as donors to WT (AG679) and *hsp104*^{T160M} (AG680) recipients. The results indicate that the [*PSI*$^+$]s were no longer Hsp104 hypersensitive. Controls were Ade$^+$ cytoductants originating in WT cells. *$P < 10^{-5}$.

tested each for ability to infect isogenic WT or mutant hosts. We found that most [*PSI*$^+$] variants arising spontaneously in this mutant could not propagate in a WT host. These variants would arise in a WT strain but most would be quickly eliminated. Nonetheless, we did find a few [*PSI*$^+$hhs] variants arising in the WT host. Like the elevated frequency of [*URE3*] in *btn2*Δ *cur1*Δ strains, we found that the frequency of [*PSI*$^+$] arising spontaneously is about 10-fold higher in *hsp104*^{T160M} strains than in isogenic WT, a phenotype that cosegregates with *hsp104*^{T160M} and is eliminated by correcting the mutation. However, although Btn2p and Cur1p selectively cure [*URE3*] variants having low seed number, there is no such correlation for Hsp104 curing, with both high and low seed variants cured and not cured.

Because the same *hsp104*^{T160M} mutant lacks both the overproduction curing activity and the ability to cure [*PSI*$^+$hhs] variants, it seemed likely that the same Hsp104 activity was carrying out each of these processes. We further tested this notion by examining the influence on the latter process of other factors known to influence the former. Sti1p, a cochaperone of Hsp90s, Hsp70s, and Hsp104, has been shown to promote the curing of [*PSI*$^+$] by overproduction of Hsp104 (46, 47), but not to be necessary for propagation of [*PSI*$^+$] (46, 47, 78). We find that Sti1p is required for efficient elimination of most [*PSI*$^+$hhs] variants by normal levels of WT Hsp104 (Table 6). Hsp90 is involved in Hsp104 overproduction curing of [*PSI*$^+$] (47) and two mutations in Hsp90 also diminish the sensitivity to normal levels of Hsp104 of [*PSI*$^+$hhs] variants (Table 7). Furthermore, the *sis1Δ338–352* mutation that eliminates Hsp104 overproduction curing (72) also reduces the ability of cells with normal levels of WT Hsp104 to cure [*PSI*$^+$hhs]. These results argue that the Hsp104 activity that cures [*PSI*$^+$hhs] is the same as that which cures all [*PSI*$^+$] variants on overproduction of Hsp104.

Although our work does not specifically address the mechanism, the fact that [*PSI*$^+$hhs] curing occurs without Hsp104 overproduction suggests that overproduction is not an inherent component of the mechanism. The *hsp104*^{T160M} mutation was originally isolated by its suppressing the [*PSI*$^+$]-destabilizing dominant *SSA1-21* mutation, and *sti1*, *hsp90*, and *sis1* mutations had a similar effect (43, 47, 72). This earlier work also suggested that Hsp104 is part of an antiprion system that does not require overproduction to be active. Likewise, our [*PSI*$^+$hss] variants are distinct from the nonsense-suppression observed by Salnikova et al. on sustained overexpression of Sup35p or Sup35NM (79).

Prion variants are central to the prion phenomenon. Properties tested for a single [*PSI*$^+$] variant, for example, may not be representative of the whole range of [*PSI*$^+$] variants. The commonly studied [*URE3-1*], originally isolated by Francois Lacroute (80), is evidently not cured by normal levels of Btn2p or Cur1p, but most [*URE3*] variants arising in the absence of these two proteins are cured by simply restoring their normal levels (64). Transmission of [*URE3*] across a species barrier also varies substantially with the prion variant (81). Similarly, transmission of [*PSI*$^+$] from one Sup35p sequence polymorph to another is a rather low frequency event for one [*PSI*$^+$] prion variant, but other [*PSI*$^+$] variants are transmitted at high efficiency (82). One [*PSI*$^+$] variant is very

Table 4. Cytoductions into CRISPR-CAS – corrected and uncorrected recipients

Donors	Recipients: wt*	*hsp104*^{T160M}*	*hsp104*^{T160M} Ura$^-$	wt-CR11	wt-CR12
	% Ade$^+$ cytoductants (total cytoductants)				
779-6A [PSI+] ss	91 (64)	97 (121)	100 (13)	94 (31)	95 (56)
779-6A [psi-]	0 (70)	8 (73)	0 (44)	0 (42)	0 (22)
wtB2	99 (167)	100 (123)	99 (71)	90 (57)	98 (55)
hspB2	100 (137)	100 (161)	95 (39)	82 (49)	99 (69)
hspB5	33 (180)	87 (230)	83 (30)	5 (80)‡	58 (45)‡
hspB7	32 (185)	98 (95)	100 (19)	13 (64)‡	21 (57)‡
wtA6 ss	100 (22)	100 (36)	100 (37)	96 (56)	100 (101)
wtA20 ss	100 (14)	100 (18)	91 (34)	97 (64)	94 (53)
hspA1 ws	100 (48)	100 (20)	100 (23)	87 (55)	100 (63)
hspA3 vwvu	23 (48)	100 (36)	83 (72)	50 (113)‡	49 (57)‡
hspA4 su	30 (27)	100 (33)	100 (29)	20 (54)‡	46 (24)‡
hspA5 vwvu	14 (65)	100 (31)	71 (49)	40 (73)‡	54 (68)†

To confirm that the loss of the hypersensitive [*PSI*$^+$] variants from WT recipients was because of their normal Hsp104, the *hsp104*^{T160M} mutant recipient was converted to WT using CRISPR-CAS gene editing (see *SI Methods*) and several [*PSI*$^+$] variants were introduced by cytoduction into the mutant strain (*hsp104*^{T160M} Ura$^-$, AG730) and two corrected WT strains (C11, C12; AG780, AG781).

*The data for the cytoductions from green and orange donors to these WT and *hsp104*^{T160M} recipients are the same as in Table 2. The statistics relate the CRISPR-corrected recipient strains wt-CR11 and wt-CR12 to the mutant parent, *hsp104*^{T160M} Ura$^-$ (strain AG730).

†$P < 10^{-5}$.

‡$P < 10^{-4}$.

Table 5. Cosegregation of elevated spontaneous [*PSI*$^+$] generation and *hsp104*^{T160M}

No.	Spore clone	MAT	*HSP104*	[PSI$^+$] per 10^6 cells*
1	1.A1	α	WT	4.7
2	1.A2	a	WT	0.3
3	1.A3	a	WT	0.3
4	1.A4	α	WT	5.3
5	3.A1†	α	WT	12
6	3.A2†	a	WT	7
7	3.A3†	a	T160M	500
8	3.A4†	α	T160M	120
9	2A	α	WT	1
10	2B	a	T160M	140
11	2C	a	WT	33
12	2D	α	T160M	80
13	6A	α	WT	2
14	6B	a	WT	59
15	6C	α	T160M	96
16	6D	a	T160M	55
17	10A	a	WT	2
18	10B	a	T160M	91
19	10C	α	T160M	89
20	10D	α	WT	1
21	11A	α	WT	31
22	11B	a	T160M	53
23	11C	α	T160M	120
24	11D	a	WT	3
25	14A	a	WT	5
26	14B	α	T160M	95
27	14C	α	WT	12
28	14D	a	T160M	140
29	17A	α	WT	5
30	17B	a	WT	12
31	17C	a	T160M	100
32	17D	α	T160M	210
33	19A	α	WT	2
34	19B	a	WT	3
35	19C	a	T160M	91
36	19D	α	T160M	110
37	8–1 [pin-]†	a	T160M	0
38	8–2 [pin-]	a	T160M	0

Meiotic tetrads of strains AG457 × AG417 (nos. 1–4) and AG457 × AG478 (nos. 5–38) were plated for Ade$^+$ colonies. The Ade$^+$ clones were tested for guanidine-curability.
*[*PSI*$^+$] is guanidine-curable Ade$^+$.
†See Fig. S1*A*.

unstable in WT cells but is stabilized by Hsp104 overproduction (83). In our study, we find that most spontaneously arising [*PSI*$^+$] isolates in the *hsp104*^{T160M} mutant are cured by normal levels of WT Hsp104. These Hsp104 hypersensitive variants include both strong and weak [*PSI*$^+$] variants and those with high or low seed number. It is not yet clear what structural aspects determine sensitivity to this antiprion system. Similarly, each of the four [*PSI*$^+$] transmission variant types included strong and weak variants (68). By culling most spontaneous [*PSI*$^+$] variants as they arise, Hsp104 evidently limits the potential damage to the cell.

We find that the Hsp104 hypersensitivity trait is not as stable as some other [*PSI*$^+$] characteristics, apparently because even in the *hsp104*^{T160M} mutant, there is selection against such variants. Conditional-lethal [*PSI*$^+$] variants are gradually either lost or convert to a less pathogenic form, even under the "permissive condition" (54). It is likely that the permissive condition does not completely avoid the prion's toxicity, so that there is continuing selection for loss or milder variants. Similarly, toxic [*URE3*] variants rapidly change or are lost as mitotic segregants with an altered variant are selected for (54). Similar instability has been noted for many other prion traits, including the transmission phenotype of [*PSI*$^+$] (68) and Btn2/Cur1-sensitivity of [*URE3*] (64).

Sse1p is a chaperone and nucleotide exchange factor that has an interesting parallel with Hsp104. Sse1p is both necessary for [*URE3*] propagation and cures [*URE3*] when overproduced (84). SseI is necessary for the propagation of weak [*PSI*$^+$], but not of strong variants (73, 84). However, overproduction of Sse1p, far from curing [*PSI*$^+$], greatly stimulates the generation of [*PSI*$^+$] (73). Completing this parallel, we note that overproduction of Hsp104 increases [*URE3*] generation (85). This is thus another example of a chaperone shaping the spectrum of prion variants.

Although there are now many means of curing yeast prions, few have been shown to be naturally acting curing mechanisms, in contrast to artificial imbalance of components involved in or affecting the prion propagation process. These antiprion systems, like DNA repair systems, are not completely effective, but certainly lower the burden of prions on the host, and shape the array of prion variants that will succeed in arising. The existence of at least two anti-[*PSI*$^+$] systems argues that the cell does not view [*PSI*$^+$] as an unalloyed blessing.

Methods

Yeast Culture and Genetic Manipulation. Strains of *S. cerevisiae* are listed in Table S4 and growth media are as described by Sherman (86). Yeast were grown at 30 °C. Knockout mutations (87) were transferred by PCR amplification, transformation of the target strain, and confirmation, using PCR, of the absence of the normal allele and the presence, in the correct location, of the mutant allele.

Cytoduction Donor Isolation. All [*PSI*$^+$] variant strains used as cytoduction donors were isolated in either WT strain AG664 or *hsp104*^{T160M} strain AG663 (both [*psi*$^-$] [*PIN*$^+$] and isogenic to 779-6A) (Table S4). To isolate spontaneously arising [*PSI*$^+$] variants, or to measure its frequency, 10^5, 10^6, and 10^7 [*psi*$^-$] cells were plated on –Ade (SD + HLU) medium. To raise the frequency of [*PSI*$^+$] generation, AG664 and AG663 were transformed with pHK006 (*LEU2* P_{Gal1}-*SUP35NM*), grown in YPAGal (1% yeast extract, 2% peptone, 0.004% adenine sulfate, 2% galactose) overnight, and plated on –Ade medium. After 5–6 d at 30 °C when most Ade$^+$ colonies were at least 2–3 mm in diameter, clones were transferred into separate wells of 96-well plates containing 15% glycerol. Using a 48-pin replicator tool Ade$^+$ isolates were stamped onto –Ade to double-check the Ade$^+$ phenotype, 1/2 YPD to estimate the strength of the (presumed) [*PSI*$^+$], 1/2 YPD with 3 mM guanidine hydrochloride to find the guanidine-curable (=[*PSI*$^+$]) isolates, and YPG to confirm an intact mitochondrial genome that would be needed for cytoduction. The 96-well plates containing the Ade$^+$ isolates were stored at –80 °C. To minimize the growth of the yeast cells, which could potentially change the [*PSI*$^+$] variant, before each set of cytoduction experiments we thawed the 96-well plates at room temperature and "stamped" the isolates to –Ade plates, grew them for 4–5 d, suspended the cells in water, and used as cytoduction donors.

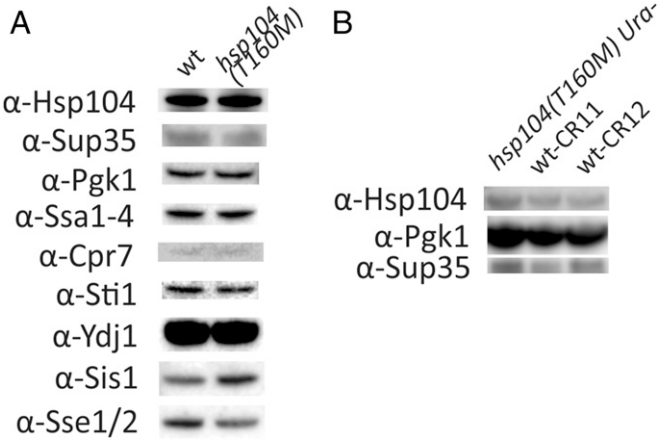

Fig. 3. Western blot analysis of the protein levels in the WT and *hsp104*^{T160M} strains. (*A*) The comparison of the protein levels in the original WT and *hsp104*^{T160M} strains. (*B*) The comparison of the protein levels in the strains in which WT allele of *HSP104* was restored using the CRISPR-Cas technique (wt-CR11 and wt-CR12) and their parent strain (*hsp104*^{T160M} Ura$^-$) (*Methods*).

Cytoduction. Donors and recipients were isogenic (to 779-6A in most cases) (Table S4). The donors of spontaneous [*PSI*$^+$] variants carried the *TRP1* vector pRS314. Donors of induced [*PSI*$^+$] variants also carried the *LEU2* plasmid pHK006 (bearing P_{Gal1}-*SUP35NM* which is not expressed on glucose-containing media) (Table S5). The recipient carried the *HIS3* plasmid pRS313 to allow selection against donors and distinction of diploids and recipients. Recipients were made ρ^0 by growth on media containing 25 μg/mL ethidium bromide, and transfer of mitochondrial DNA, as assayed by growth on glycerol, was used as an indicator that cytoplasm had been transferred from donor cells. Donor and recipients were mixed in water with a modest excess of donor cells, and spotted on a YPAD plate. After 6- to 8-h incubation at 30 °C, the mixture was streaked for single colonies on media selecting against the donor. Colonies were replica-plated to YPG, media selective for diploids (two types: one was –Leu and the other was –Trp) and –Ade. Clones growing on YPG but not on the media selective for

Table 6. Effects of other chaperones and cochaperones on Hsp104 normal level curing of [*PSI*$^+$]

	Recipients								
Donors	WT*	*hsp104 T160M*	*ssa1Δ*	*sgt2Δ*	*sgt2Δ hsp104 T160M*	*sti1Δ*	*cpr7Δ*	*cpr7Δ hsp104 T160M*	*sis1 Δ338–352*
	% Ade$^+$ cytoductants (total cytoductants)								
779-6A [*PSI*$^+$]	91 (64)	97 (121)	97 (32)	98 (51)	58(109)	95 (62)	92 (13)	100 (95)	100 (12)
779-6A [*psi*$^-$]	0 (70)	8 (73)	0 (41)	0 (25)	9 (35)	5 (39)	0 (21)	2 (57)	2 (102)
wtB2	99 (167)	100 (123)	100 (20)	93 (14)	100 (21)	100 (12)	100 (18)	100 (39)	98 (44)
hspB2	100 (137)	100 (161)	100 (19)	97 (31)	100 (11)	100 (26)	89 (18)	100 (31)	100 (45)
hspB5	33 (180)	87 (230)	0 (35)†	51 (51)†	100 (11)	20 (20)	0/2	100 (52)	80 (10)†
hspB7	32 (185)	98 (95)	10 (47)†	23 (31)	100 (28)	14 (29)	31 (13)	100 (5)	20 (49)
wtA1 ss	97 (30)	100 (20)	97 (39)	100 (13)	77 (35)	92 (26)	75 (12)	100 (25)	50 (10)
wtA2 ss	95 (41)	100 (14)	100 (33)	100 (35)	100 (16)	92 (25)	100 (15)	85 (20)	95 (43)
wtA3 wu	10 (20)	59 (17)	16 (32)	74 (19)†	30 (53)	23 (13)	13 (23)	20 (5)	30 (27)‡
wtA4 su	33 (42)	16 (62)	4 (29)	70 (43)	17 (36)	19 (26)	6 (35)	9 (22)	22 (27)
wtA5 ss	100 (16)	100 (16)	100 (32)	100 (21)	100 (48)	100 (16)	71 (14)	100 (28)	49 (33)
wtA6 ss	100 (22)	100 (36)	100 (44)	100 (9)	100 (77)	95 (58)	100 (14)	98 (60)	98 (57)
wtA7 wvu	0 (22)	18 (50)	5 (43)	48 (23)†	16 (45)	25 (16)	52 (19)	28 (29)	23 (57)†
wtA8 ss	100 (16)	100 (21)	100 (26)	100 (22)	95 (38)	73 (30)	89 (19)	96 (25)	69 (13)
wtA9 svu	17 (47)	9 (45)	3 (72)	38 (32)	18 (39)	22 (9)	5 (22)	20 (25)	20 (10)
wtA10 svu	20 (30)	17 (46)	0 (43)	27 (15)	42 (36)	90 (20)	0 (17)	100 (20)	25 (4)
wtA11 ss	95 (19)	92 (39)	100 (62)	95 (20)	100 (59)	93 (27)	60 (15)	100 (48)	94 (49)
wtA12 ss	98 (40)	100 (29)	98 (44)	89 (36)	80 (50)	67 (12)	92 (13)	56 (9)	33 (9)
wtA13 ss	81 (73)	100 (19)	100 (22)†	100 (15)†	91 (67)	90 (20)	26 (23)	94 (33)	97 (34)†
wtA14 ss	100 (18)	98 (45)	100 (24)	100 (27)	100 (71)	96 (45)	94 (17)	96 (27)	94 (16)
wtA15 wu	52 (38)	30 (37)	38 (8)	75 (4)	21 (24)	56 (16)	0 (10)	38 (24)	49 (39)
wtA16 ss	91 (21)	100 (15)	100 (35)	100 (35)	98 (44)	91 (43)	89 (18)	15 (33)	90 (20)
wtA17 su	85 (20)	100 (17)	100 (25)	88 (40)	100 (21)	62 (21)	56 (25)	59 (17)	73 (15)
wtA18 ss	94 (36)	100 (18)	97 (37)	100 (14)	97 (35)	94 (35)	48 (23)	100 (27)	58 (6)
wtA19 ss	100 (28)	100 (25)	100 (17)	73 (37)	77 (26)	81 (21)	41 (17)	88 (17)	100 (32)
wtA20 ss	100 (14)	100 (18)	100 (17)	100 (16)	100 (33)	48 (92)	52 (16)	96 (27)	100 (22)
hspA1 ws	100 (48)	100 (20)	100 (14)	100 (29)	100 (28)	98 (58)	100 (15)	99 (103)	97 (31)
hspA2 vwvu	60 (32)	41 (37)	21 (28)	78 (36)	2 (50)	71 (17)	33 (21)	97 (31)	90 (29)
hspA3 vwvu	23 (48)	100 (36)	52 (31)†	50 (26)†	91 (34)	54 (59)†	21 (62)	81 (48)	47 (76)†
hspA4 su	30 (27)	100 (33)	29 (48)	100 (14)†	96 (27)	67 (30)†	4 (24)	96 (26)	74 (38)†
hspA5 vwvu	14 (65)	100 (31)	23 (48)	100 (18)†	87 (71)	65 (89)†	8 (48)	93 (56)	86 (92)†
hspA6 vwvu	0 (25)	20 (15)	24 (17)	32 (19)	0 (17)	45 (20)†	6 (17)	19 (36)	48 (21)†
hspA7 vwvu	24 (17)	100 (18)	14 (29)	29 (21)	94 (36)	47 (43)§	4 (26)	98 (42)	100 (19)†
hspA8 ws	100 (21)	100 (23)	100 (32)	68 (44)	100 (26)	80 (39)	100 (15)	100 (22)	40 (52)
hspA9 ws	41 (32)	94 (18)	7 (31)	57 (23)	100 (24)	90 (19)†	20 (15)	94 (33)	90 (20)†
hspA10 wu	100 (16)	96 (23)	89 (18)	100 (12)	94 (34)	93 (27)	47 (15)	100 (26)	50 (58)
hspA11 wu	96 (50)	100 (30)	100 (24)	100 (17)	98 (51)	95 (38)	100 (9)	97 (36)	78 (45)
hspA12 ws	100 (24)	100 (32)	100 (21)	100 (65)	100 (53)	90 (39)	100 (11)	100 (18)	98 (48)
hspA13 wvu	64 (28)	78 (32)	49 (41)	56 (27)	68 (47)	96 (22)	60 (15)	50 (26)	76 (46)
hspA14 wvu	13 (32)	17 (23)	34 (38)	17 (23)	27 (45)	78 (27)	87 (15)	45 (20)	54 (37)
hspA15 wvu	49 (39)	100 (17)	75 (16)	100 (23)†	83 (47)	63 (32)	20 (30)	83 (29)	49 (69)
hspA16 wu	96 (46)	86 (21)	93 (15)	87 (23)	100 (49)	82 (27)	12 (17)	88 (33)	87 (31)
hspA17 su	41 (44)	100 (17)	63 (19)‡	60 (37)§	100 (18)	84 (19)	100 (18)	92 (24)	85 (27)†
hspA18 vwvu	48 (31)	24 (46)	22 (27)	60 (25)	33 (39)	70 (33)	69 (13)	4 (28)	94 (64)†
hspA19 vwvu	25 (53)	82 (22)	11 (19)	89 (26)†	87 (46)	39 (33)	17 (18)	87 (23)	94 (47)†
hspA20 vu	70 (30)	100 (11)	58 (19)	100 (20) 0.01	100 (8)	69 (16)	85 (27)	84 (25)	73 (11)

Because *sti1*Δ, *cpr7*Δ and *sis1*Δ*3338–352* each are reported to prevent curing of [*PSI*$^+$] by overproduction of Hsp104, we tested whether these mutations affect transmission of [*PSI*$^+$] variants hypersensitive to Hsp104. Some probabilities shown as, for example, "(81%).05," where 0.05 is the *P* value.

*The data for cytoductions from green and orange donors to WT and *hsp104*^{T160M} recipients are the same as in Table 2.

$^\dagger P < 10^{-5}$.

$^\ddagger P < 10^{-4}$.

$^\S P < 10^{-3}$.

Table 7. Hsp90 is necessary for efficient elimination of [*PSI*$^+$hhs] by normal levels of Hsp104

	Recipient					
			hsp82Δ hsc82Δ			
Donor	WT* (%)	*hsp104*^{T160M}* (%)	p[*HSP82*] (%)	p[*hsp82 ΔMEEVD*] (%)	p[*hsp82*^{W585T}] (%)	*hsp42Δ* (%)
779-6A [*PSI*$^+$]	91 (64)	97 (121)	72 (37)	100 (91)	98 (88)	92 (36)
779-6A [*psi*$^-$]	0 (70)	8 (73)	0 (48)	0 (76)	0 (49)	0 (53)
wtB2	99 (167)	100 (123)	80 (74)	100 (51)	99 (76)	100 (56)
hspB2	100 (137)	100 (161)	88 (60)	97 (109)	92 (82)	100 (39)
hspB5	33 (180)	87 (230)	55 (58)	79 (122)†	88 (86)†	64 (39)
hspB7	32 (185)	98 (95)	64 (50)	84 (313)†	89 (72)†	22 (23)

*The data for cytoductions from green and orange donors to WT and *hsp104*^{T160M} recipients are the same as in Table 2; the data for cytoductions from 779-6A [*PSI*$^+$] and [*psi*−] to WT and *hsp104*^{T160M} recipients are the same as in Table 6.
$^\dagger P < 10^{-5}$. Comparisons are shown between the *hsp82Δ hsc82Δ* strain complemented by WT *HSP82* and mutant *hsp82* plasmids.

diploids were cytoductants. Colonies that grew on –Ade media propagated [*PSI*$^+$]. The back-cytoduction recipients carried the *TRP1* vector pRS314 as a marker and, like the forward-cytoduction recipients, were ρ^0.

Statistics. We used the binomial distribution to assess the significance of differences of transmission frequency between strains or of frequency differences of a yes/no type between groups. For example, whether an orange variant's transmission to a WT recipient was significantly different from transmission to an isogenic hsp104 T160M recipient usually used this method. The mean transmission frequency (p), of the total population (adding cytoductants to both mutant and WT), the total number of cytoductants (N), and the binomial distribution allows calculation of the SD = sqrt[$p(1 - p)N$]. If the sample sizes are sufficiently large [i.e., if $p(1 - p)N > \sim 10$], the actual differences of the mean transmission frequency to WT and mutant would show a normal distribution, with the SD calculated above and a mean of 0 if such differences were a result of chance. Using an online normal distribution calculator, this allows calculation of the likelihood that the observed difference is a result of chance.

Seed Number Measurement. The [*PSI*$^+$] strains were streaked to single colonies on 1/2 YPD medium supplemented with 3 mM guanidine hydrochloride. Individual colonies, with the underlying agar cube, were cut out of the plates, and the cells were suspended in water and plated on –Ade medium. The number of Ade$^+$ colonies estimates the number of [*PSI*$^+$] prion seeds contained by the cell which gave rise to the colony. For each strain, at least 10 individual colonies were tested (88).

Western Blot Analysis. Yeast were grown overnight at 30 °C in YPAD, washed with water, and suspended in Disruption buffer [25 mM Tris·Cl pH 7.5, 150 mM NaCl, 1 mM DTT, 10 mM $MgCl_2$, 1.2 mg ATP/mL, 1× Halt Protease Inhibitor Mixture (Thermo), one tablet Complete Mini Protease Inhibitor EDTA free (Roche)/8 mL, and 10 mM Prefabloc]. The cell suspension was placed, with glass beads, in screw-cap 2-mL tubes, and disrupted using a Bead Beater homogenizer (3 min, 4 °C). Protein concentrations were determined using the BCA assay and equalized between the samples using Disruption buffer. The samples were analyzed on a polyacrylamide gel and transferred to a PVDF membrane. The primary and the secondary antibodies used to perform the membrane staining are listed in Table S6.

Proteins Co-IP with Hsp104 and Hsp104^{T160M}. Cells of strain AG686 (WT) and AG687 (hsp104T160M) were grown and extracts made as described above for Western blots. Forty microliters of extract was mixed with 1 μL of anti-Hsp104 antibody and 460 μL of Disruption buffer (see Western blot method described above) in a 2-mL screw-cap tube and incubated overnight at 4 °C with slow mixing. Magnetic beads from the Pierce Classic Magnetic IP/co-IP kit were washed two times with 1 mL of Washing buffer (Disruption buffer without the protease inhibitors or detergents), the extract–antibody mixture was added to these beads, and the suspension was incubated for 1 h at room temperature with rotation. Beads were then washed three times with the Washing buffer, and proteins were eluted with 8 M urea in 100 mM Tris·Cl pH 7.5. Protein components were then subjected to proteolysis, on-column postdigestion reductive di-methylation, and quantitative proteomics essentially using approaches described by others (89–91), with details provided in *SI Methods*.

ACKNOWLEDGMENTS. We thank Eric Anderson (National Institute of Diabetes and Digestive and Kidney Diseases) for mass spectometry analysis; Harold Smith (National Institute of Diabetes and Digestive and Kidney Diseases) for next-generation sequencing; Sue Liebman for strains and antibodies; and Elizabeth Craig (University of Wisconsin) and Jeff Brodsky (Pittsburgh University) for antibodies. This work was supported by the Intramural Program of the National Institute of Diabetes and Digestive and Kidney Diseases.

1. Wickner RB (1994) [URE3] as an altered URE2 protein: Evidence for a prion analog in Saccharomyces cerevisiae. *Science* 264:566–569.
2. King C-Y, et al. (1997) Prion-inducing domain 2-114 of yeast Sup35 protein transforms in vitro into amyloid-like filaments. *Proc Natl Acad Sci USA* 94:6618–6622.
3. Paushkin SV, Kushnirov VV, Smirnov VN, Ter-Avanesyan MD (1997) In vitro propagation of the prion-like state of yeast Sup35 protein. *Science* 277:381–383.
4. Glover JR, et al. (1997) Self-seeded fibers formed by Sup35, the protein determinant of [*PSI*+], a heritable prion-like factor of *S. cerevisiae*. *Cell* 89:811–819.
5. King CY, Diaz-Avalos R (2004) Protein-only transmission of three yeast prion strains. *Nature* 428:319–323.
6. Tanaka M, Chien P, Naber N, Cooke R, Weissman JS (2004) Conformational variations in an infectious protein determine prion strain differences. *Nature* 428:323–328.
7. Edskes HK, Gray VT, Wickner RB (1999) The [URE3] prion is an aggregated form of Ure2p that can be cured by overexpression of Ure2p fragments. *Proc Natl Acad Sci USA* 96:1498–1503.
8. Masison DC, Wickner RB (1995) Prion-inducing domain of yeast Ure2p and protease resistance of Ure2p in prion-containing cells. *Science* 270:93–95.
9. Brachmann A, Baxa U, Wickner RB (2005) Prion generation in vitro: Amyloid of Ure2p is infectious. *EMBO J* 24:3082–3092.
10. Derkatch IL, Bradley ME, Hong JY, Liebman SW (2001) Prions affect the appearance of other prions: The story of [PIN(+)]. *Cell* 106:171–182.
11. Derkatch IL, Bradley ME, Zhou P, Chernoff YO, Liebman SW (1997) Genetic and environmental factors affecting the de novo appearance of the [*PSI*+] prion in *Saccharomyces cerevisiae*. *Genetics* 147:507–519.
12. Shewmaker F, Wickner RB, Tycko R (2006) Amyloid of the prion domain of Sup35p has an in-register parallel β-sheet structure. *Proc Natl Acad Sci USA* 103:19754–19759.
13. Baxa U, et al. (2007) Characterization of β-sheet structure in Ure2p1-89 yeast prion fibrils by solid-state nuclear magnetic resonance. *Biochemistry* 46:13149–13162.
14. Wickner RB, Dyda F, Tycko R (2008) Amyloid of Rnq1p, the basis of the [*PIN*$^+$] prion, has a parallel in-register β-sheet structure. *Proc Natl Acad Sci USA* 105:2403–2408.
15. Gorkovskiy A, Thurber KR, Tycko R, Wickner RB (2014) Locating folds of the in-register parallel β-sheet of the Sup35p prion domain infectious amyloid. *Proc Natl Acad Sci USA* 111:E4615–E4622.
16. Wickner RB, Shewmaker F, Kryndushkin D, Edskes HK (2008) Protein inheritance (prions) based on parallel in-register β-sheet amyloid structures. *BioEssays* 30:955–964.
17. Wickner RB, et al. (2013) Amyloids and yeast prion biology. *Biochemistry* 52:1514–1527.
18. Chernoff YO, Ono B-I (1992) Dosage-dependent modifiers of PSI-dependent omnipotent suppression in yeast. *Protein Synthesis and Targeting in Yeast*, eds Brown AJP, Tuite MF, McCarthy JEG (Springer, Berlin), pp 101–107.
19. Chernoff YO, Lindquist SL, Ono B, Inge-Vechtomov SG, Liebman SW (1995) Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi+]. *Science* 268:880–884.
20. Sanchez Y, Lindquist SL (1990) HSP104 required for induced thermotolerance. *Science* 248:1112–1115.
21. Glover JR, Lindquist S (1998) Hsp104, Hsp70, and Hsp40: A novel chaperone system that rescues previously aggregated proteins. *Cell* 94:73–82.
22. Schlieker C, Tews I, Bukau B, Mogk A (2004) Solubilization of aggregated proteins by ClpB/DnaK relies on the continuous extraction of unfolded polypeptides. *FEBS Lett* 578:351–356.
23. Lum R, Niggemann M, Glover JR (2008) Peptide and protein binding in the axial channel of Hsp104. Insights into the mechanism of protein unfolding. *J Biol Chem* 283:30139–30150.
24. Lum R, Tkach JM, Vierling E, Glover JR (2004) Evidence for an unfolding/threading mechanism for protein disaggregation by *Saccharomyces cerevisiae* Hsp104. *J Biol Chem* 279:29139–29146.
25. Tuite MF, Mundy CR, Cox BS (1981) Agents that cause a high frequency of genetic change from [*psi*+] to [*psi*-] in *Saccharomyces cerevisiae*. *Genetics* 98:691–711.

26. Jung G, Masison DC (2001) Guanidine hydrochloride inhibits Hsp104 activity in vivo: A possible explanation for its effect in curing yeast prions. *Curr Microbiol* 43:7–10.
27. Ferreira PC, Ness F, Edwards SR, Cox BS, Tuite MF (2001) The elimination of the yeast [PSI+] prion by guanidine hydrochloride is the result of Hsp104 inactivation. *Mol Microbiol* 40:1357–1369.
28. Jung G, Jones G, Masison DC (2002) Amino acid residue 184 of yeast Hsp104 chaperone is critical for prion-curing by guanidine, prion propagation, and thermotolerance. *Proc Natl Acad Sci USA* 99:9936–9941.
29. Grimminger V, Richter K, Imhof A, Buchner J, Walter S (2004) The prion curing agent guanidinium chloride specifically inhibits ATP hydrolysis by Hsp104. *J Biol Chem* 279:7378–7383.
30. Wegrzyn RD, Bapat K, Newnam GP, Zink AD, Chernoff YO (2001) Mechanism of prion loss after Hsp104 inactivation in yeast. *Mol Cell Biol* 21:4656–4669.
31. Ness F, Ferreira P, Cox BS, Tuite MF (2002) Guanidine hydrochloride inhibits the generation of prion "seeds" but not prion protein aggregation in yeast. *Mol Cell Biol* 22:5593–5605.
32. Kryndushkin DS, Alexandrov IM, Ter-Avanesyan MD, Kushnirov VV (2003) Yeast [*PSI*$^+$] prion aggregates are formed by small Sup35 polymers fragmented by Hsp104. *J Biol Chem* 278:49636–49643.
33. Jung G, Jones G, Wegrzyn RD, Masison DC (2000) A role for cytosolic hsp70 in yeast [PSI(+)] prion propagation and [PSI(+)] as a cellular stress. *Genetics* 156:559–570.
34. Jones GW, Masison DC (2003) *Saccharomyces cerevisiae* Hsp70 mutations affect [PSI+] prion propagation and cell growth differently and implicate Hsp40 and tetratricopeptide repeat cochaperones in impairment of [PSI+]. *Genetics* 163:495–506.
35. Roberts BT, Moriyama H, Wickner RB (2004) [URE3] prion propagation is abolished by a mutation of the primary cytosolic Hsp70 of budding yeast. *Yeast* 21:107–117.
36. Hines JK, et al. (2011) [SWI], the prion formed by the chromatin remodeling factor Swi1, is highly sensitive to alterations in Hsp70 chaperone system activity. *PLoS Genet* 7:e1001309.
37. Winkler J, Tyedmers J, Bukau B, Mogk A (2012) Hsp70 targets Hsp100 chaperones to substrates for protein disaggregation and prion fragmentation. *J Cell Biol* 198:387–404.
38. Newnam GP, Wegrzyn RD, Lindquist SL, Chernoff YO (1999) Antagonistic interactions between yeast chaperones Hsp104 and Hsp70 in prion curing. *Mol Cell Biol* 19:1325–1333.
39. Kiktev DA, et al. (2012) Regulation of chaperone effects on a yeast prion by cochaperone Sgt2. *Mol Cell Biol* 32:4960–4970.
40. Higurashi T, Hines JK, Sahi C, Aron R, Craig EA (2008) Specificity of the J-protein Sis1 in the propagation of 3 yeast prions. *Proc Natl Acad Sci USA* 105:16596–16601.
41. Troisi EM, Rockman ME, Nguyen PP, Oliver EE, Hines JK (2015) Swa2, the yeast homolog of mammalian auxilin, is specifically required for the propagation of the prion variant [URE3-1]. *Mol Microbiol* 97:926–941.
42. Reidy M, Miot M, Masison DC (2012) Prokaryotic chaperones support yeast prions and thermotolerance and define disaggregation machinery interactions. *Genetics* 192:185–193.
43. Hung GC, Masison DC (2006) N-terminal domain of yeast Hsp104 chaperone is dispensable for thermotolerance and prion propagation but necessary for curing prions by Hsp104 overexpression. *Genetics* 173:611–620.
44. Scheufler C, et al. (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell* 101:199–210.
45. Abbas-Terki T, Donzé O, Briand P-A, Picard D (2001) Hsp104 interacts with Hsp90 cochaperones in respiring yeast. *Mol Cell Biol* 21:7569–7575.
46. Moosavi B, Wongwigkarn J, Tuite MF (2010) Hsp70/Hsp90 co-chaperones are required for efficient Hsp104-mediated elimination of the yeast [PSI(+)] prion but not for prion propagation. *Yeast* 27:167–179.
47. Reidy M, Masison DC (2010) Sti1 regulation of Hsp70 and Hsp90 is critical for curing of *Saccharomyces cerevisiae* [PSI+] prions by Hsp104. *Mol Cell Biol* 30:3542–3552.
48. Jones G, Song Y, Chung S, Masison DC (2004) Propagation of Saccharomyces cerevisiae [PSI+] prion is impaired by factors that regulate Hsp70 substrate binding. *Mol Cell Biol* 24:3928–3937.
49. Helsen CW, Glover JR (2012) Insight into molecular basis of curing of [PSI+] prion by overexpression of 104-kDa heat shock protein (Hsp104). *J Biol Chem* 287:542–556.
50. Park YN, et al. (2014) Hsp104 overexpression cures *Saccharomyces cerevisiae* [PSI+] by causing dissolution of the prion seeds. *Eukaryot Cell* 13:635–647.
51. Ness F, Cox BS, Wongwigkarn J, Naeimi WR, Tuite MF (2017) Over-expression of the molecular chaperone Hsp104 in *Saccharomyces cerevisiae* results in the malpartition of [PSI(+)] propagons. *Mol Microbiol* 104:125–143.
52. Newnam GP, Birchmore JL, Chernoff YO (2011) Destabilization and recovery of a yeast prion after mild heat shock. *J Mol Biol* 408:432–448.
53. Nakayashiki T, Kurtzman CP, Edskes HK, Wickner RB (2005) Yeast prions [URE3] and [*PSI*$^+$] are diseases. *Proc Natl Acad Sci USA* 102:10575–10580.
54. McGlinchey RP, Kryndushkin D, Wickner RB (2011) Suicidal [PSI+] is a lethal yeast prion. *Proc Natl Acad Sci USA* 108:5337–5341.
55. Kelly AC, Shewmaker FP, Kryndushkin D, Wickner RB (2012) Sex, prions, and plasmids in yeast. *Proc Natl Acad Sci USA* 109:E2683–E2690.
56. Halfmann R, et al. (2012) Prions are a common mechanism for phenotypic inheritance in wild yeasts. *Nature* 482:363–368.
57. Kelly AC, Busby B, Wickner RB (2014) Effect of domestication on the spread of the [PIN+] prion in *Saccharomyces cerevisiae*. *Genetics* 197:1007–1024.
58. Wickner RB, Kelly AC (2016) Prions are affected by evolution at two levels. *Cell Mol Life Sci* 73:1131–1144.
59. Chernoff YO, Newnam GP, Kumar J, Allen K, Zink AD (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.
60. Chacinska A, et al. (2001) Ssb1 chaperone is a [PSI+] prion-curing factor. *Curr Genet* 39:62–67.
61. Nelson RJ, Ziegelhoffer T, Nicolet C, Werner-Washburne M, Craig EA (1992) The translation machinery and 70 kd heat shock protein cooperate in protein synthesis. *Cell* 71:97–105.
62. Pfund C, et al. (1998) The molecular chaperone Ssb from *Saccharomyces cerevisiae* is a component of the ribosome-nascent chain complex. *EMBO J* 17:3981–3989.
63. Kryndushkin DS, Shewmaker F, Wickner RB (2008) Curing of the [URE3] prion by Btn2p, a Batten disease-related protein. *EMBO J* 27:2725–2735.
64. Wickner RB, Bezsonov E, Bateman DA (2014) Normal levels of the antiprion proteins Btn2 and Cur1 cure most newly formed [URE3] prion variants. *Proc Natl Acad Sci USA* 111:E2711–E2720.
65. Malinovska L, Kroschwald S, Munder MC, Richter D, Alberti S (2012) Molecular chaperones and stress-inducible protein-sorting factors coordinate the spatiotemporal distribution of protein aggregates. *Mol Biol Cell* 23:3041–3056.
66. Kryndushkin D, Ihrke G, Piermartiri TC, Shewmaker F (2012) A yeast model of optineurin proteinopathy reveals a unique aggregation pattern associated with cellular toxicity. *Mol Microbiol* 86:1531–1547.
67. Borchsenius AS, Wegrzyn RD, Newnam GP, Inge-Vechtomov SG, Chernoff YO (2001) Yeast prion protein derivative defective in aggregate shearing and production of new 'seeds'. *EMBO J* 20:6683–6691.
68. Bateman DA, Wickner RB (2013) The [PSI+] prion exists as a dynamic cloud of variants. *PLoS Genet* 9:e1003257.
69. Kimberlin RH, Cole S, Walker CA (1987) Temporary and permanent modifications to a single strain of mouse scrapie on transmission to rats and hamsters. *J Gen Virol* 68:1875–1881.
70. Collinge J, Clarke AR (2007) A general model of prion strains and their pathogenicity. *Science* 318:930–936.
71. Zuehlke AD, Wren N, Tenge V, Johnson JL (2013) Interaction of heat shock protein 90 and the co-chaperone Cpr6 with Ura2, a bifunctional enzyme required for pyrimidine biosynthesis. *J Biol Chem* 288:27406–27414.
72. Kirkland PA, Reidy M, Masison DC (2011) Functions of yeast Hsp40 chaperone Sis1p dispensable for prion propagation but important for prion curing and protection from prion toxicity. *Genetics* 188:565–577.
73. Fan Q, Park K-W, Du Z, Morano KA, Li L (2007) The role of Sse1 in the de novo formation and variant determination of the [PSI+] prion. *Genetics* 177:1583–1593.
74. Moran C, Kinsella GK, Zhang ZR, Perrett S, Jones GW (2013) Mutational analysis of Sse1 (Hsp110) suggests an integral role for this chaperone in yeast prion propagation in vivo. *G3 (Bethesda)* 3:1409–1418.
75. Sharma D, Masison DC (2008) Functionally redundant isoforms of a yeast Hsp70 chaperone subfamily have different antiprion effects. *Genetics* 179:1301–1311.
76. Borkovich KA, Farrelly FW, Finkelstein DB, Taulien J, Lindquist S (1989) hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. *Mol Cell Biol* 9:3919–3930.
77. Genest O, et al. (2013) Uncovering a region of heat shock protein 90 important for client binding in *E. coli* and chaperone function in yeast. *Mol Cell* 49:464–473.
78. Kumar N, Gaur D, Gupta A, Puri A, Sharma D (2015) Hsp90-associated immunophilin homolog Cpr7 is required for the mitotic stability of [URE3] prion in *Saccharomyces cerevisiae*. *PLoS Genet* 11:e1005567.
79. Salnikova AB, Kryndushkin DS, Smirnov VN, Kushnirov VV, Ter-Avanesyan MD (2005) Nonsense suppression in yeast cells overproducing Sup35 (eRF3) is caused by its non-heritable amyloids. *J Biol Chem* 280:8808–8812.
80. Lacroute F (1971) Non-Mendelian mutation allowing ureidosuccinic acid uptake in yeast. *J Bacteriol* 106:519–522.
81. Edskes HK, McCann LM, Hebert AM, Wickner RB (2009) Prion variants and species barriers among *Saccharomyces* Ure2 proteins. *Genetics* 181:1159–1167.
82. Bateman DA, Wickner RB (2012) [PSI+] Prion transmission barriers protect *Saccharomyces cerevisiae* from infection: Intraspecies 'species barriers'. *Genetics* 190:569–579.
83. Borchsenius AS, Müller S, Newnam GP, Inge-Vechtomov SG, Chernoff YO (2006) Prion variant maintained only at high levels of the Hsp104 disaggregase. *Curr Genet* 49:21–29.
84. Kryndushkin D, Wickner RB (2007) Nucleotide exchange factors for Hsp70s are required for [URE3] prion propagation in *Saccharomyces cerevisiae*. *Mol Biol Cell* 18:2149–2154.
85. Kryndushkin DS, Engel A, Edskes H, Wickner RB (2011) Molecular chaperone Hsp104 2can promote yeast prion generation. *Genetics* 188:339–348.
86. Sherman F (1991) Getting started with yeast. *Guide to Yeast Genetics and Molecular Biology*, eds Guthrie C, Fink GR (Academic, San Diego), pp 3–21.
87. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14:115–132.
88. Cox B, Ness F, Tuite M (2003) Analysis of the generation and segregation of propagons: Entities that propagate the [PSI+] prion in yeast. *Genetics* 165:23–33.
89. Kulak NA, Pichler G, Paron I, Nagaraj N, Mann M (2014) Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. *Nat Methods* 11:319–324.
90. Boersema PJ, Raijmakers R, Lemeer S, Mohammed S, Heck AJ (2009) Multiplex peptide stable isotope dimethyl labeling for quantitative proteomics. *Nat Protoc* 4:484–494.
91. Cox J, Mann M (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol* 26:1367–1372.
92. DiCarlo JE, et al. (2013) Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. *Nucleic Acids Res* 41:4336–4343.
93. Horwitz AA, et al. (2015) Efficient multiplexed integration of synergistic alleles and metabolic pathways in yeasts via CRISPR-Cas. *Cell Syst* 1:88–96.
94. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122:19–27.
95. Edskes HK, et al. (2014) Sporadic distribution of prion-forming ability of Sup35p from yeasts and fungi. *Genetics* 198:605–616.
96. Bagriantsev SN, Kushnirov VV, Liebman SW (2006) Analysis of amyloid aggregates using agarose gel electrophoresis. *Methods Enzymol* 412:33–48.
97. Goeckeler JL, Stephens A, Lee P, Caplan AJ, Brodsky JL (2002) Overexpression of yeast Hsp110 homolog Sse1p suppresses ydj1-151 thermosensitivity and restores Hsp90-dependent activity. *Mol Biol Cell* 13:2760–2770.
98. Yan W, Craig EA (1999) The glycine-phenylalanine-rich region determines the specificity of the yeast Hsp40 Sis1. *Mol Cell Biol* 19:7751–7758.