Nonsense-mediated mRNA decay factors cure most [PSI+] prion variants

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The yeast prion [PSI+] is a self-propagating amyloid of Sup35p with a folded in-register parallel β-sheet architecture. In a genetic screen for antiprion genes, using the yeast knockout collection, *UPF1/NAM7* and *UPF3*, encoding nonsense-mediated mRNA decay (NMD) factors, were frequently detected. Almost all [PSI+] variants arising in the absence of Upf proteins were eliminated by restored normal levels of these proteins, and [PSI+] arises more frequently in *upf* mutants. Upf1p, complexed with Upf2p and Upf3p, is a multifunctional protein with helicase, ATP-binding, and RNA-binding activities promoting efficient translation termination and degradation of mRNAs with premature nonsense codons. We find that the curing ability of Upf proteins is uncorrelated with these previously reported functions but does depend on their interaction with Sup35p and formation of the Upf1p–Upf2p–Upf3p complex (i.e., the Upf complex). Indeed, Sup35p amyloid formation in vitro is inhibited by substoichiometric Upf1p. Inhibition of [PSI+] prion generation and propagation by Upf proteins may be due to the monomeric Upf proteins and the Upf complex competing with Sup35p amyloid fibers for available Sup35p monomers. Alternatively, the association of the Upf complex with amyloid filaments may block the addition of new monomers. Our results suggest that maintenance of normal protein–protein interactions prevents prion formation and can even reverse the process.

prion | antiprion system | [PSI+] | Upf proteins | nonsense-mediated mRNA decay

The yeast prion [PSI+] is a self-propagating amyloid of Sup35p (1–7). Sup35p normally functions as a soluble subunit of the translation termination factor (8, 9). However, its conversion to the prion amyloid aggregate sequesters Sup35p from the translation termination machinery, resulting in read-through of stop codons. [URE3] is similarly an amyloid prion of Ure2p (2, 10–14), a regulator of nitrogen catabolism (15), and [PIN+] is a prion of Rnq1p, an asparagine (N)- and glutamine (Q)-rich protein with unknown function (16–18).

A given prion protein sequence can become any of many distinct prion variants/strains, with different biological properties as a result of different amyloid structures, each quite stably propagating (19, 20). The yeast prion amyloids have a folded, parallel, in-register β-sheet architecture (21–24), a structure that can explain how prion proteins can template their conformation, resulting in the stable propagation of different prion variants (25, 26). These different prion variants act as alleles of a cytoplasmic protein-based gene.

The high frequency of toxic or even lethal variants of [PSI+] and [URE3] (27), and the rare occurrence in wild strains of even the mildest variants of these prions (28), indicates that these prions are detrimental to yeast and that the potential to develop or be infected by a prion is a risk with no reproducible compensating advantage other than the normal function of the prion domain (29, 30). Like the antipathogen systems against fungi, bacteria, and viruses, yeast should have evolved systems to prevent prion generation or to eliminate them or limit their pathogenic effects after they arise. Most of the known antiprion systems are closely related to molecular chaperones, well-characterized protein quality-control systems for dealing with protein aggregates (reviewed in ref. 26). Deletion of ribosome-associated Hsp70 chaperones Ssb1p and Ssb2p leads to elevated frequency of [PSI+] formation, either spontaneously or induced by Sup35p overproduction (31). Restored Ssbs did not eliminate [PSI+] variants arising in an *ssb1*Δ *ssb2*Δ strain, indicating that the normal level of Ssb1p and Ssb2p affects [PSI+] generation but not propagation (31). Sis1p is an Hsp40 that is necessary for cell growth (32) and for propagation of [PSI+], [URE3], and [PIN+] (33). Sis1p's C-terminal domain is not necessary for cell growth in the absence of [PSI+] but becomes nearly essential in the presence of an otherwise mild [PSI+] (34). Thus, Sis1p prevents [PSI+] toxicity.

The disaggregating chaperone Hsp104 is both necessary for the propagation of [PSI+] and other amyloid-based yeast prions and cures [PSI+] on its overproduction (35, 36). Deletion or mutation (e.g., T160M) of the Hsp104 N-terminal domain eliminates the ability of overproduced Hsp104 to cure [PSI+] without affecting [PSI+] propagation, suggesting that these are different activities of Hsp104 (37). Indeed, the Hsp104 overproduction curing activity requires Sti1p and Hsp90, but neither affects [PSI+] propagation (38–40). This result was used to show that normal levels of WT Hsp104 cures many [PSI+] variants arising spontaneously in an $hsp104^{T160M}$ strain, and the frequency of spontaneous [PSI+] is >10-fold elevated in the $hsp104^{T160M}$ mutant (41). Like the curing by Hsp104 overproduction, the curing of [PSI+] by normal levels of Hsp104 is promoted by Sti1p and Hsp90.

Overproduction of either Btn2p or its paralogue Cur1p was found to cure the [URE3] prion (42). During the curing process, Btn2p colocalized with and collected Ure2p-GFP aggregates, suggesting that Btn2p sequesters prion aggregates, preventing their distribution to daughter cells (42, 43). Btn2p also partially colocalized with Sup35NM-GFP aggregates or huntingtin-like Q103-GFP aggregates, although [PSI+] was not cured by Btn2p or Cur1p overproduction (42), and Btn2p can collect other nonprion aggregates (44, 45). It was then found that almost all [URE3] variants isolated in a *btn2*Δ*cur1*Δ strain were cured by restoring

Significance

The [PSI+] prion (infectious protein) is an amyloid (filamentous polymer) of the Sup35 protein, producing detrimental effects on yeast. We find that at their normal expression levels the core components of nonsense-mediated mRNA decay for mRNA quality control, Upf1p, Upf2p, and Upf3p, block the propagation of most new [PSI+] prion variants. The curing mechanism relies upon both Sup35p-binding by each Upf protein and by the trimeric Upf complex. Our results support the notion that normal protein–protein interactions prevent abnormal interactions.

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just normal levels of either Btn2p or Cur1p, or both (46). These results indicate that normal levels of Btn2p and Cur1p cure most [URE3] variants arising in the cell.

Most recently, Siw14p, a pyrophosphatase specific for 5PP-IP5 (5-diphosphoinositol pentakisphosphate) in the inositol polyphosphate synthesis pathway, was found to play a role as an antiprion factor (47). Restored normal levels of Siw14p eliminate about half of the [PSI+] variants generated in a *siw14*Δ strain by limiting the levels of some inositol poly-/pyrophosphates. Further study of this phenomenon revealed that inositol poly-/pyrophosphates are needed for nearly all [PSI+] variants (47). These studies indicate that yeast has antiprion systems that constantly act to block the formation of prions and to prevent the propagation of most prions that do arise. These systems are working in normal cells whose physiology is not distorted by overproduction or deficiency of any components.

Here, we report that normal levels of the Upf proteins (Upf1p, Upf2p, and Upf3p), components of the nonsense-mediated mRNA decay (NMD) apparatus (48), can cure [PSI+] variants arising in the absence of each of these proteins. Further, we present evidence that prion curing involves binding to Sup35p and formation of the Upf complex. Throughout this study, we were careful to distinguish the known effects of *upf* mutations on the translational effects of [PSI+] from the effect of *upf* mutations on the propagation of [PSI+] as a genetic element.

Results

Screening for [PSI+] Prion-Curing Factors. We screened for [PSI+] prion-curing factors by a genetic method without protein overproduction (47). Pools of the *MATa* knockout collection were transformed with the centromeric plasmid p1520 (47), which carries *LEU2* as a marker for yeast, the [PSI+]-suppressible nonsense allele *ura3-14* (49), and the Sup35p-prion domain (NM) controlled by the *GAL1* promoter. [PSI+] was induced by growth for 24 h in galactose to overproduce Sup35NM, and cells were plated on −Ura plates with dextrose to select Ura^+ ([PSI+]) clones. These Ura^+ clones were crossed with an isogenic WT strain, BY4742, to complement the knockout mutation in each clone. Ura^- diploids were identified, and the corresponding original Ura^+ clone was tested for curability by transient growth on guanidine, an inhibitor of Hsp104 that specifically cures prions. Guanidine-curable candidates were not simply recessive suppressors of the *ura3-14* nonsense mutation complemented by mating with the WT strain but had to be [PSI+] clones. As shown below, this was confirmed by the transfer by cytoplasmic mixing (cytoduction) of the suppressor phenotype. From each Ura^+ candidate that produced Ura^- diploids and whose Ura^+ phenotype was curable by growth on guanidine, DNA was extracted, and the deleted gene was identified by PCR of the bar-code region of *KanMX*. Among 30 candidates, *upf1::kanMX* was found eight times, and *upf3::kanMX* was found nine times. *UPF1* and *UPF3* encode components of the NMD apparatus.

A Normal Level of Upf1p Can Cure [PSI+] Variants Isolated in a *upf1*Δ Strain. We cured [PSI+] from one *upf1*Δ isolate, replaced [PIN+] by cytoduction (cytoplasmic transfer), and then induced [PSI+] formation by overproduction of Sup35NM. Ura^+ isolates were mated with either the WT [psi−] strain BY4742 or a *upf1*Δ [psi−] strain. Almost all diploids formed with the WT were uniformly Ura^-, but not the diploids with the *upf1*Δ [psi−] strain (Fig. 1). Because *ura3-14* is expected to be a target of NMD, we took special care to determine whether [PSI+] was lost from the diploids formed in these tests or if its phenotype was merely unapparent.

Meiotic segregation of the *upf1*Δ/+ Ura^- heterozygotes showed mostly 4 Ura^-:0 Ura^+ segregation. Only a few *upf1*Δ segregants showed a Ura^+ or a very weak Ura^+ phenotype; the majority (50–100%, average 76%) of the *upf1*Δ segregants were Ura^-. Because *upf1*Δ segregants were largely Ura^-, [PSI+] must have been lost in most of the heterozygous diploids before sporulation. In contrast, if, after mating, the diploids were immediately sporulated to prevent loss of [PSI+] in the diploids, the segregation was 2 Ura^- $G418^{sensitive}$:2 Ura^+ $G418^{resistant}$ (37 tetrads). The combined meiotic analysis implies that the [PSI+] genetic element is eliminated by Upf1p, not that its phenotype is merely disguised. Based on their loss of [PSI+] on restoration of normal levels of Upf1p, these variants (all guanidine-curable and mitotically stable) are denoted "Upf1p-sensitive," [PSI+u1s].

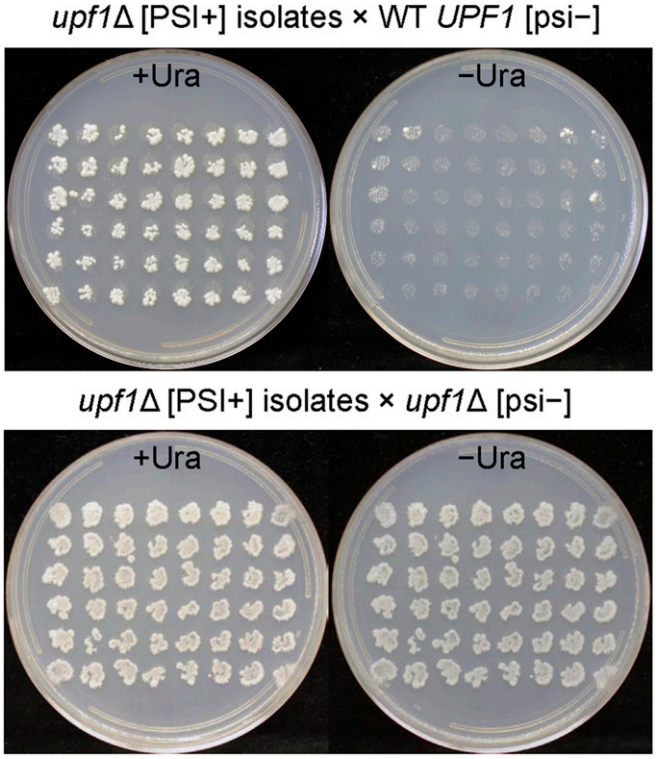

Fig. 1. Most [PSI+] prion variants isolated in a *upf1*Δ strain are lost in the presence of the WT allele of *UPF1*. [PSI+] isolates in the *upf1*Δ strain MS114 were mated for 1 d on YPAD with either isogenic WT MS173 or the *upf1*Δ [psi−] strain MS177 and were replica-plated to minimal medium with and without uracil. The presence of p1520 (*pCEN LEU2 ura3-14*) in all strains enables scoring [PSI+]. (*Upper*) Diploids formed with WT are almost all Ura^- as a result of the elimination of [PSI+]. Several isolates are not efficiently cured by mating with isogenic WT (*Lower*). Diploids formed with *upf1*Δ are Ura^+, indicating stable maintenance of [PSI+].

To confirm loss of [PSI+u1s] in WT strains, cytoduction (transfer of cytoplasm; see *Methods*) was performed using [PSI+ u1s] *upf1*Δ strains as donors and WT or *upf1*Δ [psi−] strains as recipients. As expected, almost all cytoductants showed a Ura^- phenotype in WT recipients and a Ura^+ phenotype in *upf1*Δ recipients (Table 1). To verify that Ura^- cytoductants from *upf1*Δ [PSI+u1s] strains to WT strains had indeed lost [PSI+u1s], reverse-cytoductions were conducted using Ura^- cytoductants as donors. The majority of reverse-cytoductants were Ura^- in the *upf1*Δ recipient, indicating that a large fraction of [PSI+u1s] variants was really lost in the WT recipients (Table 1). The appearance of a minority of Ura^+ cytoductants on the return of cytoplasm to the *upf1*Δ host indicates that this [PSI+] variant was mostly, but not completely, lost in the WT host. This varies with prion isolate (see Tables S2 and S3, in which it can be seen that, of 24 [PSI+] isolates in a *upf1*Δ host, two showed retention of [PSI+] in a significant fraction of WT cells).

It is well known that *upf1* and *upf3* mutations enhance the expression of genes, such as *ura3-14*, that have a premature termination codon, both by allowing longer survival of their mRNA (no NMD) and by decreasing the efficiency of termination,

Table 1. Confirmation of elimination of [PSI+] variants by normal level of Upf1p

Donor	Recipient	Cytoductants	
		Ura$^+$	Total
*upf1*Δ [PSI+u1s2]	WT ρ°	0	35
*upf1*Δ [PSI+u1s5]		0	40
*upf1*Δ [PSI+u1s6]		0	36
*upf1*Δ [PSI+u1s2]	*upf1*Δ ρ°	37	38
*upf1*Δ [PSI+u1s5]		39	40
*upf1*Δ [PSI+u1s6]		30	32
WT-2* Ura$^-$	*upf1*Δ ρ°	7	146
WT-5* Ura$^-$		33	150
WT-6* Ura$^-$		23	160
*upf1*Δ-2† Ura$^+$	*upf1*Δ ρ°	38	40
*upf1*Δ-5† Ura$^+$		37	40
*upf1*Δ-6† Ura$^+$		39	40

Upf1p-sensitive [PSI+] variants ([PSI+u1s]) were transferred by cytoduction (cytoplasmic mixing) from isolates 2, 5, and 6 into the WT strain MS173 and into the *upf1*Δ strain MS177. Cytoductants of each were used as reverse-cytoduction donors into *upf1*Δ strain MS114.
*Ura$^-$ WT cytoductants from first cytoduction.
†Ura$^+$ *upf1*Δ cytoductants from first cytoduction.

allowing more read-through of the premature termination codon (reviewed in ref. 48). However, the reverse cytoductants are Ura$^-$, while the original [PSI+u1s] isolates are Ura$^+$, both being *upf1Δ*, showing that [PSI+] can be scored in a *upf1Δ* strain and that [PSI+u1s] was lost in the WT strains rather than its phenotype simply being disguised. Additionally, the loss of [PSI+u1s] in WT strains was examined by investigating the formation of fluorescent structures. Sup35NM-GFP was expressed in Ura$^-$ WT cytoductants and Ura$^+$ *upf1*Δ cytoductants to detect prion aggregates. Like the WT [psi−][PIN+] and *upf1*Δ [psi−][PIN+] strains, single or multiple dots rarely appeared in the Ura$^-$ WT cytoductants but were seen in many cells of normal [PSI+] strains and Ura$^+$ *upf1*Δ cytoductants (Fig. S1 and Table S1). Taken together, these several lines of evidence prove that many [PSI+] variants are lost in the presence of *UPF1* in WT strains, independent of the known effect of Upf1p on the phenotypic expression of the prion.

To confirm that Upf1p plays a role in eliminating [PSI+u1s], pRS313 (*CEN* vector) or pM25 (pRS313-*UPF1*), a single-copy plasmid with the *UPF1* gene controlled by its native promoter, was transformed into [PSI+u1s] *upf1*Δ isolates. Transformants were selected in the presence of uracil and were replica-plated to plates lacking uracil. Almost none of the transformants carrying pM25 could grow on medium lacking uracil, indicating that [PSI+u1s] was eliminated by normal levels of Upf1p (Fig. S2). As shown in Table 2, three independent [PSI+u1s] variants examined were almost completely eliminated by the restoration of *UPF1,* while standard strong [PSI+] ([PSI+s]) or weak [PSI+] ([PSI+w]) variants were almost unaffected. More than 50 Ura$^-$ subclones that had lost pM25 were examined for uracil auxotrophy by replica-plating. Nearly all Ura$^-$ subclones remained Ura$^-$ after losing pM25, indicating that [PSI+u1s] variants are cured by p*UPF1* and that the *UPF1* gene does not merely affect the Ura$^+$ phenotype (Table 2).

Overexpression of Hsp104 cures all [PSI+] variants (35), and overexpression of Btn2p or Cur1p cures all [URE3] variants (42). However, we found that overexpression of Upf1p or Upf3p from a *GAL* promoter did not cure [PSI+] from strain 779-6A carrying a conventional [PSI+s] variant.

Nonsense Suppression Phenotypes Produced by [PSI+u1s] or *upf1*Δ or Other [PSI+]s Are Distinguishable, Depending on the Assay Marker. In [PSI+] cells, most of Sup35p is in filaments, producing inefficient translation termination and readthrough of termination codons, such as *ade1-14*, *ade2-1*, and *ura3-14* (for this study). We compared the phenotypes of [PSI+u1s] variants with those of [PSI+s] and [PSI+w] using strains generated by cytoduction into the background of the bank strains and carrying p1520 (with *ura3-14*). In the white/red color assay on adenine-limited half-strength yeast extract/peptone/dextrose (YPD) medium, the *upf1*Δ [PSI+s] cells show essentially the same pink color as WT [PSI+s] cells, but the former grow dramatically better on −Ura plates than the latter (Fig. 2). The *ade1-14* allele has a UGA terminator at codon 244 of the 306-residue protein (50), while *ura3-14* was constructed to have the same terminator at codon 18 of the 267-residue Ura3 protein (49). NMD is far less effective if the codon is close to the 3′ end of the ORF than if it is near the beginning (51). This could explain the larger effect of *upf1*Δ on the Ura$^-$ phenotype than on the Ade$^-$ phenotype. When compared in a *upf1*Δ strain, the efficiency of *ura3-14* nonsense suppression by [PSI+u1s] is slightly lower than that produced by [PSI+s] or [PSI+w] (Fig. 2). In addition, the efficiency of readthrough produced by [PSI+] alone is significantly higher than that resulting from *upf1*Δ in a [psi−] strain, and the strongest readthrough results from the combination in *upf1*Δ [PSI+] cells (Fig. 2). Although *upf1*Δ [psi−] cells barely grew after 3-d incubation on a plate lacking uracil, allowing them a further 7 d of growth made possible a distinction from WT [psi−] cells. Therefore, 3-d incubation was determined as a checkpoint for *ura3-14* nonsense suppression by [PSI+] or [PSI+u1s] for this study.

Upf1p-Sensitive [PSI+] Variants Have Seed Numbers Similar to Normal [PSI+]. The prion seed numbers (also called "propagons") of [PSI+u1s] variants were investigated using a method developed previously (52). Low concentrations of guanidine specifically inhibit Hsp104's filament-cleaving activity (53–55), preventing the generation of new seeds. Preexisting seeds segregate until there is one seed per cell. By measuring the number of prion-carrying cells in the colony, the number of preexisted seeds in the founder cell can be estimated (52). Using two different concentrations of guanidine, the seed number of [PSI+u1s] variants was counted. Both experiments showed that [PSI+u1s] variants have seed numbers not significantly different from those in normal [PSI+] variants (Table 3). This result contrasts with the Btn2p/Cur1p antiprion systems that cure [URE3]. Btn2p/Cur1p-hypersensitive

Table 2. Restored normal level of Upf1p can eliminate most Upf1p-sensitive [PSI+] variants

[PSI+] variant	Transformant clones				Phenotype of subclones from Ura$^-$ clones that lost p*UPF1*	
	Vector		p*UPF1*			
	Ura$^+$	Total	Ura$^+$	Total	Ura$^+$	Ura$^-$
[PSI+s]	263	265	235	265	0	50
[PSI+w]	245	247	255	280	0	55
[PSI+u1s2]	271	276	15	217	1	54
[PSI+u1s5]	255	275	11	308	1	50
[PSI+u1s6]	209	218	9	235	2	54

*upf1*Δ strains MS277, MS281, MS2, MS5, and MS6 (top to bottom) carrying [PSI+s], [PSI+w], and [PSI+u1s] were transformed with the *CEN* plasmid pRS313 or with the same plasmid carrying *UPF1* under its native promoter (pM25 = p*UPF1*). Transformants were selected in the presence of uracil and were replica-plated to a plate lacking uracil. More than 200 transformant clones were investigated in each case. Subclones of Ura$^-$ transformants that had lost pM25 were tested for uracil auxotrophy by replica-plating. The results, summed and shown in the rightmost column, show that [PSI+u1s] had been eliminated by p*UPF1*.

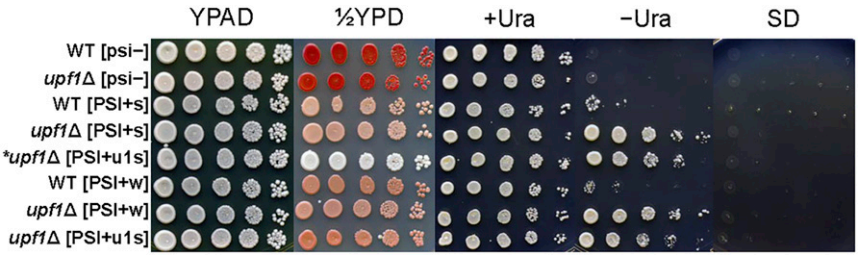

Fig. 2. Comparison of nonsense-suppression phenotypes of [PSI+u1s] or other [PSI+]s in WT or *upf1*Δ hosts. Tenfold dilutions of cells of strains MS109, MS114, MS224, MS277, MS2, MS225, MS281, and MS286 (top to bottom) carrying p1520 with *ura3-14* were plated on rich medium and minimal medium (SD) with and without uracil. [PSI+s] indicates a strong variant and [PSI+w] indicates a weak variant. In the fifth row, asterisked *upf1*Δ [PSI+u1s] is a bank-background original isolate without the *ade1-14* allele. For photographs, cells on rich medium (YPAD and 1/2YPD) were grown at 30 °C for 2 d, and cells on minimal medium were grown at 30 °C for 3 d.

[URE3] variants have substantially lower seed numbers (46), suggesting that these two different antiprion systems use distinct mechanisms to deal with yeast prions.

Spontaneous and Induced [PSI+] Generation Is Elevated in *upf* Mutants. To test whether the normal level of Upf proteins can block many [PSI+] variants as they arise, the frequency of spontaneous and induced generation of [PSI+] was investigated in WT strains and in NMD-deficient *upf1*Δ, *upf2*Δ, and *upf3*Δ mutants. For these experiments, the same [PIN+], a prion of Rnq1, was transferred to all 5 mM guanidine-treated recipient strains. For spontaneous generation of [PSI+], the frequency of [PSI+] clones per 10^7 cells from *upf1*Δ or *upf2*Δ strains was elevated sevenfold and fivefold, respectively (Table 4). Interestingly, although all strains carried the same [PIN+], the *upf3*Δ mutant did not show elevated spontaneous [PSI+] generation. In [PSI+] generation induced by Sup35NM overproduction, *upf1Δ, upf2Δ*, and *upf3Δ* mutants produced 16-, 17-, and 13-fold more [PSI+] clones, respectively, than the WT strain (Table 4).

The [PSI+] variants arising at high frequency in *upf2*Δ or *upf3*Δ strains (Table 4) were further tested by cytoduction to determine whether normal levels of Upf2p or Upf3p can cure them (Table 5). As was found for [PSI+u1s] (Table 1), cytoduction to WT recipients resulted in Ura^- cytoductants, but cytoduction into another strain with the same *upf* mutation allowed maintenance of [PSI+]. Returning cytoplasm from the WT Ura^- cytoductants to the original *upf2*Δ or *upf3*Δ host produced mostly Ura^- back-cytoductants, indicating that the Ura^- phenotype in the WT strain was due to the loss of [PSI+], not to merely affecting the phenotype (Table 5). Thus, these are Upf2p-sensitive ([PSI+u2s]) and Upf3p-sensitive ([PSI+u3s]) [PSI+] prion variants. In addition, 12 [PSI+] clones each from those spontaneously generated and induced in the *upf1*Δ host (Table 4) were analyzed in the same way as our original isolates in Table 1. Cytoplasm was transferred to WT recipients, resulting in nearly all cytoductants being Ura^- (Tables S2 and S3). Sample WT Ura^- cytoductants were then used as donors to return cytoplasm to *upf1*Δ cells, with nearly all cytoductants again being Ura^- (Tables S2 and S3), a pattern exactly similar to the previous cytoduction results using the original [PSI+u1s] isolates as a donor (Table 1) and indicating that both spontaneous and induced [PSI+] clones are indeed mostly Upf1p-sensitive [PSI+] prion variants.

Does the elevated frequency of [PSI+] in *upf*Δ strains result from the nonsense-suppression effect of these mutations allowing more residual growth on –Ura plates and thus the selection of [PSI+] from a larger effective population? The suggested mechanism would predict that the [PSI+] variants arising in a *upf* mutant would be largely the same kind as variants arising in a WT cell, just more of them because there was a bigger population of cells from which they came. In fact, nearly all the variants that arise are cured by mating with a WT strain or by cytoduction into a WT strain. Thus, this mechanism cannot explain the observed results.

Upf1p-Sensitive [PSI+] Variants Are Stabilized in *upf2*Δ or *upf3*Δ Cells. Upf1p acts with Upf2 and Upf3 in NMD, forming a trimeric Upf1p–Upf2p–Upf3p complex (i.e., the Upf complex) necessary for activity (56–58). We investigated whether [PSI+u1s] variants can propagate in *upf2*Δ or *upf3*Δ strains despite the presence of

Table 3. Seed number of [PSI+] variants

Prion variant	Seed no. ± SD
Experiment 1 (4 mM guanidine HCl)	
*upf1*Δ [PSI+s]	145.5 ± 59.3
*upf1*Δ [PSI+w]	71.8 ± 17.3
*upf1*Δ [PSI+u1s2]	45.5 ± 2.4
*upf1*Δ [PSI+u1s5]	106.0 ± 97.0
*upf1*Δ [PSI+u1s6]	59.0 ± 6.6
Experiment 2 (5 mM guanidine HCl)	
*upf1*Δ [PSI+s]	89.6 ± 15.4
*upf1*Δ [PSI+w]	47.1 ± 18.9
*upf1*Δ [PSI+u1s2]	40.6 ± 21.4
*upf1*Δ [PSI+u1s5]	66.2 ± 13.6
*upf1*Δ [PSI+u1s6]	63.4 ± 6.8

*upf1*Δ strain MS114 carrying the indicated [PSI+] variants was streaked for single colonies on YPAD medium containing 4 mM guanidine HCl (experiment 1) or 5 mM guanidine HCl (experiment 2). Single colonies were suspended in sterilized water and plated on –Ade plates. Ade^+ colonies are a relative measure of the number of prion seeds in the cell founding the colony.

Table 4. De novo generation of [PSI+] variants is enhanced in *upf* mutants

Host	Spontaneous [PSI+], total Ura^+ colonies per 10^7 cells	Induced [PSI+], total Ura^+ colonies per 10^5 cells
WT	3.7 ± 1.0	8.00 ± 3.4
*upf1*Δ	28.3 ± 7.3	130.3 ± 32.4
*upf2*Δ	19.0 ± 5.4	132.2 ± 22.5
*upf3*Δ	2.7 ± 1.5	105.7 ± 16.4

Strains MS327, MS330, MS333, and MS336 (top to bottom), used in this experiment, carry the same [PIN+]. For spontaneous [PSI+], cells were grown for 2 d in 2% glucose liquid culture at 30 °C, and 10^7 yeast cells were spread on standard synthetic complete medium (SC) plates without uracil. For induced [PSI+] formation, strains carrying p1520 with *SUP35NM* driven by a galactose-inducible promoter were grown for 2 d in 2% galactose/2% raffinose medium at 30 °C, and 10^5 yeast cells were spread on SC plates without uracil. The average number of colonies formed after 5 d of incubation at 30 °C is shown (the data from six independent experiments were combined). Numbers represent Ura^+ colonies with [PSI+] that were confirmed by GuHCl curability. The number of Ura^+ colonies ±SD is shown.

Table 5. [PSI+] variants isolated in *upf2*Δ and *upf3*Δ cells are cured by restoring Upf2p and Upf3p, respectively

Donor	Recipient	Cytoductants	
		Ura$^+$	Total
*upf2*Δ [PSI+u2s1]	WT ρ^{o}*	0	43
*upf2*Δ [PSI+u2s2]		0	40
*upf2*Δ [PSI+u2s4]		0	41
*upf2*Δ [PSI+u2s1]	*upf2*Δ ρ^{o}	39	41
*upf2*Δ [PSI+u2s2]		35	38
*upf2*Δ [PSI+u2s4]		36	39
*upf3*Δ [PSI+u3s1]	WT ρ^{o}†	0	38
*upf3*Δ [PSI+u3s2]		0	40
*upf3*Δ [PSI+u3s3]		1	35
*upf3*Δ [PSI+u3s1]	*upf3*Δ ρ^{o}	39	40
*upf3*Δ [PSI+u3s2]		34	40
*upf3*Δ [PSI+u3s3]		37	40
WT*	*upf2*Δ ρ^{o}	2	40
WT*		1	40
WT*		2	35
WT†	*upf3*Δ ρ^{o}	1	40
WT†		1	40
WT†		2	40

[PSI+] variants isolated in *upf2*Δ strain MS62 and *upf3*Δ strain MS65 (Table 4) were transferred by cytoduction into the WT strain MS327, *upf2*Δ strain MS308, and *upf3*Δ strain MS68. Ura$^-$ cytoductants of each were used as reverse-cytoduction donors. The results show the loss of [PSI+] from the WT host in each case, meaning that these variants can be referred to as Upf2p-sensitive ([PSI+u2s]) or Upf3p-sensitive ([PSI+u3s]).
*Ura$^-$ cytoductants from *upf2*Δ [PSI+u2s] to WT.
†Ura$^-$ cytoductants from *upf3*Δ [PSI+u3s] to WT.

Upf1p. As is evident from Table 6, almost all cytoductants into *upf2*Δ and *upf3*Δ recipient strains showed a Ura$^+$ phenotype, indicating that the curing of [PSI+u1s] variants requires all three Upf proteins. In each case, cytoduction from Ura$^+$ cytoductants into WT recipients confirmed that [PSI+u1s] variants in the *upf2*Δ or *upf3*Δ strains had not changed and were eliminated by the presence of normal levels of the three Upf proteins. Thus, [PSI+u1s] variants can propagate if any one of the Upf proteins is absent (Table 6).

Curing of [PSI+u1s] by Normal Levels of Upf1p Is Uncorrelated with Its Functions in Translation Termination and Nonsense-Mediated mRNA Degradation. Upf1p is a multifunctional protein consisting of a cysteine- and histidine-rich zinc-finger domain (CH domain) at the N-terminal region and a C-terminal Helicase domain (59–61). These two domains engage in various functions, including ATP hydrolysis, ATP binding, RNA binding, and functional interaction with other factors required for activating NMD. To identify which function(s) of Upf1p is important for eliminating [PSI+u1s], plasmids expressing various mutants of Upf1p were generated using pM25 as a backbone (Fig. S3*A* and Table S4).

These plasmids were transformed into a *upf1*Δ strain carrying [PSI+u1s], and transformants were replica-plated to plates lacking uracil. Consistent with the results shown in Table 2 and Fig. S2, the WT allele of *UPF1* could eliminate [PSI+u1s] from most cells, but the empty vector could not. Except for C72S and RR793KK, each of the mutant plasmids produced less efficient curing than p*UPF1*-WT, suggesting that the curing ability of Upf1p is impaired by amino acid substitutions of Upf1p (Table 7 and Fig. S3*B*); C72S and RR793KK mutations were previously reported to not affect the original functions of Upf1p (59, 61). Mutations in the CH domain (C62Y, C84S, and C125S) more severely affected curing ability than those in Helicase domain (K436Q, DE572AA, TR800AA, and RR793AA) (Table 7 and Fig. S3*B*).

Both C62Y and C84S mutations dramatically reduced [PSI+] curing ability by ∼90%, but the former stabilizes nonsense-containing mRNA (like *upf1*Δ), and the latter does not (like WT *UPF1*) (60). The K436Q mutation produces strong nonsense suppression, while DE572AA resembles the WT (59), but these mutants have similar curing ability, reduced by 70.4% and 71.8%, respectively (Table 7). In this sense, there is no clear correlation of [PSI+]-curing activity with these or other known activities of Upf1p (Table S4).

Upf1p Colocalizes with [PSI+] Prion Aggregates in Vivo. Direct binding of Upf1p and soluble Sup35p or Sup35p aggregates has been previously identified in extracts (62). To examine the interaction between Upf1p and both forms of Sup35p in vivo, strain MS307, which has RFP-tagged Upf1p, was transformed with pSL1066, a centromeric plasmid with *CUP1*-promoted Sup35NM-GFP, to decorate [PSI+] aggregates (63). After 48 h growth in medium containing 50 μM $CuSO_4$, the localization of each protein was investigated using confocal microscopy. As expected, Upf1p-RFP showed cytoplasmic localization without Sup35NM-GFP aggregates (Fig. 3*A*). In cells with Sup35NM-aggregates, Upf1p-RFP colocalized with Sup35NM-GFP aggregates and was not detected free in the cytoplasm (Fig. 3*A*, *Bottom*), indicating that Upf1p colocalizes with [PSI+] prion aggregates in vivo. Since deficiency of Upf1p is known to produce nonsense suppression [both by allowing mRNAs to survive longer and by other mechanisms (64)], it is possible that the association of Upf1p with Sup35p aggregates in [PSI+] cells contributes to the nonsense-suppression phenotype of this prion.

Upf1p Inhibits Sup35p Amyloid Formation but Not Ure2p Amyloid Formation in Vitro. The effect of Upf1p on the assembly of Sup35p into amyloid was tested in vitro to understand how normal levels of Upf1p cure [PSI+]. For this experiment, full-length recombinant Sup35p and Ure2p were expressed with His_6 tags in *Escherichia coli*, and FLAG-tagged Upf1p was purified in yeast using the published method (62). Amyloid formation of Sup35p or Ure2p was monitored using thioflavin T binding. As

Table 6. Upf1p-sensitive [PSI+] variants are stabilized in *upf2*Δ or *upf3*Δ cells

Donor	Recipient	Cytoductants	
		Ura$^+$	Total
*upf1*Δ [PSI+u1s2]	WT ρ^{o}	0	35
*upf1*Δ [PSI+u1s5]		0	40
*upf1*Δ [PSI+u1s6]		0	37
*upf1*Δ [PSI+u1s2]	*upf1*Δ ρ^{o}*	45	45
*upf1*Δ [PSI+u1s5]		33	34
*upf1*Δ [PSI+u1s6]		31	33
*upf1*Δ [PSI+u1s2]	*upf2*Δ ρ^{o}†	31	31
*upf1*Δ [PSI+u1s5]		30	30
*upf1*Δ [PSI+u1s6]		25	25
*upf1*Δ [PSI+u1s2]	*upf3*Δ ρ^{o}‡	30	30
*upf1*Δ [PSI+u1s5]		28	28
*upf1*Δ [PSI+u1s6]		38	38
*upf1*Δ [PSI+u1s]*	WT ρ^{o}	1	90
*upf2*Δ [PSI+u1s]†	WT ρ^{o}	0	90
*upf3*Δ [PSI+u1s]‡	WT ρ^{o}	0	90

Upf1p-sensitive [PSI+] variants ([PSI+u1s]) were transferred by cytoduction from strain MS2, MS5, and MS6 into the [psi−] ρ^{o} WT strain MS173 and *upf1*Δ strain MS177, *upf2*Δ strain MS62, and *upf3*Δ strain MS65. Ura$^+$ Cytoductants of each were used as reverse-cytoduction donors.
*Ura$^+$ cytoductants from *upf1*Δ [PSI+u1s] to *upf1*Δ [psi−].
†Ura$^+$ cytoductants from *upf1*Δ [PSI+u1s] to *upf2*Δ.
‡Ura$^+$ cytoductants from *upf1*Δ [PSI+u1s] to *upf3*Δ.

Table 7. Mutations in the CH domain or the helicase region of Upf1p can impair elimination of Upf1p-sensitive [PSI+] variants

UPF1 alleles in plasmid	Average % Ura$^+$ transformant clones, ± SD	Phenotype of subclones from Ura$^-$ clones that lost plasmid	
		Ura$^+$	Ura$^-$
Vector	99.9 ± 0.1[a]		
pUPF1-WT	4.7 ± 1.4[b]	2	114
pUPF1-C62Y	92.4 ± 3.5[c]	1	84
pUPF1-C72S	2.5 ± 2.2[b]	0	109
pUPF1-C84S	90.7 ± 5.7[c]	1	97
pUPF1-C125S	86.1 ± 3.3[c]	0	88
pUPF1-K436Q	70.4 ± 7.1[d]	1	121
pUPF1-DE572AA	71.8 ± 2.9[d]	1	94
pUPF1-TR800AA	68.2 ± 4.5[d]	1	113
pUPF1-RR793AA	51.9 ± 2.0[e]	0	78
pUPF1-RR793KK	4.4 ± 1.0[b]	0	81

Primary [PSI+u1s] *upf1*Δ isolates (MS2, MS5, and MS6) and three other [PSI+u1s] isolated in the *upf1*Δ strain MS114 (MS318, MS319, and MS320) were transformed with the *CEN* vector pRS313 or the vector carrying *UPF1* (pM25) or *UPF1* mutated by amino acid substitution(s) under its native promoter. Substituted amino acid(s) and their positions are shown. Transformants were selected in the presence of uracil and were replica-plated to a plate lacking uracil. More than 400 transformant clones were investigated in each case. The average percent of Ura$^+$ transformants, calculated using six independent experiments, ± SD, is shown. Numbers with different letters are significantly different at a *P* value of <0.05 based on the Tukey test. Ura$^-$ subclones that had lost each plasmid were tested for uracil auxotrophy by replica-plating. Their failure to become Ura$^+$ again shows that the prion was cured.

expected, even a 10-fold excess of Upf1p did not affect Ure2p amyloid formation (Fig. 3*B*, green line). However, a decinormal amount of Upf1p was sufficient to slow the growth of Sup35p amyloid by half (Fig. 3*C*, blue line), and Sup35p amyloid did not assemble at all in presence of an equal amount or a 10-fold excess of Upf1p (Fig. 3*C*, brown line and green line, respectively). As internal control, we tested whether FLAG peptide has an effect on amyloid formation or whether Upf1p can form amyloid by itself. However, we did not detect a substantial difference between Sup35p alone and Sup35p mixing with FLAG peptide, and there was no fluorescent signal from Upf1p alone. This indicates that Upf1p has an inhibitory effect on Sup35p amyloid formation in vitro.

Both Sup35p-Binding Activity and Upf Complex Formation Are Required for Efficient Elimination of [PSI+] Prion Variants. For the full function of NMD, both Upf complex formation and binding between each of the Upf proteins and Sup35p are necessary (58, 61, 64). The CH domain of Upf1p plays roles in both Sup35p binding and Upf complex formation by interaction with Upf2p (62, 64, 65). Upf2p, another core NMD component, has a highly acidic domain (AC, amino acids 886–938) for Sup35p binding and a Upf1p-interacting domain (U1I, amino acids 939–1,089) for Upf complex formation (58, 64, 66). To distinguish which of these functions is important for [PSI+] prion curing, a series of *UPF2* plasmids lacking the AC domain, the U1I domain, or both, were generated (Fig. S4*A*) and transformed into *upf2*Δ strains carrying [PSI+] variants. Transformants were then replica-plated on medium with and without uracil (Fig. S4*B*).

Loss of Sup35p-binding activity by deletion of the AC domain decreased the ability to cure [PSI+u1s] or [PSI+u2s] to half that of WT Upf2p, and blocking Upf complex formation by deletion of the U1I domain had a similar effect (Table 8). Deletion of both domains completely eliminated the curing ability for [PSI+u1s] or [PSI+u2s], similar to the empty vector (Table 8 and Fig. S4*B*). This result suggests that both Sup35p binding of Upf2p and formation of the Upf complex are required for efficient curing of the [PSI+] variants and also supports our previous result that each of the Upf proteins is needed for efficient curing of [PSI+] variants that arise in the absence of one Upfp.

Discussion

Using a screen for antiprion factors active against [PSI+] without overproduction, we found that some variants are cured by Upf proteins. By isolating many [PSI+] variants in a *upf1*Δ strain and then restoring the normal level of Upf1p (by mating with an isogenic WT strain, by cytoduction into a *UPF1* strain, or by transformation with the *UPF1* gene on a single-copy plasmid), we found that most [PSI+] variants (Upf1p-sensitive, u1s) are

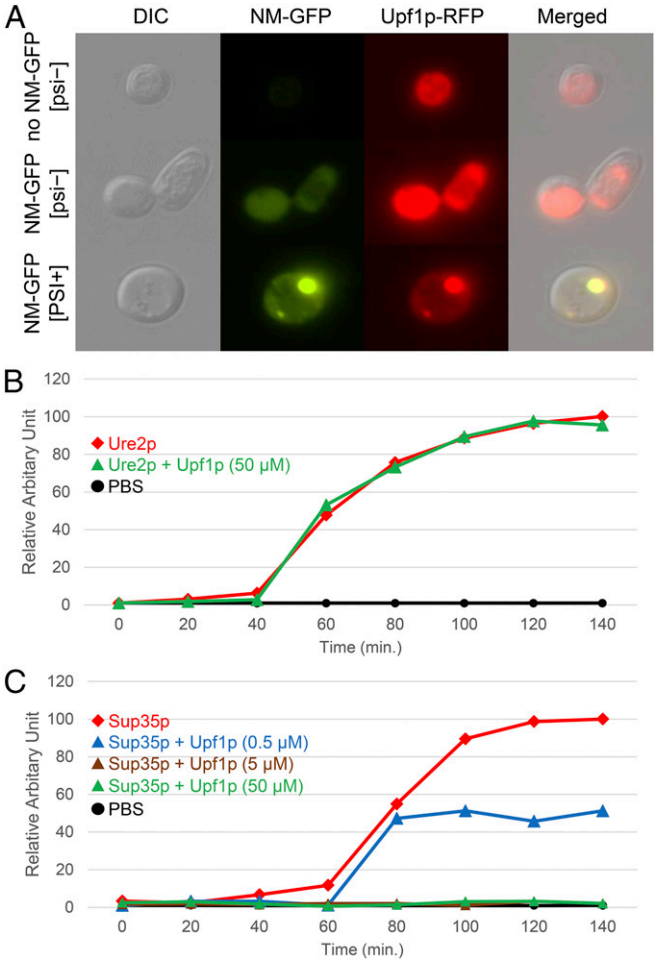

Fig. 3. Upf1p colocalizes with Sup35p prion aggregates in vivo and has an inhibitory effect on the assembly of Sup35p amyloid in vitro. (*A*) Colocalization of Upf1p-RFP and Sup35NM-GFP aggregates in strain MS307 was observed after 48 h of Sup35NM-GFP induction from pSL1066 and visualized by fluorescence confocal microscopy. (*Top*) Sup35NM-GFP not expressed in [psi–] cells. (*Middle*) Sup35NM-GFP expressed in [psi–] cells. (*Bottom*) Sup35NM-GFP expressed in [PSI+] cells. (Magnification: 1,500×.) (*B*) Kinetics of Ure2p amyloid formation with and without Upf1p. Thioflavin-T was used for monitoring amyloid formation. Purified Ure2p (5 μM) was incubated with 50 μM FLAG peptide (red line) or 50 μM Upf1p (green line), with constant shaking at 37 °C. (*C*) Kinetics of Sup35p amyloid formation with and without Upf1p. Purified Sup35p (5 μM) was incubated with 50 μM FLAG peptide (red line) or Upf1p (0.5 μM, blue line; 5 μM, brown line; or 50 μM, green line). The brown, green, and black lines are densely packed near the *x* axis. *B* and *C* show representative individual experiments; data were normalized to the maximum fluorescence level of Ure2p or Sup35p incubated alone, which was set as 100%.

eliminated by restoration of normal levels of Upf1p. Although the known effects of Upf proteins on suppression necessarily complicated the situation, our genetic analysis shows that the loss of the [PSI+] prion is occurring, rather than simply effects on the detection system. In each case, following exposure to Upf1p, the cytoplasm was returned to the *upf1*Δ state (by meiosis, reverse cytoduction, or loss of the *UPF1* plasmid), and the phenotype of [PSI+] was gone, showing that the prion had been lost when the cells had Upf1p. This curing occurs in a WT strain, indicating that Upf1p has an antiprion action. We find that [PSI+] arises at greater frequency in *upf* mutants. This is not simply the detection of weaker [PSI+] variants, as replacing the corresponding *UPF* gene cures nearly all the [PSI+] variants. Ssb1p and Ssb2p, Hsp70 family chaperones central to the ribosome-associated chaperone system, lower the frequency of [PSI+] generation, but their restoration to normal levels does not cure the [PSI+] variants arising in their absence, suggesting that the Upf proteins are not acting by affecting Ssb protein activity.

From yeasts to humans, NMD is a conserved surveillance mechanism targeting for degradation cytoplasmic mRNA containing premature termination codons (PTCs) (48, 61). Efficient activation of NMD requires the conserved core components Upf1p, Upf2p, and Upf3p. The *upf* mutants also led to inefficient translation termination (readthrough of PTCs), in part by allowing the PTC-containing mRNAs to survive longer (59, 60). Yeast and human Upf proteins interact with each other and with both the eukaryotic translation termination factors eRF1 (Sup45p) and eRF3 (Sup35p) (48, 61, 62, 64, 67). Upf1p, the central actor in NMD, is a multifunctional protein classified as a superfamily I RNA helicase with ATPase, ATP-binding, RNA-binding, and Upf2p-binding activities defined by a series of point mutants (59, 60, 65, 68). We used these mutations to examine which functions of Upf1p can affect [PSI+] curing ability. In general, all substitutions led to a decrease in curing ability to some degree (Table 8 and Fig. S3), but there was no close correlation between [PSI+] curing ability and nonsense suppression or PTC-containing mRNA decay.

Table 8. Both the Sup35p-binding activity of Upf2p and Upf complex formation are required for efficient curing of [PSI+] prion variants in a *upf2*Δ strain

Prion isolate	*UPF2* alleles in plasmid	Average % of Ura$^+$ transformant clones	Phenotype of subclones from Ura$^-$ clones that lost plasmid	
			Ura$^+$	Ura$^-$
[PSI+u1s]	Vector	96.0 ± 3.0[a]	0	20
	pUPF2-WT	4.6 ± 1.9[b]	2	52
	pUPF2-ΔAC	55.5 ± 2.5[c]	2	48
	pUPF2-ΔU1I	55.2 ± 1.8[c]	0	41
	pUPF2-ΔACU1I	89.4 ± 2.7[a]	0	45
[PSI+u2s]	Vector	95.4 ± 4.3[a]	0	20
	pUPF2-WT	5.1 ± 1.0[b]	0	36
	pUPF2-ΔAC	56.6 ± 1.6[c]	1	44
	pUPF2-ΔU1I	48.5 ± 4.5[c]	1	50
	pUPF2-ΔACU1I	93.9 ± 3.8[a]	0	41

*upf2*Δ strains (MS321, MS322, MS323) carrying [PSI+u1s] (cytoductants from Table 6) and *upf2*Δ strains carrying three [PSI+u2s] (primary isolates in *upf2*Δ strain MS62 from Table 5) (MS299, MS300, and MS302) were transformed with the *CEN* vector pRS313 or with the vector carrying the *UPF2* or *UPF2* mutants under its native promoter. Deletion mutants of *pUPF2* were denoted as ΔAC lacking the highly acidic domain (amino acids 886–938), ΔU1I lacking the Upf1p-interacting domain (amino acids 939–1,089), and ΔACU1I for the double mutant. Transformants were selected in the presence of uracil and were replica-plated to a plate lacking uracil. More than 200 transformant clones were investigated in each case. The average percent of Ura$^+$ transformants, calculated using six independent experiments, ±SD, is shown. Numbers with different letters are significantly different at a *P* value of <0.05 based on the Tukey test. Ura$^-$ subclones that had lost each plasmid were tested for uracil auxotrophy by replica-plating to confirm that the Ura$^-$ transformants had lost [PSI+].

Upf1p was detected in Sup35p aggregates in extracts of [PSI+] cells (62), and we observed colocalization of Upf1p and [PSI+] aggregates in vivo (Fig. 3*A*), suggesting that Upf1p can interact with both the soluble and amyloid forms of Sup35p in yeast cells. Although there could be substantial difference(s) between the test tube situation and living cells, our in vitro Sup35p amyloid formation assay indicates that Upf1p has an inhibitory effect on the assembly of Sup35p amyloid (Fig. 3*C*).

What is the mechanism of the curing of [PSI+] by the Upf proteins? The frequency with which [PSI+] arises is generally increased by the mutation of any of the *UPF* genes (Table 4), and all three are needed for curing the sensitive [PSI+]s (Table 6). Point mutations in *UPF1* show no clear correlation of [PSI+]-curing ability with NMD efficiency or nonsense-suppression activity or with the helicase, ATPase, ATP-binding, or RNA-binding activities of Upf1p. However, there is some weak correlation with Upf2p-binding activity (Table S4). These lines of evidence all point to the formation of the complex of Upf proteins with the Sup35p-Sup45p termination factor as key to the curing effect. More striking are the effects of Upf2p mutants defective in binding to Sup35p or to Upf1p: Each lowers curing activity by half, and the combined double mutant completely eliminates [PSI+] curing. Moreover, Upf1p alone at a 1/10 molar concentration blocks amyloid formation by Sup35p in vitro. Accordingly, both the formation of the Upf1-2–1-3/Sup35–45 complex and monomeric Upf proteins may compete with the amyloid filaments for Sup35p monomers, inhibiting fiber growth enough to allow other cellular systems to destroy the prion. Alternatively, the association of the Upf complex and Upf proteins with the filaments may block growing points (presumably the filament ends) sufficiently to prevent fiber growth. Our proposed mechanisms are reminiscent of a negative effect of another Sup35p-binding protein, Sup45p, on [PSI+], namely, that overproduction of Sup45p inhibits [PSI+] induction by overproduction of Sup35p but does not affect prion propagation (69).

The association of Upf proteins with Sup35p amyloid filaments in extracts of [PSI+] cells and the colocalization of [PSI+] filaments with Upf1p in vivo (Fig. 3) suggest that the Sup35p amyloid depletes Upf1p (and probably other Upfs) from the available pool. It is likely that this Upf protein deficiency effect contributes to the nonsense-suppression phenotype of [PSI+] strains.

Prion variants can be cured without protein overproduction by Btn2p or Cur1p for [URE3-1] (46) and by Hsp104 or Siw14p for [PSI+] (41, 47). Normal levels of the Ssb chaperones largely block [PSI+] generation (31). These previously reported antiprion systems and the current study indicate that the yeast cell has an exquisite antiprion defense system, specialized for different prions, to repress the generation of new prion variants and block their propagation once they have arisen. While the Upf system is primarily directed at NMD, it certainly also serves as a strong and natural barrier to [PSI+] prion variants.

Although we think of these systems as somewhat analogous to antiviral or antibacterial systems, there is a fundamental difference, in that viruses and bacteria represent an outside invader, whereas prions are an inside-the-cell risk. This is illustrated by the Upf proteins blocking prion formation or curing prions by their normal interactions with Sup35p.

It is remarkable that the Ssb1 system lowers [PSI+] generation frequency by ∼10-fold (31), the Hsp104 antiprion system apparently lowers [PSI+] frequency by ∼13-fold (41), and the Upf antiprion effect described here has a 5- to 10-fold or greater effect on [PSI+]. If these systems work independently, then the

Table 9. Strains used in this study

Strain	Genotype	Source
BY4741/MS157	*MATa ura3 leu2 his3 met15* [psi−][PIN+]	(77)
BY4742/MS317	*MATα ura3 leu2 his3 lys2* [psi−][PIN+]	(77)
MS2,5,6	*MATa ura3 leu2 his3 met15 upf1::kanMX* [PSI+u1s (2, 5, 6)] primary [PSI+] isolates	This study
5335	*MATα ura3 leu2 his3 lys2 kar1Δ15* [psi−][PIN+]	(47)
MS173	*MATα ura3 leu2 his3 lys2 ade1-14 kar1Δ15* [psi−][PIN+]	This study
MS177	*MATα ura3 leu2 his3 lys2 ade1-14 kar1Δ15 upf1::kanMX* [psi−][PIN+]	This study
MS109, MS327	*MATa ura3 leu2 his3 met15 ade1-14 kar1Δ15* [psi−][PIN+]	This study
MS114, MS330	*MATa ura3 leu2 his3 met15 ade1-14 kar1Δ15 upf1::kanMX* [psi−][PIN+]	This study
MS224	*MATa ura3 leu2 his3 met15 ade1-14 kar1Δ15* [PSI+s]	This study
MS225	*MATa ura3 leu2 his3 met15 ade1-14 kar1Δ15* [PSI+w]	This study
MS277	*MATa ura3 leu2 his3 met15 ade1-14 kar1Δ15 upf1::kanMX* [PSI+s]	This study
MS281	*MATa ura3 leu2 his3 met15 ade1-14 kar1Δ15 upf1::kanMX* [PSI+w]	This study
MS286	*MATa ura3 leu2 his3 met15 ade1-14 kar1Δ15 upf1::kanMX* [PSI+u1s]	This study
MS333	*MATa ura3 leu2 his3 met15 ade1-14 kar1Δ15 upf2::kanMX* [psi−][PIN+]	This study
MS336	*MATa ura3 leu2 his3 met15 ade1-14 kar1Δ15 upf3::kanMX* [psi−][PIN+]	This study
MS299, 300, 302	*MATα ura3 leu2 his3 lys2 ade1-14 kar1Δ15 upf2::kanMX* [PSI+u2s]	This study
MS303, 304, 305	*MATα ura3 leu2 his3 lys2 ade1-14 kar1Δ15 upf3::kanMX* [PSI+u3s]	This study
MS308	*MATa ura3 leu2 his3 met15 ade1-14 kar1Δ15 upf2::kanMX* [psi−][PIN+]	This study
MS68	*MATa ura3 leu2 his3 met15 ade1-14 kar1Δ15 upf3::kanMX* [psi−][PIN+]	This study
MS62	*MATα ura3 leu2 his3 lys2 ade1-14 kar1Δ15 upf2::kanMX* [psi−][PIN+]	This study
MS65	*MATα ura3 leu2 his3 lys2 ade1-14 kar1Δ15 upf3::kanMX* [psi−][PIN+]	This study
MS318, 319, 320	*MATa ura3 leu2 his3 met15 ade1-14 upf1::kanMX* [PSI+u1s]	This study
MS321	*MATa ura3 leu2 his3 met15 ade1-14 upf2::kanMX* [PSI+u1s]	This study
MS307	*Matα ura3 leu2 his3 lys2 ade1-14 kar1Δ15 UPF1-mCherry* [psi−][PIN+]	This study
MS339	*MATα ura3 leu2 his3 lys2 ade1-14 kar1Δ15 UPF1-mCherry* [PSI+][PIN+]	This study
BJ2168	*MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 gal2*	Gift of A. Jacobson, University of Massachusetts Medical School, Worcester, MA

Except for strain BJ2168, all strains were isogenic to BY4741, the knockout bank parent.

true frequency of formation of the [PSI+] prion may be quite high. Our studies of Btn2p, Cur1p, Hsp104, Siw14p, and the Upf proteins have revealed classes of prions that are not able to propagate in normal strains because of the respective antiprion systems but are able to arise when those systems are not active. It is an assumption that these curable variants arise in wild strains, justified by the ease with which infectious yeast prions arise from recombinant proteins in the absence of other factors (e.g., refs. 6, 7, 14, 70, and 71). Not included in these variants are the known frankly lethal variants of [PSI+] and the near-lethal variants of [PSI+] and [URE3], which are rarely studied (27). It remains likely that there are other lethal variants of yeast prions for which a permissive condition has not yet been discovered, making their observation impossible so far.

The existence of lethal yeast prion variants (27), the rarity of even the mildest yeast prion variants in wild strains (28), and evidence for the existence of several antiprion systems, including the present study, strongly suggest that yeast prions are not generally advantageous to the cell. The present data suggest that a potentially prion-forming protein will generally be stabilized by maintenance of its normal protein–protein interactions. This phenomenon is somewhat similar to the stabilization of transthyretin against amyloid formation by analogs of thyroid hormone that bind transthyretin even better than the hormone itself, an effect now being used in therapy of transthyretin amyloidosis (72). Enhancing normal interactions may prove to be a general route to deterring the abnormal interactions that constitute amyloidoses of humans (and yeast).

Methods

Nomenclature. Yeast prions are shown in brackets to indicate they are non-chromosomal genes, e.g., [PSI+] or [URE3]. Specific types of prion variants are indicated within the brackets, e.g., "[PSI+u1s]" for Upf1-sensitive [PSI+].

Strains and Media. Strains used in this study are listed in Table 9. Gene-disruption mutants were generated by PCR-amplifying yeast genomic DNA of the corresponding strains from the *Saccharomyces cerevisiae* knockout collection (73). Media used were as described by Sherman (74). Induction of *GAL1*-promoted genes was conducted using galactose/raffinose-containing medium as previously described (46). The *ade1-14* allele from strain 74-D694 (31) was amplified by PCR, transformed into strain 5335 (derived from BY4741) selecting for cotransformation by pRS313. Mixed transformants were plated on 1/2 YPD (0.5% yeast extract, 2% peptone, 2% dextrose, 2% agar), and red clones were isolated and confirmed by PCR and sequencing.

Plasmids. Plasmids used in this study are listed in Table 10. *UPF1*, with 500 bp upstream of the ORF, was amplified and ligated into pRS313 (*CEN HIS3*) cut with BamHI and XhoI forming pM25. Plasmids expressing Upf1p with amino acid replacements were generated from pM25 using the Q5 Site-Directed Mutagenesis Kit (New England BioLabs). *UPF2* and truncated *UPF2*s, with 500 bp upstream of the ORF, were amplified and ligated into pRS313 using Gibson Assembly Master mix (New England BioLabs). *SUP35NM-GFP* was excised from pH1329 and ligated into pH770 (2 μ *HIS3* P_{GAL1}) cut with BamHI and XhoI, forming pM18.

Cytoduction. Mutants in *kar1* can mate, but nuclear fusion does not occur. Subsequent cell divisions result in separation of the two parental nuclei, but the cytoplasm of each daughter cell is a mixture of the cytoplasm of the parents. Although this process is fundamentally symmetric, it is treated as the transfer of cytoplasmic genes from one strain (the donor) to another (the recipient). The recipient's mitochondrial DNA is eliminated by growth in the presence of ethidium bromide, and the transfer of ρ+ (as measured by ability to grow on glycerol) from the ρ+ donor to the ρ^0 recipient shows the transfer of cytoplasm from donor to recipient. Donor and recipient strains were isogenic to the knockout bank strains BY4741 and BY4742. Donors of induced [PSI+] variants and recipients carried the plasmid p1520 (*CEN LEU2 ura3-14*) bearing P_{GAL1}-*SUP35NM* (47). Recipients were made ρ^0 by growing on medium containing 25 μg/mL ethidium bromide. Donor and recipient cells were mixed in distilled water with an ∼3× excess of donor cells and were spotted on a YPAD (74) plate to allow mating. After 7 h incubation at 30 °C, the cell mixture was streaked for single colonies on medium

Table 10. Plasmids used in this study

Name	Description	Source
p1520	p*CEN LEU2 URA3-14* P_{GAL1}*:SUP35NM*	(47)
pRS313	*CEN HIS3*	(78)
pM25	pRS313 P_{UPF1}*:UPF1*	This study
pH770	pRS423 P_{GAL1}	Gift of H. Edskes, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda
pM18	pRS423 P_{GAL1}*:SUP35NM-GFP*	This study
pM28	pM25-*UPF1-K486Q*	This study
pM29	pM25-*UPF1-DE572AA*	This study
pM30	pM25-*UPF1-TR800AA*	This study
pM31	pM25-*UPF1-RR793AA*	This study
pM32	pM25-*UPF1-RR793KK*	This study
pM33	pM25-*UPF1-C62Y*	This study
pM34	pM25-*UPF1-C72S*	This study
pM35	pM25-*UPF1-C84S*	This study
pM36	pM25-*UPF1-C125S*	This study
pSL1066	p*CEN URA3* P_{CUP1}*:SUP35NM-GFP*	(63)
pM27	p*FA6a*-link-*yomCherry-HIS5*	Addgene 44841
pKT-41	pET17b-*URE2* Full-length	(11, 13)
pM41	pET13b-*SUP35* Full-length	Gift of F. Shewmaker, Uniformed Services University of the Health Sciences, Bethesda, MD
pM46	pG1-FLAG-*UPF1*	A. Jacobson, University of Massachusetts Medical Center, Worcester, MA (62)
pM54	pRS313 P_{UPF2}*:UPF2*	This study
pM55	pM54 *UPF2*-ΔAC	This study
pM56	pM54 *UPF2*-ΔU1I	This study
pM57	pM54 *UPF2*-ΔACU1I	This study

selecting against the donor. After 3 d incubation at 30 °C, single colonies were replica-plated to yeast extract, peptone, glycerol (YPG) medium, to medium selective for diploids, or to medium lacking uracil. Clones growing on YPG medium, but not those growing on diploid-selection medium, were cytoductants. Clones that grew on the –Ura plate propagated [PSI+].

Measuring [PSI+] Prion Seed Number. Following Cox et al. (52), freshly grown [PSI+] cells were streaked for single colonies on YPAD medium containing 4 mM or 5 mM guanidine HCl. Single colonies with the underlying agar block were suspended in distilled water and plated on medium lacking uracil. Colonies showing the Ura^+ phenotype were counted and were assumed to represent [PSI+] prion seeds of the cell founding that colony. For each strain, at least 10 individual colonies were tested. A sample of Ura^+ colonies was checked for guanidine curability.

Amyloid Assembly and Expression of Recombinant Proteins. Fluorescence of thioflavin-T (Sigma-Aldrich) was used to measure Sup35p and Ure2p amyloid formation as described (42, 75). To form amyloid, Sup35p and Ure2p were diluted from purified stock solutions to 5 μM in 300 μL of 1× PBS and were shaken at 200 rpm at 37 °C. Aliquots were sampled at the specific time points, further mixed with thioflavin-T (final concentration 50 μM), and incubated at room temperature for 10 min without shaking. Fluorescence signal measurement was performed using a SupraMax M5 (Molecular Devices) with an excitation of 420 nm and emission of 495–500 nm.

Proteins used in the assay were expressed in *E. coli* strain BL21-CodonPlus (DE3)-RIPL (Agilent Technologies). Full-length Sup35p and Ure2p were prepared from pM41 and pKT-41, respectively. Cells were grown at 37 °C in LB medium to an optical density of 0.6 at 600 nm and were induced with 0.4 mM isopropyl β-D-1-thiogalactopyranoside. The collected cells were lysed by sonication in buffer containing 20 mM $NaH_2PO_4{\cdot}H_2O$, 500 mM NaCl, and 20 mM imidazole (His-tag column-binding buffer). After cell debris was removed by centrifugation, proteins in the supernatant were purified using His GraviTrap (GE Healthcare Life Sciences). The eluted proteins were desalted using a PD-10 desalting column (GE Healthcare Life Sciences). The final proteins were eluted in 1× PBS buffer. FLAG-tagged full-length Upf1p was expressed in yeast strain BJ2168 using pG1-FLAG-Upf1p, as described previously (76). Total yeast protein was extracted using CelLytic Y (Sigma-Aldrich), and Upf1p was further purified using ANTI-FLAG M2 Affinity Gel (Sigma-Aldrich), following the manufacturer's manual. Purified protein was eluted in 3×FLAG peptides (Sigma-Aldrich) containing 1× PBS buffer. Protein concentrations were determined by Bradford assay or absorbance at 280 nm. In all cases, protein aliquots were frozen at −80 °C before use.

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