# Ribosome-associated peroxiredoxins suppress oxidative stress–induced de novo formation of the [*PSI*$^+$] prion in yeast

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**Peroxiredoxins (Prxs) are ubiquitous antioxidants that protect cells against oxidative stress. We show that the yeast Tsa1/Tsa2 Prxs colocalize to ribosomes and function to protect the Sup35 translation termination factor against oxidative stress–induced formation of its heritable [*PSI*$^+$] prion conformation. In a *tsa1 tsa2* [*psi*$^-$] [*PIN*$^+$] strain, the frequency of [*PSI*$^+$] de novo formation is significantly elevated. The Tsa1/Tsa2 Prxs, like other 2-Cys Prxs, have dual activities as peroxidases and chaperones, and we show that the peroxidase activity is required to suppress spontaneous de novo [*PSI*$^+$] prion formation. Molecular oxygen is required for [*PSI*$^+$] prion formation as growth under anaerobic conditions prevents prion formation in the *tsa1 tsa2* mutant. Conversely, oxidative stress conditions induced by exposure to hydrogen peroxide elevates the rate of de novo [*PSI*$^+$] prion formation leading to increased suppression of all three termination codons in the *tsa1 tsa2* mutant. Altered translational fidelity in [*PSI*$^+$] strains may provide a mechanism that promotes genetic variation and phenotypic diversity (True HL, Lindquist SL (2000) *Nature* 407:477–483). In agreement, we find that prion formation provides yeast cells with an adaptive advantage under oxidative stress conditions, as elimination of the [*PSI*$^+$] prion from *tsa1 tsa2* mutants renders the resulting [*psi*$^-$] [*pin*$^-$] cells hypersensitive to hydrogen peroxide. These data support a model in which Prxs function to protect the ribosomal machinery against oxidative damage, but when these systems become overwhelmed, [*PSI*$^+$] prion formation provides a mechanism for uncovering genetic traits that aid survival during oxidative stress conditions.**

Peroxiredoxins (Prxs) are ubiquitous, thiol-specific proteins that have multiple functions in stress protection. Typical 2-Cys Prxs are active as a dimer and contain two redox active Cys residues that are directly involved in enzyme activity (1). During catalysis, the peroxidatic cysteine is oxidized to a sulphenic acid, which then condenses with a resolving cysteine (from the other subunit of the dimer) to form a disulphide. This disulphide is reduced by thioredoxin in a redox cycle that accepts electrons from NADPH. In addition to their peroxidase function, Prxs have proposed roles in diverse cellular processes including differentiation, proliferation, modulation of intracellular signaling, apoptosis, and gene expression, and have become increasingly implicated in many disease processes (2).

Tsa1 is the major 2-Cys Prx in yeast and has best been characterized as an antioxidant in the detoxification of hydroperoxides (3, 4). More recently, it has been shown to be particularly required as an antioxidant that protects against oxidative damage arising as a result of endogenous reactive oxygen species (ROS) generated during normal aerobic metabolism (5). Mutants lacking *TSA1* accumulate significant levels of DNA damage, causing genome instability and cell death during normal aerobic growth conditions. Tsa1 can also act as a molecular chaperone that promotes resistance to heat and reductive stresses (6, 7). Loss of *TSA1* results in aggregation of ribosomal proteins, suggesting that Tsa1 functions to protect the translation apparatus against oxidative damage (7).

Tsa1 functions as a peroxidase on ribosomes, as mutation of its peroxidatic cysteine residue, which inactivates its peroxidase but not its chaperone activity, results in sensitivity to translation inhibitors such as cycloheximide and hygromycin. Approximately 5% of cellular Tsa1 is associated with ribosomes during normal growth conditions (8). However, the function of this pool is unclear, as loss of *TSA1* does not affect the translation process during normal aerobic growth conditions.

The Tsa2 Prx is highly homologous to Tsa1 (86% amino acid identity) and possesses similar peroxidase and chaperone activities, although it is normally expressed at significantly lower levels compared with Tsa1 (4, 6). We show here that the majority of cellular Tsa2 localizes to ribosomes. This prompted us to examine whether the simultaneous deletion of *TSA1* and *TSA2* causes any defect in translation, and we find that cultures of *tsa1 tsa2* mutants display increased suppression of all three translation termination codons. Elevated readthrough of termination codons is a well known phenotype of the yeast [*PSI*$^+$] prion, a conformationally altered form of the Sup35 translation termination factor (9). Sup35 is an essential protein, which, together with its interacting partner Sup45, facilitates the termination of translation and polypeptide chain release (10). Sup35 forms [*PSI*$^+$] prion aggregates that sequester it away from its normal function in translation termination, resulting in suppression of all three stop codons. [*PSI*$^+$] prion formation is known to increase in response to environmental stress conditions, including $H_2O_2$ stress (11). We therefore examined [*PSI*$^+$] prion formation in the *tsa1 tsa2* mutant and we show that this mutant shows a significantly elevated frequency of de novo formation of [*PSI*$^+$] that would account for the nonsense suppression observed in a culture of a *tsa1 tsa2* strain. As [*PSI*$^+$] prion formation can provide a mechanism for uncovering hidden genetic variation that promotes phenotypic diversity (12, 13), the ability to form the [*PSI*$^+$] prion in the *tsa1 tsa2* mutant may provide an adaptive advantage, as *tsa1 tsa2* [*pin*$^-$] mutants, which are unable to form [*PSI*$^+$] prions, are hypersensitive to $H_2O_2$ stress.

## Results

**The Tsa1 and Tsa2 Peroxiredoxins Localize with Ribosomes.** Approximately 5% of Tsa1 is associated with ribosomes (8). We therefore examined Tsa2 to determine whether it is similarly ribosome-associated. Cell extracts were treated with cycloheximide to inhibit ribosomal translocation, and ribosomal fractions separated from soluble components by centrifugation through sucrose cushions. Known ribosomal markers including elongation factor

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Tef1 (EF-1A), termination factor Sup35 (eRF3), and ribosomal protein Rps3 were detected in ribosomal fractions as expected (Fig. 1*A*). Tsa1 was present in both ribosomal and soluble fractions, whereas another Prx, Ahp1, was only detected in the soluble fraction as previously described (8). Interestingly, Tsa2 was exclusively localized in ribosomal fractions and was not detected in the soluble fraction.

Tsa1 and Tsa2 are thought to have overlapping functions in protecting cells against oxidative stress (4), and their colocalization on the ribosome suggests that they play a common role in protecting the protein synthetic machinery against oxidative stress conditions. Although loss of *TSA1* alone does not affect translational fidelity as measured by the readthrough of a UAA stop codon (8), stop codon readthrough was observed in a *tsa1 tsa2* mutant of W303 as quantified using a reporter plasmid that carries the *lacZ* gene with a premature UAA, UAG, or UGA stop codon (14). Readthrough of all three stop codons was elevated in this *tsa1 tsa2* mutant compared with the WT W303 strain ranging between threefold (UAA), fivefold (UAG), and 20-fold (UGA) (Fig. 1*B*). A similar increase in UAG codon readthrough was observed in the *tsa1* single mutant, which may indicate that Tsa1 has an additional function on ribosomes distinct from that of Tsa2.

**Tsa1 and Tsa2 Peroxiredoxins Protect Against Sup35 Prion Formation.** Given that elevated readthrough of termination codons is a well known phenotype of the yeast [*PSI*$^+$] prion, we examined whether the termination readthrough detected in the *tsa1 tsa2* mutant culture correlated with an increase in [*PSI*$^+$] prion formation in cultures of this mutant. The subcellular distribution of Sup35 was examined in exponential phase cultures of WT and Prx mutants, and this showed that Sup35 was in the soluble fraction in WT and *tsa1* and *tsa2* single mutants, whereas in the *tsa1 tsa2* double mutant, a significant proportion was present in an SDS-insoluble high molecular weight form (Fig. 2*A*). The de novo formation of Sup35 aggregates was also observed in *tsa1 tsa2* cells using a fusion between the aminoterminal domain of Sup35 and GFP, under the control of the *CUP1* promoter (15). In WT cells, strong cytoplasmic fluorescence comparable with a [*psi*$^-$] control strain was seen (Fig. 2*B*), whereas many different-sized aggregates

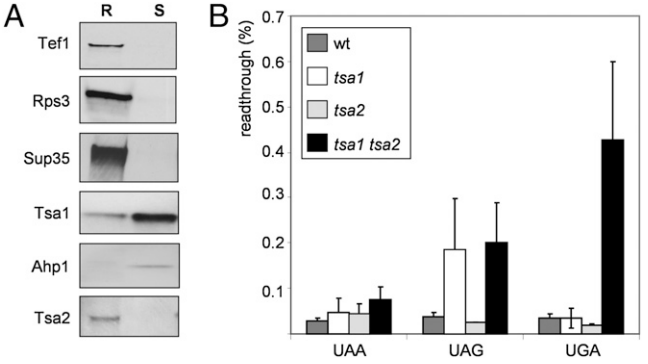


**Fig. 1.** Tsa1 and Tsa2 colocalize with ribosomes and prevent suppression of translation termination. (*A*) Ribosomal fractions (R) were separated from soluble components (S) by centrifugation through sucrose cushions. Proteins were separated by SDS/PAGE and Tef1, Rps3, Sup35, Tsa1, Ahp1, and Tsa2 detected by immunoblot analysis. (*B*) The levels of termination codon readthrough were measured using a β-gal reporter system in cultures of WT (W303), *tsa1, tsa2,* and *tsa1 tsa2* mutants grown to exponential phase in minimal SD media. Readthrough was quantified using plasmids that carry the *lacZ* gene that bears a premature termination codon. Values shown are means ± SE mean from at least three independent determinations. Readthrough is expressed as a proportion of control β-gal levels, measured in transformants carrying the control plasmid that carries the WT *lacZ* gene.

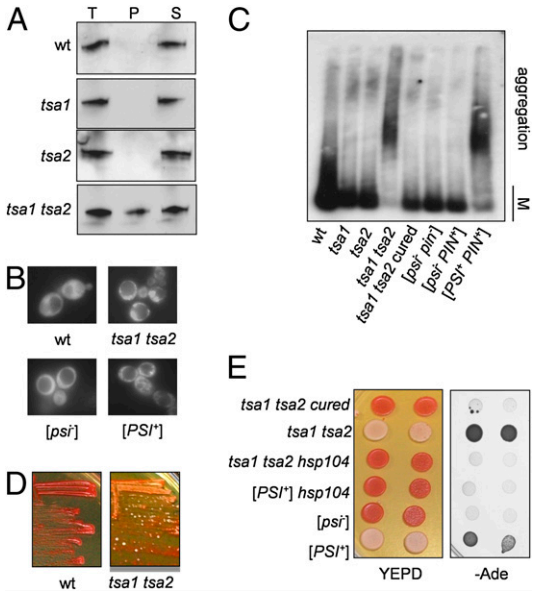


**Fig. 2.** The Tsa1 and Tsa2 Prxs protect against Sup35 prion formation. (*A*) Subcellular distribution of Sup35 in WT (W303), *tsa1, tsa2,* and *tsa1 tsa2* mutant cells grown to exponential phase in minimal SD media. Subcellular fractionation analysis of Sup35 was performed as described in *Experimental Procedures* using an anti-Sup35 polyclonal antibody. (T, total crude extract; S, soluble fraction; P, pellet fraction.) Representative gels are shown from at least three independent repeats. (*B*) Fluorescence micrographs of the W303 WT and *tsa1 tsa2* mutant and [*PSI*$^+$] and [*psi*$^-$] derivatives of 74D-694 carrying plasmid p6442 (CUP1-SUP35NM-GFP). (*C*) Detection of SDS-resistant Sup35 aggregates by SDD-AGE in cell lysates from WT (W303) *tsa1, tsa2,* and *tsa1 tsa2* mutants, and [*psi*$^-$] [*pin*$^-$], [*psi*$^-$] [*PIN*$^+$], and [*PSI*$^+$] [*PIN*$^+$] derivatives of 74D-694. The *tsa1 tsa2* mutant was also grown in the presence of 3 mM GdnHCl. (*D*) WT (74D-694) and *tsa1 tsa2* mutants were streaked on YEPD media and colony color visualized after 3 d growth. (*E*) [*PSI*$^+$] prion formation was assayed in the WT (74D-694) and mutant strains by pink/white colony formation and growth on minimal media in the absence of adenine.

of Sup35 were detected in the majority (>95% of 200 cells examined) of *tsa1 tsa2* mutants examined in two repeat experiments, similar to those seen in a [*PSI*$^+$] strain (15).

One genetic criterion for a yeast prion is reversible curability (9). Guanidine hydrochloride (GdnHCl) blocks the propagation of yeast prions by inhibiting Hsp104, a molecular chaperone that is absolutely required for yeast prion propagation (16, 17). [*PSI*$^+$] prions form SDS-resistant high molecular weight aggregates that can be detected using semidenaturing detergent agarose gel electrophoresis (SDD-AGE) (18). Such Sup35 aggregates were also observed in a *tsa1 tsa2* mutant of W303 and in the control [*PSI*$^+$] strain 74D-694 (Fig. 2*C*). Growth of the *tsa1 tsa2* mutant in the presence of GdnHCl shifted Sup35 back to its monomeric size, confirming the requirement for Hsp104 to propagate [*PSI*$^+$] prion formation in this strain.

The reversible curability of the [*PSI*$^+$] prion in the W303 *tsa1 tsa2* mutant was confirmed using a genetic assay in a different strain background. *TSA1* and *TSA2* were deleted in a [*psi*$^-$] derivative of the 74D-694. This strain contains an *ade1-14*, a nonsense (UGA) mutant allele that confers adenine autotrophy, and 74D-694 [*psi*$^-$] cells accumulate a red pigment. Suppression of the *ade1-14* nonsense mutation by [*PSI*$^+$] gives rise to white/pink Ade$^+$ colonies. The *tsa1 tsa2* mutant of 74D-694 gave rise to white/pink Ade$^+$ colonies at a very high frequency (Fig. 2*D*) and the Ade$^+$ phenotype was eliminated by growth in the presence of 3 mM GdnHCl, suggesting that this was a result of the de novo formation of [*PSI*$^+$] in these cells (Fig. 2*E*). GdnHCl also caused loss of the diagnostic SDS-resistant Sup35 polymers in this

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putative [*PSI*$^+$] *tsa1 tsa2* strain (Fig. S1), and expression of the ATPase-deficient allele of Hsp104 (*hsp104::K218T, K620T*) also generated red Ade$^-$ colonies (Fig. 2*E*), as has been previously described for [*PSI*$^+$] cells (19).

Using the 74D-694 *tsa1 tsa2* mutant we differentiated between [*PSI*$^+$] prion formation and any spontaneous nonsense suppressor mutations that might have arisen as a result of the previously described mutator phenotype displayed by *tsa1 tsa2* mutants (5). Colony-purified red Ade$^+$ 74D-694 *tsa1 tsa2* mutants were streaked for single colonies on Yeast Extract Peptone Dextrose (YEPD) media (Fig. 2*D*) and 120 of the independently arising white/pink colonies were checked for curability of the Ade$^+$ phenotype by growth in the presence of 3 mM GdnHCl. Eighty-five percent of the mutants examined were curable, indicating that these were [*PSI*$^+$] strains rather than nuclear-encoded nonsense suppressors. GdnHCl treatment also cures the [*PIN*$^+$] prion, which is absolutely required for de novo formation of [*PSI*$^+$] (20), and so we also tested 108 of the cured 74D-694 *tsa1 tsa2* mutants for their ability to form [*PSI*$^+$] prions. In all cases, few white/pink colonies were detected and all 13 such independent Ade$^+$ revertants tested were noncurable on 3 mM GdnHCl, i.e., were not [*PSI*$^+$]. These data also show that the absence of Tsa1 and Tsa2 does not override the need for the [*PIN*$^+$] prion for de novo formation of [*PSI*$^+$].

As *tsa1 tsa2* mutants were sterile, we were unable to carry out genetic crosses or cytoduction experiments to confirm the presence of a transmissible infectious entity in the spontaneous [*PSI*$^+$] *tsa1 tsa2* mutants. We therefore resorted to the "protein transformation" method recently described by Tanaka et al. (21) to demonstrate that one of the spontaneous *tsa1 tsa2* [*PSI*$^+$] strains derived from W303 contained such infectious entities that can be transmitted to a [*psi*$^-$] strain. Of 49 transformants derived using this extract, 13 were [*PSI*$^+$], and 11 of 54 transformants obtained with an extract of the 74D-694 [*PSI*$^+$] strain were [*PSI*$^+$]. No [*PSI*$^+$] cells were obtained among 74 transformants using an extract from a [*psi*$^-$] 74D-694 derivative.

**Prion Formation in the *tsa1 tsa2* Mutant Requires Oxygen.** As Tsa1 can function as both a peroxidase in the detoxification of hydroperoxides, and as a molecular chaperone that protects against protein misfolding, we determined which of these activities are important for preventing Sup35 aggregation and de novo formation of the [*PSI*$^+$] prion. Introduction of a WT plasmid copy of *TSA1* into the Ade$^-$ W303 *tsa1 tsa2* mutant resulted in Sup35 being largely redistributed into the soluble fraction (Fig. 3*A*). Mutants lacking the peroxidatic (Cys47) or the resolving (Cys170) cysteine residue cannot function as peroxidases, but maintain chaperone activity (6). Sup35 aggregation was still detected cells expressing various Tsa1 cysteine mutants (Tsa1$^{C47S}$, Tsa1$^{C170S}$, Tsa1$^{C47S/C170S}$), indicating that the peroxidase activity, rather than the chaperone activity, protects against de novo [*PSI*$^+$] prion formation (Fig. 3*A*).

To determine whether oxygen is required for the spontaneous de novo formation and propagation of the [*PSI*$^+$] prion in a *tsa1 tsa2* mutant, we exploited a *can1-100* nonsense (UAA) allele of the arginine permease gene *CAN1*, which is present in W303 and confers resistance to the toxic arginine analogue canavanine (Fig. 3*B*). Suppression of the UAA codon leads to a canavanine-sensitive phenotype, and this was observed with cultured cells of W303 *tsa1 tsa2* mutant. That this canavanine sensitivity was dependent on [*PSI*$^+$] prion formation was confirmed by comparing with a parallel culture grown in the presence of 3 mM GdnHCl (Fig. 3*B*). Interestingly, the same *tsa1 tsa2* strain grown in low oxygen conditions was far less resistant to canavanine, suggesting that [*PSI*$^+$] prion formation in this strain might be dependent on the presence of oxygen. We confirmed this hypothesis by examining the subcellular distribution of Sup35 in the W303 *tsa1 tsa2* mutant grown under anaerobic conditions, in the absence of atmospheric oxygen. Subculturing the cells five times under anaerobic growth conditions resulted in Sup35 shifting from an SDS-insoluble high molecular weight form into a soluble form (Fig. 3*C*). This behavior was specific to the *tsa1 tsa2* mutant, as the propagation of the [*PSI*$^+$] prion in a control [*PSI*$^+$] *TSA1 TSA2* strain was unaffected by anaerobic growth. These results suggest that the accumulation of ROS in the *tsa1 tsa2* mutant specifically triggers [*PSI*$^+$] prion formation.

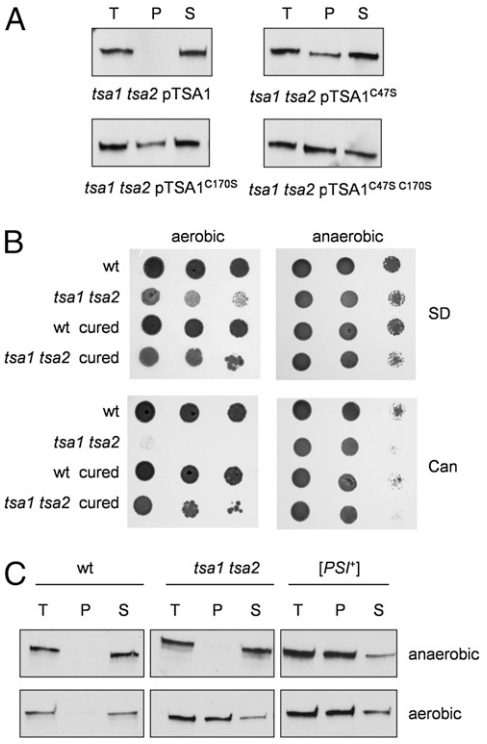


**Fig. 3.** Prion formation in the *tsa1 tsa2* mutant requires oxygen. (*A*) Subcellular distribution of Sup35 in the *tsa1 tsa2* mutant cells containing WT Tsa1 (*pTSA1*) or cysteine mutants of Tsa1 (*pTSA1$^{C47S}$*, *pTSA1$^{C170S}$*, *pTSA1$^{C47S,\ C170S}$*) as the sole source of *TSA1*. (*B*) [*PSI*$^+$] prion formation assayed in cured [*psi*$^-$] [*pin*$^-$] and noncured WT (W303) [*psi*$^-$] [*PIN*$^+$] and *tsa1 tsa2* [*PSI*$^+$] [*PIN*$^+$] mutant strains assayed by growth on canavanine. The *can1-100* allele in W303 contains a UAA nonsense mutation and translation termination at this stop codon confers resistance to canavanine. Cells were grown to stationary phase, diluted to $A_{600}$s of 1.0, 0.1, and 0.01, and spotted onto minimal SD plates containing 80 μg/mL canavanine under aerobic or anaerobic conditions. (*C*) Subcellular distribution of Sup35 in the WT W303 [*psi*$^-$] [*PIN*$^+$], *tsa1 tsa2* [*PSI*$^+$] [*PIN*$^+$] mutant, and [*PSI*$^+$] [*PIN*$^+$] derivatives of 74D-694. Cells were grown under aerobic or anaerobic conditions in minimal SD media as described in the text.

**Curable Prion Formation Is Induced by Oxidative Stress.** Tsa1 and Tsa2 have best been characterized as antioxidants that protect cells against oxidative stress induced by $H_2O_2$. Additionally, several unrelated environmental stress conditions including $H_2O_2$ have been reported to increase the frequency of [*PSI*$^+$] prion formation de novo (11). We therefore examined whether [*PSI*$^+$] prion formation is elevated in a *tsa1 tsa2* mutant exposed to $H_2O_2$. WT W303 and a *tsa1 tsa2* mutant derivative were grown to exponential phase and treated with sublethal levels (100 μM) of $H_2O_2$ for 20 h. The WT strain underwent approximately five doublings during this treatment, whereas the *tsa1 tsa2* mutant was more sensitive and divided only approximately three times. Sup35 remained largely soluble in the WT strain following this $H_2O_2$ treatment, although a small amount was detectable in the high molecular weight fraction (Fig. 4*A*). Growth of the *tsa1 tsa2* mutant in the presence of $H_2O_2$ resulted in a large increase in the

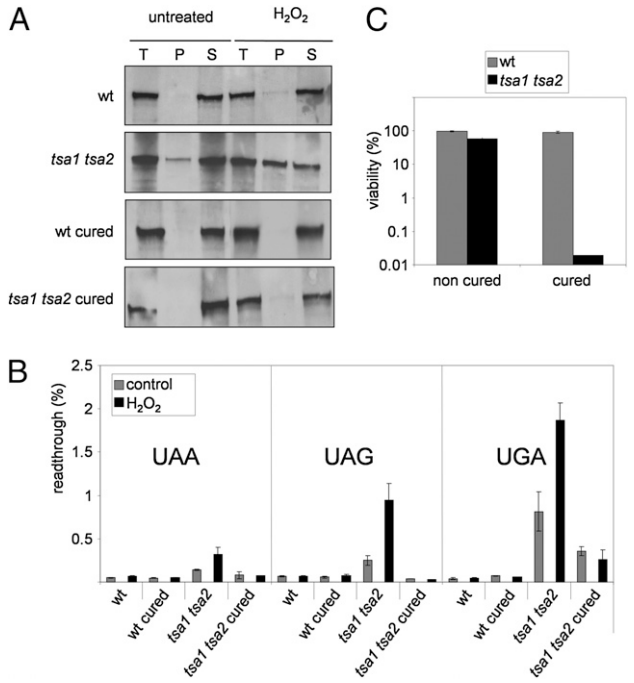


**Fig. 4.** [*PSI*$^+$] prion formation is induced by oxidative stress. (*A*) Subcellular distribution of Sup35 in cured and noncured WT (W303) and *tsa1 tsa2* mutants grown in minimal SD media and treated with 100 μM $H_2O_2$ for 20 h. The levels of termination codon readthrough (*B*) and cell viability (*C*) were measured for the same strains and growth conditions as described in *A*. Percentage survival is expressed relative to untreated cultures.

proportion of Sup35 in the high molecular weight fraction. To confirm that $H_2O_2$ stress promotes [*PSI*$^+$] prion formation, we repeated the experiment but this time in GdnHCl-cured [*pin*$^-$] derivatives. Now the Sup35 was largely absent from high molecular weight fractions of the *tsa1 tsa2* [*pin*$^-$] strains. These data indicate that oxidative stress promotes de novo [*PSI*$^+$] prion formation rather than any suppressor mutations that might affect Sup35 aggregation (Fig. 4*A*).

Termination readthrough was assayed to confirm that the predicted [*PSI*$^+$]-associated nonsense suppression correlated with the presence of Sup35 in soluble versus insoluble fractions. Readthrough of stop codons was unaffected by oxidative stress in the WT W303 strain (Fig. 4*B*). In contrast, nonsense suppression was elevated by approximately two to three fold for all three stop codons in the W303 *tsa1 tsa2* mutant. Whereas readthrough of the UAA and UAG stop codons was restored to WT levels in the GdnHCl cured *tsa1 tsa2* [*pin*$^-$] mutant, readthrough of the UGA stop codon remained elevated compared with the WT strain. That there was no further increase in response to $H_2O_2$ in this mutant suggests that there may also be a [*PSI*$^+$] prion–independent translation termination defect that specifically affects UGA nonsense suppression in a *tsa1 tsa2* mutant.

Taken together, our data indicate that the Tsa1 and Tsa2 Prxs protect against oxidative stress-induced [*PSI*$^+$] prion formation. One possible consequence of [*PSI*$^+$] prion formation is that it can alter gene expression patterns through altered stop codon recognition. We therefore examined whether the ability to form the [*PSI*$^+$] prion influences the sensitivity of the *tsa1 tsa2* mutant to oxidative stress conditions. The Tsa1 and Tsa2 Prxs are active as antioxidants and growth of the *tsa1 tsa2* mutant in the presence of 100 μM $H_2O_2$ for 20 h lowered viability by approximately 50% compared with the WT strain (Fig. 4*C*). Strikingly, the GdnHCl cured *tsa1 tsa2* [*pin*$^-$] mutant cells, which are unable to form [*PSI*$^+$] de novo, was hypersensitive to $H_2O_2$ and lost greater than 99% viability following peroxide treatment.

## Discussion

The [*PSI*$^+$] prion is an infectious, self-perpetuating form of Sup35, an essential translation termination factor. When Sup35 shifts to its transmissible prion conformation, it becomes unavailable for translation termination, resulting in readthrough of nonsense codons (9). The molecular basis of how yeast prions such as [*PSI*$^+$] nucleate de novo into the infectious amyloid-like structures that underlie the [*psi*$^-$]-to-[*PSI*$^+$] transition is poorly understood at present.

Here, we show that the Tsa1/Tsa2 Prxs suppress oxidative stress–induced de novo formation of the [*PSI*$^+$] prion. This raises the question how oxidation influences the formation of the Sup35 prion conformation. Hsp104 and a number of molecular chaperones are required for the continued propagation of [*PSI*$^+$] and other yeast prions (22). Hsp104 expression is induced in response to oxidative stress conditions, but this cannot account for the high frequency of de novo [*PSI*$^+$] prion formation in the *tsa1 tsa2* mutant as elevated levels of Hsp104 promote [*PSI*$^+$] loss rather than facilitating its de novo formation (19). However, it is possible that oxidant-induced changes in expression of the protein homeostasis network may account for [*PSI*$^+$] prion formation. Alternatively, direct oxidation of Sup35 may generate structural conformations that favor a shift to the transmissible amyloid-like form. This would be analogous to various environmental factors which have been proposed to trigger misfolding of the mammalian prion protein ($PrP^C$) into the aggregated scrapie form ($PrP^{Sc}$) (23).

All amino acids in proteins are potential targets for oxidation, but cysteine and methionine residues are particularly susceptible to oxidation by ROS. Sup35 can be divided into three functional regions demarked by methionine residues: an N-terminal region (residues 1–123) that is essential for prion formation, a highly charged middle (M) region (residues 124–253), and a C-terminal region (residues 254–685) that is required for translation termination activity (24). The N and M regions contain no cysteine residues, but the M region is flanked by methionine residues. Interestingly, oxidation of methionine residues in purified $PrP^C$ may cause the α-to-β structural conversion that underlies the sporadic formation of $PrP^{Sc}$ (25). Direct oxidation of Sup35 methionine residue (or other residues) may therefore facilitate the conformational conversion to the prion form.

That Tsa1 and Tsa2 are required to maintain genome stability during growth on glucose-based media suggests that sufficient endogenous ROS are generated to damage cellular macromolecules during normal aerobic growth (5). Loss of *TSA1* has been correlated with protein damage and aggregation during similar growth conditions (6, 7), and we show here that [*PSI*$^+$] prion formation increases significantly in the *tsa1 tsa2* mutant during normal aerobic growth conditions. We confirmed that this is an oxygen-dependent mechanism, as growth in the absence of molecular oxygen significantly reduces the de novo appearance of the [*PSI*$^+$] prion in a *tsa1 tsa2* mutant. Oxidative stress conditions induced by exposure to $H_2O_2$ were found to further elevate the frequency of de novo formation of the [*PSI*$^+$] prion, suggesting that the extent of Sup35 aggregation correlates with the degree of oxidative stress suffered by the cell.

The loss of the nonsense suppression phenotype from the *tsa1 tsa2* [*PSI*$^+$] strains when Hsp104 activity is inhibited by GdnHCl is consistent with a self-replicating prion state. For example, cells expressing an aggregating but noninheritable form of Sup35p (e.g., those formed by an Aβ–Sup35p fusion) do not show loss of the nonsense suppression phenotype in the presence of GdnHCl (26). The demonstration that a transmissible prion entity is present in a *tsa1 tsa2* [*PSI*$^+$] coupled with the presence of SDS-resistant

aggregates of Sup35 in the same strain confirms that transmissible forms of the [*PSI*$^+$] prion are formed in the *tsa1 tsa2* mutant.

Tsa1 and Tsa2 are the only antioxidants that have so far been shown to localize on ribosomes. Despite the finding that Tsa1/Tsa2 apparently function to protect Sup35 on ribosomes, it is not clear why the protein synthetic machinery should be a particular target of oxidative damage. It may simply reflect the relative abundance of ribosomes and ribosome-associated proteins. There is also increasing evidence that the translational apparatus is a target of oxidative damage and such damage has been implicated in the pathogenesis of a number of major neurodegenerative disorders, including Alzheimer disease and other protein misfolding disorders (27, 28).

Tsa1 is the major yeast Prx (4), and we show here that loss of both *TSA1* and *TSA2* results in a 50% decrease in cell viability following exposure to a normally nonlethal treatment with $H_2O_2$. $H_2O_2$ treatment also leads to a significant elevation in the frequency of de novo [*PSI*$^+$] prion formation (11), which in turn increases the readthrough of all translation termination codons. The elimination of [*PSI*$^+$] by GdnHCl from the *tsa1 tsa2* mutant caused the mutant to become hypersensitive to $H_2O_2$, reducing cell viability to less than 0.1% of the WT strain following the same oxidant treatment. This suggests that the ability to form the [*PSI*$^+$] prion provides a selective advantage to the *tsa1 tsa2* mutant during exposure to oxidative stress conditions. However, it remains possible that [*PIN*$^+$] or even other GdnHCl-curable prions could be responsible for relieving the peroxide sensitivity of the *tsa1 tsa2* mutant without the appearance of [*PSI*$^+$]. Resistance to several environmental stress conditions does correlate with the [*PSI*$^+$] versus [*psi*$^-$] status of cells (12, 13). Additionally, [*PSI*$^+$]-induced effects on frame shifting increase the levels of antizyme, a negative regulator of polyamine biosynthesis. The resulting alterations in polyamine synthesis can account for many of the reported changes in sensitivity to environmental insults which have been correlated with the [*PSI*$^+$] prion status of cells (29). The shift to the [*PSI*$^+$] prion state may therefore allow cells to reprogram gene expression such that new genetic traits become uncovered that aid survival during the altered conditions (11). Our findings support the hypothesis that prion-induced changes in gene expression provide a mechanism to uncover normally hidden genetic traits, which in this case aid survival under oxidative stress conditions. Tsa1 and Tsa2 normally act to prevent oxidant-induced [*PSI*$^+$] prion formation, but overwhelming this defense system provides an evolvable mechanism to survive the ensuing oxidative stress conditions.

## Experimental Procedures

**Yeast Strains, Plasmids, and Growth Conditions.** The WT strain W303 (*MATa ura3-52 leu2-3 leu2-112 trp1-1 ade2-1 his3-11 can1-100,* [*PIN*$^+$]) and its isogenic derivative deleted for *TSA1* (*tsa1::LEU2*) have been described previously (7). Strains deleted for *TSA2* (*tsa2::KANMX*) were constructed using standard yeast methodology. [*PSI*$^+$], [*psi*$^-$], and *hsp104::K218T, K620T* derivatives of 74D-694 (*MATa ade1-14 trp1-289 his3-200 ura3-52 leu2-3,112,* [*PIN*$^+$]) have been described previously (17). The centromeric plasmids *pFL-TSA1* and *pFL-TSA1*$^{C47S}$ have been described previously (30) and pFL-*TSA1*$^{C170S}$ and *pFL-TSA1*$^{C47S,C170S}$ plasmids were constructed as part of this study using the QuickChange II site-directed mutagenesis kit (Stratagene). Strains were grown in rich YEPD medium (2% wt/vol glucose, 2% wt/vol Bacto-Peptone, 1% wt/vol yeast extract) or minimal SD medium (0.17% wt/vol yeast nitrogen base without amino acids, 5% wt/vol ammonium sulfate, 2% wt/vol glucose) supplemented with appropriate amino acids and bases at 30 °C and 180 rpm. Strains were cured by growth on YEPD agar plates containing 3 mM GdnHCl. For anaerobic plate growth, plates were maintained in an anaerobic jar (Oxoid) containing a gas generating kit (anaerobic system BR38; Oxoid). Anaerobic culture conditions were established using SD media, which was degassed with nitrogen gas as previously described (31).

**Translation Termination Assays.** The levels of termination codon readthrough were measured using a β-gal reporter system (10). For genetic assays of termination codon readthrough, strains were grown to stationary phase, serially diluted ($OD_{600}$ = 1.0, 0.1, 0.01, 0.001) and spotted onto appropriate plates. Readthrough of the UAA stop codon in the *can1-100* allele was determined by examining growth on SD media containing various concentrations of canavanine. Readthrough of the UGA stop codon in the *ade1-114* allele was determined by examining growth on SD media lacking adenine and red/white colony color visualized on 1/4 YEPD media.

**Protein Analysis.** Ribosomal fractions were separated from soluble components by centrifugation through sucrose cushions (8). The analysis of Sup35 aggregates by subcellular fractionation was performed as described previously (32). Analysis of Sup35 aggregates by SDD-AGE was performed as described elsewhere (33). De novo [*PSI*$^+$] prion formation was visualized using the CUP1-SUP35NM-GFP plasmid (34). Cells were grown to early exponential phase ($A_{600}$ = 0.5) in SD minimal media, and 50 μM copper sulfate added for 5 h for induction of the *CUP1* promoter.

**Protein Transfection.** The protocol described by Tanaka et al. (21) was followed essentially as described. Crude extracts were prepared from yeast strains using the protocol described by Kryndushkin et al. (33). Spheroplasts were made from the strain 74D-694 [*psi*$^-$] [*PIN*$^+$] using Lyticase (Sigma-Aldrich) and transformations carried out with plasmid p6442 (SUP35NM-GFP) selecting for the *URA3* marker carried by this plasmid.

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