Normal levels of ribosome-associated chaperones cure two groups of [PSI+] prion variants

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Contributed by Reed B. Wickner, September 9, 2020 (sent for review August 11, 2020; reviewed by Chih-Yen King and Susan Liebman)

The yeast prion [PSI+] is a self-propagating amyloid of the translation termination factor, Sup35p. For known pathogenic prions, such as [PSI+], a single protein can form an array of different amyloid structures (prion variants) each stably inherited and with differing biological properties. The ribosome-associated chaperones, Ssb1/2p (Hsp70s), and RAC (Zuo1p (Hsp40) and Ssz1p (Hsp70)), enhance de novo protein folding by protecting nascent polypeptide chains from misfolding and maintain translational fidelity by involvement in translation termination. Ssb1/2p and RAC chaperones were previously found to inhibit [PSI+] prion generation. We find that most [PSI+] variants arising in the absence of each chaperone were cured by restoring normal levels of that protein. [PSI+] variants hypersensitive to Ssb1/2p have distinguishable biological properties from those hypersensitive to Zuo1p or Ssz1p. The elevated [PSI+] generation frequency in each deletion strain is not due to an altered [PIN+], another prion that primes [PSI+] generation. [PSI+] prion generation/propagation may be inhibited by Ssb1/2/RAC chaperones by ensuring proper folding of nascent Sup35p, thus preventing its joining amyloid fibers. Alternatively, the effect of RAC/Ssb mutations on translation termination and the absence of an effect on the [URE3] prion suggest an effect on the mature Sup35p such that it does not readily join amyloid filaments. Ssz1p is degraded in *zuo1Δ* [psi-] cells, but not if the cells carry any of several [PSI+] variants. Our results imply that prions arise more frequently than had been thought but the cell has evolved exquisite antiprion systems that rapidly eliminate most variants.

prion | antiprion system | [PSI+] | Ssb | ribosome-associated complex

Like the [PSI+] prion, an amyloid of the translation termination factor Sup35p, [URE3] is a prion of Ure2p, a repressor of transcription of genes for catabolism of poor nitrogen sources (1–16). The majority of prion variants/strains are lethal or highly pathogenic (17), and the absence of these prions in 70 wild yeast strains indicates that even the mildest variants are net deleterious to the host (18, 19). Evidently, the loss of precise translation termination (in [PSI+] strains) or of intrinsic nitrogen regulation (as in [URE3] cells) by conversion of soluble Sup35p or Ure2p to prion amyloid aggregates, is detrimental to the host cell. In addition, most variants of these two prions have detrimental effects beyond mere deficiency of the normal form (17; reviewed in ref. 20). To prevent these harmful diseases, yeast has evolved systems to repress prion generation, to cure prions after they arise ("antiprion systems"), or to limit prion toxicity, like the antipathogen systems in mammals targeting fungi, bacteria, and viruses. But, unlike invaders from outside, most cases of prion disease are sporadic, arising spontaneously for no known reason. So, it is inferred that, in a normal cell, cellular antiprion systems are continuously blocking prion generation, propagation, and toxicity (reviewed in refs. 20–22). In the absence of an antiprion factor prions will appear with elevated frequency and many of those prion variants that arose in its absence are cured when the antiprion factor is restored to normal levels.

The disaggregating chaperone Hsp104 was first found to function in the propagation of [PSI+] (23) and other amyloid-based prions (24), and in the curing of [PSI+] by its overproduction (23). These two different activities of Hsp104 were then separated by showing that disruption of the Hsp104 N-terminal region eliminates the Hsp104 overproduction curing ability without any effect on [PSI+] propagation (25). In fact, spontaneous [PSI+] generation was elevated by over 10-fold in an N-terminal mutant, *hsp104*^{T160M}, and the normal level of WT Hsp104 was able to cure many of the [PSI+] variants arising in the mutant strain (26).

Overproduced endosomal sorting factor Btn2p and its paralogue Cur1p were shown to cure the [URE3] prion, in the case of Btn2p by sequestering [URE3] prion aggregates, preventing distribution of aggregates to daughter cells (27, 28). It was then found that most of the [URE3] prion variants arising in a *btn2Δ cur1Δ* strain were cured by reintroduction of normal levels of either Btn2p or Cur1p or both (29). Although these three prion-curing systems were initially identified by using overproduction curing, it was then shown that these systems are working constantly in a normal cell whose cellular environment is not altered by intentional overproduction or deficiency of any components.

Most recently, a simple genetic screen for finding antiprion factors identified two novel anti-[PSI+] components, Siw14p, a pyrophosphatase specific for 5PP-IP5 (5-diphosphoinositol pentakisphosphate) in the inositol polyphosphate synthesis pathway, and Upf proteins (Upf1p, Upf2p and Upf3p), core components of the nonsense-mediated mRNA decay (NMD) apparatus, respectively (30, 31). By lowering the levels of some inositol poly-/pyro-phosphates (PP-IPs), restored normal levels of Siw14p cure many [PSI+] prion variants that were generated in its absence and are dependent on the resulting higher PP-IP levels (30). Normal levels of Upf proteins constantly repress [PSI+] occurrence and

Significance

[PSI+] is a prion (infectious protein) form of the yeast Sup35 protein, propagating as an amyloid filamentous polymer. We find that each of the ribosome-associated chaperones—Ssb1/2p, Zuo1p, and Ssz1p—at their normal expression levels, blocks [PSI+] generation and the propagation of most new [PSI+] prion variants generated in their absence. The curing mechanism involves the functional triad of ribosome-associated chaperones. Our results suggest that cells do not want to have a prion: Cells reduce the chance of a prion emerging at the polypeptide level, and usually immediately cure those prions that do arise perhaps by limiting fiber growth.

Author contributions: M.S. and R.B.W. designed research; M.S. performed research; M.S. contributed new reagents/analytic tools; M.S. and R.B.W. analyzed data; and M.S. and R.B.W. wrote the paper.

Reviewers: C-Y.K., Institute of Molecular Biology, Academia Sinica; and S.L., University of Nevada.

The authors declare no competing interest.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2016954117/-/DCSupplemental.

First published October 5, 2020.

block the propagation of most [PSI+] prion variants formed in their absence. The curing mechanism involves Sup35p-binding by each Upf protein and by the trimeric Upf complex (31). These studies of Btn2p, Cur1p, Hsp104, Siw14p, and the Upf proteins have uncovered new classes of prion variants, unable to arise and further propagate in normal strains with the respective antiprion systems, but able to arise and further propagate in cells without those systems (reviewed in ref. 20).

The Hsp70 chaperones Ssb1p and Ssb2p (Ssb1/2p) bind directly to translating ribosomes and newly synthesized nascent polypeptide chains (32, 33). Zuo1p (Hsp40/DnaJ homolog) and Ssz1p (Hsp70/DnaK homolog) form a stable heterodimeric ribosome-associated complex (RAC) that is necessary for the ribosome-association of Ssb1/2p (34–38). Moreover, deletion of both *SSB1* and *SSB2* or of *ZUO1* or *SSZ1* showed similar phenotypes, such as growth defects, cold sensitivity, and severe sensitivity to translation inhibitors (32, 34, 36). Ssb1/2p and RAC act in concert on nascent polypeptides and protect them from misfolding and aggregation (39, 40; reviewed in refs. 41 and 42).

Chernoff aptly described *ssb1/2* mutations as "protein mutators," when he showed that they increased the frequency of the cytoplasmic gene [PSI+] arising (43). In accordance with the cellular functions of the Ssb1/2p-RAC system, deletion of *SSB1/2* or *ZUO1* or *SSZ1* lead to increased frequency of [PSI+] generation, either spontaneously or induced by Sup35p overproduction (43–46). Restored Ssb1p on a *CEN* plasmid was unable to cure [PSI+] variants arising in an *ssb1/2*Δ strain (43). Curing of [PSI+] by overproduction of Hsp104 was impaired by *ssb1/2*Δ, but made more efficient in *ssz1*Δ or *zuo1*Δ (43, 44). The observed release of Ssb1/2p from ribosomes in *ssz1*Δ or *zuo1*Δ mutants led to the inference that soluble Ssb1/2p impairs [PSI+] propagation, while ribosome-bound Ssb1/2p impairs [PSI+] generation (44).

Here, we find that most [PSI+] prion variants arising in the absence of Ssb1p or RAC are stably propagated in the mutant strain, but can be cured by restoring normal levels. Furthermore, we show that different [PSI+] variants arise in mutants of different ribosome-associated chaperones.

Results

Spontaneous and Induced [PSI+] Generation Is Elevated in Ribosome-Associated Chaperone-Deficient Mutants. Both spontaneous and induced [PSI+] generation (by Sup35 N or NM overproduction) were previously reported to be elevated in *ssb1/2*Δ, *zuo1*Δ, or *ssz1*Δ strains (43–47). To confirm these results and obtain [PSI+] isolates in each mutant strain for further use, [PSI+] generation was investigated. [PIN+] ([PSI]-inducibility) is a prion form of Rnq1p that strongly increases the frequency of spontaneous or induced [PSI+] appearance (48–51). Because [PIN+] variants differ dramatically in their ability to stimulate [PSI+] appearance (52), comparison of [PSI+] generation rates must be done in cells that have the same [PIN+] variant. For these experiments, each strain to be tested for [PSI+] generation was cured of prions by growth on 5 mM guanidine HCl (GuHCl), an inhibitor of Hsp104 that cures all amyloid-based prions (53–56). Into each resulting [psi−][pin−] recipient strain (WT and mutants), [PIN+] was transferred by cytoduction from a single donor (BY4742) (*SI Appendix*, Table S1) (57). The resulting [PIN+] recipient strains were transformed with centromeric plasmid p1520 (*SI Appendix*, Table S2) (30, 31) carrying the [PSI+]-suppressible nonsense allele *ura3-14* (58), and the Sup35 prion-forming domain (NM) driven by the *GAL1* promoter. Strains were grown 2 d in glucose or galactose, and cells were plated on −Ura plates to select Ura$^+$ ([PSI+]) clones. To check that these Ura$^+$ clones are [PSI+], they were then tested for GuHCl curability by transient growth on 1 mM or 5 mM GuHCl.

For spontaneous [PSI+] generation, the frequency of [PSI+] clones in *ssb1/2*Δ, *zuo1*Δ, or *ssz1*Δ was increased 19-, 16-, or 13-fold, respectively, compared to the WT (Table 1). For [PSI+] generation induced by Sup35NM overexpression, *ssb1/2*Δ, *zuo1*Δ, and *ssz1*Δ strain showed 40-, 37-, and 51-fold increased [PSI+] clones compared with the WT strain (Table 1).

Normal Levels of Ssb1p, Zuo1p, or Ssz1p can Cure Most [PSI+] Variants Isolated in Absence of Each Component. Altered levels or alteration of Sis1, Hsp104, Ssa, Ssb, or Sup35 proteins can destabilize [PSI+] (8, 23, 59–63), but the levels of these and Ydj1 are unaltered in *ssz1, zuo1*, or *ssb1/2* mutants (43, 44, 64). We tested for Ssb1/2p, Zuo1p, or Ssz1p as prion-curing factors without protein overproduction. GuHCl-curable Ura$^+$ [PSI+] clones (from Table 1) were crossed with the isogenic [psi−] WT strain MS173 to complement the deletion in each strain. For *ssb1*Δ*ssb2*Δ [PSI+], 60 of 72 isolates gave Ura$^-$ diploids on mating with WT. For *zuo1*Δ [PSI+] and *ssz1*Δ [PSI+], 32 of 50 and 46 of 60 isolates, respectively, similarly produced Ura$^-$ diploids with the WT [psi−] strain. Among the isolates from each mutant strain that gave Ura$^-$ diploids, 30 were mated with either the WT [psi−] strain MS173 or a *ssb1/2*Δ [psi−] strain MS520, *zuo1*Δ [psi−] strain MS528, and *ssz1*Δ [psi−] strain MS514. All of the diploids formed with the WT (heterozygotes) were Ura$^-$, but the diploids with the same deletion mutant [psi−] strains were Ura$^+$ (Fig. 1). Among 30 isolates in each case, 12 isolates were randomly selected and tested for [PSI+] stability in the haploid, heterozygotes, or homozygotes by subcloning on YPAD medium. Both the haploid and diploid subclones were then replica-plated to –Uracil media. All [PSI+] prion variants were more stable in the original haploid and homozygous diploid than in the complemented heterozygous diploid (Table 2).

Meiotic segregation of each Ura$^-$ heterozygote showed mostly 4 Ura$^-$: 0 Ura$^+$ segregation, 20 of 24 tetrads for *ssb1ssb2*Δ/$^+$, 8 of 12 tetrads for *zuo1*/$^+$, and 21 of 26 tetrads for *ssz1*/$^+$, respectively. Only a few mutant segregants showed a Ura$^+$ or very weak Ura$^+$ phenotype, but the majority of mutant segregants were Ura$^-$: 75 to 100% (average 92.6%) for *ssb1/2*Δ segregants, 33 to 100% (average 68.2%) for *zuo1*Δ segregants, 50 to 100% (average 83.7%) for *ssz1*Δ segregants. This shows that [PSI+] is gradually lost in the heterozygous diploids. Although *ssb1/2*Δ, *ssz1*Δ, and *zuo1*Δ mutants have a slight nonsense-suppression phenotype (43, 65), the meiotic analysis shows that the Ura$^-$ phenotype of the heterozygous diploids is due to loss of [PSI+], not simply an effect on translation termination (these are, after all, ribosomal proteins). However, if, after mating with the isogenic WT [psi−] strain MS173, the diploids were immediately sporulated before loss of

Table 1. De novo generation of [PSI+] variants is elevated in *ssb-rac* deficient cells

Host	Total Ura$^+$/10^6 cells	
	Spontaneous [PSI+]	Induced [PSI+]
WT	0.36 ± 0.1	43 ± 6
*ssb1*Δ*ssb2*Δ	7.2 ± 0.5	1,740 ± 200
*zuo1*Δ	6.0 ± 0.7	1,600 ± 160
*Ssz1*Δ	4.5 ± 0.4	2,200 ± 230

Strains MS327, MS515, MS527, and MS510 (top to bottom) carry the same [PIN+]. For spontaneous [PSI+], cells were grown 2 d in 2% glucose liquid culture at 30 °C, and 10^7 yeast cells were spread on standard SC plates without uracil. For induced [PSI+] formation, strains carrying p1520 with *SUP35NM* driven by a galactose-inducible promoter were grown 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 at least three independent experiments were combined). Numbers represent Ura$^+$ colonies with [PSI+] that were confirmed by GuHCl curability. Ura$^+$ colonies ± SD is shown.

GENETICS

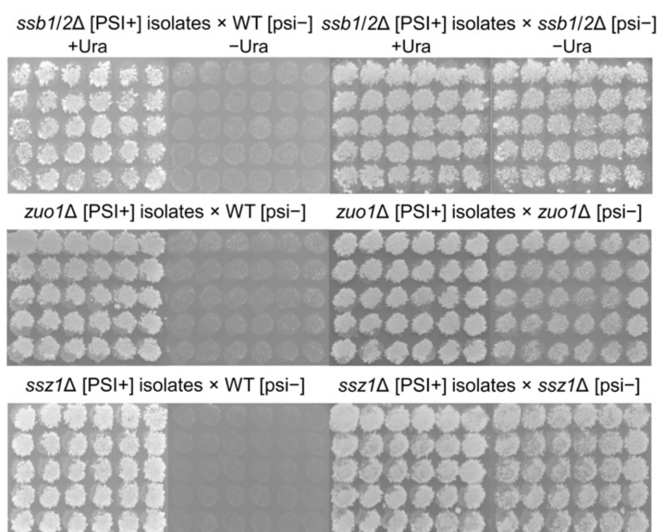

Fig. 1. Most [PSI+] prion variants isolated in *ssb1/2*Δ, *zuo1*Δ, and *ssz1*Δ strains are lost in the presence of the WT allele of *SSB1*, *ZUO1*, and *SSZ1*, respectively (see text). Such [PSI+] isolates in each deletion strain MS515, MS527, and MS510 were mated for 2 d on YPAD with either isogenic WT MS173 or each deletion strain MS520, MS528, or MS514 and replica-plated to minimal media with and without uracil. The presence of p1520 (p*CEN LEU2 ura3-14*) in all strains enables scoring [PSI+]. Diploids formed with WT are almost all Ura^- as a result of elimination of [PSI+] (*Left*). Diploids formed with deletion strains are Ura^+ indicating stable maintenance of [PSI+] (*Right*).

[PSI+] variants could happen in the diploids, segregations were two Ura^- *SSZ1*: two Ura^+ *ssz1*Δ (20 of 21 tetrads) or two Ura^- *ZUO1*: two Ura^+ *zuo1*Δ (14 of 15 tetrads). In case of *ssb1ssb2*Δ/$^+$, all of the 22 Ura^+ spore clones were *ssb1*Δ *ssb2*Δ. This confirms that it is the *ssb* or *zuo1* or *ssz1* mutation that allows propagation of these [PSI+] variants. The combined results from meiotic analysis show that each of these [PSI+] prion variants is eliminated by normal levels of Ssb1/2p, Zuo1p, or Ssz1p.

Based on their loss of [PSI+] on restoration of normal levels of Ssb1/2p, Zuo1p, or Ssz1p, these variants (all guanidine-curable and mitotically stable in the mutant) are denoted [PSI+sbs] for Ssb1/2p-sensitive, [PSI+zos] for Zuo1p-sensitive, and [PSI+szs] for Ssz1p-sensitive, respectively. To confirm loss of [PSI+] variants in all different Ura^- segregants that showed 4 Ura^-: 0 Ura^+ segregation, formation of aggregate structures was examined. To detect prion aggregates, Sup35NM-GFP was transiently overexpressed in Ura^- segregants and [PSI+] or [psi−] strains with the same backgrounds. Like the WT [psi−][PIN+] or mutant [psi−][PIN+] strain, most cells of each Ura^-segregant examined showed a diffuse GFP signal (Fig. 2). Single or multiple foci were rarely detected in in the Ura^- segregants, but were detected in many cells of different [PSI+]-carrying strains (Fig. 2). In addition, to double-check loss of [PSI+sbs], [PSI+zos], or [PSI+szs] in WT strains, cytoduction was conducted using each [PSI+] variant-carrying mutant strain as a donor and WT [psi−] or mutant [psi−] strain as a recipient. The majority of WT recipients in each case showed a Ura^- phenotype (*SI Appendix*, Table S3). To verify that these Ura^- phenotypes are due to loss of [PSI+] in the WT cytoductants, reverse-cytoductions were performed using Ura^- WT cytoductants as donors and each mutant [psi−] strain as a recipient. Almost all reverse-cytoductants still showed a Ura^- phenotype in each mutant recipient strain. For unknown reasons, too few cytoductants were obtained in the combination of mutant donor and mutant recipient, to enable analysis of such matings. Once again loss of different [PSI+] variants in WT strains was confirmed by examining the formation of fluorescent structures. As expected, mainly a diffuse GFP signal was observed in each Ura^- WT cytoductant like the control WT [psi−] strain (*SI Appendix*, Fig. S1). Taken together, these combined results demonstrated that many [PSI+] variants isolated in each mutant strain are indeed lost in the presence of normal levels of the corresponding *SSB*, *ZUO1*, or *SSZ1* in WT strains.

To further confirm that Ssb1, Zuo1p, or Ssz1p function in eliminating the [PSI+] prion variant in each mutant strain, pRS313 (*CEN* vector) or pRS313 with the corresponding gene (pM76 = pRS313-*SSB1*, pM75 = pRS313-*ZUO1*, pM78 = pRS313-*SSZ1*) controlled by its own promoter, were transformed into each [PSI+] isolate, respectively. Transformants were selected

Table 2. [PSI+] isolates are more stable in *ssb-rac*Δ strains than in the *ssb-rac*Δ/+ diploid strains

	Ura^+/total subclones								
	[PSI+sbs] in			[PSI+zos] in			[PSI+szs] in		
Isolate	*ssb1/2*Δ	*ssb1/2*Δ/+	*ssb1/2*Δ/−	*zuo1*Δ	*zuo1*Δ/+	*zuo1*Δ/−	*ssz1*Δ	*ssz1*Δ/+	*ssz1*Δ/−
1	30/30	1/20	20/20	28/30	3/20	18/20	30/30	1/20	18/20
2	30/30	2/20	20/20	30/30	0/20	19/20	30/30	0/20	19/20
3	25/30	1/20	20/20	22/30	1/20	19/20	29/30	0/20	20/20
4	30/30	0/20	18/20	24/30	1/20	19/20	30/30	0/20	20/20
5	30/30	2/20	17/20	28/30	1/20	20/20	30/30	0/20	20/20
6	30/30	1/20	18/20	28/30	1/20	19/20	30/30	0/20	20/20
7	26/30	0/20	19/20	27/30	2/20	19/20	30/30	2/20	20/20
8	30/30	3/20	20/20	26/30	2/20	20/20	27/30	0/20	19/20
9	30/30	0/20	20/20	21/30	1/20	18/20	25/30	7/20	18/20
10	27/30	1/20	19/20	24/30	1/20	20/20	27/30	6/20	18/20
11	30/30	2/20	20/20	29/30	2/20	18/20	27/30	4/20	18/20
12	30/30	1/20	19/20	30/30	1/20	19/20	30/30	2/20	19/20
Total	348/360	14/240	232/240	317/360	16/240	228/240	349/360	22/240	229/240
% Ura+	96.7%	5.8%	96.7%	88.1%	6.7%	95.0%	96.9%	9.2%	95.4%

Twelve [PSI+] isolates in each haploid strain were obtained and were either subcloned on YPAD medium or were mated with isogenic WT strain MS173 and the each of diploids formed were subcloned on YPAD medium. Each haploid strain, heterozygous- and homozygous-diploids were replica-plated to −Ura plates to test the stability of [PSI+]. Each prion variant was more stable in its original host (haploids) or homozygous diploids than in the complemented (heterozygous) diploids.

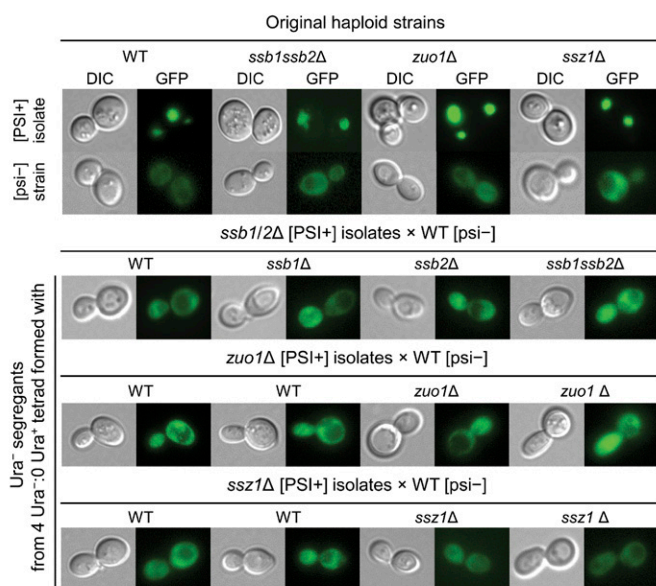

Fig. 2. [PSI+] prion variants are lost in heterozygous diploids formed with an isogenic WT strain. (*Top*) Original haploids of [PSI+] strains (WT MS224, [PSI+] isolates in each deletion strain MS515, MS527, and MS510: left to right), and [psi−] strains (MS327, MS520, MS528, and MS514: left to right), and Ura$^-$ segregants (*Top* to *Bottom*) were transformed with a 2-μ plasmid pH770/pM18 encoding Sup35NM-GFP controlled by the GAL1 promoter. Each Ura$^-$ segregant was obtained from a 4 Ura$^-$: 0 Ura$^+$ tetrad of the indicated heterozygous diploid, and its genotype was examined by replica-plating. The genotype of cells examined is shown above the image. After GAL induction for 16 h in 2% (wt/vol) raffinose, 2% (wt/vol) galactose minimal medium, Sup35NM-GFP aggregates or diffused GFP signal were observed using fluorescence confocal microscopy (magnification 1500×).

in media containing uracil and then replica-plated to plates lacking uracil to analyze the stability of [PSI+] variants. Most of the transformants carrying p*SSB1*, p*ZUO1*, or p*SSZ1* were unable to grow on media lacking uracil, indicating that each [PSI+] prion variant was eliminated by the restored normal levels of Ssb1, Zuo1p, or Ssz1p (Table 3). From Ura$^-$ transformants, 50 subclones for each case that had lost p*SSB1*, p*ZUO1*, or p*SSZ1* were found to remain Ura$^-$ in 86%, 92%, or 100% of cases. The maintenance of Ura$^-$ phenotype in each subclone after plasmid loss indicates that [PSI+] prion variants are indeed eliminated by each plasmid, not that its phenotype is merely disguised (Table 3).

Ssb1/2p-Sensitive [PSI+sbs] Variants Differ from Zuo1p-Sensitive or Ssz1p-Sensitive [PSI+] Variants in Propagation Ability. Yeast strains lacking any one or all three of the components of the ribosome-bound tripartite Ssb1/2p-RAC system show similar phenotypes, such as slow growth, hypersensitivity to several translation-inhibitors, and sensitivity to high salt or low temperature (32–34, 36–38). Thereby, all three appear to be functionally equivalent in protecting newly synthesized nascent polypeptide chains from misfolding or aggregation (38–40). First, we tried to test whether each [PSI+] prion variant can propagate in each other deletion strain even in presence of the originally missing protein, for example, infecting [PSI+sbs] into a *zuo1*Δ [psi−] or *ssz1*Δ [psi−] recipient strain. However, like our result described above and in *SI Appendix*, Table S3, we were unable to obtain enough cytoductants to analyze the combination of mutant donor and mutant recipient, and this was not caused by [PSI+]. We then examined the stability of each [PSI+] variant in different backgrounds using doubly heterozygous diploids. The *ssb1/2*Δ [PSI+sbs] isolates were mated with *zuo1*Δ [psi−], *ssz1*Δ [psi−], or *ssb1/2*Δ*zuo1*Δ [psi−] strains, and each were subcloned on YPAD and further tested for [PSI+] by assaying uracil auxotrophy. Almost all diploids formed with *ssb1/2*Δ*zuo1*Δ were Ura$^+$ (96 of 100) like *ssb1/2*Δ/$^-$ homozygotes (Fig. 1 and Table 2), but not the diploids with *zuo1*Δ [psi−] or *ssz1*Δ [psi−]. The latter diploids grew on −Ura media, but only slowly (Fig. 3), and were mixtures of Ura$^+$ (35of 100 and 11 of 100, respectively) and Ura$^-$ phenotype (*SI Appendix*, Table S4). The diploids formed with either *zuo1*Δ [PSI+zos] or *ssz1*Δ [PSI+szs] and *ssb1/2*Δ [psi−] were nearly all Ura$^-$ (Fig. 3 and *SI Appendix*, Table S4), like diploids with the WT (Fig. 1 and Table 2), while the diploids with a strain lacking the other partner of RAC were faithfully Ura$^+$ (Fig. 3 and *SI Appendix*, Table S4). Propagation abilities of [PSI+sbs] and [PSI+zos or szs] in identical doubly heterozygous diploids (*ssb1ssb2*Δ/+ *zuo1*Δ/+ [or *ssz1*Δ/+]) are different. Note that the same two parent strains were used in these crosses; in one case, [PSI+sbs] was generated in the *ssb1ssb2*Δ parent, while in the other cross, [PSI+zos] (or [PSI+szs]) was generated in the other parent. The nuclear genotypes of the diploids were the same; only the [PSI+] prion variant was different. The behavior of [PSI+zos] and [PSI+szs] were exactly similar. These results indicate that [PSI+] isolates in the *ssb1/2*Δ strain and those in *zuo1*Δ or *ssz1*Δ strains have distinct characteristics, even though these three molecular chaperones are part of the same complex on the ribosomes and their mutants show similar characteristics for cellular functions.

The Effects of Deletion of *SSB1/2*, *ZUO1*, *SSZ1*, or of [PSI+] on the Ribosome Association and Stability of Chaperones. The deletion of a RAC component gene leads to the release of at least a portion of Ssb1/2p from the ribosome to the cytosol (44). This seems to affect prion generation and propagation. To understand the mechanisms of inhibition of prion generation or propagation by Ssb1/2p or RAC, the levels and soluble fraction of Ssb1/2p, Zuo1p, or Ssz1p was analyzed by SDS/PAGE and immunoblotting with antibodies. Total extracts (T) of each strain with/without

Table 3. Restored normal level of components of ribosome-associated chaperone can eliminate each of [PSI+] variants

	*ssb1/2*Δ [PSI+sbs] transformants (Ura+/total transformants)		*zuo1*Δ [PSI+zos] transformants (Ura+/total transformants)		*ssz1*Δ [PSI+szs] transformants (Ura+/total transformants)	
Isolate	Vector	p*SSB1*	Vector	p*ZUO1*	Vector	p*SSZ1*
1	49/50	16/50	49/50	25/50	48/50	5/30
2	48/50	18/50	46/50	16/50	47/50	6/30
3	47/50	17/50	48/50	30/50	48/50	5/30
4	47/50	16/30	49/50	27/50	48/50	6/30
5	48/50	14/40	50/50	14/50	48/50	7/30
6	49/50	18/45	48/50	31/50	50/50	15/50
7	26/26	4/30	26/27	7/50	50/50	9/40
8	23/24	14/50	29/29	7/50	46/50	5/50
9	28/29	16/50	32/34	32/50	45/50	7/40
10	27/28	14/50	52/57	14/50	44/50	5/31
11	24/28	9/50	38/44	18/50	36/40	8/30
12	28/30	3/50	32/33	6/50	34/40	11/50
Total	444/465	159/545	499/524	227/600	544/580	81/441
% Ura+	95.48%	29.17%	95.23%	37.8%	93.79%	20.18%

Twelve strains carrying [PSI+sbs], [PSI+zos], or [PSI+szs] were transformed with the *CEN* plasmid pRS313 or the same plasmid carrying *SSB1*, *ZUO1*, or *SSZ1*, respectively, under their native promoters (pM76 = p*SSB1*, pM75 = p*ZUO1*, pM78 = p*SSZ1*). Transformants were selected in the presence of uracil and were replica-plated to a plate lacking uracil to test the stability of [PSI+]. Subclones of Ura$^-$ transformants that had lost pM76, pM75, or pM78 were tested for uracil auxotrophy by replica-plating; 86–100% of such subclones in each case showed Ura$^-$ phenotype, indicating that the [PSI+] variants had been largely eliminated by pM76, pM75, and pM78.

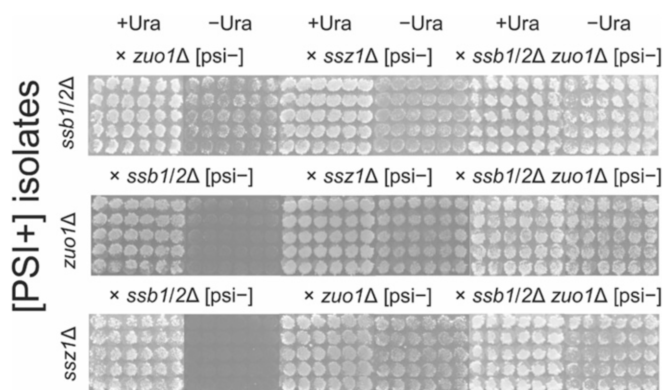

Fig. 3. [PSI+sbs], [PSI+zos], and [PSI+szs] prion variants are differentiated by their propagation ability in doubly heterozygous diploids. [PSI+sbs], [PSI+-zos], and [PSI+szs] isolates were mated on YPAD with *ssb1/2*Δ [psi−] MS520, *zuo1*Δ [psi−] MS528, *ssz1*Δ [psi−] MS514, or *ssb1/2*Δ*zuo1*Δ [psi−] MS560. Doubly heterozygous diploids were replica-plated to minimal media with and without uracil, supplemented with adenine and histidine for diploid selection.

[PSI+] were prepared and soluble (S) and pellet (P) fractions were separated using ultracentrifugation (*Material and Methods*).

In WT [psi−] or WT [PSI+] cells, Ssb1/2p was found in both soluble and pellet fraction, with a lower amount in the pellet (Fig. 4). However, in either *zuo1*Δ or *ssz1*Δ cells, Ssb1/2p was found only in the soluble fraction, not in the pellet fraction (below the detection level), in both [psi−] and [PSI+] cells (Fig. 4). This confirms again that ribosome-associated Ssb1/2p is released from ribosomes in either *zuo1*Δ or *ssz1*Δ cells (of our strain background) (44). As a ribosome-associated protein, both Zuo1p and Ssz1p in WT cells or *ssb1/2*Δ cells are detected only in the pellet fraction like ribosomal protein Rpl15a (Fig. 4). Zuo1p (expected size 49 kDa) was present, but slightly decreased in size in *ssz1*Δ [psi−] cells, and decreased in amount and appearing as two bands in *ssz1*Δ [PSI+] cells (Fig. 4 and *SI Appendix*, Fig. S2*A*). Ssz1p was completely destabilized in *zuo1*Δ [psi−] cells, but not destabilized in *zuo1*Δ cells with any of several [PSI+] variants (Fig. 4 and *SI Appendix*, Fig. S2*B*). This undegraded Ssz1p remained in the soluble fraction, not in the pellet, indicating that Ssz1p was released from ribosome, but not destabilized. These Western blots indicate that the stability of Zuo1p (both in [psi−] and [PSI+]) or Ssz1p (only in [psi−]) is severely decreased by *ssz1*Δ and *zuo1*Δ, respectively. However, in [PSI+] cells, the stability of Ssz1p is not changed by *zuo1*Δ, but the ribosome binding of Ssz1p is severely impaired.

Taking these data together, we find that the location of Ssb1/2p, not its stability, is altered by absence of Zuo1p or Ssz1p. However, both the fraction and the stability of each RAC protein is altered by absence of its partner, and not by *ssb1/2*Δ. This can suggest that the difference between [PSI+sbs] and [PSI+zos or szs] has resulted from different cellular environments based on the location and stability of Ssb1/2p, Zuo1p, or Ssz1p.

[PSI+] Variant Sensitivity to Overproduced Ribosome-Associated Chaperones. Overproduced Ssb1p is known to antagonize propagation of weak [PSI+] variants in WT cells without changing Hsp104 levels (44, 60, 64, 66). In both *zuo1*Δ and *ssz1*Δ, the spontaneous loss of weak and strong [PSI+] variants are increased (44). To test whether each of our [PSI+] is destabilized by excess Ssb1p, two plasmids carrying *SSB1* driven by its own promoter, one single copy (pRS313) and the other high copy (pRS423), were transformed into each [PSI+] variant-carrying strain. Transformants were then replica-plated on media with and without uracil.

Of [PSI+sbs] colonies transformed with the single-copy pRS313-*SSB1*, 77% lost the prion (as in Table 3), as did 97% of those transformed with the high-copy pRS423-*SSB1* (*SI Appendix*, Table S5), indicating again that the [PSI+sbs] variant is sensitive to Ssb. The meiotic crosses mentioned above support the same conclusion. [PSI+zos] variants were not affected by an extra copy of *SSB1* on a *CEN* plasmid, but were slightly destabilized when Ssb1p was overproduced from pRS423-*SSB1*. Like [PSI+zos] variants, [PSI+szs] variants are not affected by an extra copy of normally expressed *SSB1*. However, about half of [PSI+szs] transformants carrying pRS423-*SSB1* showed a Ura$^-$ phenotype, indicating that the [PSI+szs] variant is somewhat sensitive to overproduced Ssb1p (*SI Appendix*, Table S5).

Previously, either overproduced Ssb1p or Zuo1p was found to partially rescue the growth and antibiotic sensitivity phenotypes of a deletion of *SSZ1* (38). As mentioned above, high-copy *SSB1* destabilizes [PSI+szs] in an *ssz1*Δ strain (*SI Appendix*, Tables S5 and S6). As expected, overproduced Ssz1p efficiently cures [PSI+szs] variants (*SI Appendix*, Table S6). Interestingly, overproduced Zuo1p also cures [PSI+szs] partly; about 30% of transformants were Ura$^-$, indicating that the prion curing function of Ssz1p is also partially rescued by its overproduced partners. Although overproduction of these chaperones does not actually occur in normal cells while our variant is being cured, these results indicate that the prion curing ability of Ssz1p does not require those of Ssb1p and Zuo1p, but all three are needed for their common cellular function (38).

Is the [PIN+] Prion Variant Altered in the Absence of Ssb or RAC? Deletion of Hsp90 and related cochaperone genes leads to alteration of [PIN+] prion variants, changing their efficiency of [PSI+] induction, Rnq1-GFP aggregate formation, and dominance of prion variant, even after the deleted gene is restored (67). We tested whether [PSI+] inducibility of [PIN+] was altered by each deletion. [PIN+] in each deletion strain and the WT strain (MS327) were transferred into same background, a [psi−][pin−] WT recipient strain (MS173), produced by transient GuHCl treatment. These WT strains were grown in galactose, and cells were plated on −Ura plates to score [PSI+] clones.

The frequency of Ura$^+$ guanidine-curable clones induced by Sup35NM overproduction per 10^6 cells in WT [PIN+]$^{ssb1/2\Delta}$, WT [PIN+]$^{zuo1\Delta}$, or WT [PIN+]$^{ssz1\Delta}$ was increased about 2-, 2.5-, or 1.5-fold, respectively, compared to BY4742 (WT [PIN+]^{original}) or WT [PIN+] (Fig. 5*A*). To examine formation of fluorescence structures, Rnq1-GFP was transiently overexpressed in the WT strain with different sources of [PIN+] in Fig. 5*A*. In the WT [pin−] strain, all of cells tested for this study showed diffused GFP signal (Fig. 5*B* and Table 4). In WT [PIN+]^{original} or WT [PIN+]^{WT} strain, the frequency of diffuse signal, single foci, and

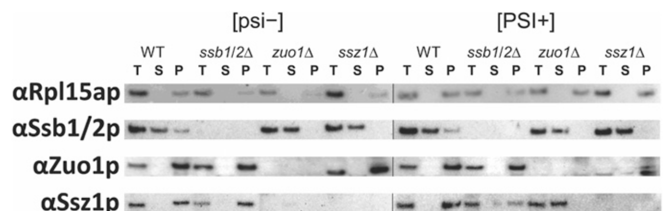

Fig. 4. The fractionation of cell extracts from WT, *ssb1/2*Δ, *zuo1*Δ, and *ssz1*Δ with and without [PSI+]. Total cell extracts (T) from each strain were separated into a postribosomal supernatant (S) and a ribosomal pellet (P) using ultracentrifugation. Corresponding amounts of T, S, and P fraction were separated by SDS/PAGE, one gel for [psi−] strain extracts and one for [PSI+] extracts, and further analyzed by Western blot, with the use of antibodies specific to Rpl15a, Ssb1/2p, Zuo1p, and Ssz1p, respectively. For better detection of Zuo1p from pellet fractions, both S and P fractions were overloaded by two-fold compared to the T fraction.

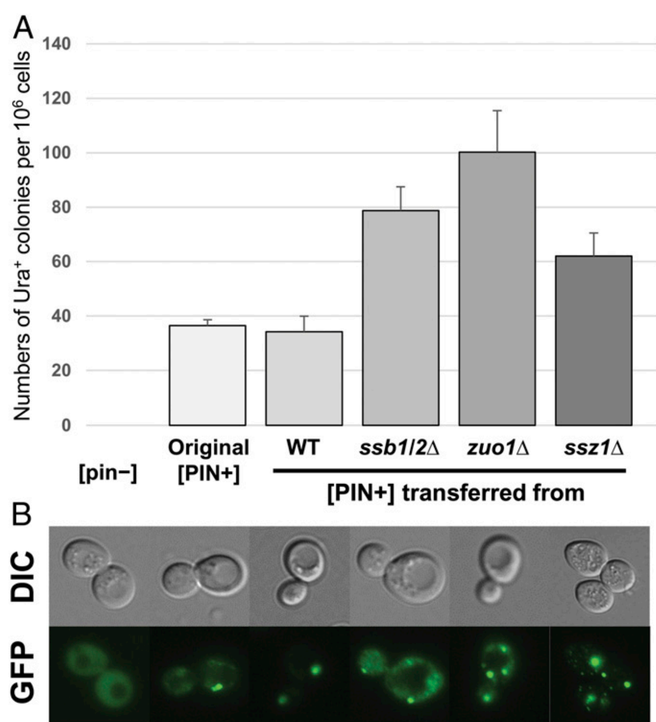

Fig. 5. Alteration of [PIN+] prion by *ssb1/2*Δ, *zuo1*Δ, and *ssz1*Δ. (*A*) The efficiency of [PSI+] induction was slightly elevated in a WT strain carrying [PIN+] from *ssb1/2*Δ, *zuo1*Δ, or *ssz1*Δ. Into each mutant strain (MS515, MS527, MS510) and the WT (MS327), all made [pin−], the [PIN+] from from BY4742 (="Original [PIN+]") was introduced by cytoduction. The mutants showed high [PSI+] generation (Table 1). Then the [PIN+] in each mutant and WT strain was transferred back into MS173 (previously made [pin-] by guanidine curing) by cytoduction. The "[pin−]" strain is MS173 cured of [PIN+]. In these strains, all with WT genome but [PIN+] from different sources, [PSI+] was induced by overproduction of Sup35NM in 2% (wt/vol) raffinose, 2% (wt/vol) galactose minimal medium. Cells were plated on −Ura plates, and arising Ura$^+$ ([PSI+]) clones were tested for GuHCl curability using transient growth on plates containing 5 mM guanidine. (*B*) The formation of Rnq1-GFP aggregates in different [PIN+]-carrying strains. BY4742 [pin−], BY4742 [PIN+], MS562, MS563, MS564, and MS565 were transformed with pM60 (p*CEN LEU2*) expressing *ADH1* promoted Rnq1-GFP. Transformants were directly used for fluorescence confocal microscopy observation (magnification 1500×).

multiple foci were about 35%, 47%, and 17%, respectively (Fig. 5*B* and *SI Appendix*, Table S5). WT [PIN+]$^{ssb1/2\Delta}$ cells had slightly different content of single and multiple foci, 42.6% and 22.9%, respectively (Table 4). [PIN+]$^{zuo1\Delta}$ or [PIN+]$^{ssz1\Delta}$ carrying cells showed very similar frequency consisting of ∼19% of diffuse, 41% of single foci, and 40% of multiple foci (Fig. 5*B* and Table 4). The combined results indicate that deletion of *SSB1/2*, *ZUO1*, or *SSZ1* leads to slight alteration of [PIN+] prion in [PSI+] induction and Rnq1-GFP aggregate formation. However, these modest changes do not account for the dramatic increases in [PSI+] generation frequency in these mutants.

Induced [URE3] Generation Is Not Elevated in *ssz1*Δ Cells. In BY241, the Ure2p-regulated *DAL5* promoter drives *ADE2*, so that [URE3] cells are Ade$^+$, but [ure-o] cells are Ade$^-$. The frequency of [URE3] induced by overproduction of Ure2 N (amino acids 1 to 65) was investigated in WT [ure-o] and *ssz1*Δ [ure-o] strains. The average of Ade$^+$ clones in WT and *ssz1*Δ strains was 846 ± 163 and 940 ± 43 per 10^5 cells, respectively. These Ade$^+$ [URE3] clones were then tested for GuHCl curability by transient growth on 4 mM GuHCl for WT or 1.5 mM GuHCl for *ssz1*Δ. Thirty of 32 Ade$^+$ clones in WT and 28 of 32 Ade$^+$ clones in the mutant were GuHCl curable, indicating that they carried a [URE3]. Eleven randomly selected [URE3] isolates, each from WT or *ssz1*Δ strains, were tested for [URE3] stability in the haploid or heterozygous diploid by subcloning on YPAD media and replica-plating to −Ade plates. All [URE3] isolates in *ssz1*Δ strains were mostly stable in both the original *ssz1*Δ haploid and in the heterozygous diploid (*SI Appendix*, Fig. S3 and Table S7), behaving the same as variants isolated in the WT host. This result was confirmed by transforming *ssz1*Δ or WT [URE3] isolates with a single-copy plasmid carrying *SSZ1* expressed by its own promoter (pM89). These transformants were no more likely to lose [URE3] than those transformed with the vector whether the host was *ssz1*Δ or WT (*SI Appendix*, Table S8).

Discussion

Ssb1/2p, Zuo1p, and Ssz1p were previously shown to repress prion generation (43–45), and we have now shown that the majority of the prions arising in their absence are cured by normal levels of the respective protein; these latter prions are the focus of this work. We confirm previous reports that an increased frequency of "insensitive" [PSI+] variants also occurs in the mutants, but we find that the majority of [PSI+] variants arising are cured in the normal environment. We show by genetic analyses and cell biological analysis that curing of the [PSI+] prion variants by normal levels of the WT allele is occurring, not simply an effect on the phenotype. Thus Ssb1/2p, Zuo1p, and Ssz1p are components of an antiprion system. We show that [PSI+sbs] variants differ from [PSI+szs] or [PSI+zos] variants based on consistently different stability in the identical doubly heterozygous *sbs1/2*Δ/+ *ssz1*Δ/+ (or *zuo1*Δ/+) host. In investigating the mechanisms involved in the elevated generation of the new prion variants and their curing, we find that Ssz1p is very unstable in the absence of Zuo1p, and Zuo1p is somewhat unstable in the absence of Ssz1p. Interestingly, in the presence of [PSI+], Ssz1p is stable in the *zuo1*Δ strain, but soluble- , no longer ribosome-bound.

Why Is Prion Generation Elevated in ssb1/2 and RAC Mutants? A large part of the increase in [PSI+] frequency was the appearance of the novel curable variants that could not propagate in the WT strain. The most straight-forward explanation for the appearance of these variants as well as increased incidence of variants not cured in the WT is that Ssb1/2 and RAC chaperones are important for proper folding of nascent proteins, and that improperly folded Sup35p is preferred or required for the growth of the [PSI+] amyloid filaments (43). This mechanism might suggest that other prions would be affected similarly, but we find that [URE3] generation is not increased in an *ssz1Δ* strain and that *ssz1Δ* [URE3] isolates are not cured by restored Ssz1p, suggesting that alternative explanations should be considered.

Table 4. The frequency of cells with different numbers of Rnq1-GFP foci

Strain	GFP signals		
	Diffused, %	Single foci, %	Multiple foci, %
WT [pin−]	100	0	0
WT [PIN+]^{original}	35.6	47.1	17.3
WT [PIN+]^{WT}	35.0	47.4	17.6
WT [PIN+]$^{ssb1/2\Delta}$	34.5	42.6	22.9
WT [PIN+]$^{zuo1\Delta}$	19.5	40.8	39.7
WT [PIN+]$^{ssz1\Delta}$	18.9	41.9	39.2

Strains MS317, MS562-MS565 (top to bottom), transformed with plasmids encoding RNQ1–GFP were grown on SD-Leu. The frequencies of cells with different number of aggregates were scored in 100 to 150 cells. Superscripts in the strain column indicate different sources of [PIN+].

GENETICS

One known effect of deletion of *SSB1/2, ZUO1,* or *SSZ1* is slight nonsense suppression (43, 65), too weak to explain the elevated [PSI+] appearance (43), and our genetic and cytologic analysis shows that the [PSI+sbs], [PSI+szs], and [PSI+zos] prions are indeed lost in the WT, not just phenotypically masked. But the existence of this nonsense suppression suggests some functional interaction of the Ssb and RAC components with the Sup35/Sup45 termination apparatus, through the ribosome structure. Thus, it is possible that it is an effect on the mature Sup35p (rather than only nascent Sup35p) that is responsible for elevated [PSI+] formation. We previously explained the antiprion action of Upf proteins by their specific interactions with Sup35p (31). Similarly, direct interactions of Ssb1/2 with Sup35p have been described previously (64), and the [PSI+] specificity of ribosome associated chaperones might be a result of a direct interaction of these components with mature Sup35p.

Deficiency of specific chaperones (Hsc82, Aha1p, Cpr6p, Cpr7p, Sba1p, Tah1p, and Sse1p) was reported to alter [PIN+] variant phenotypes, namely, [PSI+]-inducibility, Rnq1-GFP aggregate formation and variant dominance (67). In that study, the [PIN+] prion transferred to a WT strain conferred the elevated or reduced [PSI+] inducing activity of the mutant, indicating that the [PIN+] prion had been altered in the mutant strain. In our study, [PSI+] inducing efficiency of the [PIN+] prion that had experienced the *ssb1/2, ssz1*, or *zuo1* deletion strain was increased by only about twofold in each case when transferred to the WT (Fig. 5*A*). However, compared to the >30-fold effect of the deletion itself on [PSI+] induction, it is not likely that alteration of [PIN+] by each deletion is important in determining the inducing efficiency. The small differences in inducing activity, and the modest variation of the pattern of Rnq1-GFP foci in each case suggests that $[PIN+]^{zuo1\Delta}$, $[PIN+]^{ssz1\Delta}$, and $[PIN+]^{ssb1/2\Delta}$ are essentially the same as their common parent (Fig. 5*B* and Table 4).

What Controls Generation and Curing of [PSI+sbs] vs. [PSI+szs] or [PSI+zos]? The existence of prion variants, with different biological properties, results from different amyloid structures of a single prion protein (68–70). The architecture of amyloid-based yeast prions (71–73) led to an explanation of how a protein could template its own conformation and the stable propagation of many distinct prion variants from one single protein sequence (74, 75). [PSI+] prion variants previously isolated in *ssb1/2*Δ were not cured by restored *SSB1* on a *CEN* plasmid (43). [PSI+] isolates in either *zuo1*Δ or *ssz1*Δ were more often unstable in their own mutant strain (44). These previous studies used the [PSI+]-suppressible *ade1-14* nonsense allele in a different strain background. We confirm the existence of variants not cured in a WT cell but, unlike previous studies, find a majority of variants that are cured in a normal WT strain. This difference may be due to the different strain backgrounds and reporters used.

[PSI+sbs] variants differ from both [PSI+szs] and [PSI+zos] prion variants, because the latter two are rapidly lost in doubly heterozygous *ssb1,2*Δ/+ *ssz1*Δ (or *zuo1*Δ)/+ diploids, while [PSI+sbs] propagates and is only slowly lost from the very same diploids (Fig. 3 and *SI Appendix*, Tables S3 and S4). Beyond the prion variant differences, one would like to understand what component is causing prion loss in each case. This prion variant difference must result from the conditions under which variants were isolated. As shown in Fig. 4 (Fig. 4, *Left*: [psi−]), [PSI+zos] and [PSI+szs] were generated under the condition with no ribosomal Ssb1/2p, but with soluble Ssb1/2p, indicating that both variants are resistant to the soluble (cytosolic) Ssb1/2p. [PSI+sbs] must be inhibited by ribosome-bound or soluble Ssb1/2p, because Ssz1p and Zuo1p are ribosome-bound even without Ssb1/2p. While *ssb1/2*Δ [PSI+sbs] × WT produces rapid curing, *ssb1/2*Δ [PSI+sbs] × *ssz1*Δ (or *zuo1*Δ) show slower curing, even though the latter diploids have more soluble Ssb1/2p than the former, being partially deficient (haplo-insufficient) in Ssz1p (or Zuo1p). We infer that it is ribosome-bound Ssb1/2p that inhibits propagation of [PSI+sbs]. Because *ssz1*Δ (or *zuo1*Δ) strains lack ribosome bound Ssb1/2, but have elevated soluble Ssb1/2p, [PSI+szs] and [PSI+zos] may be inhibited by ribosome-bound Ssb1/2 or by Ssz1p (or Zuo1p). Just because [PSI+sbs] differs from [PSI+szs] and [PSI+zos], we speculate that it is not ribosomal Ssb1/2p, but rather Ssz1p (or Zuo1p) itself that blocks [PSI+szs] and [PSI+zos].

Deletion of *SSB1/2*, *ZUO1*, *SSZ1*, or all show the same cellular phenotypes, and the same stimulation of [PSI+] formation, suggesting a common function (32–34, 36–38, 44). Here we found that RAC deletions lead to the generation of substantially different prion variants than *ssb1/2*Δ strains. Most recently, RAC was found to bind to translating nascent peptide chains at the ribosomal tunnel exit like ribosomal Ssb1/2p (76). Ribosome-associated Zuo1p, Ssz1p, and Ssb1/2p sequentially contact growing nascent chains of minimum length 40, 45, and 50 residues, respectively, and hand over chains to the next chaperone in a relay for cotranslational de novo protein folding. Accordingly, the absence of different components may differently affect the site-specific contact with the translating nascent chain, inducing distinct misfolding of Sup35p and thus different arrays of prion variants. Replacing the missing chaperone should prevent the occurrence of a specifically misfolded Sup35p needed for a specific amyloid structure, thus explaining the curing of [PSI+sbs], [PSI+szs], and [PSI+zos] by limiting the growth of the amyloid fibers. Inhibition of Sup35 NM fibrilization by purified RAC and Ssb1p in vitro was previously reported, suggesting that nascent Sup35 polypeptides are likely shielded from possible prion conformations upon emerging from the ribosome (77).

In earlier work, Ssb1/2p was suggested to prevent formation of the misfolded intermediates for Sup35p prion aggregates and stimulate degradation of these intermediates, but not final [PSI+] prion aggregates (43). Especially, ribosomal bound Ssb1/2p was also reported to repress formation of [PSI+] prion aggregates, and the relocation of Ssb1/2p from ribosome to cytosol enhanced prion formation, but antagonized prion propagation by competing with cytosolic Ssa for binding to prion aggregates (44–46). In this work we studied a different class of prion variants, not detected in the earlier work, and so the roles of Ssb1/2p and RAC in preventing generation and in curing these prions could be different.

Why Does [PSI+] Stabilize Ssz1p in zuo1Δ Cells? Zuo1p is directly bound to the ribosome and Ssz1p is bound to the ribosome through Zuo1p (41). Ssb1/2p relies for its ribosome association on Zuo1p and Ssz1p. We find that in *zuo1*Δ [psi−] cells Ssz1p is degraded, but not in [PSI+] cells. Although stable in [PSI+] cells, Ssz1p is not ribosome-associated, so it is unlikely to interact with the normal ribosome-bound Sup35p, or sense the frequent absence of Sup35p on ribosomes in [PSI+] cells. It is possible that the [PSI+] prion leads to the partial inactivation of some E3 ligase or autophagy factor that would otherwise lead to degradation of Ssz1p in *zuo1*Δ.

This study confirms that prions are more abundant and more differentiated than was previous thought (reviewed in ref. 20). Yeast has evolved a wide (and widening) array of systems to prevent prion formation, to cure most prion variants, and to limit the damage caused by those variants that survive those measures. It is hoped that the variety of antiprion systems found in yeast will stimulate a similar search in mammals, and provide tools useful in treatment of the common human amyloidoses, increasingly found to have infectious aspects.

Materials and Methods

Nomenclature. Yeast prions are shown in brackets to indicate they are nonchromosomal genes (e.g., [PSI+], [URE3], or [PIN+]). Specific types of prion variants are indicated within the brackets (e.g., [PSI+sbs]). Strains of origin of [PIN+] prion are indicated with a superscript (e.g., "WT $[PIN+]^{zuo1\Delta}$" for [PIN+] transferred from a *zuo1*Δ strain into the WT strain).

Strains and Media. Strains used in this study are listed in *SI Appendix*, Table S1. Gene disruption mutants were generated by PCR-amplifying yeast genomic DNA of the corresponding strains from the *Saccharomyces cerevisiae* knockout collection or mating with isogenic strains from the knockout collection, and further confirmed using PCR-amplification (78). Media used were as described by Sherman (79). Induction of *GAL1*-promoted genes was conducted using galactose-raffinose–containing media as previously described (31). The *ade1-14* allele carrying parental strains were derivatives of strains used for previous studies (31). Scoring [PSI+] with the suppressible *ura3-14* mutation and inducing prion formation routinely used p1520 (*CEN LEU2 ura3-14 $GAL1_{promoter}$-SUP35NM*). For all of the [PSI+] scoring experiments, p1520 was maintained in strains/colonies without leucine to avoid loss of this plasmid.

Plasmids. Plasmids used in this study are listed in *SI Appendix*, Table S2. *SSB1* with 357 bp and *ZUO1* with 500 bp upstream of the ORF were amplified and ligated into pRS313 (*CEN HIS3*) cut with BamHI and XhoI forming pM76 and pM75, respectively. *SSZ1* with 390 bp upstream of the ORF was amplified and ligated into pRS313 cut with BamHI and SalI forming pM78. The same strategy was used for generating pRS423 (2μ *HIS3*)-based *SSB1*, *ZUO1*, and *SSZ1* plasmids, forming pM85, pM87, and pM86, respectively. An *ADH1*-promoted Rnq1p-GFP expressing plasmid was generated previously (18).

Cytoduction. In mating *kar1* mutants fail in nuclear fusion so daughter cells have a mixture of the parental cytoplasms. Cytoduction donors are ρ^+, recipients are ρ^o from ethidium exposure, both isogenic to the knockout bank strains, BY4741 and BY4742, both carrying p1520 (*CEN LEU2 ura3-14*) bearing P_{GAL1}*-SUP35NM* (30). In effect, cytoplasm is transferred from donors (with induced [PSI+] variants) and recipients. After mating, mixtures were streaked on media selecting against the donor. Colonies were replica-plated to YPG, media selective for diploids and media lacking uracil. Clones growing on YPG but not on diploid selection media were cytoductants. Ura^+ clones propagated [PSI+]. When donor and recipient strains are both *ssb-racΔ*, resulting cytoductants rarely appeared making this type of analysis impractical.

Antibodies. Antibodies to Ssb1/2p, Zuo1p, and Ssz1p were generous gifts from Elizabeth A. Craig, University of Wisconsin–Madison, Madison, WI. Rpl15p antibody was purchased from Bio-Rad.

Data Availability. All study data are included in the main text and *SI Appendix*.

ACKNOWLEDGMENTS. We especially thank Thomas Ziegelhoffer (University of Wisconsin–Madison) for detailed guidance and kind advice about protein analysis. This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases.

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