Peroxiredoxin Chaperone Activity Is Critical for Protein Homeostasis in Zinc-deficient Yeast**

Colin W. MacDiarmid, Janet Taggart, Kittikhun Kerdsonomboon, Michael Kubisiak, Supawee Panascharoen, Katherine Schelble, and David J. Eide

From the Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706 and the Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

Supported by grants GM56285 and GM56285 (to D. E.). This work was also supported by a grant from the Office of Commission on Higher Education Thailand (to K. K.).

Received for publication, August 28, 2013, and in revised form, September 6, 2013.Published, JBC Papers in Press, September 10, 2013, DOI 10.1074/jbc.M113.512384

Zinc is required for the folding and function of many proteins. In Saccharomyces cerevisiae, homeostatic and adaptive responses to zinc deficiency are regulated by the Zap1 transcription factor. One Zap1 target gene encodes the Tsa1 peroxiredoxin, a protein with both peroxidase and protein chaperone activities. Consistent with its regulation, Tsa1 is critical for growth under low zinc conditions. We previously showed that Tsa1’s peroxidase function decreases the oxidative stress that occurs in zinc deficiency. In this report, we show that Tsa1 chaperone, and not peroxidase, activity is the more critical function occurring in zinc deficiency. Thus, despite not being redox-active, zinc plays an antioxidant role. The source of the increased ROS in deficient cells is unknown. Some Zap1 target genes may be induced to counteract oxidative stress; for example, CTT1 encodes cytosolic catalase, and TSA1 is a Zap1-regulated gene that encodes a cytosolic peroxidase (9). Tsa1 is a member of the peroxiredoxin family that reduces hydrogen peroxide and organic hydroperoxides using electrons supplied by the thioredoxin/thioredoxin reductase pathway (13). Consistent with a role for Tsa1 in counteracting oxidative stress, a zinc-deficient tsa1Δ mutant accumulated more ROS (9).

All peroxiredoxins have a conserved cysteine (Cys) at their N terminus (the “peroxidatic” cysteine) that reacts with hydrogen peroxide and is oxidized to the sulfinic acid (–SOH) form during the peroxidase catalytic cycle. Peroxiredoxins can be divided into subclasses depending on the number of cysteines that act in peroxide reduction (14). Tsa1 and its paralog in yeast Tsa2 are members of the 2-Cys subclass (15, 16). 2-Cys peroxiredoxins have a second cysteine near their C terminus, the

mediating the folding of many protein domains (1). Approximately 10% of proteins encoded by the human genome are estimated to require zinc for their folding and function (2). Critical zinc-containing proteins include zinc finger transcription factors such as TFIIIA (3). Another such regulator is the yeast Zap1 protein, which activates gene expression in response to zinc deficiency. Zap1 regulates expression of ~80 genes grouped into two broad categories as follows: those involved in maintaining zinc homeostasis (e.g. zinc transporters), and those that help the cell adapt to the stress of zinc deficiency (4–6).

Adaptive responses include altering phospholipid synthesis pathways to maintain their production (7) and suppressing sulfate assimilation to preserve NADPH for use in antioxidant pathways (8).

We previously reported that zinc-limited yeast show increased oxidative stress (9, 10). Increased levels of reactive oxygen species (ROS)2 have been observed in zinc-deficient cultured mammalian cells as well as whole animals (11, 12). Thus, despite not being redox-active, zinc plays an antioxidant role. The source of the increased ROS in deficient cells is unknown. Some Zap1 target genes may be induced to counteract oxidative stress; for example, CTT1 encodes cytosolic catalase, and TSA1 is a Zap1-regulated gene that encodes a cytosolic peroxidase (9). Tsa1 is a member of the peroxiredoxin family that reduces hydrogen peroxide and organic hydroperoxides using electrons supplied by the thioredoxin/thioredoxin reductase pathway (13). Consistent with a role for Tsa1 in counteracting oxidative stress, a zinc-deficient tsa1Δ mutant accumulated more ROS (9).

All peroxiredoxins have a conserved cysteine (Cys) at their N terminus (the “peroxidatic” cysteine) that reacts with hydrogen peroxide and is oxidized to the sulfinic acid (–SOH) form during the peroxidase catalytic cycle. Peroxiredoxins can be divided into subclasses depending on the number of cysteines that act in peroxide reduction (14). Tsa1 and its paralog in yeast Tsa2 are members of the 2-Cys subclass (15, 16). 2-Cys peroxiredoxins have a second cysteine near their C terminus, the

footnote:

* This work was supported, in whole or in part, by National Institutes of Health Grant GM56285 (to D. E.). This work was also supported by a grant from the Office of Commissioner on Higher Education Thailand (to K. K.).

** This article was selected as a Paper of the Week.

1 To whom correspondence should be addressed: Dept. of Nutritional Sciences, University of Wisconsin-Madison, 1415 Linden Dr., Madison, WI 53706. Tel.: 608-263-1613; Fax: 608-262-5860; E-mail: eide@nutrisci.wisc.edu.

2 The abbreviations used are: ROS, reactive oxygen species; MPEG, methoxy-polyethylene glycol.

Significance: Disrupted protein homeostasis is a major and previously unrecognized stress of zinc deficiency.
Although the chaperone activity of 2-Cys peroxiredoxins has shows a dramatic increase in chaperone activity (19, 22). The superchaperone form lacks peroxidase activity but and decamers) to a higher order “superchaperone” structure. Although forms of peroxidase activity, thioredoxin reductase pathway to reactivate the peroxidase (17, 18). Thus, thioredoxin reductase and thioredoxin are essential for Tsa1 and Tsa2 peroxidase activity.

In addition to their peroxidase activities, 2-Cys peroxiredoxins like Tsa1 and Tsa2 can also act as molecular chaperones (19, 20), possessing a “holdase”-type activity that binds unfolded proteins and prevents their aggregation. Although forms of peroxidase activity, thioredoxin catalytic cycle have low chaperone activity, hyperoxidation of the peroxiredoxin cysteine from sulfenic acid to either sulfenic (–SO₂H) or sulfonic (–SO₃H) acid results in a transition from lower order complexes (i.e. dimers and decamers) to a higher order “superchaperone” structure (21). The superchaperone form lacks peroxidase activity but shows a dramatic increase in chaperone activity (19, 22). Although the chaperone activity of 2-Cys peroxiredoxins has been well characterized in vitro, and they may contribute to tolerance of heat stress (19), their physiological role is not yet clear.

With the goal of identifying the source of ROS in zinc-deficient cells, we isolated mutations that suppressed the growth defect of tsa1Δ mutations in zinc-deficient conditions, believing such mutations might disrupt pathways responsible for ROS generation. Surprisingly, one such strain carried a loss-of-function mutation in the TRR1 gene, encoding thioredoxin reductase. Trr1 is an essential component of thioredoxin-dependent antioxidant pathways, and cells lacking Trr1 function are more sensitive to oxidative stress. For this reason, we re-examined the role of Tsa1 in zinc-deficient cells. Our analysis revealed that although Tsa1 peroxidase activity decreases oxidative stress in low zinc, the Tsa1 chaperone function is the more critical activity for growth under those conditions. Our observations indicate that Tsa1 protects zinc-deficient cells from defective protein homeostasis.

### Experimental Procedures

#### Yeast Strains, Growth Media, and Standard Methods

All yeast strains used in this work are listed in Table 1. Yeast strains were routinely grown in rich or synthetic medium as described previously (23). For zinc-deficient conditions, synthetic low zinc medium (LZM) was prepared as described previously (24). LZM is zinc-limiting because it contains 1 mM EDTA and 20 mM citrate as metal buffers. In all experiments, LZM was prepared as described previously (24).

In addition, to aid growth of S288c-derived mutant strains with strong growth defects (e.g. trr1Δ), LZM was supplemented with amino acids and inositol as described (25). Yeast transformation was performed using standard methods (26). β-Galactosidase activity was measured by the method of Guarente (27). Cells were harvested during exponential growth, and activity was calculated as follows: (A₄₂₀ × 1000)/(min × ml of culture used × culture A₄₉₅). lacZ reporter genes with very low activity (e.g. pHSE-lacZ) were assayed using Beta-Glo (Promega).

#### Construction of Yeast Mutant Strains

The tsa1Δ::LEU2 allele was originally generated by transformation of CWY8 (tsa1Δ::KanMX4) with a LEU2 marker swap plasmid (kanMX::LEU2) (28). The tsa1Δ::LEU2 marker was transferred to other strains via mating or by PCR amplification and transformation. The tsa2Δ::HphMX4 strains were generated by transformation with a PCR product generated by amplification of the HphMX4 gene from the pAG32 plasmid (29) using oligonucleotides designed to add 82 bases of homology to regions directly flanking the Tsa2A-coding sequence. The tsa1Δ::KanMX4 marker was amplified from a diploid mutant (Invitrogen) and transferred to other strains by transformation.
Plasmid Constructions—Plasmids used in this work are listed in Table 2. All plasmids constructed were assembled by gap repair in yeast (30). To construct pHA-TSA2, the TSA2 5′-intergenic region and the combined TSA2-coding sequence and 3′-intergenic region were amplified from CWY2 genomic DNA as separate PCR products. Primers were designed to include 30 bases of homology to a yeast vector (pFL38) at the 5′ end of the TSA2 promoter fragment and at the 3′ end of the coding DNA sequence intergenic fragment. The oligonucleotide used to amplify the 5′ end of the coding sequence fragment included a region of homology to the 5′ fragment, followed by an ATG start codon, and two repeats of the HA tag sequence fused 5′ to the TSA2 coding DNA sequence lacking the native TSA2 start codon. Both fragments were combined with restriction-digested vector and used to transform a yeast strain (CWY2), selecting for URA3 clones. The intact recombinant vector was recovered from the resulting transformants. Two other versions of this plasmid were constructed using the same strategy. To generate pHA-TSA2Δ, the 5′-intergenic fragment was amplified from genomic DNA of a strain carrying the TSA2Δ allele. To generate pYREΔ-HA-TSA2, a mutant version of the TSA2 promoter fragment lacking all three YRE sequences was amplified from the pTSA2mYRE1,2,3-lacZ plasmid. pHSP104-GFP was constructed by amplifying the HSP104-GFP fusion from genomic DNA of the EY0986/HSP104-GFP yeast strain (Invitrogen) (31), using primers that included homology to the pFL38 vector. Similarly, to construct pDR-HSP26 and pDR-HSP42, pDR195 was digested with XhoI and BamHI and co-transformed into yeast with ORF-containing PCR products to generate PM1A1 promoter-driven alleles.

Isolating Transposon-linked tsa1Δ Suppressors—Mutant tsa1Δ strains in the S288c background (CWY8) or the W303 background (CW48) were transformed with independently constructed pools of yeast genomic DNA fragments containing random transposon insertions marked with the LEU2 gene (32). Library DNA was digested with NotI before transformation. Insertion mutants were selected on plates lacking leucine and incubated until the appearance of colonies (2 days). Colonies were recovered in liquid SC-leucine medium for 4 h and then used to inoculate cultures in zinc-deficient medium (LZM + 1 μM zinc) at an initial A595 of 0.1. Cultures were grown until A595 reached ~1.0 and then re-diluted to an A595 of 0.02 and grown for 5 days. Transformant pools reaching a final density of >0.3 A595 were diluted and plated on SC-leucine plates to isolate single clones. Growth of independent clones in zinc-deficient conditions was assayed to identify suppressed strains. Independent clones were backcrossed to tsa1Δ mutant strains to follow segregation of the suppression trait and the LEU2-marked transposon in tetrads. Clones showing 2:2 segregation of a strong suppressor trait linked to LEU2 were selected for further analysis. The location of the transposon insertion in the genome of suppressor mutant strains was determined using an inverse PCR method. Briefly, genomic DNA was isolated from the mutant strains and digested with an enzyme (TaqI) that cuts at a known site close to the end of the transposon and at unknown sites nearby in flanking DNA. Genomic DNA fragments were recircularized with DNA ligase, and a fragment containing the insertion site was amplified by inverse PCR from divergent primer sites within the transposon sequence. The PCR fragment was then sequenced to identify the flanking sequence and insertion site.

Pooled Linkage Analysis and Whole Genome Sequencing—Several suppressor mutations segregated as single loci unrelated to the LEU2 transposon marker. These mutations were first classed as recessive or dominant by constructing tsa1Δ/tsa1Δ sup/+ diploid strains and assaying their growth under zinc-deficient conditions. Most of the suppressor mutations were recessive. To identify one recessive suppressor mutation (clone 23-2), we used pooled linkage analysis with whole genome sequencing (33). The original mutant strain was backcrossed twice to isogenic tsa1Δ mutant strains to reduce the number of unrelated mutations. Fourteen tetrads from the second cross were dissected and genotyped to identify 28 haploid segregants each of the tsa1Δ and tsa1Δ sup double mutant genotypes. Segregants were grown separately in culture, and an equal number of cells from each class were pooled to give separate tsa1Δ and

**Table 2**

| Plasmid        | Description                                                                 | Ref. |
|----------------|------------------------------------------------------------------------------|-----|
| pSC99          | TRX2 promoter-lacZ reporter                                                   | 78  |
| pFL38          | URA3/CEN low copy shuttle vector                                             | 79  |
| pJAW79         | Synthetic lacZ reporter gene, YRE site from YCF1 promoter inserted in minimal | 80  |
| pTSA2-lacZ     | Wild-type TSA2-lacZ reporter                                                 | 39  |
| pYREΔ-TSA2-lacZ| TSA2-lacZ reporter with mutated YRE sites (pTSA2mYRE1,2,3-lacZ in reference)| 39  |
| pRS316-TRR1    | Low copy wild-type TRR1 clone                                               | 81  |
| pTSA1          | Low copy untagged wild-type TSA1 clone (pFL-TSA1 in reference)              | 9   |
| pmyc-Tsa1      | Low copy Myc-tagged wild-type TSA1 clone (pRS316-Myc-TSA1 in reference)     | 82  |
| pTsa1-C48S     | Low copy Myc-tagged C48S TSA1 mutant clone (pRS316-Myc-TSA1C48S in reference)| 82  |
| pTsa1-C171S    | Low copy Myc-tagged C171S Tsa1 mutant clone                                 | 82  |
| pDR195         | High copy yeast expression vector (PMA1 promoter)                           | 83  |
| pHA-TSA2       | Low copy 2 × HA-tagged TSA2 clone                                           | This work |
| pHA-TSA2Δ      | Low copy 2 × HA-tagged TSA2 clone with transposon insertion in promoter      | This work |
| pHSE-lacZ      | Synthetic lacZ reporter gene, HSE + PDS containing fragment from the SSA4 promoter inserted in minimal | This work |
| pSSA3-lacZ     | Synthetic lacZ reporter gene, HSE + PDS containing fragment from the SSA3 promoter inserted in minimal | 84  |
| pHSP104-GFP    | C-terminal GFP fusion to HSP104 in low copy vector                          | This work |
| pDR-HSP42      | HSP42 expressed from PMA1 promoter in high copy vector                       | This work |
| pDR-HSP26      | HSP26 expressed from PMA1 promoter in high copy vector                       | This work |
*Tsa1 Chaperone Is Essential in Zinc Deficiency*

tsa1Δ sup pools. Genomic DNA was extracted from the pools and subjected to whole genome sequencing on an Illumina HiSeq2000 machine using 1× 100-bp reads. Sequence image analysis and base calling were performed using the CASAVA 1.7.0 pipeline (Illumina). Primary analysis of the data (mapping and trimming) was performed with CLC Genomics Workbench 4.7.1 and mapped to the reference S288c sequence build (as of June 2011). Average sequence coverage was ~250 reads/base for both pools. We identified 19 single nucleotide polymorphisms that differed from the reference sequence and were only present in the suppressed DNA pool. Examination of these suppressor-specific single nucleotide polymorphisms indicated that most were likely to be suppressor-specific due to low sequence coverage (10 or fewer reads) at that position. Only one suppressor-specific single nucleotide polymorphism (chromosome IV T1184128A, trr1L277*) showed sufficient sequence coverage (204 reads) to be a reliable candidate (data not shown).

**Preparation of Protein Extracts**—Yeast protein extracts were prepared using a TCA extraction protocol. Cells from 5-ml cultures were collected, washed with water, and resuspended in 1 ml of ice-cold 10% TCA. The cells were collected by centrifugation and either frozen at −80 °C or immediately processed. Cell pellets were resuspended in 200 μl of 10% TCA; a 100-μl volume of glass beads was added to the tube and the suspension vortexed for 10 min at 4 °C. The suspension was removed from the beads and crude protein collected by centrifugation at 16,000 × g for 5 min at 4 °C. TCA was removed and the pellet washed twice in 1 ml of acetone followed by centrifugation (16,000 × g for 5 min at 4 °C). Proteins were solubilized in 200 μl of buffer A (100 mM Tris-Cl, pH 8, 1% SDS, 1 mM EDTA) containing 1× complete protease inhibitors (Roche Applied Science) and 1 mM PMSF and incubated for 1 h at 37 °C. Insoluble debris was removed by centrifugation (12,000 × g/5 min) and supernatant transferred to a new tube. Protein concentration was determined using a Bio-Rad DC assay kit.

**Maleimide-PEG Modification of Protein Extracts**—Yeast protein extracts for methoxypolyethylene glycol maleimide M₄ 5000 (mPEG) modification (34) were prepared using the TCA extraction procedure detailed above, with the following modifications. The concentration of DTT in buffer A was increased to 50 mM to ensure complete reduction of disulfide bonds. After the last step, the supernatant containing the solubilized protein was re-precipitated with an equal volume of 20% TCA. The protein was collected by centrifugation (12,000 × g for 5 min at 4 °C) and the pellet washed twice with acetone. After the last wash, the tube was centrifuged again and residual acetone removed. The pellet was immediately re-dissolved in 200 μl of buffer A (without DTT) + 2 mM mPEG (4 μl of 100 mM mPEG stock, made fresh by dissolving 50 mg in 100 μl of buffer A). Reactions were incubated for 30 min at 37 °C in the dark to prevent inactivation of mPEG. Aggregates were dispersed with a pipette, and the reaction was incubated an additional 12 h. mPEG addition to specific target proteins was detected by immunoblotting.

**SDS-PAGE and Immunoblotting**—SDS-PAGE and immunoblotting experiments were performed, as described previously (23), except that signal was detected using the Li-Cor infrared dye system. Immunoblots were detected with Li-Cor Odyssey Classic and quantified using Image Studio Version 2.1.10 (Li-Cor). Rabbit α-Myc (ab9106), mouse monoclonal α-HA (ab9110), and rabbit anti-GFP (ab290) were obtained from Abcam. Secondary IR dye-labeled antibodies were obtained from Li-Cor. Anti-Tsa1 Cys-48-SO₃H (22), anti-Hsp42 (35), and anti-Ssa3/4 were kind gifts of their respective laboratories.

**Preparation of Soluble and Insoluble Proteins from Yeast Cells**—Insoluble protein aggregates were separated from soluble proteins using a centrifugation method based on two previously published protocols (36, 37). Thirty-five-ml cultures were grown to a density of 4 × 10⁶ cells/ml (A₆₅₀ = 0.4). Cultures were grown for sufficient time to allow at least three generations of growth before harvest. Strains with growth defects in zinc-deficient conditions (e.g. tsa1Δ) were inoculated to an initial A₆₅₀ of 0.2 and maintained below 0.6 A₆₅₀ by dilution with fresh medium. At harvest, the cells were transferred to a 50-ml conical tube and chilled on ice. Cells were collected by centrifugation (5 min/3000 × g at 4 °C) and resuspended in 1 ml of ice-cold Tris-Cl (50 mM, pH 8.5), transferred to 1.5-ml microcentrifuge tubes, and centrifuged again. Cell pellets were frozen in liquid nitrogen and stored at −80 °C. Protein extracts were prepared under nondenaturing conditions. The frozen cell pellets were thawed on ice and resuspended in 50 μl of Soluble Protein Buffer (SPB) (50 mM Tris-Cl, pH 8.5, 500 mM NaCl, 1 mM PMSF, 1× Complete Mini EDTA-free protease inhibitor (Roche Applied Science)). For each sample, a 2-ml round-bottom microcentrifuge tube was racked under liquid N₂, and a 7-mm stainless steel ball (Retsch 05.368.0035) was placed inside. Thawed cells were transferred to tubes half-filled with liquid N₂ to ensure the sample did not freeze the ball to the bottom of the tube. The tube was removed to a rack to allow all the N₂ to evaporate and then closed and placed back in liquid N₂. Tubes were racked into a PTFE 2-ml tube adaptor for the Retsch Mixer Mill MM400 (Retsch 22.008.0005) and agitated for four times for 90 s at 30 Hz, returning the sample holder to chill in liquid N₂ between sessions. Tubes were then removed and placed on ice, and 950 μl of ice-cold SPB was added to each tube. After the samples had thawed, the tubes were gently mixed by inversion, and the ball was removed with a magnet. Lysates were centrifuged at 3000 × g for 30 s at 4 °C to remove unbroken cells. The supernatant was carefully removed (avoiding the pellet) and transferred to a 1.5-ml polylallomer conical centrifuge tube (Beckman Coulter). Tubes were centrifuged at 100,000 × g for 20 min; the supernatant (representing the soluble fraction) was removed to a 1.5-ml microcentrifuge tube, frozen in liquid N₂, and stored at −80 °C. After removal of any residual supernatant, 500 μl of wash buffer (50 mM Tris-Cl, pH 8.5, 150 mM NaCl, 1 mM PMSF, 1× Complete Mini EDTA-free protease inhibitor (Roche Applied Science)) was added to the tube, and the pellet was resuspended by repeated pipetting until homogeneous. One hundred μl of 10% Nonidet P-40 was added and the sample gently rotated at 4 °C for 30 min. Tubes were centrifuged at 100,000 × g for 20 min, and the supernatant was discarded. One hundred μl of insoluble protein buffer (IPB, 50 mM Tris-Cl, pH 8.5, 50 mM NaCl, 8 mM urea, 2% SDS, 0.5 mM DTT, 1 mM PMSF, 1× Complete Mini EDTA-free protease inhibitor (Roche Applied Science)) was added to the pellet, and the samples were boiled for 5 min. The pellet was resuspended...
with a pipette until homogeneous, and the sample was vortexed for 15 min and then boiled again for 5 min. Samples were centrifuged at 16,000 \( \times g \) for 10 min, and the supernatant (the insoluble fraction) was removed to a new 1.5-ml microcentrifuge tube, avoiding the pellet. Hsp104-GFP was detected in the soluble and insoluble fractions by immunoblotting.

**Fluorescence Microscopy**—GFP fluorescence was visualized using an epifluorescence microscope. Cells were harvested, washed once with PBS, fixed for 2 h with 1% paraformaldehyde, washed with PBS, and collected by centrifugation. Cells were resuspended in 50 \( \mu l \) of PBS and transferred to a 0.2-mm thick pad of 1% agarose in PBS on a microscope slide. A coverslip was applied, and the edges were sealed with paraffin wax. High resolution Z-stacks were obtained with Velocity Version 6.11 and processed to Z-projections using ImageJ Version 10.2.

**RESULTS**

**Genetic Screen for tsa1\( \Delta \) Suppressors**—To identify processes that might produce ROS in zinc-deficient cells, we used a transposon mutagenesis strategy to isolate tsa1\( \Delta \) strains carrying suppressor mutations that improved growth in zinc-deficient conditions. One suppressor was the result of a transposon insertion into the promoter of the TSA2 gene 336 bp upstream of the start codon (TSA2\( ^{Tn} \)). The TSA2\( ^{Tn} \) allele conferred improved growth on a tsa1\( \Delta \) mutant, as shown here using pooled haploid segregants from a tsa1\( \Delta \)/tsa1\( \Delta \) TSA2\( ^{Tn} \) (LEU2)/+ (leu2) diploid parent (Fig. 1A). Suppression by the TSA2\( ^{Tn} \) allele was confirmed by cloning the wild-type and transposon insertion TSA2 alleles and testing their ability to suppress tsa1\( \Delta \) (Fig. 1B). Although an increased copy number of wild-type TSA2 improved growth to a small degree \((p < 0.01)\), the transposon insertion allele conferred strong suppression. Tsa2 is a close ortholog of Tsa1, but its expression is much lower (38, 39). The TSA2\( ^{Tn} \) allele increased Tsa2 accumulation \(-7\)-fold (Fig. 1C). These results indicated that elevated Tsa2 could substitute for Tsa1 function in zinc-deficient cells.

**A TRR1 Loss-of-Function Mutation Suppresses tsa1\( \Delta \)**—Analysis of additional suppressed strains indicated that many carried single recessive mutations unlinked to an inserted transposon (data not shown). Consistent with this observation, tsa1\( \Delta \) is known to cause a higher frequency of spontaneous mutations (40, 41). We identified one suppressor mutation by pooled linkage analysis and whole genome sequencing (33). This analysis suggested that suppression was caused by a nonsense mutation at codon 277 of the TRR1 gene (trr1\( ^{277\Delta} \)). In support of this identification, suppression by trr1\( ^{277\Delta} \) was complemented (i.e. eliminated) by the wild-type TRR1 gene (Fig. 2A). In addition, complete deletion of TRR1 also suppressed the growth defect of tsa1\( \Delta \) under deficient conditions (see Fig. 5B), confirming that trr1\( ^{277\Delta} \) was a loss of function allele.

Trr1 is required for function of the thioredoxin antioxidant pathway and resistance to oxidative stress (17, 42). Because previous evidence suggested that the growth defect of zinc-deficient tsa1\( \Delta \) cells resulted from increased ROS, we were surprised to find that trr1 loss-of-function alleles suppressed tsa1\( \Delta \). One possible explanation was that trr1 mutation somehow increased oxidant tolerance in a tsa1 background. How-

![FIGURE 1. Transposon-mediated activation of TSA2 suppresses tsa1\( \Delta \). A dominant suppressor mutation tightly linked to the transposon LEU2 marker was identified as an insertion in the TSA2 promoter (TSA2\( ^{Δ} \)). A, Growth of leu2 (transposon-negative) and LEU2 (transposon-positive) tsa1\( \Delta \) haploid segregants from four tetrads was compared with wild-type and tsa1\( \Delta \) control strains (CWY2 and CWY8, respectively) under zinc-deficient conditions (LZM + 1 \( \mu M \) ZnCl\( _2 \)). Shown are the averages of three (WT) or eight (tsa1\( \Delta \)) independent cultures for control strains, and the averages of single cultures of eight segregant clones for each suppressor genotype. Cultures were inoculated at an \( A_{660} \) of 0.01, and cell density was determined after 3 days of growth. Error bars indicate ±1 S.D. B, Suppression was verified by cloning the wild-type and TSA2\( ^{Tn} \) alleles into a single-copy plasmid vector and comparing their effect on growth of a tsa1\( \Delta \) strain under deficient conditions. Wild-type (CWY2) or tsa1\( \Delta \) (CWY20) cells bearing either the pFL38 vector (Vec) or epitope-tagged wild-type (pHA-TSA2) or transposon-modified (pHA-TSA2\( ^{Tn} \)) TSA2 alleles were grown in zinc-deficient medium as in A. Error bars indicate ±1 S.D. C, Elevated Tsa2 accumulation in TSA2\( ^{Tn} \) strains. Immunoblot of Tsa2 protein from a wild-type strain (CWY2) carrying pFL38 (Vec), wild-type HA-tagged TSA2, or transposon-modified HA-tagged TSA2 plasmids after growth in zinc-deficient conditions. All panels were from the same blot with equal exposure times. The average fold change in Tsa2 levels was calculated from five independent experiments and standard deviation (SD) is shown. Pgk1 was used as a loading control.

However, we confirmed that trr1\( \Delta \) strongly decreased resistance to hydrogen peroxide, either alone or in combination with tsa1\( \Delta \) (Fig. 2B).

**Alternative Model for Tsa1 Function in Deficient Cells**—Identifying trr1\( \Delta \) as a tsa1\( \Delta \) suppressor argued that the tsa1\( \Delta \) growth defect was not a consequence of elevated oxidative stress. Therefore, we re-examined the role of peroxiredoxins in zinc-deficient cells. In addition to peroxidase activity, Tsa1 has holdase-type chaperone activity that maintains solubility of unfolded proteins (19). We used a genetic strategy to examine the relative importance of these two activities. Tsa1 contains two cysteines, the Cys-48 peroxidatic and Cys-171 resolving cysteines, that are both critical for peroxidase activity (17). Thus, Tsa1-C48S and C171S mutants lack peroxidase function (19), and neither allele complemented the hydrogen peroxide sensitivity of tsa1\( \Delta \) (Fig. 3A). In contrast, full chaperone function requires only Cys-48 because it is highly activated by hyperoxidation of Cys-48 to the sulfonic acid (–SO\(_2\)H) or fur-
tther to the sulfonic acid (–SO₃H) forms (22, 43). Cys-171 is not required for chaperone function. Although tsa1Δ cells expressing Tsa1C₄₈₅ (peroxidase⁻ chaperone⁻) grew poorly in low zinc, Tsa1C¹⁷¹S (peroxidase⁺ chaperone⁺) complemented nearly as effectively as wild-type Tsa1 (Fig. 3B). The ineffectiveness of Tsa1C₄₈₅ was not due to reduced stability, as both mutant proteins accumulated to similar levels (data not shown). Thus, zinc-deficient cells primarily require Tsa1 chaperone activity.

Given these results, we suspected that other holdase-type chaperones might be capable of substituting for Tsa1 in zinc-deficient cells. Hsp26 and Hsp42 are heat shock-inducible chaperones with holdase activity (35, 44–47). Unlike Tsa1, neither protein is known to have peroxidase activity. Overexpression of these proteins from the strong PMA1 promoter suppressed the tsa1Δ growth defect (Fig. 3C), further indicating that Tsa1’s holdase chaperone activity alone is critical under zinc-deficient conditions.

trr1Δ Activates Yap1 in tsa1Δ Mutants—The above observations suggested a model to explain suppression by trr1Δ mutations. Previous work showed that trr1Δ mutations constitutively activate the Yap1 oxidative stress-responsive transcription factor (42). Yap1 binds to the YRE promoter elements and is activated by oxidation and deactivated by thioredoxin-dependent reduction (48). In a trr1Δ mutant lacking reduced thioredoxin, Yap1 is constitutively active and up-regulates a number of genes, including TSA2 (42). We reasoned that in a trr1Δ tsa1Δ mutant, increased Yap1-driven expression of Tsa2 chaperone function might suppress the tsa1Δ growth defect. Although Yap1 regulates expression of other protein chaperones (49), we had already observed that increased Tsa2 could substitute for Tsa1 (Fig. 1). Investigating the role of Tsa2 in suppression by trr1Δ also allowed us to further test the importance of peroxiredoxin chaperone activity in deficient cells, because in a trr1Δ strain only Tsa2 chaperone activity is functional.

When we examined the effect of trr1Δ mutations on Yap1 activity in zinc-deficient cells, trr1Δ mutations strongly elevated Yap1-responsive gene expression, either alone or in combination with tsa1Δ. Both the Yap1-responsive TRX2-lacZ reporter (Fig. 4A) and a synthetic YRE-lacZ reporter (Fig. 4B) were induced indicating that trr1Δ mutation specifically activated Yap1. Consistent with TSA2 contributing to suppression, sub-

FIGURE 2. trr1Δ mutation suppresses the tsa1Δ low zinc growth defect. A, tsa1Δ low zinc growth defect is suppressed by the trr1Δ mutation, and suppression is complemented by wild-type TRR1. Wild-type (DY1457), tsa1Δ (CWM48), and tsa1Δ trr1Δ (CWM123) strains transformed with either the pFL38 vector (Vec) or a low copy wild-type TRR1 plasmid (pRS316-TRR1) were grown in zinc-deficient (ZnD, 3 days) or zinc-replete (ZnR, 1 day) media prior to measuring cell density. Shown are averages of three independent cultures, and error bars denote ± 1 S.D. B, trr1Δ decreases resistance to hydrogen peroxide. 5-Fold serial dilutions of wild-type (CWM2), tsa1Δ (CWM20), trr1Δ (CWM170), and tsa1Δ trr1Δ (CWM83) cultures were spotted onto YPD plates prepared with or without 4 mM H₂O₂ and incubated for 3 days.

FIGURE 3. Tsa1 chaperone activity is required for zinc-deficient growth. A, Tsa1 cysteine mutations do not confer peroxide tolerance. A tsa1Δ strain (CWM20) was transformed with pFL38 (Vec), a wild-type Tsa1 plasmid (pmyc-Tsa1), or plasmids encoding Tsa1C⁴₈₅ or Tsa1C¹⁷¹S mutant alleles. 5-Fold serial dilutions (left to right) of saturated cultures were spotted onto YPD plates prepared with or without 4 mM H₂O₂ and incubated for 3 days. B, strains of the indicated genotypes were transformed with pFL38 (Vec), a wild-type Tsa1 plasmid (pmyc-Tsa1), or plasmids with Tsa1C₄₈₅ or Tsa1C¹⁷¹S mutant alleles and grown in zinc-deficient media as described for Fig. 1A. Shown are averages of three independent cultures, and error bars denote ± 1 S.D. C, mutant tsa1Δ (CWM20) cells were transformed with the vector pDR195 (Vec), a wild-type Tsa1 plasmid (pmyc-Tsa1), or plasmids to overexpress HSP26 (pDR-HSP26) or HSP42 (pDR-HSP42) from the strong PMA1 promoter. Cultures were inoculated at an A₅₇₈ of 0.01 in deficient medium and incubated for 10 days (HSP26 set) or 7 days (HSP42 set). Shown are the averages of three independent cultures, and error bars denote ± 1 S.D.
FIGURE 4. *trr1* mutation activates Yap1 and increases Tsa2 expression. A–C, strains of the indicated genotypes bearing pSC99 (TRX2-*lacZ*) (A), pJAW79 (YRE-*lacZ*) (B), or pTSA2-*lacZ* (TSA2-*lacZ*) (C) reporter genes were grown to log phase in zinc-deficient medium prior to β-galactosidase assays. Shown are the averages of at least three replicates, and the error bars denote ± 1 S.D. MU, Miller units. Strains used in A were DY1457, CWM48, CWM115, and CWM113; strains used in B were CWY2, CWM20, CWM163, and CWM83, and strains used in C were CWY2, CWM20, CWM170, and CWM83. D, *trr1*Δ mutation induces Tsa2 protein accumulation. Strains of the indicated genotypes were grown to log phase in zinc-replete and zinc-deficient medium before protein extraction. One representative immunoblot of three independent experiments is shown. Values below (Tsa2 signal) are the average fold change in Tsa2 band intensity relative to the corresponding wild-type/pHA-Tsa2 sample (S.D. = standard deviation, three replicates). Pgk1 was used as a loading control. Vec, vector.

Tsa1 Chaperone Is Essential in Zinc Deficiency

stantial induction of a TSA2-*lacZ* reporter was also observed in both *trr1*Δ and *trr1*Δ *tsa1*Δ strains under deficient conditions (Fig. 4C). Immunoblotting confirmed a similar effect of *trr1*Δ on Tsa2 protein accumulation in zinc-replete and deficient cells (Fig. 4D). Thus, *trr1* alleles constitutively activated Yap1 and induced its target genes, including Tsa2.

We then tested whether Tsa2 activity was required for suppression in *trr1*Δ *tsa1*Δ mutants. Growth of strains carrying combinations of *trr1*Δ, *tsa1*Δ, and *tsa2*Δ mutations was compared in zinc-replete and deficient conditions. In zinc-replete conditions, the *trr1*Δ single mutant displayed a growth defect, but all other strains grew well (Fig. 5A). In deficient conditions, however, growth of the *trr1*Δ *tsa1*Δ *tsa2*Δ triple mutant was reduced ~50% relative to the *trr1*Δ *tsa1*Δ double mutant (Fig. 5B). Similar results were obtained with the *trr1*Δ*277* allele (data not shown). These observations establish that in a zinc-deficient *trr1*Δ *tsa1*Δ mutant, Tsa2 is both markedly induced and required for full suppression. The observation that the *tsa2*Δ mutation did not completely eliminate suppression by *trr1*Δ suggested that activation of other Yap1 target genes, or perhaps other *trr1*-associated changes in gene expression, also contributes to suppression.

If the induction of Yap1-regulated target genes was responsible for suppression in *trr1*Δ *tsa1*Δ mutants, we predicted that inactivating Yap1 would block this suppression. However, we could not isolate *yap1 trr1* strains due to synthetic lethality. Instead, we determined whether Yap1-mediated induction of TSA2 was required for full suppression. A TSA2-*lacZ* reporter lacking YREs was previously shown to be unresponsive to Yap1 (39). We introduced wild-type and YREΔ mutant TSA2-*lacZ* reporters into wild-type, *tsa1*Δ, *trr1*Δ, and *trr1*Δ *tsa1*Δ strains. In zinc-deficient cells, the *trr1*Δ mutation activated the wild-type TSA2-*lacZ* construct ~10-fold (comparing *tsa1*Δ and *trr1*Δ *tsa1*Δ strains, Fig. 5C). In contrast, expression from the YREΔ mutant promoter increased only ~2-fold. This effect of the YREΔ promoter on TSA2 expression was also observed at the protein level (Fig. 5D) confirming that Yap1 is needed for full *trr1*Δ-induced up-regulation of TSA2 expression.

We then determined the effect of the TSA2 YREΔ mutations on suppression by *trr1*Δ (Fig. 5E). When TSA2 was expressed from its wild-type promoter, the *trr1*Δ mutation conferred an 11-fold increase in growth yield under low zinc conditions. This contribution of TSA2 to suppression was greater than we observed when comparing deletion mutants (Fig. 5B), perhaps...
**Tsa1 Chaperone Is Essential in Zinc Deficiency**

Due to additional suppression effects from increased TSA2 copy number. When TSA2 was expressed from the Yap1-insensitive YREΔ mutant promoter, growth of the suppressed strain was reduced by ~30%. These results indicate that the Yap1-mediated up-regulation of Tsa2 contributes to a significant proportion of tsa1Δ suppression in a trr1Δ mutant. The observation that the YREΔ mutations did not totally eliminate TSA2 induction (Fig. 5, C and D) or Tsa2’s contribution to suppression (Fig. 5E) in trr1Δ cells suggests that other regulatory factors may also contribute to TSA2 activation by trr1Δ (e.g. Msn2/4) (39).

**Detection of the Hyperoxidized Peroxiredoxin Chaperone Form**—These results indicated that the chaperone function of Tsa1 (and of Tsa2 in a trr1Δ tsa1Δ strain) is critical for low zinc growth. Because the activated chaperone form of Tsa1 is hyperoxidized on Cys-48, we predicted that hyperoxidized Tsa1 would be present in zinc-limited cells. To test this prediction, we first determined whether zinc-deficient cells accumulated Cys-48 sulfinic acid-Tsa1. Using a specific antibody (22), sulfinic acid-Tsa1 was readily detectable on immunoblots of protein from cells treated with H₂O₂ (data not shown). However, it was not detected in zinc-deficient protein extracts, indicating zinc deficiency did not generate sufficient oxidative stress to cause terminal hyperoxidation of Tsa1.

To determine whether Tsa1 accumulated as the Cys-48 sulfinic acid form, we used a thiol-reactive modifying reagent (mPEG) (34). Treatment with mPEG adds ~25 kDa to the apparent molecular mass of a protein per modified cysteine residue. Because hyperoxidized cysteine sulfinic and sulfonic acids cannot be reduced by DTT, DTT treatment of proteins followed by mPEG modification can distinguish Tsa1 hyperoxidized at Cys-48 (single-modified) from the fully reduced, disulfide, or sulfenic acid forms (double-modified). Accordingly, mPEG treatment of samples from unstressed cells generated two slower migrating forms of Tsa1 (Fig. 6A). Control experiments indicated modification was specific to cysteines, as Tsa1 C48S and C171S mutant proteins were modified only once (data not shown). Moreover, we found that mPEG modification of cysteines that are not hyperoxidized is highly efficient, as ~98% of the single cysteine present in the Pgk1 protein was modified (Fig. 6A and data not shown).

The trr1Δ suppression phenotype prompted us to determine its effect on Tsa1 and Tsa2 hyperoxidation. No trr1Δ-depen-
Tsa1 Chaperone Is Essential in Zinc Deficiency

undetectable in wild-type and tsα1Δ cells but very abundant in trr1Δ strains. This observation supports our hypothesis that an increase in Tsa2 chaperone activity contributes to suppression by trr1Δ.

Zinc-deficient tsα1Δ Mutants Exhibit Unfolded Protein Stress—The requirement for Tsa1 chaperone function in zinc-deficient cells strongly suggested that they are challenged by the accumulation of unfolded proteins, and the Tsa1 chaperone was required to stabilize these proteins. In yeast, unfolded protein stress activates the Hsf1 and Msn2/4 transcription factors, which induce expression of chaperones to facilitate folding and prevent aggregation (50). We predicted that in zinc-deficient tsα1Δ cells, accumulation of unfolded proteins would activate Hsf1 and/or Msn2/4, leading to accumulation of their target chaperones. To test this prediction, we measured the accumulation of the Hsp70-type foldase isozymes Ssa3 and Ssa4, the holdase proteins Hsp26 and Hsp42, and the Hsp104 disaggregase under zinc-replete and -deficient conditions (Fig. 7, A and B). Zinc deficiency had little effect on chaperone accumulation in wild-type cells. In contrast, the tsα1Δ mutation increased the accumulation of all these chaperones under zinc-deficient conditions, from ~2-fold for Hsp104 to more than 100-fold for Hsp26. This result suggested that the loss of Tsa1 activity caused induction of an unfolded protein stress response in low zinc.

Because oxidative stress caused by loss of Tsa1 peroxidase activity might also activate Hsf1 or Msn2/4 (37, 46, 51), we determined whether this response was due to the absence of the Tsa1 peroxidase or its chaperone activity. Ssa3/4 accumulation was compared in tsα1Δ expressing no Tsa1 (vector only), wild-type Tsa1, Tsa1C48S (peroxidase− chaperone−), or Tsa1C171S (peroxidase− chaperone+) (Fig. 7C). No difference in Ssa3/4 accumulation between these genotypes was observed under zinc-replete conditions. However, a zinc-deficient tsα1Δ strain showed a 9-fold increase in Ssa3/4 accumulation compared with the same strain expressing wild-type Tsa1. A strain expressing Tsa1C48S showed a substantial 5-fold increase in Ssa3/4, while the strain expressing Tsa1C171S was similar to wild-type Tsa1. These observations argue that tsα1Δ-associated Ssa3/4 induction primarily occurs due to loss of Tsa1 chaperone activity and provides further evidence that zinc-deficient cells are challenged by unfolded protein stress.

The increased chaperone accumulation in tsα1Δ could result from activation of the Hsf1 or Msn2/4 factors or from some post-transcriptional change, e.g. in chaperone stability. To determine whether transcription was activated in zinc-deficient cells, we compared the activity of a lacZ reporter gene derived from the SSA3 promoter in wild-type and tsα1Δ strains. Approximately 5-fold more SSA3-lacZ expression was detected in zinc-deficient tsα1Δ cells (Fig. 7D). The induction was not due to cells undergoing diauxic shift (which activates Msn2/4 and Hsf1), as they were maintained at low density throughout the growth period. Thus, Ssa3 accumulation in tsα1Δ cells was (at least in part) due to the induction of the SSA3 promoter, indicating that the Hsf1 and/or Msn2/4 regulators are activated.

If the tsα1Δ growth defect was related to unfolded protein accumulation, we predicted that trr1Δ might reverse this effect

---

**FIGURE 6. Tsa1 chaperone form is abundant in zinc-deficient cells and Tsa2 chaperone levels increase in trr1Δ mutants.** A, mPEG modification of Tsa1 cysteine residues. CWM20 (tsα1Δ) was transformed with plasmids for wild-type untagged Tsa1 (pTsa1) or Myc-tagged Tsa1 (pmyc-Tsa1). Strains were grown under zinc-replete conditions, and protein samples were extracted and treated as described under “Experimental Procedures.” Some control samples were left untreated with DTT and/or mPEG as indicated. Samples were then analyzed by immunoblotting, and Tsa1 and Pgk1 were detected with anti-Myc and anti-Pgk1 antibodies, respectively. B, representative immunoblot of Tsa1 and Pgk1 detected in total yeast protein samples after treatment with DTT and mPEG, tsα1Δ and trr1Δ tsα1Δ strains (CWM20 and CWM83, respectively) expressing epitope-tagged Tsa1 (pmyc-Tsa1) were grown in zinc-replete (R) or -deficient (D) media to log phase before analysis. Duplicate zinc-replete cultures were treated with 5 mM H2O2 for 20 min before harvest, DTT/mPEG treatment, and analysis by immunoblotting. C, proportion of hyperoxidized Tsa1 accumulated by cells was calculated from three independent replicates of the experiment shown in B. Band intensities of the modified Tsa1 forms (singly modified/hyperoxidized or doubly modified/fully reduced) were quantified, and the hyperoxidized Tsa1 signal was expressed as percent of total Tsa1. Error bars denote ± 1 S.D. D, effect of zinc supply and trr1 mutation increased the expression of chaperone Hsc70 and Tsa2. E, effect of zinc supply and trr1 mutation increased the expression of chaperone Hsp26. This result suggested that the loss of Tsa1 activity caused induction of an unfolded protein stress response in low zinc.

---

**TABLE 1.** Strains and Constructs

| Strain Name | Genotype | Description |
|-------------|----------|-------------|
| WC830       | Δtsa1Δ   | tsα1Δ mutant |
| WC20        | Δtsa1Δ   | tsα1Δ mutant |
| WC21        | Δtsa1Δ   | tsα1Δ mutant |
| WC22        | Δtsa1Δ   | tsα1Δ mutant |
| WC23        | Δtsa1Δ   | tsα1Δ mutant |
| WC24        | Δtsa1Δ   | tsα1Δ mutant |
| WC25        | Δtsa1Δ   | tsα1Δ mutant |
| WC26        | Δtsa1Δ   | tsα1Δ mutant |
| WC27        | Δtsa1Δ   | tsα1Δ mutant |
| WC28        | Δtsa1Δ   | tsα1Δ mutant |

---

**TABLE 2.** Plasmids

| Plasmid | Description |
|---------|-------------|
| pTsa1   | Tsa1 expression plasmid |
| pmyc-Tsa1 | Myc-tagged Tsa1 expression plasmid |
| pTsa1C171S | Tsa1C171S expression plasmid |
| pTsa1C48S | Tsa1C48S expression plasmid |
| pTsa1Δ | Δtsa1Δ expression plasmid |

---

**TABLE 3.** Proteins

| Protein | Description |
|---------|-------------|
| Tsa1    | Chaperone protein |
| Tsa2    | Chaperone protein |
| Pgk1    | Glyceraldehyde 3-phosphate dehydrogenase |
| Ssa3/4  | Chaperone proteins |
| Hsp26   | Heat shock protein |
| Hsc70   | Chaperone proteins |
| Hsp42   | Heat shock protein |

---

**FIGURE 7. Tsa1 expression in zinc-deficient cells.** A, mPEG modification of Tsa1 cysteine residues. CWM20 (tsα1Δ) was transformed with plasmids for wild-type untagged Tsa1 (pTsa1) or Myc-tagged Tsa1 (pmyc-Tsa1). Strains were grown under zinc-replete conditions, and protein samples were extracted and treated as described under “Experimental Procedures.” Some control samples were left untreated with DTT and/or mPEG as indicated. Samples were then analyzed by immunoblotting, and Tsa1 and Pgk1 were detected with anti-Myc and anti-Pgk1 antibodies, respectively. B, representative immunoblot of Tsa1 and Pgk1 detected in total yeast protein samples after treatment with DTT and mPEG, tsα1Δ and trr1Δ tsα1Δ strains (CWM20 and CWM83, respectively) expressing epitope-tagged Tsa1 (pmyc-Tsa1) were grown in zinc-replete (R) or -deficient (D) media to log phase before analysis. Duplicate zinc-replete cultures were treated with 5 mM H2O2 for 20 min before harvest, DTT/mPEG treatment, and analysis by immunoblotting. C, proportion of hyperoxidized Tsa1 accumulated by cells was calculated from three independent replicates of the experiment shown in B. Band intensities of the modified Tsa1 forms (singly modified/hyperoxidized or doubly modified/fully reduced) were quantified, and the hyperoxidized Tsa1 signal was expressed as percent of total Tsa1. Error bars denote ± 1 S.D. D, effect of zinc supply and trr1 mutation increased the expression of chaperone Hsc70 and Tsa2. E, effect of zinc supply and trr1 mutation increased the expression of chaperone Hsp26. This result suggested that the loss of Tsa1 activity caused induction of an unfolded protein stress response in low zinc.
Tsa1 Chaperone Is Essential in Zinc Deficiency

and lower chaperone accumulation. Surprisingly, however, the trr1Δ mutation increased Ssa3/4 accumulation, particularly in zinc-deficient tsa1Δ trr1Δ cells (Fig. 7C, ZnD). This effect was likely mediated by activation of Hsf1, because an Hsf1-specific HSE-lacZ reporter gene was induced in trr1Δ (Fig. 7E). Activation of Hsf1 in trr1Δ is a novel observation, and the mechanism of this effect is unclear. Given that Hsf1 regulates many different chaperones, its activation in trr1Δ may contribute to the observed Tsa2-independent component of tsa1Δ suppression (Fig. 5B). The observation that the magnitude of trr1Δ-mediated Hsf1 activation was similar to that produced by sustained heat shock (Fig. 7E) is consistent with this hypothesis.

Tsa1 Protects Zinc-deficient Cells from Protein Aggregation—Our observations strongly suggested that zinc-deficient tsa1Δ cells accumulate unfolded proteins, which are prone to aggregate. Without knowing the specific identity of the affected proteins, we could not test this hypothesis directly. However, indirect evidence for protein aggregation could be obtained by examining chaperones that associate with such aggregates. For example, Hsp104 binds to the surface of aggregates and disassembles their component proteins for refolding (52). Accordingly, both native (53) and GFP-tagged (54) versions of Hsp104 were reported to coalesce into foci after heat stress, as we also observed (data not shown). We therefore investigated if zinc deficiency and tsa1Δ affected Hsp104-GFP behavior at optimal growth temperature. In most zinc-replete wild-type and tsa1Δ cells, Hsp104-GFP was evenly distributed in the cytosol (Fig. 8A), but 2–3% of cells displayed small fluorescent foci, suggesting the rare presence of chaperone-associated protein aggregates (Fig. 8, A and B). Zinc deficiency had little effect on the frequency of wild-type cells showing foci, suggesting little change in protein solubility. In contrast, Hsp104-GFP foci were present in ~70% of zinc-deficient tsa1Δ cells (Fig. 8B). A single aggregate was normally present in each cell, with greatly increased size and fluorescence intensity compared with the few observed in wild-type cells. Zinc deficiency had no effect on the distribution of GFP alone in tsa1Δ cells (data not shown), indicating that the effect was a property of the Hsp104 chaperone. Foci formation was not likely to result simply from increased Hsp104 accumulation because we saw only a 2-fold increase in deficient tsa1Δ cells (Fig. 7B). To determine whether the Hsp104-GFP aggregation phenotype was due to loss of Tsa1 chaperone or peroxidase activity, we examined cells expressing the cysteine mutants of Tsa1. Expressing Tsa1C371S in tsa1Δ almost completely complemented the Hsp104 aggregation phenotype, although expression of Tsa1C495S had little effect (Fig. 8C), indicating a requirement for chaperone function. The trr1Δ suppressor mutation also effectively suppressed the tsa1Δ Hsp104-GFP aggregation phenotype (Fig. 8D), supporting a correlation between the tsa1Δ growth defect and chaperone aggregation phenotype.
The Hsp26 holdase chaperone also associates with protein aggregates after heat stress (55). Therefore, we examined the effect of zinc supply on the distribution of Hsp26-GFP in tsα1/H9004 cells. In zinc-replete cells, no Hsp26-GFP fluorescence was detectable, consistent with the low expression of Hsp26 under these conditions (Fig. 7B). Approximately 18% of zinc-deficient wild-type cells displayed Hsp26-GFP fluorescence, predominantly as multiple small foci per cell (Fig. 8, E and F). In zinc-limited tsα1 cultures, however, the proportion of cells with Hsp26-GFP foci increased dramatically, and the protein aggregated further to form 1–2 large foci/cell. The change in Hsp104 and Hsp26 distribution in tsα1/H9004 suggests that these proteins are trafficked to organized sites of misfolded protein deposition, which are thought to arise when protein homeostasis is disrupted (55, 56).

**tsα1 Mutation Decreases Hsp104 Solubility**—We suspected that the large Hsp104-GFP foci seen in zinc-deficient tsα1 cells contained aggregated insoluble protein. If so, we expected that the solubility of Hsp104 would also be reduced. To test this prediction, we isolated soluble and insoluble protein fractions from wild-type, tsα1/H9004, trr1/H9004, and trr1 tsα1/H9004 double mutant strains grown under zinc-replete and -deficient conditions, and we examined the distribution of Hsp104-GFP in these fractions. Hsp104-GFP solubility in zinc-replete and -deficient cultures described for A. Fluorescence images were captured and quantified as described for B, G. Hsp104-GFP solubility in zinc-replete and -deficient cultures of strains described in D. Cultures were grown for at least four generations, maintaining cell density below 0.4 A595 by dilution. Lysates were prepared, separated into soluble and insoluble fractions, and analyzed by immunoblotting with anti-GFP antibody. Hsp104-GFP band density was quantified and normalized to protein loaded, and the ratio of insoluble/soluble Hsp104 was calculated. Shown are the averages of three independent replicates, and error bars denote ± 1 S.D.

**FIGURE 8. Effect of tsα1 and trr1 mutations on chaperone aggregation and solubility.** A, wild-type and tsα1 mutant cells expressing Hsp104-GFP (strains HSP104-GFP and CWM180, respectively) were grown in zinc-replete or -deficient media for at least four generations, maintaining cell density below 0.4 A595 by dilution. Cells were fixed with paraformaldehyde, and images were captured using fluorescence (GFP) and differential interference contrast (DIC) microscopy. B, foci prevalence was measured in replicate cultures grown as in A. Cells containing 1–2 or >2 Hsp104-GFP foci were counted and are presented as the percentage of total cells. For all foci quantitations in this figure, the averages of three independent replicates are shown, and error bars denote ± 1 S.D. C, tsα1 mutant expressing Hsp104-GFP (CWM180) was transformed with vector alone (Vec, pFL38) or plasmids expressing wild-type, C48S, or C171S alleles of Tsa1. Cells were grown in zinc-deficient medium, and foci frequency was determined. D, wild-type (CWY2), tsα1 (CWM20), trr1 (CWM170), and trr1 tsα1 (CWM83) cells expressing Hsp104-GFP (pHSP104-GFP) were grown in deficient medium, and foci frequency was determined. E and F, wild-type (HSP26-GFP) and tsα1 (CWM188) strains expressing Hsp26-GFP were grown in zinc-deficient medium as described for A. Fluorescence images were captured and quantified as described for B, G. Hsp104-GFP solubility in zinc-replete and -deficient cultures described in D. Cultures were grown for at least four generations, maintaining cell density below 0.4 A595 by dilution. Lysates were prepared, separated into soluble and insoluble fractions, and analyzed by immunoblotting with anti-GFP antibody. Hsp104-GFP band density was quantified and normalized to protein loaded, and the ratio of insoluble/soluble Hsp104 was calculated. Shown are the averages of three independent replicates, and error bars denote ± 1 S.D.
Tsa1 Chaperone Is Essential in Zinc Deficiency

Tsa1's chaperone function was shown to contribute to heat reductase gene strongly suppressed the expression of Tsa1 protects these cells from protein aggregation. Although the Hsp70/Hsp40 systems, are termed "foldases" and directly mediate folding. Other chaperones, e.g. Hsp26, Hsp42, and Tsa1-like peroxiredoxins, are "holdases" that bind to unfolded proteins to prevent their aggregation. In this study, we discovered that zinc-limited cells experience a severe disruption of protein homeostasis and that the holdase chaperone function of Tsa1 protects these cells from protein aggregation. Although peroxiredoxin chaperone activity has been well characterized in vitro (19, 20, 22), its relevance in vivo has been more elusive. Tsa1's chaperone function was shown to contribute to heat tolerance (19), and it was suggested that the Tsa1 chaperone protects ribosomal subunits from aggregation in DTT-treated cells (60), but this was not tested. It appears that Tsa1 plays a uniquely important role in zinc deficiency, because the other holdase chaperones Hsp26 and Hsp42 cannot substitute for Tsa1 unless highly overexpressed (61).

Our initial goal was to identify the source(s) of oxidative stress in zinc-limited cells by isolating mutations that suppressed the growth defect of a tsa1Δ mutant. A key discovery was that loss-of-function mutations in the TRR1 thioredoxin reductase gene strongly suppressed the tsa1Δ growth defect in zinc-limited cells. In low zinc, single trr1Δ mutants grow much better than tsa1Δ mutants (Fig. 5B). A trr1Δ mutant lacks Tsa1 peroxidase activity, but retains the hyperoxidized (chaperone) form (Fig. 6). These observations alone suggest it is Tsa1's chaperone function that is vital for deficient cells. This hypothesis was confirmed by the observation that the Tsa1C171S mutant, which lacks peroxidase function but retains chaperone activity, complemented the tsa1Δ growth defect, although the Tsa1C48S allele, which lacks peroxidase activity and has much less chaperone function, does not.

DISCUSSION

The mechanisms of protein homeostasis include protein folding, degradation, and sequestration. Protein chaperones play roles in all three by mediating folding (57), aiding in ubiquitination and proteasomal degradation (58), and controlling sequestration of protein aggregates (59). Some chaperones, e.g. the Hsp70/Hsp40 systems, are termed "foldases" and directly mediate folding. Other chaperones, e.g. Hsp26, Hsp42, and Tsa1-like peroxiredoxins, are "holdases" that bind to unfolded proteins to prevent their aggregation. In this study, we discovered that zinc-limited cells experience a severe disruption of protein homeostasis and that the holdase chaperone function of Tsa1 protects these cells from protein aggregation. Although peroxiredoxin chaperone activity has been well characterized in vitro (19, 20, 22), its relevance in vivo has been more elusive.

Tsa1's chaperone function was shown to contribute to heat tolerance (19), and it was suggested that the Tsa1 chaperone protects ribosomal subunits from aggregation in DTT-treated cells (60), but this was not tested. It appears that Tsa1 plays a uniquely important role in zinc deficiency, because the other holdase chaperones Hsp26 and Hsp42 cannot substitute for Tsa1 unless highly overexpressed (61).

Our initial goal was to identify the source(s) of oxidative stress in zinc-limited cells by isolating mutations that suppressed the growth defect of a tsa1Δ mutant. A key discovery was that loss-of-function mutations in the TRR1 thioredoxin reductase gene strongly suppressed the tsa1Δ growth defect in zinc-limited cells. In low zinc, single trr1Δ mutants grow much better than tsa1Δ mutants (Fig. 5B). A trr1Δ mutant lacks Tsa1 peroxidase activity, but retains the hyperoxidized (chaperone) form (Fig. 6). These observations alone suggest it is Tsa1's chaperone function that is vital for deficient cells. This hypothesis was confirmed by the observation that the Tsa1C171S mutant, which lacks peroxidase function but retains chaperone activity, complemented the tsa1Δ growth defect, although the Tsa1C48S allele, which lacks peroxidase activity and has much less chaperone function, does not.

Additional confirmation came from the fact that overexpression of Hsp26 and Hsp42 suppressed the tsa1Δ growth defect. These are holdase-type chaperones like Tsa1 but have no known peroxidase function (35, 44). A final argument for the essentiality of the chaperone came from the observation that suppression in trr1Δ tsa1Δ double mutants is partially dependent on the Tsa2 paralog of Tsa1. Because Tsa2 also requires Trr1 for its peroxidase function, increased ROS metabolism cannot explain its contribution to suppression.

The critical importance of Tsa1 chaperone activity to zinc-limited growth indicates that zinc deficiency is associated with a major disruption in protein homeostasis. Several of our observations support this hypothesis. First, severe zinc deficiency caused by mutating ZAP1 induces activity of several Hsf1-responsive promoters (62), although we recognize that this effect may reflect decreased Tsa1 expression. Second, the behavior of chaperones Hsp104 and Hsp26 in zinc-deficient tsa1Δ cells is consistent with the accumulation and aggregation of unfolded proteins. Hsp104 is a "disaggregate"-type chaperone that disassembles protein aggregates for folding by other chaperones (e.g. the Hsp70/Hsp40 systems) (63–65). In response to heat shock, Hsp104 associates with aggregated proteins in cytosolic foci to mediate their refolding or degradation (56, 65). In zinc-limited tsa1Δ cells, we observed an increase in Hsp104-GFP aggregation and a decrease in its solubility, suggesting it was associated with insoluble protein aggregates. Hsp26 also associates with protein aggregates (65, 66) and showed similar aggregation in deficient tsa1Δ cells. Two distinct quality control compartments, called the JUNQ and the IPOD, in cells with defective protein homeostasis (56). Recent work argues that Hsp104-GFP primarily associates with the IPOD (54), whereas Hsp26 associates with diverse protein aggregates (55, 67). Interestingly, we observed that Hsp104 primarily formed single foci in zinc-deficient tsa1Δ cells (suggesting this compartment represents the IPOD), whereas Hsp26-GFP primarily formed two (Fig. 8). The decrease in Hsp104-GFP solubility that we observed would also be consistent with its location in the IPOD, as this compartment is thought to accumulate terminally misfolded and insoluble components (56). However, the identity of the tsa1Δ-associated chaperone foci and their relationship to the IPOD and JUNQ remain to be determined.

The switch from peroxidase to full chaperone function involves hyperoxidation of Cys-48 to the sulfonic (–SO3H) or sulfonyl (–SO2H) acid forms (19, 20, 22). In zinc-replete cells, we estimate that ~25% of Tsa1 was in the sulfenic chaperone form. As about 400,000 Tsa1 molecules are normally present in unstressed cells (31), they therefore contain ~100,000 chaperone molecules. Zinc deficiency did not increase the proportion of Tsas in the chaperone form. However, given that total Tsa1 protein levels increase about 3-fold in zinc-limited cells by Zap1 transcriptional activation (9), we predict that ~300,000 Tsa1 molecules are present in the chaperone form in low zinc. By comparison, the Hsp42 and Hsp26 holdases are estimated to be much less abundant (31), and their expression is not strongly induced by zinc deficiency in wild-type cells (Fig. 7B). A supply of 300,000 holdase molecules represents a substantial chaperone capacity for the protection of proteins from misfolding and irreversible aggregation. What stress to protein homeostasis in low zinc would require such a large capacity? One intriguing possibility is the failure to metallate zinc-dependent proteins. An estimated 10% of proteins in eukaryotes require zinc for folding, and many of these are very abundant in...
cells (2, 68). For example, cytosolic superoxide dismutase and alcohol dehydrogenase normally accumulate to several hundred thousand molecules per cell (31). A failure to metallate even a fraction of zinc-binding proteins would result in a large burden of incompletely folded proteins. In support of this model, we have previously shown that at least some zinc sites are poorly metallated in cells grown under the same zinc-deficient conditions used here (10, 69, 70).

Although this hypothesis is intriguing, other potential mechanisms also warrant consideration. For example, the 26 S proteasome includes an essential zinc-dependent subunit, Rpn11 (71). In zinc-deficient cells, decreased Rpn11 activity might increase the accumulation of ubiquitinated proteasome substrates. Alternatively, the critical lesion in zinc-deficient cells could result from more general defects in protein folding. The Ydj1 protein is the major cytosolic DnaJ-type Hsp40 co-chaperone, which helps bind and present substrate proteins to Hsp70 for folding. Ydj1 contains two C4-type zinc binding domains, which may play a role in presenting some substrates to Hsp70 (72). Thus, under-metallation of Ydj1 could disrupt the efficient folding of many proteins.

Considerable effort has been directed toward understanding how potentially promiscuous and toxic metal ions are delivered to specific apoproteins to enable their correct folding and function. Our observations emphasize that metal-deficient cells must deal with another and just as critical an issue, i.e. how to maintain apoproteins in a folding-competent state in the absence of their essential metal cofactor. To our knowledge, the importance of adequate zinc to protein homeostasis has not previously been documented. Regardless of the cause of disrupted protein homeostasis, it is likely that zinc deficiency may have similar effects in vertebrate cells. This is especially true if the stress is due to unmetallated zinc apoproteins, because zinc is a common structural cofactor in all organisms. If zinc deficiency in humans also disrupts protein homeostasis, it may be an important environmental factor in the etiology of diseases of protein misfolding, such as Alzheimer, Parkinson, and Huntington diseases or prion diseases such as Creutzfeldt-Jakob. For example, failure to metallate Cu,Zn superoxide dismutase with zinc has been linked to the neurotoxicity associated with the familial form of amyotrophic lateral sclerosis (73). Moreover, given that the chaperone function of Tsa1 is conserved in human 2-Cys peroxiredoxins (20), it seems likely that these proteins play key roles in maintaining protein homeostasis in zinc-deficient cells of vertebrates. The World Health Organization estimates that more than 15% of the world’s population is at risk for zinc deficiency. Our results suggest that this high prevalence of zinc deficiency may have a major unforeseen impact on human health.

Acknowledgments—We thank Johannes Buchner, Ho Zoon Chae, Chris Grant, Shusuke Kuge, and Elizabeth Craig for their generous gifts of antibodies and Mikael Molin, Dong-Yan Jin, Gary Merrill, Dennis Thiele, and Scott Maye-Rowley for providing plasmid constructs. We thank Jeremy Glasner and Eric Cabot for their help with analysis of the whole genome sequence data, Guy Groblewski and Diana Thomas for their assistance with microscopy, and Josh Coon for the use of his mixer mill.

REFERENCES
1. Andreini, C., Bertini, L., and Cavallaro, G. (2011) Minimal functional sites allow a classification of zinc sites in proteins. PLoS One 6, e26325
2. Andreini, C., Banci, L., Bertini, I., and Rosato, A. (2006) Counting the zinc-proteins encoded in the human genome. J. Proteome Res. 5, 196–201
3. Klug, A. (2010) The discovery of zinc fingers and their development for practical applications in gene regulation and genome manipulation. Q. Rev. Biophys. 43, 1–21
4. Eide, D. J. (2009) Homeostatic and adaptive responses to zinc deficiency in Saccharomyces cerevisiae. J. Biol. Chem. 284, 18565–18569
5. Lyons, T. J., Gasch, A. P., Gaither, L. A., Botstein, D., Brown, P. O., and Eide, D. J. (2000) Genome-wide characterization of the Zap1p zinc-responsive regulon in yeast. Proc. Natl. Acad. Sci. U.S.A. 97, 7957–7962
6. Wu, C.-Y., Bird, A. J., Chung, L. M., Newton, M. A., Winge, D. R., and Eide, D. J. (2008) Differential control of Zap1-regulated genes in response to zinc deficiency in Saccharomyces cerevisiae. BMC Genomics 9, 370
7. Soto-Cardalda, A., Fakas, S., Pascual, F., Choi, H.-S., and Carman, G. M. (2012) Phosphatidate phosphatase plays role in zinc-mediated regulation of phospholipid synthesis in yeast. J. Biol. Chem. 287, 968–977
8. Wu, C.-Y., Roje, S., Sandoval, F. J., Bird, A. J., Winge, D. R., and Eide, D. J. (2009) Repression of sulfate assimilation is an adaptive response of yeast to the oxidative stress of zinc deficiency. J. Biol. Chem. 284, 27544–27556
9. Wu, C.-Y., Bird, A. J., Winge, D. R., and Eide, D. J. (2007) Regulation of the yeast TSA1 peroxiredoxin by ZAP1 is an adaptive response to the oxidative stress of zinc deficiency. J. Biol. Chem. 282, 2184–2195
10. Wu, C.-Y., Steffen, J., and Eide, D. J. (2009) Cytosolic superoxide dismutase (SOD1) is critical for tolerating the oxidative stress of zinc deficiency in yeast. PLoS One 4, e7061
11. Powell, S. R. (2000) The antioxidant properties of zinc. J. Nutr. 130, 1447S–1454S
12. Eide, D. J. (2011) The oxidative stress of zinc deficiency. Metallomics 3, 1124–1129
13. Manhoz, D. C., and Netto, L. E. (2004) Cytosolic thioredoxin peroxidase I and II are important defenses of yeast against organic hydroperoxide insult: catalases and peroxiredoxins cooperate in the decomposition of H2O2 by yeast. J. Biol. Chem. 279, 35219–35227
14. Hofmann, B., Hecht, H.-J., and Flohé, L. (2002) Peroxiredoxins. Biol. Chem. 383, 347–364
15. Hall, A., Karplus, P. A., and Poole, L. B. (2009) Typical 2-Cys peroxiredoxins—structures, mechanisms and functions. FEBS J. 276, 2469–2477
16. Park, S. G., Cha, M. K., Jeong, W., and Kim, I. H. (2000) Distinct physiological functions of thiol peroxidase isoenzymes in Saccharomyces cerevisiae. J. Biol. Chem. 275, 5723–5732
17. Chae, H. Z., Chung, S. J., and Rhee, S. G. (1994) Thioredoxin-dependent peroxide reductase from yeast. J. Biol. Chem. 269, 27670–27678
18. Chae, H. Z., Uh, T. B., and Rhee, S. G. (1994) Dimerization of thiol-specific antioxidant and the essential role of cysteine 47. Proc. Natl. Acad. Sci. U.S.A. 91, 7022–7026
19. 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) Two enzymes in one: two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecular chaperone function. Cell 117, 625–635
20. Moon, J. C., Hah, Y.-S., Kim, W. Y., Jung, B. G., Jang, H. H., Lee, J. R., Kim, S. Y., Lee, Y. M., Jeon, M. G., Kim, C. W., Cho, M. J., and Lee, S. Y. (2005) Oxidative stress-dependent structural and functional switching of a human 2-Cys peroxiredoxin isoform that enhances HeLa cell resistance to H2O2-induced cell death. J. Biol. Chem. 280, 28775–28784
21. Barranco-Medina, S., Lázaro, J.-I., and Dietz, K.-F. (2009) The oligomeric conformation of peroxiredoxins links redox state to function. FEBS Lett. 583, 1809–1816
22. Lim, J. C., Choi, H.-I., Park, Y. S., Nam, H. W., Woo, H. A., Kwon, K.-S., Kim, Y. S., Rhee, S. G., Kim, K., and Chae, H. Z. (2008) Irreversible oxidation of the active-site cysteine of peroxiredoxin to cysteine sulfonic acid for enhanced molecular chaperone activity. J. Biol. Chem. 283,
Tsa1 Chaperone Is Essential in Zinc Deficiency

28873–28880
23. Pisat, N. P., Pandey, A., and Macdiarmid, C. W. (2009) MNR2 regulates intracellular magnesium storage in *Saccharomyces cerevisiae*. Genetics 183, 873–884
24. Macdiarmid, C. W., Gaither, L. A., and Eide, D. (2000) Zinc transporters that regulate vacuolar zinc storage in *Saccharomyces cerevisiae*. *EMBO J.* 19, 2845–2855
25. Hanscho, M., Ruckerbauer, D. E., Chauhan, N., Hofbauer, H. F., Kraulec, S., Niedetzky, B., Kohlwein, S. D., Zanghellini, J., and Natter, K. (2012) Nutritional requirements of the BY series of *Saccharomyces cerevisiae* strains for optimum growth. *FEMS Yeast Res.* 12, 796–808
26. Gietz, R. D., St. Jean, A., Woods, R. A., and Schiestl, R. H. (1992) Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res.* 20, 1425
27. Guarente, L. (1983) Yeast promoters and lacZ fusions designed to study expression of cloned genes in yeast. *Methods Enzymol.* 101, 181–191
28. Voth, W. P., Jiang, Y. W., and Stillman, D. J. (2003) New marker swap plasmids for converting selectable markers on budding yeast gene disruptions and plasmids. *Yeast* 20, 985–993
29. Goldstein, A. L., and McCusker, J. H. (1999) Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* 15, 1541–1553
30. Muhldorf, H., Hunter, R., and Parker, R. (1992) A rapid method for localized mutagenesis of yeast genes. *Yeast* 8, 79–82
31. Ghaemmaghami, S., Huh, W.-K., Bower, K., Howson, R. W., Belle, A., Dehoure, N., O’Shea, E. K., and Weissman, J. S. (2003) Global analysis of protein expression in yeast. *Nature* 425, 737–741
32. Burns, N., Grimwade, B., Ross-Macdonald, P. B., Choi, E. Y., Finberg, K., Roeder, G. S., and Snyder, M. (1994) Large-scale analysis of gene expression, protein localization, and gene disruption in *Saccharomyces cerevisiae*. *Genes Dev.* 8, 1087–1105
33. Birkeland, S. R., Jin, N., Ozdemir, A. C., Lyons, R. H. Jr., Weisman, L. S., and Wilson, T. E. (2010) Discovery of mutations in *Saccharomyces cerevisiae* by pooled linkage analysis and whole-genome sequencing. *Genetics* 186, 1127–1137
34. Makmura, L., Hamann, M., Areopagita, A., Furuta, S., Muñoz, A., and Momand, J. (2001) Development of a sensitive assay to detect reversibly oxidized protein cysteine sulfhydryl groups. *Antioxid. Redox Signal.* 3, 1105–1118
35. Hasieck, M., Braun, N., Strome, T., Richter, B., Model, N., Weinkauf, S., and Buchner, J. (2004) Hsp42 is the general small heat shock protein in the cytosol of *Saccharomyces cerevisiae*. *EMBO J.* 23, 638–649
36. Geller-Samerotte, K. A., Dion, M. F., Budnik, B. A., Wang, S. M., Hartl, D. L., and Drummmond, D. A. (2011) Misfolded proteins impose a dosage-dependent fitness cost and trigger a cytosolic unfolded protein response in *Saccharomyces cerevisiae*. *EMBO J.* 30, 738–747
37. Wang, Y., Gibney, P. A., West, J. D., and Morano, K. A. (2012) The yeast Hsp70 Ssa1 is a sensor for activation of the heat shock response by thiol-reactive compounds. *Mol. Biol. Cell* 23, 3290–3298
38. Hong, S.-K., Cha, M.-K., Choi, Y.-S., Kim, W.-C., and Kim, I.-H. (2002) Msn2p/Msn4p act as a key transcriptional activator of yeast cytoplasmic Mss2p/Msn4p. *Mol. Microbiol.* 43, 595–605
39. Moon, J. C., Kim, G. M., Kim, E.-K., Lee, H. N., Ha, B., Lee, S. Y., and Jang, H. H. (2013) Reversal of 2-Cys peroxiredoxin oligomerization by sulfiredoxin. *Biochem. Biophys. Res. Commun.* 432, 291–295
40. Haslbeck, M., Walke, S., Stromer, T., Ehrnsperger, M., White, H. E., Chen, S., Saibil, H. R., and Buchner, J. (1999) Hsp26: a temperature-regulated chaperone. *EMBO J.* 18, 6744–6751
41. Chen, J., and Pederson, D. S. (1993) A distal heat shock element promotes the rapid response to heat shock of the Hsp26 gene in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* 268, 7442–7448
42. Amorós, M., and Estruch, F. (2001) Hsf1 and Msn2/Msn4 cooperate in the expression of *Saccharomyces cerevisiae* genes. *Cell* 111, 471–481
43. Lee, J., Godon, C., Lagniel, G., Spector, D., Garin, J., Labarre, J., and Toledano, M. B. (1999) Yap1 and Snf1? control two specialized oxidative stress response regulons in yeast. *J. Biol. Chem.* 274, 16040–16046
44. Morano, K. A., Grant, C. M., and Moyer-Rowley, W. S. (2012) The response to heat shock and oxidative stress in *Saccharomyces cerevisiae*. *Genetics* 190, 1157–1195
45. Boisnard, S., Lagniel, G., Garmendia-Torres, C., Molin, M., Boy-Marcotte, E., Jacquet, M., Toledano, M. B., Labarre, J., and Chédin, S. (2009) H2O2 activates the nuclear localization of Msn2 and Ma1 through thioredoxins in *Saccharomyces cerevisiae*. *Eukaryot. Cell* 8, 1429–1438
46. Doyle, S. M., and Wickner, S. (2009) Hsp104 and ClpB: protein disaggregating machines. *Trends Biochem. Sci.* 34, 40–48
47. Kawai, R., Fujita, K., Iwashashi, H., and Komatsu, Y. (1999) Direct evidence for the intracellular localization of Hsp104 in *Saccharomyces cerevisiae* by immunoelectron microscopy. *Cell Stress Chaperones* 4, 46–53
48. Specht, S., Miller, S. B., Mogk, A., and Bukau, B. (2011) Hsp42 is required for sequestration of protein aggregates into deposition sites in *Saccharomyces cerevisiae*. *J. Cell Biol.* 195, 617–629
49. Kaganovich, D., Kopito, R., and Frydman, J. (2008) Misfolded proteins partition between two distinct quality control compartments. *Nature* 454, 1088–1095
50. Arndt, V., Rocon, C., and Höfeld, J. (2007) To be, or not to be—molecular chaperones in protein degradation. *Cell. Mol. Life Sci.* 64, 2525–2541
51. Chen, B., Retzlaff, M., Roos, T., and Frydman, J. (2011) Cellular strategies of protein quality control. *Cold Spring Harb. Perspect. Biol.* 3, a004374–a004374
52. Rand, J. D., and Grant, C. M. (2006) The thioredoxin system protects ribosomes against stress-induced aggregation. *Mol. Biol. Cell* 17, 387–401
53. North, M., Steffen, J., Loguinov, A. V., Zimmerman, G. R., Vulpe, C. D., and Eide, D. J. (2012) Genome-wide functional profiling identifies genes and processes important for zinc-limited growth of *Saccharomyces cerevisiae*. *PLoS Genet.* 8, e1002899
54. Frey, A. G., and Eide, D. J. (2011) Roles of two activation domains in Zap1 in the response to zinc deficiency in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 286, 6844–6854
55. Parsell, D. A., Kowal, A. S., Singer, M. A., and Lindquist, S. (1994) Protein disaggregation mediated by heat-shock protein Hsp104. *Nature* 372, 475–478
56. Glover, J. R., and Lindquist, S. (1998) Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell* 94, 73–82
57. Haslbeck, M., Miess, A., Stromer, T., Walter, S., and Buchner, J. (2005) Disassembly of protein aggregates in the yeast cytosol. The cooperation of Hsp26 with Ssa1 and HsP104. *J. Biol. Chem.* 280, 23861–23868
66. Cashikar, A. G., Duennwald, M., and Lindquist, S. L. (2005) A chaperone pathway in protein disaggregation. Hsp26 alters the nature of protein aggregates to facilitate reactivation by Hsp104. *J. Biol. Chem.* **280**, 23869–23875

67. Liu, I. C., Chiu, S. W., Lee, H. Y., and Leu, J. Y. (2012) The histone deacetylase Hos2 forms an Hsp42-dependent cytoplasmic granule in quiescent yeast cells. *Mol. Biol. Cell* **23**, 1231–1242

68. Andreini, C., Bertini, I., Cavallaro, G., Holliday, G. L., and Thornton, J. M. (2009) Metal-MACiE: a database of metals involved in biological catalysis. *Bioinformatics* **25**, 2088–2089

69. Qiao, W., Ellis, C., Steffen, J., Wu, C.-Y., and Eide, D. J. (2009) Zinc status and vacuolar zinc transporters control alkaline phosphatase accumulation and activity in *Saccharomyces cerevisiae*. *Mol. Microbiol.* **72**, 320–334

70. Qiao, W., Mooney, M., Bird, A. J., Winge, D. R., and Eide, D. J. (2006) Zinc binding to a regulatory zinc-sensing domain monitored in vivo by using FRET. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 8674–8679

71. Verma, R., Aravind, L., Oania, R., McDonald, W. H., Yates, J. R., 3rd, Koonin, E. V., and Deshaies, R. J. (2002) Role of Rpn11 metalloprotease in the yeast replicative, integrative and single-stranded *S. cerevisiae/E. coli* shuttle vectors. *Mol. Cell. Biol.* **22**, 4379–4388

72. Cyr, D. M. (1995) Cooperation of the molecular chaperone Ydj1 with specific Hsp70 homologs to suppress protein aggregation. *FEBS Lett.* **359**, 129–132

73. Roberts, B. R., Tainer, J. A., Getzoff, E. D., Malencik, D. A., Anderson, S. R., Bomben, V. C., Meyers, K. R., Karplus, P. A., and Beckman, J. S. (2007) Structural characterization of zinc-deficient human superoxide dismutase and implications for ALS. *J. Mol. Biol.* **373**, 877–890

74. Zhao, H., and Eide, D. (1996) The yeast ZRT1 gene encodes the zinc transporter protein of a high-affinity uptake system induced by zinc limitation. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 2454–2458

75. Winzeler, E. A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J. D., Bussey, H., Chu, A. M., Connelly, C., Davis, K., Dietrich, F., Dow, S. W., El Bakkoury, M., Foury, F., Friend, S. H., Gentzol, E., Giaever, G., Hegemann, J. H., Jones, T., Laub, M., Liao, H., Liebundguth, N., Lockhart, D. J., Lucas-Danila, A., Lussier, M., M’Rabet, N., Menard, P., Mittmann, M., Pai, C., Rebischung, C., Revuelta, J. L., Riles, L., Roberts, C. J., Ross-MacDonald, P., Scherens, B., Snyder, M., Sookhai-Mahadeo, S., Storms, R. K., Véronneau, S., Voet, M., Volckaert, G., Ward, T. R., Wysocki, R., Yen, G. S., Yu, K., Zimmermann, K., Philippens, P., Johnston, M., and Davis, R. W. (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* **285**, 901–906

76. Huh, W. K., Falvo, J. V., Gerke, L. C., Carroll, A. S., Howson, R. W., Weissman, J. S., and O’Shea, E. K. (2003) Global analysis of protein localization in budding yeast. *Nature* **425**, 686–691

77. Breslow, D. K., Cameron, D. M., Collins, S. R., Schuldiner, M., Stewart-Ornstein, J., Newman, H. W., Braun, S., Madhani, H. D., Krogan, N. J., and Weissman, J. S. (2008) A comprehensive strategy enabling high-resolution functional analysis of the yeast genome. *Nat. Methods* **5**, 711–718

78. Coleman, S. T., Epping, E. A., Steggerda, S. M., and Moye-Rowley, W. S. (1999) Yap1p activates gene transcription in an oxidant-specific fashion. *Mol. Cell. Biol.* **19**, 8302–8313

79. Bonneaud, N., Ozier-Kalogeropoulos, O., Li, G. Y., Labouesse, M., Minvielle-Sebastia, L., and Lacroute, F. (1991) A family of low and high copy replicative, integrative and single-stranded *S. cerevisiae/E. coli* shuttle vectors. *Yeast* **7**, 609–615

80. Wemmie, J. A., Szczypka, M. S., Thiele, D. J., and Moye-Rowley, W. S. (1994) Cadmium tolerance mediated by the yeast AP-1 protein requires the presence of an ATP-binding cassette transporter-encoding gene, YCF1. *J. Biol. Chem.* **269**, 32592–32597

81. Pearson, G. D., and Merrill, G. F. (1998) Deletion of the *Saccharomyces cerevisiae* TRR1 gene encoding thioredoxin reductase inhibits p53-dependent reporter gene expression. *J. Biol. Chem.* **273**, 5431–5434

82. Molin, M., Yang, J., Hanzén, S., Toledano, M. B., Labarre, J., and Nyström, T. (2011) Life span extension and H2O2 resistance elicited by caloric restriction require the peroxiredoxin Tsa1 in *Saccharomyces cerevisiae*. *Mol. Cell* **43**, 823–833

83. Rentsch, D., Laloi, M., Rouhara, I., Schmelzer, E., Delrot, S., and Frommer, W. B. (1995) *NTR1* encodes a high affinity oligopeptide transporter in *Arabidopsis*. *FEBS Lett.* **370**, 264–268

84. Liu, X. D., Liu, P. C., Santoro, N., and Thiele, D. J. (1997) Conservation of N-terminal reporter gene expression.