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Methionine Oxidation of Sup35 Protein Induces Formation of the [*PSI*$^+$] Prion in a Yeast Peroxiredoxin Mutant

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The frequency with which the yeast [*PSI*$^+$] prion form of Sup35 arises *de novo* is controlled by a number of genetic and environmental factors. We have previously shown that in cells lacking the antioxidant peroxiredoxin proteins Tsa1 and Tsa2, the frequency of *de novo* formation of [*PSI*$^+$] is greatly elevated. We show here that Tsa1/Tsa2 also function to suppress the formation of the [*PIN*$^+$] prion form of Rnq1. However, although oxidative stress increases the *de novo* formation of both [*PIN*$^+$] and [*PSI*$^+$], it does not overcome the requirement of cells being [*PIN*$^+$] to form the [*PSI*$^+$] prion. We use an anti-methionine sulfoxide antibody to show that methionine oxidation is elevated in Sup35 during oxidative stress conditions. Abrogating Sup35 methionine oxidation by overexpressing methionine sulfoxide reductase (*MSRA*) prevents [*PSI*$^+$] formation, indicating that Sup35 oxidation may underlie the switch from a soluble to an aggregated form of Sup35. In contrast, we were unable to detect methionine oxidation of Rnq1, and *MSRA* overexpression did not affect [*PIN*$^+$] formation in a *tsa1 tsa2* mutant. The molecular basis of how yeast and mammalian prions form infectious amyloid-like structures *de novo* is poorly understood. Our data suggest a causal link between Sup35 protein oxidation and *de novo* [*PSI*$^+$] prion formation.

Prions are novel protein-only infectious agents associated with a group of transmissible neurodegenerative diseases (1). Prion formation also underlies the unusual genetic behavior of several non-Mendelian traits found in fungi (2, 3). The most extensively studied yeast prion [*PSI*$^+$] is an infectious, self-perpetuating form of Sup35, an essential translation termination factor (4). When Sup35 shifts to its transmissible prion conformation, it becomes unavailable for translation termination, resulting in translation of nonsense codons by near cognate tRNAs (5, 6). Although mammalian prions are associated with disease, yeast prions can cause epigenetic changes in protein activity resulting in genetic variability, which can promote adaption under certain growth conditions (7, 8).

How prions form spontaneously into infectious amyloid-like structures is poorly understood at the molecular level. The frequency of *de novo* formation of the [*PSI*$^+$] prion in yeast is known to be regulated by a number of genetic and environmental factors (5, 8, 9). For example, for [*PSI*$^+$] to form *de novo*, there is an absolute requirement for the cells to carry the [*PIN*$^+$] prion (10). [*PIN*$^+$] is the prion form of Rnq1, which is a protein of unknown function. [*PIN*$^+$] prion aggregates appear to act as imperfect templates on which Sup35 molecules misfold and assemble into transmissible prion aggregates (11, 12). Clues as to how the initial misfolding event translates into a stable and transmissible prion have begun to emerge through the analysis of a Sup35NM-GFP fusion protein that can be switched to a heritable prion form by wild-type [*PSI*$^+$] prions. As with wild-type Sup35, the Sup35NM-GFP fusion protein retains the unstructured N-terminal prion-forming domain that has a high propensity to misfold (13). Any Sup35 molecules that do misfold are transferred to the recently discovered cellular site known as the insoluble protein deposit, a component of the quality control machinery in eukaryotic cells that is located adjacent to the vacuole. The insoluble protein deposit is a site of accumulation of two different prion proteins (Sup35 and Rnq1) as well as oxidatively damaged proteins (14, 15). The resulting localized concentration of such misfolded proteins in a [*PIN*$^+$] cell together with the aggregated Rnq1 is believed to facilitate the nucleation of prion protein polymerization in a [*PIN*$^+$]-dependent manner. The transmissible form of the prion is then formed via a two-stage process that initially involves the formation of non-transmissible extended polymers of the prion protein followed by their fragmentation into shorter transmissible prion protein polymers that most likely define the transmissible entities that are required for continued propagation, *i.e.* propagons (15).

Increasing evidence suggests a possible causal link between protein oxidation and aggregate formation. All organisms are exposed to reactive oxygen species (ROS)[4] during the course of normal aerobic metabolism or following exposure to radical-generating compounds (16). ROS are toxic agents that can damage a wide variety of cellular components, and oxidative damage to amino acid residues in proteins is a well established trigger of protein misfolding (17). For example, oxidative damage to Met residues in purified PrP^C may cause the α-to-β structural conversion, which underlies the sporadic formation of PrP^{Sc} (18, 19). Oxidation of Met-35 has been detected in βamyloid-β peptide from Alzheimer disease patients, which may contribute to the neuronal cytoskeletal disruption that is

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[4] The abbreviations used are: ROS, reactive oxygen species; PrP, prion protein; Prx, peroxiredoxin; MSR, methionine sulfoxide reductase; GdnHCl, guanidine hydrochloride; MetO, methionine sulfoxide; TAP, tandem affinity purification; SD, synthetic defined; SDD-AGE, semidenaturing detergent-agarose gel electrophoresis.

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characteristic of the disease (20). Oxidative stress may also play a role in modulating α-synuclein aggregation in Parkinson disease because formation of α-synuclein-soluble oligomers has been shown to require methionine oxidation (21), and clearance of α-synuclein oligomers is inhibited in methionine sulfoxide reductase mutants (22). Protein oxidation may therefore be a common mechanism underlying the aggregation of mammalian amyloidogenic proteins.

Oxidative stress induced by exposure to H_2O_2 elevates the frequency of *de novo* [*PSI*$^+$] formation (8). Additionally, the frequency of [*PSI*$^+$] prion formation is significantly elevated in mutants lacking the *TSA1* and *TSA2* peroxiredoxins (Prxs) (23). Prxs are ubiquitous, thiol-specific proteins that have multiple functions in stress protection, including oxidative stress (24, 25). In yeast, Tsa1 is the major 2-Cys Prx and acts as an antioxidant in the detoxification of hydroperoxides (26, 27), particularly as a result of endogenous ROS generated during normal aerobic metabolism (28). The Tsa2 peroxiredoxin is highly homologous to Tsa1 (86% amino acid identity) and possesses similar peroxidase activity, although it is normally expressed at significantly lower levels when compared with Tsa1 (27, 29). Tsa1 and Tsa2 co-localize to ribosomes and function to protect Sup35 against oxidative stress-induced formation of its heritable [*PSI*$^+$] prion conformation (23). Molecular oxygen is required for [*PSI*$^+$] prion formation because 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 (23). Taken together, these data suggest oxidative damage as being one of the triggers of *de novo* [*PSI*$^+$] prion formation. However, it is unclear whether ROS directly oxidize Sup35 or whether ROS cause alterations in the protein homeostasis network, which result in increased [*PSI*$^+$] prion formation.

In this study, we show that Tsa1 and Tsa2 function to suppress the formation of another unrelated prion, [*PIN*$^+$], the prion form of the glutamine and asparagines-rich Rnq1 protein, but increased protein aggregation *per se* does not appear to be a common feature of prion and amyloidogenic proteins in *tsa1 tsa2* mutants. Although oxidative stress increases the *de novo* formation of the [*PSI*$^+$] prion, it does not overcome the requirement of cells being [*PIN*$^+$] to form the [*PSI*$^+$] prion. We further show a correlation between methionine oxidation in Sup35 and [*PSI*$^+$] prion formation, confirming a link between Sup35 oxidative damage and *de novo* prion formation.

EXPERIMENTAL PROCEDURES

Yeast Strains and Plasmids—The wild-type strain W303 (*MATa ura3-52 leu2-3 leu2-112 trp1-1 ade2-1 his3-11 can1-100*) and its isogenic derivatives deleted for *TSA1* (*tsa1*::*LEU2* or *tsa1*::*URA3*) and *TSA2* (*tsa2*::*KANMX*) have been described previously (23, 30). [*PSI*$^+$], [*psi*$^-$], *tsa1*::*LEU2 tsa2*::*HIS*, and *hsp104*::*K218T*, *K620T* derivatives of 74D-694 (*MATa ade1-14 trp1-289 his3-200 ura3-52 leu2-3,112*) have been described previously (23). Strains were deleted for *RNQ1* (*rnq1*::*TRP1* or *rnq1*::*URA3*) in 74D-694 using standard yeast methodology. Sup35 and Rnq1 were tagged at their C termini in 74D-694 with a tandem affinity purification (TAP) tag (31). Overexpression of methionine sulfoxide reductase (*MSRA*) was achieved using a *GAL1-MSRA-GST* plasmid (pEGH) supplied by Open Biosystems.

Growth and Stress Conditions—Strains were grown at 30 °C with shaking at 180 rpm in rich YEPD medium (2% w/v glucose, 2% w/v bactopeptone, 1% w/v yeast extract) or minimal SD (0.67% w/v yeast nitrogen base without amino acids, 2% w/v glucose) supplemented with appropriate amino acids and bases. SGal media contained 2% w/v galactose in place of glucose. Media were solidified by the addition of 2% (w/v) agar. Visual differentiation of red/white colony formation was enhanced by growth on quarter YEPD (qYEPD: 4% w/v glucose, 1% w/v bactopeptone, 0.25% w/v yeast extract). Strains were cured by growth on YEPD agar plates containing 5 mM guanidine hydrochloride (GdnHCl). Sensitivity to hydrogen peroxide was determined by growing cells to early exponential phase (A_{600} ~0.1) and treating cultures with 100 μM H_2O_2 for 20 h. Aliquots of cells were diluted in fresh YEPD medium and plated in triplicate on YEPD plates to obtain viable counts after 3 days of growth.

Visualization of Aggregation of Prion and Amyloidogenic Proteins—De novo [*PSI*$^+$] prion formation was visualized as described previously using the CUP1-SUP35NM-GFP plasmid (23). Similarly, Cyc8 was visualized using the CUP1-CYC8-YFP plasmid (32). 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. *GAL1*-controlled expression plasmids were used for Htt (pYES2-Htt25Q-GFP, pYES2-Htt103Q-GFP), α-synuclein (pHY314-αSyn-GFP), Ure2 (pYe2T-URE2GFP), and Rnq1 (GAL1-RNQ1-EGFP) (33–35). Cultures were grown in non-repressing sucrose media or inducing galactose media for 2 h to induce *GAL1* expression. Swi1 was visualized using the p416TEF-NQYFP plasmid, which expresses the NQ region of Swi1 as a fusion with YFP under the control of the constitutive *TEF1* promoter (36). Plasmids were transformed into wt (74D-694 or W303) and *tsa1 tsa2* mutant cells and visualized using an Olympus widefield microscope and MetaVue software (Bioimaging Facility, Faculty of Life Science, University of Manchester).

Protein Analysis—The analysis of Sup35 aggregates by subcellular fractionation was performed as described previously (37). Rnq1 subcellular fractionation was analyzed using a slightly modified procedure. Briefly, exponential phase cells (A_{600} ~0.5) were collected by centrifugation, washed once with distilled water, and resuspended in 50 μl of lysis buffer (74 mM Tris-HCl, pH 8.0, 150 mM KCl, 50 mM EDTA, 1 mM DTT, 0.2% w/v SDS, 1% v/v Triton, 1 mM PMSF, Complete mini protease inhibitor mixture, Roche Applied Science). Cell breakage was achieved by vortexing at 4 °C with an equal volume of glass beads. Cell debris was removed by centrifugation at 3000 rpm for 3 min at 4 °C. Total cell extracts were fractionated by ultracentrifugation for 1 h at 85,000 × *g* in a Beckman MLA-130 rotor to generate supernatant and pellet fractions. Pellets were resuspended in an equivalent volume of lysis buffer to the supernatant and fractions subjected to SDS-PAGE and Western blot analysis. The analysis of Sup35 amyloid polymers by

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semidenaturing detergent-agarose gel electrophoresis (SDD-AGE) was performed as described previously (38).

Determination of Spontaneous [PIN$^+$] Prion Formation—To assay spontaneous [*PIN*$^+$] prion formation, wild-type (74D-694) and *tsa1 tsa2* [*psi*$^-$] mutant strains were first transformed with the CUP1-SUP35NM-GFP plasmid and grown in the presence of 3 mM GdnHCl to eliminate the [*PIN*$^+$] prion. The resulting [*pin*$^-$] transformed colonies were grown in SD-Ura media to early exponential phase (A_{600} ~0.1) and treated with 100 μM H_2O_2 for 20 h. Cultures were then diluted to A_{600} = 0.0001, and 100 μl of diluted cultures were plated onto SD-Ura plates to generate single colonies following growth at 30 °C for 3 days. 96 individual colonies were inoculated into 150 μl of SD-Ura media containing 25 μM CuSO4 in 96-well plates and incubated overnight at 30 °C/180 rpm. 5 μl from each of the 96 cultures were spotted onto qYEPD, qYEPD + 4 mM GdnHCl, or SD-Ade (containing 1% YEPD to improve growth) plates. Overexpression of Sup35NM-GFP generates [*PSI*$^+$] colonies, which were scored as white/pink colonies and by their ability to grow in the absence of adenine, with both phenotypes being curable with GdnHCl. The rate of [*PIN*$^+$] formation was inferred from the rate of [*PSI*$^+$] formation, which is entirely dependent on cells being [*PIN*$^+$] (10).

TAP Affinity Chromatography—Sup35-TAP and Rnq1-TAP affinity purification was based on the methods described by Ref. 31. TAP-tagged strains were grown to exponential phase in minimal SD media. Cultures were harvested by centrifugation, washed with ice-cold water, and resuspended in 300 μl of buffer D (20 mM HEPES, pH 7.9, 50 mM KCl, 0.2 mM EDTA, 20% v/v glycerol, 1 mM PMSF, 1× Complete EDTA-free protease inhibitor mixture tablet, Roche Applied Science, catalog number 04 693 159 001). Cells were broken with glass beads using a Minibead beater (Biospec Scientific, Bartlesville, OK) for 4 × 30-s cycles (1-min cool-down between each cycle) at 4 °C. An additional 200 μl of buffer D were added to the lysed cells, and cell debris were removed by centrifugation at 10,000 rpm for 10 min at 4 °C. IgG-Sepharose beads (300 μl) (GE Healthcare) were placed in a 10-ml column (Thermo Fisher Scientific) and washed with 5 ml of IPP150 (10 mM Tris, pH 8, 1% v/v Nonidet P-40, 150 mM NaCl) buffer. Cell extract (400 μl) was added to the washed IgG beads in a final volume of 3 ml containing 10 mM Tris, pH 8, 1% v/v Nonidet P-40, 150 mM NaCl and incubated on a rotator for 2 h at 4 °C. Following incubation, columns were washed with 3 × 10 ml of IPP150 buffer. Washed beads were resuspended in 333 μl of buffer D and 67 μl of 6× protein loading buffer and boiled for 10 min at 95 °C, and 10–20 μl were used for SDS-PAGE and Western blotting. Methionine oxidation of Sup35 and Rnq1 was detected using αMetO antibodies (Novus Biologicals).

RESULTS

Do Novo Aggregation of Rnq1 and Sup35 Is Increased in a tsa1 tsa2 Mutant—We previously used a Sup35::GFP fusion protein to demonstrate that the *de novo* formation of Sup35 aggregates is increased in a *tsa1 tsa2* mutant (23). In this study, we examined a range of known prion and amyloidogenic proteins to determine whether aggregation is a common feature of such proteins in *tsa1 tsa2* mutants. Prion-forming proteins, including Sup35, Swi1, Cyc8, Ure2, and Rnq1, were all expressed under the control of heterologous promoters as chimeras with GFP or YFP (see "Experimental Procedures" for details). In agreement with previous observations, strong diffuse cytoplasmic fluorescence of Sup35::GFP was observed in wild-type cells, whereas many different sized aggregates of Sup35 were detected in the majority (~95%) of *tsa1 tsa2* mutant cells (Fig. 1*A*).

Swi1 is a chromatin-remodeling factor that has been shown to form numerous fluorescent foci in [*SWI*$^+$] cells, but not in [*swi*$^-$] cells (36). Fluorescence was diffuse, and no foci were detected in the *tsa1 tsa2* mutant, indicating that Swi1 does not form aggregates in this mutant (Fig. 1*A*). Cyc8 is a transcriptional co-repressor that normally shows distinct nuclear fluorescence but forms punctate fluorescent dots in its prion form (32). Nuclear localization of Cyc8::YFP was observed in wild-type cells, whereas fluorescence was more diffuse and less pronounced in a *tsa1 tsa2* mutant (Fig. 1*A*). It is unclear why fluorescence was altered in this manner in the *tsa1 tsa2* mutant; nevertheless we were unable to detect any aggregates of Cyc8::YFP that would be indicative of prion formation. Formation of the [*URE3*$^+$] prion by the Ure2 transcriptional repressor has been extensively characterized as a model prion (2). Ure2::GFP can form large fluorescent foci indicative of aggregation in [*URE3*$^+$] strains. However, Ure2::GFP was detected as diffuse cytoplasmic fluorescence in both the wild-type and the *tsa1 tsa2* mutant strains (Fig. 1*A*).

Rnq1 can form the [*PIN*$^+$] prion, which is known to be required for the *de novo* formation of [*PSI*$^+$] (10). We found that the wild-type strain W303 used in this study is [*PIN*$^+$], but only ~18% of cells examined showed distinct formation of Rnq1 aggregates (data not shown). As expected, the *tsa1 tsa2* mutant derived from W303 was also [*PIN*$^+$], although all *tsa1 tsa2* mutant cells examined contained distinct Rnq1::GFP aggregates (data not shown). To determine whether Rnq1 shows a higher propensity to aggregate in a [*pin*$^-$] *tsa1 tsa2* mutant, the W303-based strains were cured of [*PIN*$^+$] by treatment with GdnHCl, which inhibits Hsp104, a molecular chaperone that is absolutely required for yeast prion propagation (39, 40). The resulting [*pin*$^-$] strains were then grown for ~40 generations in liquid culture, and Rnq1::GFP aggregation was examined. Under these conditions, Rnq1 fluorescence showed diffuse cytoplasmic staining in the wild-type [*pin*$^-$] strain, whereas distinct aggregates were detected in all *tsa1 tsa2* mutant cells examined (Fig. 1*A*).

A number of mammalian proteins that have amyloidogenic potential have been expressed in yeast and shown to retain their propensity to form amyloid. These include proteins that are associated with neurodegenerative diseases such as Parkinson disease (α-synuclein) and Huntington disease (huntingtin) (41). We therefore examined whether loss of *TSA1* and *TSA2* influences the aggregation of α-synuclein and huntingtin-GFP fusions. α-Synuclein showed diffuse cytoplasmic fluorescence in both the wild-type strain and the *tsa1 tsa2* [*PIN*$^+$] mutant (Fig. 1*B*). Expansion of the poly(Q)-encoding region of the Htt gene beyond the critical length of ~35 glutamines causes the Htt protein to become neurotoxic and prone to aggregation. Htt25Q::GFP showed diffuse cytoplasmic fluorescence in both

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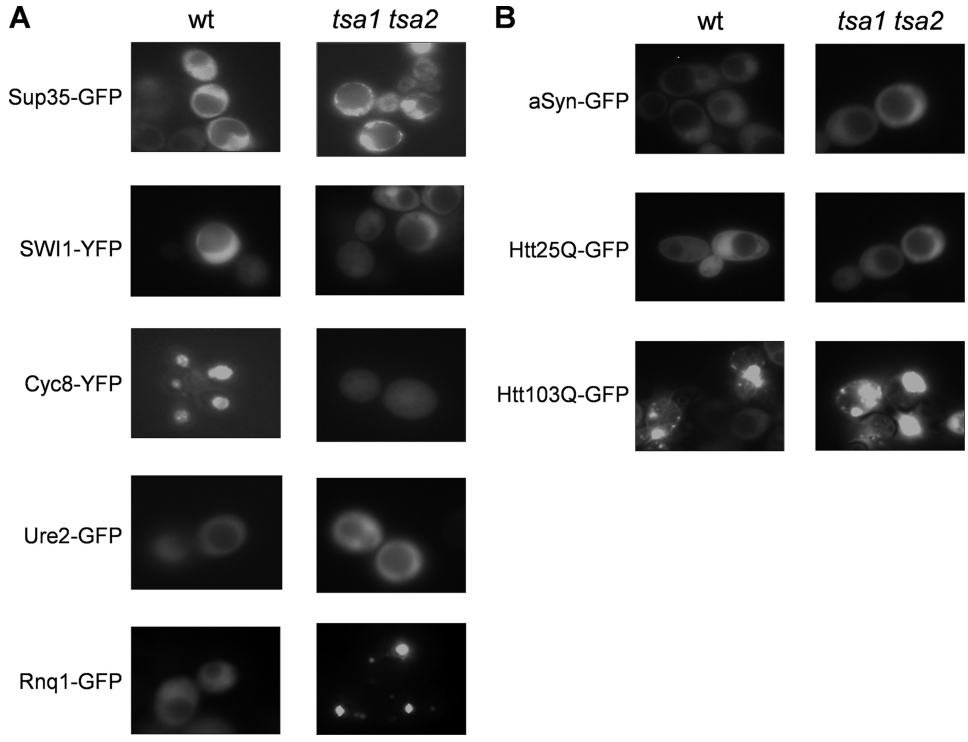


FIGURE 1. **Aggregation of prion and amyloidogenic proteins in a *tsa1 tsa2* mutant.** Representative fluorescence micrographs are shown for the *TSA1*$^+$/*TSA2*$^+$ wild-type and *tsa1 tsa2* mutant strains containing plasmid expression fusion proteins of Sup35 (Sup35NM-GFP), Swi1 (p416TEF-NQYFP), Cyc8 (CUP1-CYC8-YFP), Ure2 (pYe2T-URE2GFP), Rnq1 (GAL1-RNQ1-EGFP), Htt (pYES2-Htt25Q-GFP and pYES2-Htt103Q-GFP) and α-synuclein (pHY314-αSyn-GFP). All strains are [*PIN*$^+$].

the wild-type and the *tsa1 tsa2* [*PIN*$^+$] mutant strain. Htt103Q::GFP was seen to aggregate in the wild-type strain as expected, and ~26% of cells were found to contain numerous fluorescent foci (Fig. 1*B*). However, in the *tsa1 tsa2* [*PIN*$^+$] mutant, a similar number of cells containing aggregates (~27%) were detected. Taken together, these data indicate that Tsa1 and Tsa2 normally function to suppress the formation of both the [*PSI*$^+$] and the [*PIN*$^+$] prions, but increased protein aggregation does not appear to be a common feature of prion and amyloidogenic proteins in *tsa1 tsa2* mutants.

Tsa1 and Tsa2 Protect against de Novo Formation of the [PIN$^+$*] Prion*—Given that aggregation of Rnq1::GFP was detected in the *tsa1 tsa2* mutant, the subcellular distribution of Rnq1 was examined in exponential phase cultures of the wild-type and the peroxiredoxin mutant strains. [*pin*$^-$] derivatives of these strains were generated using GdnHCl and then grown for ~40 generations in liquid culture to allow the *de novo* formation of new prions. Analysis of the resulting subcellular distribution of Rnq1 revealed that the protein was in the soluble fraction in wild-type cells but was predominantly in an SDS-insoluble high molecular weight form in the *tsa1 tsa2* mutant (Fig. 2*A*). Subsequently, growing these *tsa1 tsa2* mutants with 3 mM GdnHCl or overexpressing an ATPase-deficient allele of Hsp104 (*hsp104::K218T, K620T*) in that strain resulted in Rnq1 switching back to the soluble fraction (Fig. 2*A*). These data confirm the requirement for wild-type Hsp104 for maintaining the Rnq1 aggregates in the *tsa1 tsa2* mutant; this is an important genetic criterion for yeast prions (2).

The 74D-694 strain used for these studies contains the *ade1-14* nonsense (UGA) mutant allele, which confers adenine autotrophy. 74D-694 [*psi*$^-$] cells accumulate a red pigment, and suppression of the *ade1-14* nonsense mutation gives rise to white/pink Ade$^+$ colonies. This phenotypic change was used to quantify the *de novo* formation of [*PIN*$^+$] as follows. Sup35NM-GFP was overexpressed in [*psi*$^-$] [*pin*$^-$] wild-type and *tsa1 tsa2* mutant cells to detect cells that will generate [*PSI*$^+$] *de novo*. Because [*PSI*$^+$] formation is dependent on cells being [*PIN*$^+$] (10), one can estimate the rate of [*PIN*$^+$] formation based on the number of white/pink Ade$^+$ cells that arise. The rate of *de novo* formation of [*PIN*$^+$] colonies was ~3% in the wild-type strain, and this was elevated 7-fold in the *tsa1 tsa2* mutant (Fig. 2*B*). Furthermore, culturing strains in the presence of 100 μM H_2O_2 for 20 h further increased the rate of *de novo* [*PIN*$^+$] prion formation in wild-type and *tsa1 tsa2* mutant cells, reaching a rate of ~27% in the peroxiredoxin-deficient mutant (Fig. 2*B*).

De Novo Formation of the [PSI$^+$*] Prion Still Depends on the [PSI*$^+$*] Prion in a tsa1 tsa2 Mutant*—Because the *de novo* formation of both [*PSI*$^+$] (23) and [*PIN*$^+$] (this study) prions is elevated in *tsa1 tsa2* mutants, we examined whether the loss of *TSA1* and *TSA2* abrogates the requirement of cells being [*PIN*$^+$] for *de novo* formation of the [*PSI*$^+$] prion. This was achieved by deleting the *RNQ1* gene in the *tsa1 tsa2* mutant to prevent cells from forming the [*PIN*$^+$] prion. The wild-type, *tsa1 tsa2*, and *tsa1 tsa2 rnq1* strains were grown with 3 mM GdnHCl before being grown for ~40 generations in liquid culture in the absence of GdnHCl to allow the formation of new prions. Although white/pink Ade$^+$ [*PSI*$^+$] colonies were generated at high frequency in the *tsa1 tsa2* mutant, no such colonies were generated in the *tsa1 tsa2 rnq1* mutant, confirming that

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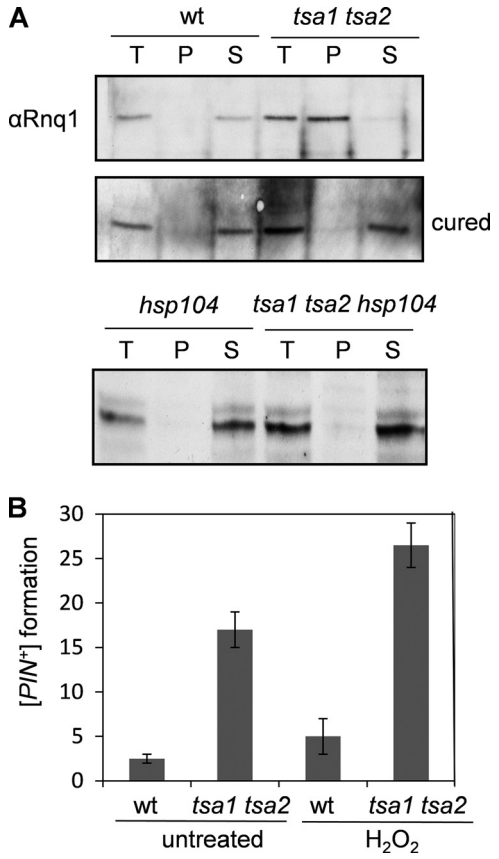


FIGURE 2. ***De novo* formation of the [*PIN*$^+$][rsqb] prion is increased in a *tsa1 tsa2* [*pin*−] mutant.** *A*, subcellular localization of Rnq1 was examined in wild-type (74D-694, *wt*) and *tsa1 tsa2* mutant cells and in the same cells following growth in the presence of GdnHCl (*i.e.* cured) or expressing an ATPase-deficient allele of Hsp104 (*hsp104*::*K218T*, *K620T*). Cells were grown to exponential phase in SD minimal media, and subcellular fractionation analysis of Rnq1 was performed as described under "Experimental Procedures," using an anti-Rnq1 polyclonal antibody. *T*, total crude extract; *S*, soluble fraction; *P*, pellet fraction. *B*, the rate of *de novo* [*PIN*$^+$] formation was determined in the wild type (74D-694, *wt*) and *tsa1 tsa2* mutant as described under "Experimental Procedures" and is expressed as the number of [*PIN*$^+$] colonies formed per 96 colonies examined. The rate of *de novo* [*PIN*$^+$] formation is significantly different in the *tsa1 tsa2* mutant when compared with the wild-type strain (p value <0.05). Hydrogen peroxide exposure increased the rate of *de novo* [*PIN*$^+$] formation in both the wild-type and the *tsa1 tsa2* mutant strains but was not significant (p value = 0.34 and 0.052, respectively). *Error bars* indicate S.E.

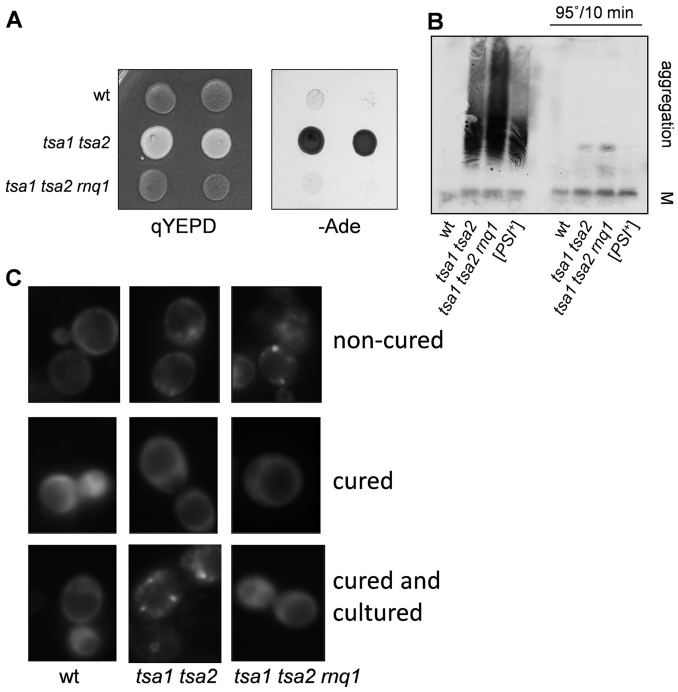


FIGURE 3. ***De novo* formation of the [*PSI*$^+$] prion still depends on the [*PIN*$^+$] prion in a *tsa1 tsa2* mutant.** *A*, [*PSI*$^+$] prion formation was assayed in the wild-type (74D-694, *wt*), *tsa1 tsa2*, and *tsa1 tsa2 rnq1* mutant strains by pink/white colony formation on qYEPD medium (*white/gray* in black and white) and growth on minimal medium in the absence of adenine (−*Ade*). *B*, SDS-resistant Sup35 aggregates were detected in wild-type (W303), *tsa1 tsa2*, *tsa1 tsa2 rnq1*, and a [*PSI*$^+$] control strain using SDD-AGE. Heating cell extracts to 95 °C for 10 min collapses aggregates to the monomer form (*M*). *C*, representative fluorescence micrographs are shown for the wild type, *tsa1 tsa2*, and *tsa1 tsa2 rnq1* mutants containing the Sup35NM-GFP plasmid. *Non-cured* refers to strains that have been routinely cultured in rich growth medium, whereas *cured* refers to strains that were analyzed following growth with 3 mM GdnHCl. *Cured and cultured* refers to strains that were examined following the growth of cured strains in the absence of GdnHCl for ~40 generations in liquid culture to allow the formation of new prions.

the [*PIN*$^+$] prion is still required for [*PSI*$^+$] prion formation in *tsa1 tsa2* mutants (Fig. 3*A*).

This result was further confirmed by examining the *de novo* formation of Sup35 aggregates in [*PIN*$^+$] and [*pin*$^-$] strains using the Sup35NM-GFP reporter construct in a W303 genetic background. The *tsa1 tsa2 rnq1* [*pin*$^-$] mutant of W303 was constructed by deleting *RNQ1* in a *tsa1 tsa2* [*PIN*$^+$] mutant. The resulting *tsa1 tsa2 rnq1* [*pin*$^-$] mutant still generated [*PSI*$^+$] cells at high frequency that contained the diagnostic high molecular weight SDS-resistant aggregates of Sup35 that were also observed in the *tsa1 tsa2* [*PIN*$^+$] and control 74D-694 [*PIN*$^+$] [*PSI*$^+$] strains (Fig. 3*B*). In agreement with this, strong cytoplasmic fluorescence of Sup35::GFP was observed in the [*psi*$^-$] [*PIN*$^+$] wild-type cells, whereas many different sized aggregates of Sup35 were detected in the majority of *tsa1 tsa2* and *tsa1 tsa2 rnq1* mutant cells (Fig. 3*C*, *non-cured*). As expected, growth in the presence of 3 mM GdnHCl resulted in Sup35::GFP shifting to cytoplasmic fluorescence in the two mutant strains (Fig. 3*C*, *cured*), but following growth of these cured strains in the absence of GdnHCl for ~40 generations, Sup35NM-GFP aggregates were re-formed in the *tsa1 tsa2* mutant but not the *tsa1 tsa2 rnq1* mutant (Fig. 3*C*, *cured and cultured*), further confirming the requirement for Rnq1/[*PIN*$^+$] for *de novo* [*PSI*$^+$] prion formation (Fig. 3*B*).

Sup35 Protein Oxidation Is Increased in a tsa1 tsa2 Mutant and Correlates with Protein Aggregation—Given that oxidation of methionine residues in purified PrP^C may cause the α-to-β structural conversion, which underlies the sporadic formation of PrP^{Sc} (18, 19), we examined methionine oxidation of Sup35 and Rnq1 in the *tsa1 tsa2* mutant. Sup35 and Rnq1 were genomically tagged to enable purification from wild-type and *tsa1 tsa2* mutant strains using TAP. We first confirmed that genomically tagging Sup35 and Rnq1 does not affect reversible prion formation in the tagged 74D-694 *tsa1 tsa2* mutant strains. Colony-purified red Ade$^+$ 74D-694 *tsa1 tsa2 SUP35-TAP* and *RNQ1-TAP* mutant strains were streaked for single colonies on YEPD media. For the *SUP35-TAP* mutant, 110 out of 120 independently arising pink/white colonies were curable with GdnHCl. Similarly, 108 out of 120 independently arising pink/white colonies from the *RNQ1-TAP* mutant were curable with GdnHCl, confirming reversible prion formation. Methionine oxidation was detected using an antibody that recognizes

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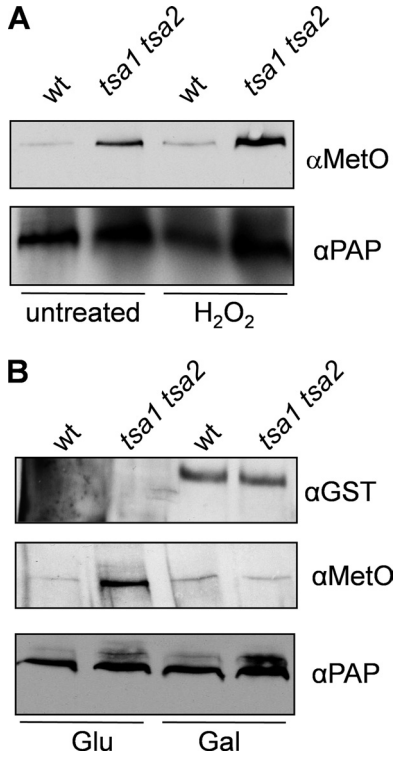


FIGURE 4. **Overexpression of *MSRA* protects Sup35 against methionine oxidation.** *A*, Sup35 was affinity-purified using TAP chromatography from a wild-type and *tsa1 tsa2* mutant strain treated with 1 mM H_2O_2 for 1 h. Western blots were probed with $\alpha\alpha$-PAP to confirm that similar amounts of Sup35 were purified from each strain. Sup35 oxidation was detected using antibodies that recognize methionine sulfoxide (*αMetO*). *B*, methionine sulfoxide reductase (*MSRA*) was overexpressed using plasmid *GAL1-MSRA-GST* in wild-type and *tsa1 tsa2* mutant strains. Overexpression was confirmed under inducing (*Gal*) *versus* repressing (*Glu*) conditions using an anti-GST antibody (*αGST*). *MSRA* expression prevented methionine oxidation of Sup35 detected using the α-MetO antibody.

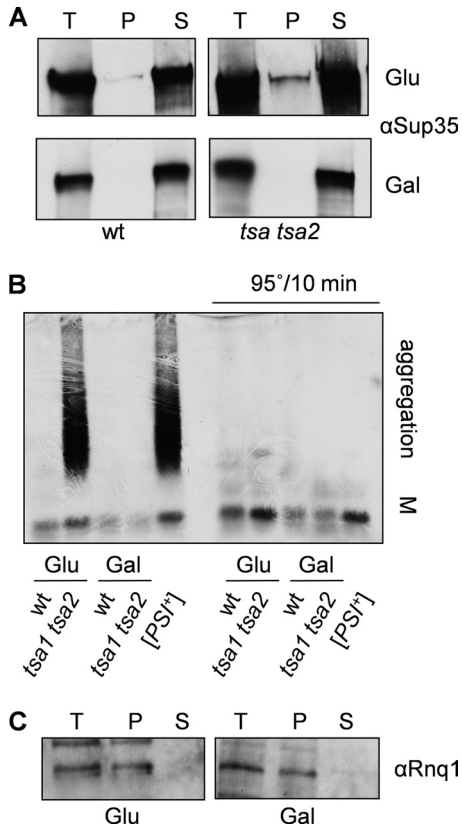


FIGURE 5. **Overexpression of *MSRA* prevents [*PSI*$^+$] prion formation.** *A*, the subcellular distribution of Sup35 was determined in wild-type (74D-694, wt) and *tsa1 tsa2* mutant cells containing plasmid *GAL1-MSRA-GST* grown under inducing (*Gal*) and repressing (*Glu*) conditions. *T*, total crude extract; *S*, soluble fraction; *P*, pellet fraction. *B*, SDS-resistant Sup35 aggregates were detected in the same strains using SDD-AGE. A [*PSI*$^+$] derivative of 74D-694 is shown for comparison. Heating cell extracts to 95 °C for 10 min collapses aggregates to the monomer form (*M*). *C*, the subcellular distribution of Rnq1 was determined in the *tsa1 tsa2* mutant containing plasmid *GAL1-MSRA-GST* grown under inducing (*Gal*) and repressing (*Glu*) conditions.

methionine sulfoxide (MetO), and basal levels of MetO were significantly elevated in the *tsa1 tsa2* mutant when compared with a wild-type strain (Fig. 4*A*). Methionine oxidation was also further elevated following exposure to 1 mM H_2O_2 for 1 h. In contrast, we could not detect any elevated methionine oxidation in Rnq1 purified from the *tsa1 tsa2* mutant (data not shown).

To determine whether methionine oxidation correlates with Sup35 aggregation, we examined whether overexpression of methionine sulfoxide reductase (MSR) could protect against methionine oxidation and reduce the frequency of *de novo* formation of [*PSI*$^+$]. MSRs are antioxidants that protect against Met oxidation by catalyzing thiol-dependent reduction of oxidized Met residues (42). Wild-type (74D-694) and *tsa1 tsa2* mutant strains were first transformed with a *GAL1-MSRA-GST* plasmid, and then [*pin*$^-$] derivatives were generated by growth with 3 mM GdnHCl. The resulting [*pin*$^-$] transformed colonies were then grown in SD or SGal media for ~40 generations in liquid culture to allow the formation of new prions. Growth on galactose was confirmed to induce the expression of *MSRA* as determined by Western blot analysis, and the resulting elevation in the levels of *MSRA* prevented the increased methionine oxidation normally observed in the *tsa1 tsa2* mutant (Fig. 4*B*). In comparison, no *MSRA* expression was detected in glucose-grown cells, and elevated MetO formation was still detected in the *tsa1 tsa2* mutant (Fig. 4*B*).

The subcellular distribution of Sup35 was used to determine whether methionine oxidation correlates with Sup35 aggregation. Strikingly, concomitant *MSRA* expression prevented the formation of SDS-resistant Sup35 aggregates in the *tsa1 tsa2* mutant (Fig. 5*A*). Similarly, SDD-AGE was used to confirm that Sup35 does not form high molecular weight SDS-resistant aggregates when *MSRA* is overexpressed in the *tsa1 tsa2* mutant (Fig. 5*B*). In comparison, overexpression of *MSRA* did not affect [*PIN*$^+$] formation because Rnq1 was largely present in an SDS-insoluble high molecular weight form in the *tsa1 tsa2* mutant grown in glucose or galactose media (Fig. 5*C*). These data indicate that methionine oxidation in Sup35 may play a critical role in *de novo* formation of the [*PSI*$^+$] prion in *tsa1 tsa2* mutants.

DISCUSSION

We show here that formation of the [*PIN*$^+$] prion is increased in response to oxidative stress conditions similar to the previously reported increase for the [*PSI*$^+$] prion (23). However, this does not appear to be a common feature of prion and amyloidogenic proteins because increased aggregation was not observed

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for Swi1, Cyc8, Ure2, Htt, and α-synuclein in *tsa1 tsa2* mutants. The specificity for Sup35 and Rnq1 may reflect the localization and/or catalytic activity of the two relevant peroxiredoxins. Tsa1/Tsa2 are cytoplasmic antioxidants that detoxify hydroperoxides (26, 27), and they are particularly important during normal aerobic growth conditions because their absence results in an increased rate of spontaneous mutations. This indicates that Tsa1/Tsa2 normally function to protect against endogenous ROS (28). Tsa1/Tsa2 and Sup35 co-localize to ribosomes, which may explain the role of these Prxs in protecting against [*PSI*$^+$] prion formation (23). It is not yet known whether Rnq1 is similarly localized, but ribosomal association may also explain the role of Tsa1/Tsa2 in protecting against [*PIN*$^+$] prion formation. More studies will be required to determine the general oxidant sensitivity of prion and amyloidogenic proteins and whether particular antioxidants provide compartment- or ROS-specific protection against protein oxidation.

[*PSI*$^+$] arises *de novo* at a frequency of $\sim 5 \times 10^{-7}$ per generation (43). Our data indicate that the *de novo* rate of [*PIN*$^+$] formation ($\sim 3 \times 10^{-2}$) is several orders of magnitude greater than that of [*PSI*$^+$]. A [*pin*$^-$] derivative of the wild-type *TSA1*$^+$/*TSA2*$^+$ readily formed the [*PIN*$^+$] prion, and the rate of formation was further enhanced in the *tsa1 tsa2* mutant and under oxidative stress conditions. It is known that heterologous prions can interact; for example, [*PIN*$^+$] promotes and enhances the *de novo* appearance of [*PSI*$^+$] through a direct protein-protein interaction (44). The [*PIN*$^+$] prion is essential for the formation of [*PSI*$^+$] in *tsa1 tsa2* mutants because deletion of *RNQ1* prevented [*PSI*$^+$] prion formation in response to oxidative stress conditions. Although other proteins can form [*PIN*$^+$] when overexpressed (11), in the majority of laboratory strains, [*PIN*$^+$] is the prion form of Rnq1. The observed high rate of [*PIN*$^+$] formation is consistent with the finding that nearly all laboratory strains and a significant proportion of natural strains are [*PIN*$^+$] prions (45).[5] Consequently, oxidation may only normally function to increase [*PSI*$^+$] prion formation.

All amino acids are potentially susceptible to oxidation by ROS (17). Methionine residues are particularly sensitive, forming a racemic mixture of methionine-*S*-sulfoxide and methionine-*R*-sulfoxide in cells (17, 46). Sup35 and Rnq1 are rich in methionine residues; Sup35 contains 19 methionine residues, and Rnq1 contains 14 methionine residues, respectively. We were able to detect increased Sup35 methionine oxidation in *tsa1 tsa2* mutants using an antibody that recognizes methionine sulfoxide, and oxidation was further increased following exposure to hydrogen peroxide. Most organisms contain MSRs, which protect against methionine oxidation by catalyzing thiol-dependent reduction of oxidized Met residues. This is particularly important because it means that methionine oxidation is readily reversible and may play an antioxidant role in scavenging ROS (47). Yeast contains three MSR enzymes (fRMsr/MsrA/MsrB) (42). Overexpression of *MSRA* protected Sup35 against methionine oxidation and also prevented [*PSI*$^+$] prion formation, indicating that Sup35 methionine oxidation may induce [*PSI*$^+$] prion formation. Taking these observations

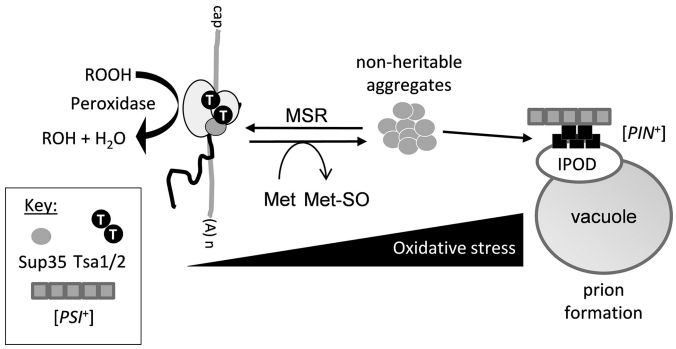


FIGURE 6. **Model depicting the role of Tsa1/Tsa2 in protecting Sup35 against protein oxidation and *de novo* [*PSI*$^+$] prion formation.** Tsa1/2 function as ribosome-associated antioxidants, presumably to protect the translation machinery and the emerging nascent polypeptide chain against ROS. An oxidative stress can cause oxidation of methionine residues to methionine sulfoxide (*Met-SO*), which may underlie the switch from a soluble to an aggregated form of Sup35. It remains to be established whether Met oxidation occurs on the nascent Sup35 polypeptide chain or in pre-existing Sup35 proteins. MSR catalyzes thiol-dependent reduction of Met-SO, protecting against Sup35 oxidation and aggregation. Oxidized Sup35 misfolds, and the resulting aggregates can be targeted to the insoluble protein deposit (*IPOD*) where [*PIN*$^+$]/Rnq1 aggregates promote *de novo* formation of the [*PSI*$^+$] prion (15). In the absence of [*PIN*$^+$]/Rnq1, Sup35 aggregates can still be formed but are not converted to the [*PSI*$^+$] prion, *i.e.* they are non-heritable. For details, see under "Results."

together, we propose a model for ROS-induced [*PSI*$^+$] prion formation shown in Fig. 6. In contrast, we were unable to detect any elevated methionine oxidation in Rnq1, and *MSRA* overexpression did not prevent [*PIN*$^+$] formation. Because Rnq1 is known to directly interact with Sup35, one possibility is that the oxidation of Sup35 directly affects the formation of [*PIN*$^+$] from Rnq1. We have addressed this possibility by examining whether oxidants can induce formation of the [*PIN*$^+$] prion in the absence of [*PSI*$^+$] prion formation. [*pin*$^-$] [*psi*$^-$] derivatives of the peroxiredoxin mutant were generated using GdnHCl and then grown in the presence of H_2O_2 to promote new prion formation. However, 6 out of 10 [*PIN*$^+$] strains identified in this analysis were found to still be [*psi*$^-$], indicating that [*PSI*$^+$] formation is not necessarily required to promote [*PIN*$^+$] formation in response to oxidant exposure. Further studies will be required to determine whether oxidative modifications, or alternatively, oxidant-induced changes in the protein homeostasis network, promote [*PIN*$^+$] formation in the *tsa1 tsa2* mutant.

Methionine oxidation may be a common feature in the aggregation of prion and amyloidogenic proteins. For example, similar MetO antibodies have been used to detect increased methionine oxidation in aged mouse samples and samples from patients with Alzheimer disease (48). The MetO content of proteins is also known to increase with age in various model systems (49). Methionine oxidation of mammalian PrP has been proposed to underlie the α-β structural switch that converts PrP^C to PrP^{Sc} (18, 19). Our findings with yeast prions together with evidence suggesting up-regulation of peroxiredoxins in the brain of mice infected with prions (50, 51) point to Prxs playing an important antioxidant role in maintaining the solubility of prion-forming proteins. Additionally, peroxiredoxins may be important in other pathologies involving protein aggregation as they are up-regulated in human brain postmortem samples of Huntington disease (52), and peroxiredoxin-6 is

[5] G. L. Staniforth and M. F. Tuite, unpublished data.

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increased in brain tissue of patients with Alzheimer disease (53).

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REFERENCES

1. Prusiner, S. B. (1998) *Proc. Natl. Acad. Sci. U.S.A.* **95,** 13363–13383
2. Wickner, R. B. (1994) *Science* **264,** 566–5699
3. Alberti, S., Halfmann, R., King, O., Kapila, A., and Lindquist, S. (2009) *Cell* **137,** 146–158
4. Stansfield, I., Jones, K. M., Kushnirov, V. V., Dagkesamanskaya, A. R., Poznyakovski, A. I., Paushkin, S. V., Nierras, C. R., Cox, B. S., Ter-Avanesyan, M. D., and Tuite, M. F. (1995) *EMBO J.* **14,** 4365–4373
5. Tuite, M. F., and Cox, B. S. (2007) *Prion* **1,** 101–109
6. Shorter, J., and Lindquist, S. (2005) *Nat. Rev. Genet.* **6,** 435–450
7. True, H. L., and Lindquist, S. L. (2000) *Nature* **407,** 477–483
8. Tyedmers, J., Madariaga, M. L., and Lindquist, S. (2008) *PLoS Biol.* **6,** e294
9. Tuite, M., Stojanovski, K., Ness, F., Merritt, G., and Koloteva-Levine, N. (2008) *Biochem. Soc. Trans.* **36,** 1083–1087
10. Derkatch, I. L., Bradley, M. E., Masse, S. V., Zadorsky, S. P., Polozkov, G. V., Inge-Vechtomov, S. G., and Liebman, S. W. (2000) *EMBO J.* **19,** 1942–1952
11. Derkatch, I. L., Bradley, M. E., Hong, J. Y., and Liebman, S. W. (2001) *Cell* **106,** 171–182
12. Derkatch, I. L., Uptain, S. M., Outeiro, T. F., Krishnan, R., Lindquist, S. L., and Liebman, S. W. (2004) *Proc. Natl. Acad. Sci. U.S.A.* **101,** 12934–12939
13. Scheibel, T., and Lindquist, S. L. (2001) *Nat. Struct. Biol.* **8,** 958–962
14. Tyedmers, J., Mogk, A., and Bukau, B. (2010) *Nat. Rev. Mol. Cell Biol.* **11,** 777–788
15. Tyedmers, J., Treusch, S., Dong, J., McCaffery, J. M., Bevis, B., and Lindquist, S. (2010) *Proc. Natl. Acad. Sci. U.S.A.* **107,** 8633–8638
16. Halliwell, B. (2006) *Plant Physiol.* **141,** 312–322
17. Dean, R. T., Fu, S., Stocker, R., and Davies, M. J. (1997) *Biochem. J.* **324,** 1–18
18. Canello, T., Frid, K., Gabizon, R., Lisa, S., Friedler, A., Moskovitz, J., Gasset, M., and Gabizon, R. (2010) *PLoS Pathog.* **6,** e1000977
19. Wolschner, C., Giese, A., Kretzschmar, H. A., Huber, R., Moroder, L., and Budisa, N. (2009) *Proc. Natl. Acad. Sci. U.S.A.* **106,** 7756–7761
20. Boutte, A. M., Woltjer, R. L., Zimmerman, L. J., Stamer, S. L., Montine, K. S., Manno, M. V., Cimino, P. J., Liebler, D. C., and Montine, T. J. (2006) *FASEB J.* **20,** 1473–1483
21. Leong, S. L., Pham, C. L., Galatis, D., Fodero-Tavoletti, M. T., Perez, K., Hill, A. F., Masters, C. L., Ali, F. E., Barnham, K. J., and Cappai, R. (2009) *Free Radic. Biol. Med.* **46,** 1328–1337
22. Oien, D. B., Shinogle, H. E., Moore, D. S., and Moskovitz, J. (2009) *J. Mol. Neurosci.* **39,** 323–332
23. Sideri, T. C., Stojanovski, K., Tuite, M. F., and Grant, C. M. (2010) *Proc. Natl. Acad. Sci. U.S.A.* **107,** 6394–6399
24. Veal, E. A., Day, A. M., and Morgan, B. A. (2007) *Mol. Cell* **26,** 1–14
25. Wood, Z. A., Schröder, E., Robin Harris, J., and Poole, L. B. (2003) *Trends Biochem. Sci.* **28,** 32–40
26. Garrido, E. O., and Grant, C. M. (2002) *Mol. Microbiol.* **43,** 993–1003
27. Wong, C. M., Siu, K. L., and Jin, D. Y. (2004) *J. Biol. Chem.* **279,** 23207–23213
28. Iraqui, I., Faye, G., Ragu, S., Masurel-Heneman, A., Kolodner, R. D., and Huang, M. E. (2008) *Cancer Res.* **68,** 1055–1063
29. Jang, H. H., Lee, K. O., Chi, Y. H., Jung, B. G., Park, S. K., Park, J. H., Lee, J. R., Lee, S. S., Moon, J. C., Yun, J. W., Choi, Y. O., Kim, W. Y., Kang, J. S., Cheong, G. W., Yun, D. J., Rhee, S. G., Cho, M. J., and Lee, S. Y. (2004) *Cell* **117,** 625–635
30. Rand, J. D., and Grant, C. M. (2006) *Mol. Biol. Cell* **17,** 387–401
31. Puig, O., Caspary, F., Rigaut, G., Rutz, B., Bouveret, E., Bragado-Nilsson, E., Wilm, M., and Séraphin, B. (2001) *Methods* **24,** 218–229
32. Patel, B. K., Gavin-Smyth, J., and Liebman, S. W. (2009) *Nat. Cell Biol.* **11,** 344–349
33. Dixon, C., Mathias, N., Zweig, R. M., Davis, D. A., and Gross, D. S. (2005) *Genetics* **170,** 47–59
34. Ripaud, L., Maillet, L., and Cullin, C. (2003) *EMBO J.* **22,** 5251–5259
35. Meriin, A. B., Zhang, X., He, X., Newnam, G. P., Chernoff, Y. O., and Sherman, M. Y. (2002) *J. Cell Biol.* **157,** 997–1004
36. Du, Z., Crow, E. T., Kang, H. S., and Li, L. (2010) *Mol. Cell. Biol.* **30,** 4644–4655
37. Ness, F., Ferreira, P., Cox, B. S., and Tuite, M. F. (2002) *Mol. Cell. Biol.* **22,** 5593–5605
38. Alberti, S., Halfmann, R., and Lindquist, S. (2010) *Methods Enzymol.* **470,** 709–734
39. Ferreira, P. C., Ness, F., Edwards, S. R., Cox, B. S., and Tuite, M. F. (2001) *Mol. Microbiol.* **40,** 1357–1369
40. Jung, G., and Masison, D. C. (2001) *Curr. Microbiol.* **43,** 7–10
41. Outeiro, T. F., and Lindquist, S. (2003) *Science* **302,** 1772–1775
42. Le, D. T., Lee, B. C., Marino, S. M., Zhang, Y., Fomenko, D. E., Kaya, A., Hacioglu, E., Kwak, G. H., Koc, A., Kim, H. Y., and Gladyshev, V. N. (2009) *J. Biol. Chem.* **284,** 4354–4364
43. Lancaster, A. K., Bardill, J. P., True, H. L., and Masel, J. (2010) *Genetics* **184,** 393–400
44. Bradley, M. E., Edskes, H. K., Hong, J. Y., Wickner, R. B., and Liebman, S. W. (2002) *Proc. Natl. Acad. Sci. U.S.A.* **99,** Suppl. 4, 16392–16399
45. Resende, C. G., Outeiro, T. F., Sands, L., Lindquist, S., and Tuite, M. F. (2003) *Mol. Microbiol.* **49,** 1005–1017
46. Stadtman, E. R., and Levine, R. L. (2003) *Amino Acids* **25,** 207–218
47. Stadtman, E. R., Moskovitz, J., and Levine, R. L. (2003) *Antioxid. Redox Signal.* **5,** 577–582
48. Oien, D. B., Canello, T., Gabizon, R., Gasset, M., Lundquist, B. L., Burns, J. M., and Moskovitz, J. (2009) *Arch Biochem. Biophys* **485,** 35–40
49. Stadtman, E. R., Van Remmen, H., Richardson, A., Wehr, N. B., and Levine, R. L. (2005) *Biochim. Biophys. Acta* **1703,** 135–140
50. Kopacek, J., Sakaguchi, S., Shigematsu, K., Nishida, N., Atarashi, R., Nakaoke, R., Moriuchi, R., Niwa, M., and Katamine, S. (2000) *J. Virol.* **74,** 411–417
51. Zabel, C., Sagi, D., Kaindl, A. M., Steireif, N., Kläre, Y., Mao, L., Peters, H., Wacker, M. A., Kleene, R., and Klose, J. (2006) *J Proteome Res.* **5,** 1948–1958
52. Sorolla, M. A., Reverter-Branchat, G., Tamarit, J., Ferrer, I., Ros, J., and Cabiscol, E. (2008) *Free Radic. Biol. Med.* **45,** 667–678
53. Power, J. H., Asad, S., Chataway, T. K., Chegini, F., Manavis, J., Temlett, J. A., Jensen, P. H., Blumbergs, P. C., and Gai, W. P. (2008) *Acta Neuropathol.* **115,** 611–622

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