Misfolded Proteins are Competent to Mediate a Subset of the Responses to Heat Shock in *Saccharomyces cerevisiae* 1*

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**Running Head:** Misfolded Proteins and Heat Shock
SUMMARY

Cells may sense heat shock via the accumulation of thermally-misfolded proteins. To explore this possibility, we determined the effect of protein misfolding on gene expression in the absence of temperature changes. The imino acid analogue azetidine 2-carboxylic acid (AZC) is incorporated into protein competitively with proline and causes reduced thermal stability or misfolding. We find that adding AZC to yeast, at sublethal concentrations sufficient to arrest proliferation, selectively induces expression of heat shock factor-regulated genes to a maximum of 27 fold and that these inductions are dependent on heat shock factor. AZC treatment also selectively represses expression of the ribosomal protein genes, another heat shock factor-dependent process, to a maximum of 20 fold. AZC treatment thus strongly and selectively activates heat shock factor. AZC treatment causes this activation by misfolding proteins: induction of HSP42 by AZC treatment required protein synthesis; treatment with ethanol, which can also misfold proteins, activates heat shock factor, but treatment with canavanine, an arginine analogue less potent than AZC at misfolding proteins, does not. However, misfolded proteins do not strongly induce the STRE regulon. We conclude that misfolded proteins are competent to specifically trigger activation of heat shock factor in response to heat shock.
Eukaryotic cells respond to heat shock by the induction of a conserved set of proteins, the heat shock proteins, via transcriptional activation of the corresponding genes (1). In the budding yeast, *Saccharomyces cerevisiae*, two distinct promoter elements mediate transcriptional activation in response to heat shock (reviewed in 2). Heat shock elements (HSEs) are found upstream of many heat-induced genes, *e.g.* *HSP42* and *SSA4*. Heat-shock factor binds to HSEs and is required for the induction of HSE-driven genes in response to heat shock. Stress response elements (STREs) are also found upstream of many heat-inducible genes, *e.g.* *CTT1* and *DDR1*, and bind the transcription factors Msn2 and Msn4 (2). Loss of both transcription factors compromises heat shock-induced expression of STRE-containing genes. Some heat shock-inducible genes contain both HSEs and STREs in their promoters, *e.g.* *HSP12, HSP30* and *HSP104*. The HSE and STRE regulons constitute the majority, if not all, of the genes that are specifically induced by heat shock (3).

Rapid upshifts in temperature within the permissive growth range of yeast (so-called "temperature upshifts"), *e.g.* 23 to 36 degrees Celsius, result in the transient and selective induction of the heat shock genes (both HSE- and STRE-containing) and in the transient and selective repression of the 137 ribosomal protein genes (4 and references therein). Heat shocks to non-permissive temperature (*e.g.* to 42 degrees Celsius) also cause a global repression of gene expression not seen upon temperature upshift (4). Repression of the ribosomal protein genes by heat shock is dependent on activation of heat shock factor. Because these genes do not contain HSEs, the repression is thought to be indirect (4).

The current model for how cells sense heat shock is as follows (6, and references therein). Heat shock is proposed to cause the thermal misfolding of a fraction of cell protein. Because activation of heat shock factor requires protein synthesis, it is thought that nascent proteins are the most susceptible to thermal denaturation. Misfolded proteins then bind to cytoplasmic Hsp70 chaperones. Prior to heat shock, these chaperones are believed to equilibrate between being bound to heat shock factor (and inactivating it) and being free in the cytoplasm. Because misfolded proteins bind Hsp70s very tightly, their accumulation upon heat shock is proposed to titrate Hsp70 chaperones resulting in liberated and active heat shock factor. Consistent with this model, misfolded proteins have been detected in
mammalian cells upon heat shock (7). In addition, some HSE-containing genes have been shown to be induced by the accumulation of nascent proteins (8), by failure to degrade misfolded and short-lived proteins (9) and by reduced cytoplasmic Hsp70 function (10). Unfortunately, the extent, specificity, potency and mechanism of the above inductions are not known. Alternative triggers for activation of heat shock factor upon heat shocks have also been proposed, such as heat shock-induced oxidative stress and membrane changes (5). The trigger for induction of the STRE-containing genes upon heat shock is not known (2,5).

If thermally-misfolded proteins indeed trigger some or all of the transcriptional responses to heat shock, then induction of misfolded proteins by artificial means in the absence of temperature changes should 1) cause many or all of the gene expression changes caused by heat-shock, 2) cause these changes as strongly as does heat shock, 3) selectively cause these changes and 4) cause these changes by the same mechanism as does heat shock.

L-azetidine 2-carboxylic acid (AZC) is a toxic analogue of proline and is incorporated into proteins competitively with proline (11). Because the analogue has one less carbon atom in its ring than does proline, the conformation of the polypeptide backbone is altered when AZC is incorporated in place of proline. Thus, incorporation of AZC into proteins causes reduced thermal stability or misfolding (12-14). Indeed, AZC-containing proteins bind avidly to Hsp70 chaperones in vivo (7). AZC thus affords us an opportunity to study the cell’s response to misfolded proteins in the absence of temperature changes.

We have recently shown that AZC reversibly inhibits proliferation of budding yeast cells, causing arrest in the G1 phase of the cell cycle within one to two cell generations (15). AZC treatment and temperature upshift cause G1 arrest by the same mechanism; arrest is due to lowered G1 cyclin activity and is dependent on proper activation of heat shock factor in both cases (15). AZC treatment may therefore activate heat shock factor via misfolding a fraction of cellular protein. Phenotypic analyses indicate that AZC treatment does not activate other heat shock-induced responses such as activation of the cell integrity pathway and accumulation of glycogen and trehalose (15). Thus AZC treatment appears to induce some, but not all, of the phenotypic responses to heat shock.

In this study, we examine the effect of AZC treatment on gene expression.
EXPERIMENTAL PROCEDURES

Materials and chemicals

All chemicals were from Sigma-Aldrich Company (Dorset, UK) Components of growth media were from Becton Dickinson (Sparks, Maryland, USA) and Fisher Scientific UK (Leicester, UK). D/L-AZC was used throughout this work but L-AZC is the active agent in this racemic mixture. Any given concentration of AZC herein refers to the concentration of the racemic mixture.

Plasmids, yeast strains and manipulations

Liquid media, both rich and minimal, were prepared as described previously (16). Solid media contained 2% agar. Strains were routinely grown on YPD agar plates at 25°C. Liquid cultures were grown in YPD broth.

The strains used in this study were W303-1A (WT: MATα.ura3-1.lys2.trp1-1.leu2-3,112.his3-11.can1-100.ade2-1) from the laboratory collections, JVG961 (S288c WT: MATα.ura3-52.lys2-801.his3-Δ200.leu2.ade2-101.ho::LacZ) from the laboratory collections, MH297 (EXA3-1 strain: MATα.leu2-3,112.lys2.ura3-52,his3-11,15,trp1-ΔI,EXA3-1) and a corresponding WT, DS10 (MATα), both kind gifts from E. Craig (4). WT (W303-1A-STRE-LacZ) and congenic msn2Δ.msn4Δ double mutant (MATα.STRE-LacZ,msn2Δ::HIS3,msn4Δ::TRP1) were kind gifts from C. Schüller.

For drug treatments, cells were grown at 30°C to an OD₆₀₀nm value of 0.05, unless stated otherwise. AZC and canavanine were dissolved in water to a stock concentration of 500 mM and added to growth medium to achieve the final concentrations specified in individual experiments. Ethanol was added to the growth medium to a final concentration of 8% v/v. Cycloheximide (stock solution of 10 mg/mL in ethanol) was added to a final concentration of 10 µg/mL (and hence the vehicle, ethanol, to 0.1% v/v).

For temperature upshift experiments, cells were grown at 23°C to an OD₆₀₀nm value of 0.2 and added to an equal volume of YPD in a conical flask preheated at 36°C in a water bath. Incubation was continued at 36°C with agitation for the timecourse of each experiment.

All optical density measurements were made on a Milton Roy Spectronic 601 spectrophotometer.
Microarray Analysis: RNA preparation

Yeast strain W303-1A was grown to early log phase (OD\textsubscript{600} ~ 0.4) in 100 mL YPD medium. AZC was added to a final concentration of 50 mM and the cells were incubated for up to an additional 5 hr. The control sample was treated identically but did not contain AZC. Cells were harvested by centrifugation for 3 min at 1500 x g (7000 rpm in a Beckman tabletop centrifuge). The pellet was resuspended in 1 mL ice-cold water and microcentrifuged for 10 sec at 4°C. The pellet was then resuspended in 400 µL TES solution and 400 µL acid phenol was added with vortexing for 10 sec. After 60 min incubation at 65°C with occasional, brief vortexing the sample was placed on ice for 5 min and then microcentrifuged at 14,000 rpm for 5 min at 4°C. The aqueous (top) phase was transferred to a clean 1.5 mL microcentrifuge tube and the phenol extraction, incubation and microcentrifugation were repeated. The aqueous phase was then transferred to a clean 1.5 mL microcentrifuge tube and 400 µL chloroform was added with vigorous vortexing. The total RNA sample was then microcentrifuged for 5 min at 14,000 rpm at 4°C. The aqueous phase was transferred to a new tube, 40 µL of 3 M sodium acetate, pH 5.3 and 1 mL of ice-cold 100% ethanol were added, followed by microcentrifugation at 4°C at 14,000 rpm for 5 min to precipitate the RNA. The RNA pellet was washed by vortexing briefly in ice-cold 70% ethanol and spun down as before. After resuspension in 50 µL H\textsubscript{2}O the concentration of RNA was determined spectrophotometrically. mRNA was selected from total RNA by means of the PolyA Olygotex Kit (QIAGEN, Inc.).

Microarray Analysis: Expression profiling

For each mRNA sample, 2 µg was used for the cDNA microarray experiments. Changes in the mRNA transcript levels for the 6219 protein-encoding genes of budding yeast were measured by comparing transcript abundance at t=1 hr and t=5 hr relative to t=0 (untreated). Fluorescent t=0 RNA was prepared by reverse transcription in the presence of Cy3-labelled dUTP, which fluoresces green (maximum 532 nm) and was used as the common hybridization reference for the remaining samples. Fluorescent cDNA from the 1 hr and 5 hr samples was synthesised using Cy5-labeled dUTP, which fluoresces red (maximum 635 nm). The Cy5-labeled cDNA representing mRNA from each time point was
mixed with Cy3-labeled t=0 cDNA, and the mixture hybridised onto a DNA microarray containing approximately 6200 yeast ORFs. The resulting fluorescence intensities across the array were measured by a laser scanning microscope. For a given array spot, the ratio of Cy3 and Cy5 intensities reflects the transcript levels of the corresponding gene at the time in question relative to t=0, after adjustment by a normalisation factor that sets the average of the log transformed ratios from one array to zero.

Images were analysed with ScanAlyze (M. Eisen; http://www.microarrays.org/software), and fluorescence ratios (along with numerous quality parameters; see ScanAlyze manual) were stored in a custom database. Single spots or areas of the array with obvious blemishes were flagged and excluded from subsequent analysis.

**Northern probes**

Probes were amplified from yeast amplified ORFs (Research Genetics, Huntsville, Alabama, USA) using universal yeast primers (Research Genetics, Huntsville, Alabama, USA) by PCR using Reddy-load PCR mix (Advanced Biotechnologies, Surrey, UK) according to the manufacturer's instructions. The genes that were probed (and their ORF numbers) are as follows: *ACT1* (YFL039c); *HSP42* (YDR171w); *SSA4* (YER103w); *HSP12* (YFL014w); *HSP30* (YCR021c); *CTT1* (YGR088w); *RPL3* (YOR063w); *RPL30* (YGL030w); *RPS1a* (YLR441c).

Amplified yeast ORFs were run on 1% agarose gels and purified using Concert Rapid Gel Extraction System (Life Technologies, Naperville, Illinois, USA) according to the manufacturer's instructions. Amplified yeast ORFs were labelled with $[^{32}P]$dCTP (New England Nuclear, Boston, Massachusetts, USA) using the Prime-It II random primer labelling kit (Stratagene, La Jolla, California, USA) and purified using NucTrap purification columns (Stratagene, La Jolla, California, USA) according to the manufacturer's instructions.

**Northern analysis**

Extraction of total RNA was performed as previously described (Ogas *et al*, 1991). For Northern analysis, 10 µg of total RNA was denatured at 65°C for 5 min before separation on a 1.3% agarose gel containing 10% formaldehyde. The RNA was transferred
overnight to biodyne-b (0.45 µm) membrane (Pall Corporation., Ann Arbor, Michigan, USA) in 10X SSC. Membranes were baked for 2 hours at 80°C. Membranes were prehybridised in 50% formamide, 10X Denhardt's solution, 2% SDS, 5X SSC and 100 µg/ml denatured salmon sperm DNA for 2 hr. The labelled probe was boiled for 5 min before addition to the membrane in the presence of prehybridisation mix. Membranes were hybridised overnight at 42°C. After stringent washing (2 x 15 min in 1% SDS, 0.25X SSC at room temperature, 15 min in 0.1% SDS, 0.1% SSC at room temperature and 30 min in 0.1% SDS, 0.1% SSC at 65°C membranes were exposed to x-ray film (Konica Corporation, Tokyo, Japan) at –70°C with intensifying screens for an appropriate length of time.

RESULTS

In this study, we set out to determine if AZC treatment mimics the effect of heat shock on genome-wide gene expression and, if so, to explore the mechanism by which the analogue causes these effects.

Laboratory strain backgrounds differ in their sensitivity to AZC, as judged by colony formation on plates (15). The concentrations used herein are those just sufficient to permanently inhibit proliferation of each strain background under study, unless stated otherwise. All experiments were conducted in the presence of normal amounts of proline in the growth medium.

We determined the expression changes of the 6129 protein-encoding genes by microarray analysis (see experimental procedures) using wild-type W303-1A cells treated with a growth-inhibitory concentration of AZC (50 mM) for five hours. We have also performed an equivalent analysis after one hour of AZC treatment (50 mM). The result of the latter experiment was qualitatively similar to the five hour data set but with less potent expression changes. Our subsequent analysis has therefore focused on the five hour data set.

AZC treatment does not cause starvation.

AZC is an analogue of proline and may thereby interfere with proline uptake or metabolism, resulting in starvation. Nutrient-starved cells or cells treated with rapamycin, an inhibitor of the TOR proteins, stop proliferation in the G1 phase of the cell cycle and enter
quiescence-like, non-proliferating states (20) superficially reminiscent of AZC-arrested cells (15). We find by microarray analysis that AZC treatment (5 hr) does not induce the gene expression changes characteristic of starving cells (21-24), e.g. GAP1, PUT4, SNZ1 and SNZ2 are not strongly induced by AZC treatment (0.8 fold, 1.4 fold, 1.2 fold and 1.1 fold respectively). We conclude that AZC is not arresting proliferation by causing starvation. Furthermore, expression of the proline utilisation genes, PRO3 and PRO1, is not significantly induced by AZC treatment (1.02-fold and 1.26-fold respectively)\(^2\), indicating that AZC treatment does not grossly interfere with proline metabolism.

**AZC treatment selectively causes most of the genome-wide gene expression changes caused by temperature upshift.**

Does AZC treatment mimic heat shock? The expression level of 91.8% of genes did not vary within a factor of three upon AZC treatment, indicating that the analogue does not induce the dramatic global changes in gene expression characteristically caused by severe heat shock. Our subsequent analysis focussed on a comparison with temperature upshift.

We determined the correlations between the microarray-derived expression profiles of AZC-arrested cells (5 hr, this work) and temperature-upshifted cells (23°C to 37°C for 10 min, 20 min and 40 min) (18). This analysis focused on those genes (2470 in total) of known function because data for this subset upon temperature upshift are publicly available (18). We find that the correlation between AZC (5 hr) and temperature upshift microarray data sets is highest for the 20 min timepoint of temperature upshift, peaking at 0.576. This correlation compares well with those between temperature upshift timepoints: 10 min versus 20 min - correlation of 0.793; 10 min versus 40 min - correlation of 0.582 (18)\(^3\). These data suggest that AZC treatment selectively causes the majority, but not all, of the gene expression changes characteristic of temperature upshift. We conclude that AZC treatment partially mimics temperature upshift.

**AZC treatment selectively causes heat shock factor-dependent gene expression changes.**

What genes are selectively induced by AZC treatment? Expression of a subset of genes was strongly induced by treatment with AZC (see Table 1 for a partial list and
supplementary data for the complete list). Dramatic induction (3-27 fold) was observed for 217 genes. Of the 50 most strongly-induced genes of known function, 46 are also strongly induced after 20 min of temperature upshift (18). Most conspicuous among these highly-induced genes are those known, or suspected, to be part of the HSE regulon, e.g., *HSP104*, *HSP82*, *HSP78*, *HSP42*, *HSP30*, *HSP12*, *HSP26*, *SSA3*, *SSA4* and *SSE2* (3). These data indicate that AZC treatment mimics temperature upshift in selectively inducing expression of the HSE regulon. Thus, AZC treatment may selectively activate heat shock factor.

Activation of heat shock factor directly or indirectly causes repression of the ribosomal protein genes (4). If AZC treatment indeed activates heat shock factor, then we expect treatment with the analogue to also repress expression of the ribosomal protein genes. Expression of a subset of genes (293 in total) was repressed by a factor of three or more (to a maximum of 20 fold) by AZC treatment (see Table 1 for a partial list and supplementary data for the complete list). Of the 50 genes of known function that are repressed most strongly by AZC treatment, 47 are also strongly repressed by temperature upshift (18). The majority of these (42 out of 47) encode ribosomal proteins and repression of these genes by severe heat shock is dependent on heat shock factor (4). Our data suggest that AZC treatment, like temperature upshift, selectively activates heat shock factor and thereby causes increased expression of HSE-containing genes and repression of the ribosomal protein genes.

Of the 50 genes of known function whose expression is repressed most strongly by temperature upshift (18), 46 are also strongly repressed by AZC treatment. Again, the majority of these genes encode ribosomal proteins. Thus AZC treatment selectively causes the vast majority of the repressions caused by temperature upshift.

**AZC treatment fails to strongly induce expression of the STRE regulon.**

Almost all of the genes whose expression is induced by temperature upshift are components of the HSE or STRE regulons, or both (3). Does AZC treatment also activate the STRE regulon? Expression of a significant number of genes is strongly induced by temperature upshift (18) but not by AZC treatment. Of the 50 genes encoding proteins of known function that are most strongly induced after 20 min of temperature upshift (18), only
25 were strongly induced by AZC treatment. Most notable amongst the genes whose expression is strongly induced by temperature upshift but not AZC treatment are targets of the STRE pathway, e.g. CTT1 and DDR1 (induced only 1.1-fold and 2.4-fold respectively in response to AZC treatment) and also HXK1, GLK1, TPS1, TPS3, PYK2, HXT2, HXT5, HXT6, HXT7, PYC1, SDH1, SDH2, SDH4, ZWF1, ALD4, ALD6, GIP2, GSY1 and GPH1 (3). We conclude that the STRE regulon is at best weakly activated by AZC treatment. This possibility is supported by our previous finding that AZC treatment fails to cause accumulation of glycogen and trehalose, phenomena dependent on activation of the STRE pathway (15). We conclude that the STRE regulon is much less sensitive to AZC treatment than is the HSE regulon.

To a first approximation, temperature upshift selectively induces the STRE regulon and the HSE regulon (and thereby represses expression of the ribosomal protein genes), whereas AZC treatment selectively induces only the latter.

**AZC treatment causes few gene expression changes not caused by temperature upshift.**

Although incorporation of AZC into any given protein molecule is likely to cause dysfunction of that protein, the efficiency of incorporation of AZC in place of proline in our experiments is likely to be low (15) (see discussion). Hence, most molecules of any particular protein in AZC-treated cells are unlikely to contain AZC and thus are functional. However, proteins that are large, proline-rich and short-lived are most likely to incorporate at least one residue of AZC in place of a proline. Thus AZC treatment may preferentially inactivate a particular subset of proteins within the cell and thereby directly cause gene expression changes not caused by temperature upshift. Surprisingly, we find only a handful of genes in this set: CUP1-1, CUP1-2 and ICS3 are induced by AZC treatment and not temperature upshift; ACS2, RPN8, CTS1 and BUD2 are repressed by AZC treatment but not by temperature upshift (18, see Table 1 for a partial list and supplementary data for the complete list of genes whose expression is affected by a factor of 3 or more by AZC treatment). Therefore, the majority of the gene expression changes caused by AZC treatment are not due to selective inactivation of any particular subset of proteins by the analogue.

**AZC treatment, like temperature upshift, does not activate the ER-UPR.**
If AZC treatment and temperature upshift cause widespread misfolding of cell proteins, then we would expect these treatments to activate the unfolded protein response pathway of the endoplasmic reticulum (the ER-UPR pathway). This signalling pathway is activated by the accumulation of misfolded proteins in the endoplasmic reticulum and promotes transcription (and thus expression) of genes containing unfolded protein response elements (UPREs) in their promoters (19). Significantly, we find that genes that are strongly induced by the ER-UPR pathway, e.g., *EUG1*, *PDI1*, and *LHS1*, are not strongly induced by AZC treatment (1.15-fold, 1.7-fold and 1.63-fold respectively). Expression of these genes is also not strongly induced by temperature upshift (18). These findings are consistent with AZC treatment and temperature upshift causing only a low level of protein misfolding in the endoplasmic reticulum (and presumably throughout the cell).

**AZC treatment strongly and robustly causes heat shock factor-dependent gene expression changes.**

We wished to confirm the salient features of the gene expression changes detected from microarray profiling by Northern blot analysis. We also wished to compare the magnitudes of these expression changes with those caused by temperature upshift. For this analysis, we used a wild-type haploid strain of the S288C background whose proliferation in YPD is inhibited by 10 mM AZC (15). We determined the expression level of the following genes as a function of time after addition of AZC (10 mM) or upon temperature upshift (23°C to 36°C): *ACT1* as a loading control and a gene sensitive to the global repression characteristic of severe heat shock; the HSE-containing genes *HSP42* and *SSA4*; the HSE- and STRE-containing genes, *HSP12* and *HSP30*; the STRE-containing gene *CTT1*; the ribosomal protein genes *RPS1a* and *RPL30* containing Rap1 binding sites in their promoters; the ribosomal protein gene *RPL3* containing an Abf1 binding site in its promoter. The mechanism(s) regulating expression of the ribosomal protein genes are poorly understood. However, the promoters of these genes fall into two classes, those containing potential binding sites for the Rap1 transcription factor and those containing potential binding sites for the Abf1 transcription factor. We included representatives of both subclasses for completeness. Equal amounts of total RNA were loaded in each lane, and blots were...
hybridised with the same probe at the same time, and exposed to the same film for equal amounts of time for each probing.

The results of the Northern analysis are shown in Fig. 1a,b. In agreement with the microarray analysis above, we find that 1) expression of the HSE-containing genes HSP42, SSA4, HSP12 and HSP30 is strongly induced by AZC treatment, comparable to, if not more profoundly than, the peak transient induction of each gene upon temperature upshift, 2) expression of the STRE-driven gene CTT1 is at best weakly induced by AZC treatment but is more strongly induced by temperature upshift, 3) expression of the ribosomal protein genes RPL3, RPL30 and RPS1a (the probe also detects the homologous gene RPS1b) is strongly repressed by AZC treatment, comparable to, if not more profoundly than, the peak transient repression caused by temperature upshift. Our Northern analysis thus confirms the observations made by microarray analysis on the gene expression changes caused by AZC treatment. Our data also demonstrate that the gene expression changes caused by treatment with a growth-inhibiting concentration of AZC are at least as strong as the peak inductions caused by temperature upshift, which transiently inhibits proliferation. Treatment with AZC thus causes heat shock factor-dependent gene expression changes as strongly as does temperature upshift.

As expected for continuous incorporation of AZC into newly synthesised proteins, expression changes caused by treatment with the analogue are persistent and not transient (as is the case for temperature upshift) and develop slowly. Induction of HSP42, SSA4 and HSP30 in response to temperature upshift peaks at the 15 minute timepoint. In contrast, induction of HSP42 and SSA4 by AZC treatment has only begun at the 30 min timepoint. Curiously, HSP30 is significantly induced only after 60 minutes of analogue treatment. Such differences in the kinetics of induction of HSE-containing genes may reflect different sensitivities of the promoters to the activity of heat shock factor revealed only when activation of the transcription factor is slow.

The microarray and Northern analyses were performed on different strain backgrounds, yet the results are in excellent agreement. Although different concentrations of AZC are required to permanently arrest proliferation of the two different strain backgrounds
used (50 mM for W303 versus 10 mM for S288c), the cellular responses appear identical in both strain backgrounds. We infer that the different sensitivities of the strain backgrounds to AZC does not reflect any fundamental differences in how the cells respond to the analogue once it is incorporated into protein. Rather, the different sensitivities to the analogue are likely to result from differences in the rate of uptake or efflux of the analogue, in the efficiency of incorporation of the analogue into protein or in the size of the intracellular pool of proline.

**Induction of HSP42 and SSA4 by AZC treatment is dependent on heat shock factor.**

Does AZC treatment, like temperature upshift, activate heat shock factor? Heat shock factor is an essential protein, abrogating the possibility of treating mutants deleted for *HSF1* with AZC. Temperature-sensitive alleles of *HSF1* are not useful for probing responses in the absence of temperature change. The *EXA3-1* allele of *HSF1* encodes a mutant form of heat shock factor with a single amino acid residue change in the DNA-binding domain of the protein (4). *EXA3-1* mutants display delayed transcriptional activation of heat shock factor-regulated genes (by 20 minutes) and delayed repression of the ribosomal protein genes upon heat shock to non-permissive temperatures (4). The slow timecourse of AZC incorporation would preclude accurate detection of such subtle kinetic delays. However, *EXA3-1* mutants are also altered in the extent of induction of HSE-driven transcripts: some transcripts are induced more strongly in the mutant than in wild type while others are induced more weakly (25). If AZC treatment activates heat shock factor, then we expect the *EXA3-1* allele to alter the extent, and possibly the persistence, of activation of HSE-driven transcripts in response to treatment with the analogue.

Curiously, the strain background containing the *EXA3-1* mutation appears to be resistant to AZC even up to a concentration of 100 mM, as judged by colony formation on YPD plates. As noted above, different strain backgrounds have different sensitivities to AZC treatment. However, treatment of the equivalent WT strain with AZC leads to a transient inhibition of proliferation in liquid culture reminiscent of the transient arrest caused by temperature upshift (15). We therefore monitored expression of *HSP42* and *SSA4* in the *EXA3-1* mutant and in its wild type as a function of time upon AZC treatment (40 mM) and...
upon temperature upshift. As shown in Figure 2a,b, we find that these HSE-containing genes are indeed transiently induced in this strain background in response to AZC treatment. Furthermore, we find that expression of HSP42 is induced more strongly in the EXA3-1 mutant compared to the wild-type strain in response to both AZC treatment and temperature upshift. Expression of HSP42 is also more persistent in the mutant in response to both treatments. Although there may be subtle effects of the EXA3-1 mutation on the extent of SSA4 induction in response to both AZC treatment and temperature upshift, expression of this gene is clearly more persistent in the mutant in response to both treatments. The EXA3-1 mutation in HSF1 thus affects the extent or persistence, or both, of induction of HSP42 and SSA4 in response to AZC treatment and temperature upshift. These data indicate that AZC treatment and temperature upshift cause induction of HSP42 and SSA4, and by inference all HSE-containing genes, by the same mechanism, namely activation of heat shock factor.

The induction of HSP42 and SSA4 by AZC treatment is clearly transient for the wild-type strain used in Figure 2, in contrast to the equivalent data for the S288c wild-type strain shown in Figure 1. The persistence of heat shock factor activation in response to AZC treatment in these strain backgrounds clearly parallels the persistence of proliferation arrest caused by the analogue, which gratifyingly is also dependent on heat shock factor (15). Although the reason for the different responses of these two strain backgrounds to the analogue are not known, it is likely to be due to some combination of differences in the rate of uptake or efflux of the compound, to different efficiencies of incorporation of the analogue into protein or to different capacities to degrade analogue-containing peptides. From our data in Figures 1 and 2, we cannot compare the relative expression levels of HSP42 and SSA4 between these two strain backgrounds since different batches of labelled probes were used for each experiment.

**Induction of HSP12 by AZC treatment is partly dependent on Msn2 and Msn4.**

From the microarray analysis above, STRE-containing genes are at best poorly induced by AZC treatment (Table 1 and supplementary data). This observation suggests that Msn2 and Msn4, the redundant transcription factors required for induction of the STRE regulon, are relatively insensitive to protein misfolding in the cell. It is known that HSP12 is
a member of both the HSE and STRE regulons. Given that \textit{HSP12} expression is very strongly induced by AZC treatment (Table 1; Figure 1a,b), we set out to determine if Msn2 and Msn4 contribute to induction of \textit{HSP12} upon AZC treatment. We find that the induction of \textit{HSP12} by AZC treatment and by temperature upshift is significantly reduced in a strain deleted for both \textit{MSN2} and \textit{MSN4} relative to its congenic wild-type strain (Figure 3a,b). We conclude that Msn2 and Msn4 (and by inference the STRE regulon) may indeed be activated by AZC treatment, but only partially.

**Induction of \textit{HSP42} by AZC treatment is dependent on protein synthesis.**

If AZC exerts its effects \textit{in vivo} by misfolding proteins into which it is incorporated, then the expression changes caused by AZC treatment should require ongoing protein synthesis. We therefore determined the effect of cycloheximide addition on the ability of AZC treatment to induce expression of \textit{HSP42}. Treatment of S288c wild-type cells with cycloheximide alone or vehicle alone does not alter expression of \textit{HSP42}. In contrast, we find that the presence of cycloheximide prevents induction of \textit{HSP42} by AZC treatment (Figure 4a). We infer that ongoing protein synthesis is required for AZC treatment to induce the HSE regulon, consistent with the analogue functioning via misfolding nascent proteins into which it is incorporated.

**Ethanol treatment mimics AZC treatment.**

If AZC acts via misfolding cellular proteins, then we expect other treatments that misfold proteins in the cell to induce the same spectrum of gene expression changes as that caused by AZC treatment.

Ethanol can disrupt protein folding by a mechanism distinct from that of AZC (5). The results of the Northern analysis of ethanol-treated cells are shown in Fig 4a. These blots, and those for canavanine-treated cells (see next section, Figure 4b) were prepared identically to, and probed with the same batch of labelled probe at the same time and exposed to the same film for the same length of time as, the blots shown in Figure 1a,b. Thus, the data shown in Figures 1 and 4 are directly comparable. The concentration of ethanol used in this experiment (8% v/v) is just sufficient to stop proliferation of the strain used (S288C wild type)\textsuperscript{2}. We find that ethanol treatment mimics AZC treatment: 1) in strongly inducing the
expression of genes regulated by heat shock factor, e.g., *HSP42*, *SSA4*, *HSP12* and *HSP30*; 
2) in failing to strongly induce *CTT1* (a STRE-driven gene) in agreement with the very weak 
activation of STRE-driven genes by ethanol reported previously (26); 3) in failing to repress 
*ACT1* (i.e., no global repression); 4) in strongly repressing the expression of the ribosomal 
protein genes tested. We conclude that ethanol and AZC treatments, both of which can 
misfold proteins but by very distinct mechanisms, cause similar gene expression changes 
attributable to activation of heat shock factor.

**Canavanine treatment does not mimic AZC treatment.**

Canavanine is an arginine analogue that, like AZC, is incorporated into protein 
competitively with the corresponding natural amino acid (27). Canavanine differs from 
arginine in the structure of its sidechain and, as such, is not expected to significantly alter the 
conformation of the polypeptide backbone into which it is incorporated (relative to the same 
protein containing arginine at the equivalent position(s)). Thus, treatment with canavanine is 
not expected to cause protein misfolding, at least not to the same extent as does AZC 
treatment.

We examined the effect of canavanine at a sub-lethal concentration (10 mM) just 
sufficient to inhibit cell proliferation, on gene expression by Northern analysis. Our results 
are shown in Figure 4b. In contrast to ethanol and AZC treatments, canavanine treatment 
does not significantly induce expression of the HSE-containing genes *HSP42* and *SSA3*. 
Canavanine treatment also fails to significantly repress expression of the ribosomal protein 
genes. We infer that canavanine treatment does not strongly activate heat shock factor. 
Canavanine treatment also fails to induce expression of *CTT1* and is thus unlikely to 
strongly activate the STRE regulon. It should be noted that in our experiments, both AZC 
and canavanine are present in the growth medium at concentrations sufficient to inhibit 
proliferation^2, yet only AZC treatment induces the HSE regulon. Thus, activation of heat 
shock factor by AZC treatment is not a consequence of growth inhibition *per se*. Rather, the 
ability of the analogues, AZC and canavanine, to activate heat shock factor correlates with 
their relative capacity to misfold proteins into which they are incorporated.
Curiously, canavanine treatment robustly induces expression of $HSP12$ and $HSP30$, the latter only slowly and weakly. Although we do not know the mechanistic basis for these inductions, it is tempting to speculate that canavanine treatment weakly activates both heat shock factor and Msn2/4, sufficiently to induce expression of genes regulated by both pathways ($HSP12$ and $HSP30$) but too weakly to drive expression of genes dependent on one or other system.

**DISCUSSION**

**AZC causes protein misfolding in vivo.**

Multiple lines of evidence indicate that AZC exerts its effects on yeast cells via incorporation into cellular protein. First, $L$-AZC, which can be incorporated into cellular protein, is the active agent in racemic mixtures and not $D$-AZC, which cannot be incorporated$^1$. Second, AZC inhibits growth of many, but not all, organisms. AZC-sensitive organisms contain aminoacyl tRNA synthetases that can charge tRNA$^{pro}$s with the analogue, whereas AZC-resistant organisms do not (reviewed in 27). Third, the ability of AZC to arrest cell proliferation is not determined by the absolute concentration of the analogue, but rather by the ratio of AZC to proline in the medium$^2$. Our microarray data indicate that AZC does not interfere with proline metabolism per se, consistent with the analogue competing with proline for incorporation into cellular protein. Fourth, induction of the heat shock factor-regulated gene $HSP42$ by AZC treatment is abolished in the presence of cycloheximide. Thus activation of heat shock factor by AZC treatment requires ongoing protein synthesis consistent with the analogue acting via incorporation into cell protein. Finally, $ubc4.ubc5$ mutants, which are defective in the ubiquitin-dependent degradation of short lived and analogue-containing polypeptides, are hypersensitive to AZC treatment (15). The simplest explanation for this observation is that, at a given concentration of AZC, the amount of analogue-containing protein in the cell is higher when these proteins are stable than when these proteins are unstable. Thus, a concentration of AZC that is insufficient to arrest cell proliferation in a wild-type cell would be sufficient to arrest a cell lacking Ubc4 and Ubc5. Taken together, these arguments strongly indicate that AZC acts via incorporation into cellular protein.
Given that incorporation of AZC into protein is known to cause reduced thermal stability or misfolding (12-14), the effects of the analogue on gene expression are most likely due to its misfolding proteins. Two lines of evidence support this notion. First, induction of the HSE regulon is also strongly and selectively caused by treatment with ethanol, another agent capable of misfolding proteins but by a mechanism different to that of AZC. Second, canavanine, an arginine analogue that is incorporated into protein competitively with arginine, does not induce the HSE regulon, whereas AZC does so efficiently. Canavanine incorporation is not expected to disrupt protein folding as efficiently as does incorporation of AZC (27). Thus, the relative of the analogues to induce the HSE regulon correlates with their capacity to misfold proteins into which they are incorporated.

**AZC treatment selectively activates heat shock factor.**

AZC treatment selectively causes the gene expression changes attributable to activation of heat shock factor. First, the expression level of only a small fraction of the protein-encoding genes are altered by a factor of three or more after five hours of treatment with an inhibitory concentration of AZC (8.2% affected in total: 3.5% induced; 4.7% repressed). Hence, AZC treatment does not cause any global changes in gene expression, but selectively affects expression of a discrete subset of genes. Second, HSE-containing transcripts predominate amongst those induced by AZC treatment. Third, the ribosomal protein genes (and co-regulated genes encoding components of the translation apparatus) comprise the vast majority of the genes that are strongly repressed by AZC treatment. Repression of the ribosomal protein genes by heat shock is known to be dependent on activation of heat shock factor (4).

We have confirmed that AZC treatment activates the HSE regulon (induction of HSE-containing genes and consequent repression of the ribosomal protein genes) by Northern analysis. Furthermore, we find that AZC treatment activates the HSE regulon as strongly if not more strongly than does temperature upshift. We have also shown that a mutation in heat shock factor affects induction of the HSE-containing genes HSP42 and SSA4 in response to AZC treatment. Critically, the mutation alters the induction of these genes in the same way in response to either AZC treatment or temperature upshift. Thus,
AZC treatment strongly and selectively induces the HSE regulon by the same mechanism as does temperature upshift, namely by activating heat shock factor.

**Misfolded proteins are competent to be mediate selective activation of heat shock factor in response to heat shock.**

Based on the above arguments, we conclude that the misfolding of a fraction of cell protein in the absence of temperature change mimics heat shock in selectively and strongly activating heat shock factor. Therefore, misfolded proteins illicit the appropriate cellular response, and do so sufficiently strongly and selectively, for them to be intermediates in the cellular response to heat shock. Given that misfolded proteins are known accumulate in heat shocked cells (7), They are competent to mediate at least part of the cellular response to heat shock.

Unfortunately, it is not yet known if misfolded proteins are kinetically competent to be intermediates in the heat shock response, i.e., that misfolded proteins accumulate sufficiently rapidly upon heat shock and that misfolded proteins cause activation of heat shock factor sufficiently quickly. Although activation of heat shock factor is slow in response to AZC treatment, it is likely that equilibration of the analogue into the cellular proline pool prior to incorporation into protein is slow. The issue of kinetic competence remains unresolved.

If misfolded proteins are intermediates in the cellular response to heat shock, then heat shock factor must be very sensitive to protein misfolding in cytoplasmic space. Temperature upshift is a very mild environmental change and is unlikely to cause extensive protein misfolding. In addition, AZC treatment at concentrations sufficient to activate heat shock factor does not appear to cause widespread protein dysfunction: 1) AZC treatment almost exclusively affects the expression of a small and discrete subset of genes that are also induced by temperature upshift; 2) AZC-arrested cells are viability (15); 3) AZC arrest is reversible (15); 4) AZC-arrested cells are responsive to subsequent treatments, e.g. heat shocks (15) and rapamycin². Indeed, neither AZC treatment nor temperature upshift strongly activates the ER-UPR, even though both treatments should misfold proteins throughout the cell, including in the endoplasmic reticulum.
The fraction of protein containing AZC (when cells are treated with a concentration of the analogue just sufficient to activate heat shock factor) should constitute an upper limit for the fraction of cellular protein whose misfolding is just sufficient to activate heat shock factor. We are attempting to determine this number.

**The STRE regulon is relatively insensitive to protein misfolding.**

Although AZC treatment profoundly activates the HSE regulon and genes whose expression is dependent thereon (e.g., the ribosomal protein genes), it at best weakly induces the STRE regulon. This possibility is supported by our observation that AZC does not lead to the accumulation of glycogen and trehalose, a STRE regulon-dependent phenomenon (15), nor does it significantly activate expression of $STRE$-$LacZ$ reporter constructs$^3$. However, AZC incorporation into cell protein does not appear to affect the activateability of the STRE regulon (15). Rather, AZC treatment simply fails to strongly activate this regulon. The primary signal for activation of the STRE regulon by heat shock may be the misfolding of cellular protein, but with the STRE regulon requiring higher levels of protein misfolding than those sufficient to activate heat shock factor (and inhibit proliferation). Indeed, the timecourse of activation of $CTT1$ expression by temperature upshift parallels that of the heat shock factor-dependent transcripts, consistent with the notion of a common trigger. Alternatively, the STRE pathway may primarily respond to heat-induced oxidative stress or some other stress that coincides with protein misfolding upon heat shock (2,5). It is clear that Msn2 and Msn4 contribute to the induction of $HSP12$ by AZC treatment. Given that the STRE regulon is activated by multiple stresses to the cell, it is possible that any partial activation of the regulon in response to analogue treatment is caused by an indirect mechanism, e.g., because of proliferation arrest. The mechanism by which the STRE regulon is activated by heat shocks remains elusive.

**How do cells sense heat shocks?**

Thermally-misfolded protein likely triggers activation of heat shock factor in response to heat shocks. The sensor for activation of the STRE regulon upon heat shock remains unclear. However, misfolded protein is not the sole sensor of heat shock in yeast. The cell integrity pathway, which is required for acquired thermotolerance and for
maintenance of the cell surface, is activated by heat shocks, including temperature upshift (28). This pathway is not activated by protein misfolding (15). Rather, the cell integrity pathway is activated upon heat shock by thermal stress to the cell surface (28) detected by the plasma membrane sensors Hcs77 (29) and Mid2 (30). In summary, misfolded protein is competent to be an intermediate in the cellular response to heat shock, but it is clearly not the only mechanism by which a cell can detect thermal stress.
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FOOTNOTES

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1ABBREVIATIONS

HSE, heat shock element; STRE, stress response element; AZC, a racemic mixture of D-AZC and L-AZC; ER-UPR, endoplasmic reticulum unfolded protein response; UPRE, unfolded protein response elements responsive to the ER-UPR; TOR, target of rapamycin.

2 E.W.T and J.V.G., unpublished observation.

3G.A.P. and J.V.G., unpublished observation.
REFERENCES

1. Parsell, D.A., and Lindquist, S. (1993) *Annu. Rev. Genet.* **27**, 437-496
2. Ruis, H., and Schüller, C. (1995) *BioEssays* **17**, 959-965
3. Boy-Marcotte, E., Lagiel, G., Perrot, M., Bussereau, F., Boudsocq, A., Jacquet, M. and Labarre, J. (1999) *Mol. Microbiol.* **33**, 274-283
4. Lopez, N., Halladay, J., Walter, W. and Craig, E.A. (1999) *Journal of Bacteriology* **181**, 3136-3143
5. Piper P.W. (1995) *FEMS Microbiol. Lett.* **134**, 121-127
6. Craig, E.A., and Gross, C.A. (1991) *TIBS* **16**, 135-140
7. Beckmann, R.P., Mizzen, L.A. and Welch, W.J. (1990) *Science* **248**, 850-854
8. Grant, C.M., Firoozen, M. and Tuite, M.F. (1989) *Mol. Microbiol.* **3**, 213-220
9. Seufert, W., and Jantsch, S. (1990) *EMBO J.* **9**, 543-550
10. Craig, E.A., and Jacobsen, K. (1984) *Cell* **38**, 841-849
11. Fowden, L., and Richmond, M.H. (1963) *Biochim. Biophys. Acta.* **71**, 459-461
12. Lane, J.M., Parkes, L.J. and Prockup, D.J. (1971) *Biochim. Biophys. Acta.* **236**, 528-541
13. Zagari, A., Nemethy, G. and Scheraga, H.A. (1990) *Biopolymers* **30**, 951-959
14. Zagari, A., Nemethy, G. and Scheraga, H.A. (1994) *Biopolymers* **34**, 51-60
15. Trotter, E.W., Berenfeld, L., Krause, S.A., Petsko, G.A. and Gray, J.V. (2001) *Proc. Natl. Acad. Sci. U.S.A.* **98**, 7313-7318
16. Ogas, J., Andrews, B.J. and Herskowitz, I. (1991) *Cell* **66**, 1015-1026
17. Barbet, N.C., Schneider, U., Helliwell, S.P., Stansfield, I., Tuite, M.F. and Hall, M.N. (1996) *Mol. Biol. Cell.* **7**, 25-42
18. Eisen, M.B., Spellman, P.T., Brown, P.O. and Botstein, D. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 14863-14868
19. Sidrauski, C., Chapman, R. and Walter, P. (1998) *Trends Cell Biol.* **8**, 245-249
20. Thomas, G., and Hall, M.N. (1997) *Curr. Opin. Cell Biol.* **9**, 982-787
21. Powers, T., and Walter, P. (1999) *Mol. Biol. Cell* **10**, 987-1000
22. Beck, T., and Hall, M.N. (1999) Nature 40, 689-692

23. Cardenas, M.E., Cutler, N.S., Lorenz, M.C., DiComo, C.J. and Heitman, J. (1999) Genes Dev. 13, 3271-3279

24. Hardwick, J.S., Kurvilla, F.G., Tong, J.K., Shamji, A.F. and Schreiber, S.L. (1999) Proc. Natl. Acad. Sci. USA. 96, 14866-14870

25. Halladay, J.T., and E.A. Craig. 1995. A heat shock transcription factor with reduced activity suppresses a yeast HSP70 mutant. Molecular and Cellular Biology 15, 4890-4897

26. Schüller, C., Brewster, J.L., Alexander, M.R., Gustin, M.C. and Ruis, H. (1994) EMBO J. 13, 4382-4389

27. Fowden, L., Lewis, D. and Tristram, H. (1967) Adv. Enzymol. Relat. Areas Mol. Biol. 29, 89-163

28. Kamada, Y., Jung, U.S., Piotrowski, J. and Levin, D.E. (1995) Genes Dev. 9, 1559-1571

29. Gray, J.V., Ogas, J.P., Kamada, Y., Stone, M., Levin, D.E. and Herskowitz, I. (1997) EMBO J. 16, 4924-4937

30. Verna, J., Lodder, A., Lee, K., Vagts, A. and Ballester, R. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 13804-13809
Table 1. Selected results from microarray-derived expression changes of known genes after AZC treatment for 5 hr (see supplementary material for more data):

| ORF     | Gene Name | Protein Function   | Fold Induction by AZC |
|---------|-----------|--------------------|-----------------------|
| Most strongly induced by AZC (and strongly induced by temperature upshift) |
| YFL014W | HSP12     | Heat-Shock Protein | 27.96                 |
| YLL026W | HSP104    | Heat-Shock Protein | 22.00                 |
| YDR171W | HSP42     | Heat-Shock Protein | 19.35                 |
| YBL075C | SSA3      | Heat-Shock Protein | 17.52                 |
| YER103W | SSA4      | Heat-Shock Protein | 16.63                 |
| YDR258C | HSP78     | Heat-Shock Protein | 14.74                 |
| YBR169C | SSE2      | Heat-Shock Protein | 14.12                 |
| YCR021C | HSP30     | Heat-Shock Protein | 13.67                 |
| Most strongly repressed by AZC (and strongly repressed by temperature upshift) |
| YLR441C | RPS1a     | Ribosomal Protein  | 0.05                  |
| YJL148W | RPA34     | RNA Pol Subunit    | 0.05                  |
| YML063W | RPS1b     | Ribosomal Protein  | 0.05                  |
| YPL220W | RPL1a     | Ribosomal Protein  | 0.05                  |
| YJL190C | RPS22a    | Ribosomal Protein  | 0.05                  |
| YGR148C | RPL24b    | Ribosomal Protein  | 0.05                  |
| Strongly induced by temperature upshift but not AZC |
| YGR088W | CTT1      | Catalase T         | 1.70                  |
| YIL101C | XBP1      | Transcriptional Repressor | 0.49 |
| Strongly induced by AZC but not temperature upshift |
| YJL077C | ICS3      | Copper Homeostasis | 6.11                  |
FIGURE LEGENDS

Figure 1. AZC-treatment mimics temperature upshift. (A) AZC treatment strongly induces expression of HSF-dependent genes and strongly represses expression of the ribosomal protein genes. JVG961 (S288c WT) was grown to logarithmic phase in YPD and treated with AZC (10 mM) at 30°C. Total RNA was prepared from samples collected as a function of time after AZC addition. Northern blots (10 µg RNA per sample) were probed for the expression of the indicated genes. (B) Temperature upshift induces HSF-dependent genes, induces STRE-dependent genes and represses the ribosomal protein genes. For temperature upshift, JVG961 was grown to logarithmic phase in YPD at 23°C and subjected to temperature upshift (23°C to 36°C). Samples were prepared and analysed as for the AZC treatment above. Blots were probed with the same probes and exposed to the same film and for the same times for each probe.

Figure 2. Induction of HSP42, SSA4 and HSP30 by AZC treatment is dependent on heat shock factor. (A) The EXA3-1 mutation affects the extent and timing of induction of HSF-dependent genes upon temperature upshift. WT (DS10) and EXA3-1 (MH297) strains were grown to logarithmic phase in YPD and subjected to temperature upshift (23°C to 36°C). Total RNA was prepared as a function of time. Samples (10 µg RNA each) were Northern blotted and the blots probed for the expression of the indicated genes. (B) The EXA3-1 mutation affects the extent and timing of induction of HSF-dependent genes upon AZC treatment. WT (DS10) and EXA3-1 (MH297) strains were grown to logarithmic phase in YPD and treated with AZC (40 mM) at 23°C. Samples were prepared and analysed as in A.

Figure 3. Induction of HSP12 expression by AZC treatment or temperature upshift is partially dependent on Msn2 and Msn4. (A) Induction of HSP12 expression by AZC treatment partially requires Msn2 and Msn4. The msn2Δ.msn4Δ double mutant and its congenic WT (W303-1A-STRE-LacZ) were grown to logarithmic phase in YPD at 23°C and treated with AZC (50 mM). Total RNA was prepared as a function of time. Samples (10 µg
RNA each) were Northern blotted and the blots probed for the expression of the indicated genes. (B) Induction of *HSP12* expression by temperature upshift partially requires Msn2 and Msn4. The *msn2Δ,msn4Δ* double mutant and its congenic WT (W303-1A-*STRE-LacZ*) were grown to logarithmic phase in YPD at 23°C and subjected to temperature upshift (23°C to 36°C). Total RNA was prepared as a function of time. Samples (10 µg RNA each) were Northern blotted and the blots probed for the expression of the indicated genes.

Figure 4. Induced expression of HSF-dependent genes correlates with protein misfolding. (A) Induction of *HSP42* expression by AZC treatment requires protein synthesis. JVG961 was grown to logarithmic phase in YPD at 30°C and treated with AZC (10 mM) or cycloheximide (10 µM) or both or vehicle (0.1% ethanol v/v) alone for 2 hr at 30°C. Total RNA was prepared. The northern blot (10 µg RNA per lane) was probed for the expression of the indicated genes. (B) Ethanol treatment causes similar gene expression changes as does AZC treatment. JVG961 was grown to logarithmic phase in YPD at 30°C and treated with 8% v/v ethanol at 30°C and total RNA prepared as a function of time. Northern blots (10 µg RNA per lane) were probed for the expression of the indicated genes. (C) Expression changes caused by canavanine treatment do not closely mimic those caused by AZC treatment. JVG961 (S288c WT) was grown to logarithmic phase in YPD at 30°C and treated with an inhibitory concentration of canavanine (10 mM) at 30°C and total RNA was prepared from samples as a function of time. Samples were prepared and analysed as for ethanol treatment. Blots were probed with the same radioactive probes at the same time and autoradiographed on the same film for the same length of time as each other and as for the blots in Figure 1.
Figure 1
Figure 2
Figure 3
Figure 4
Supplementary table legend

Table S1: Genes whose expression is altered upon AZC treatment by a factor of three or more. Microarray analysis was performed on wild-type cells (W303) grown to mid-logarithmic phase in YPD at 30°C and treated with AZC (50 mM) for 5 hours. Expression changes were determined by probing DNA microarrays as outlined in the main text. Genes whose expression was induced or repressed by a factor of three or more (and with a spot correlation of 0.75 or more) are shown in decreasing order of induction or repression. When a gene was arrayed more than once, that gene is positioned in the table at the average fold induction, with the component measurements shown. For these multiply-arrayed genes, the average difference between measurements was 25%. For comparison purpose, the fold induction of each gene upon temperature upshift is qualitatively indicated: +++, very strong induction or repression; ++, strong induction or repression; +, weak induction or repression; -, no change in expression. * indicates information derived from published work (18). = indicates unpublished observations (D.B.).
| ORF | NAME   | Molecular Function                                | Fold induction | Temp. Upshift |
|-----|--------|-------------------------------------------------|----------------|---------------|
| YFL014W | HSP12  | Heat shock protein                              | 27.96 | +++=          |
| YLL026W | HSP104 | Heat shock protein                              | 22   | +++=          |
| YDR533C | Unknown | Unknown                                         | 19.51 | +++*         |
| YDR171W | HSP42  | Heat shock protein                              | 19.35 | +++=         |
| YGR008C | STF2   | ATPase stabilising factor                        | 18.14 | +=           |
| YGR142W | BTN2   | Unknown                                         | 18.12 | +++*          |
| YBL075C | SSA3   | Heat shock protein                              | 17.52 | +++=         |
| YER103W | SSA4   | Heat shock protein                              | 16.63 | +++=         |
| YBR169C | SSE2   | Heat shock protein                              | 14.12 | +=           |
| YKL026C | GPX1   | Putative glutathione peroxidase                 | 14.06 | ++*          |
| YML128C | Unknown | Unknown                                         | 13.73 | +++*         |
| YER053C | Unknown | Unknown                                         | 16.23/11.07 | ++*          |
| YML130C | ERO1   | Protein disulfide bond formation                | 13.29 | +=           |
| YLR149C | Unknown | Unknown                                         | 12.61 | +++*         |
| YHR138C | Unknown | Unknown                                         | 12.25 | +++*         |
| YCR021C | HSP30  | Heat shock protein                              | 13.67/11.72 | +=          |
| YLR327C | Unknown | Unknown                                         | 9.9   |              |
| YLR217W | Unknown | Unknown                                         | 11.23 | +++*         |
| YMR040W | Unknown | Unknown                                         | 10.89 | -            |
| YNL015W | Unknown | Protease Inhibitor                              | 10.88 | +++*         |
| YDR258C | HSP78  | Heat shock protein                              | 14.74/6.00 | +++=         |
| YLR109W | AHP1   | Alkyl hydroperoxide reductase                   | 10.09 |              |
| YPR158W | Unknown | Unknown                                         | 9.8   | +++*         |
| YOR120W | Unknown | Unknown                                         | 9.35 | +++*         |
| YER150W | Unknown | Unknown                                         | 9.3   | +++*         |
| YLR216C | CPR6   | Peptidylprolyl isomerase                        | 9.28  | +++          |
| YOR052C | Unknown | Unknown                                         | 9.08  | +++*         |
| YNL160W | YGP1   | Unknown                                         | 8.81  | ++           |
| YLR270W | Unknown | Unknown                                         | 8.57  | ++*          |
| YKL091C | Unknown | Unknown                                         | 8.17  | +++*         |
| YLR142W | PUT1   | Proline oxidase                                 | 8.09  | ++           |
| YJ L016W | Unknown | Unknown                                         | 7.88  |              |
| YJ R107W | Unknown | Unknown                                         | 7.82  | -            |
| YHR053C | CUP1-1 | Copper binding metallothionein                 | 7.7   | -            |
| NORF  |        | Unknown                                         | 7.7   |              |
| YNL134C | Unknown | Unknown                                         | 7.62  | +*           |
| YOL031C | Unknown | Unknown                                         | 7.5   | -            |
| YNR068C | Unknown | Unknown                                         | 7.47  | +++*         |
| YOR220W | Unknown | Unknown                                         | 7.38  | ++*          |
| YGR136W | Unknown | Unknown                                         | 7.29  | ++*          |
| YJ L015C | Unknown | Unknown                                         | 7.27  | +*           |
| YBR056W | Unknown | Unknown                                         | 7.27  | ++*          |
| YKL035W | UGP1   | UTP--glucose-1-phosphate uridylyltransferase    | 7.23  | +++          |
| YOR242C | SSP2   | Unknown                                         | 7.17  |              |
| YBL078C | AUT7   | Microtubule binding                            | 7.13  | ++*          |
| YHR055C | CUP1-2 | Copper binding metallothionein                 | 6.97  | -=           |
| YGR250C | Unknown | Unknown                                         | 6.84  | +++*         |
| YBR082C | UBC4   | Ubiquitin conjugating enzyme                    | 6.68  | +=           |
| Gene      | Description                                           | Expression |
|-----------|-------------------------------------------------------|------------|
| YCL035C   | GRX1 Glutaredoxin                                     | 6.6 ++*    |
| YJR008W   | Unknown Unknown                                      | 6.60/6.41 ++* |
| YOL048C   | Unknown Unknown                                      | 6.48 +**   |
| YDR426C   | Unknown Unknown                                      | 6.47 +**   |
| YNL007C   | SIS1 Unknown Unknown                                 | 6.47 +++=  |
| YER142C   | MAG1 DNA-3-methyladenine glycosidase II               | 6.4 -=     |
| YMR173W   | DDR48 Unknown Unknown                                 | 7.71/6.16/6 ++* |
| YLR247C   | Unknown Unknown                                      | 6.38 +*    |
| YBR072W   | HSP26 Heat shock protein                              | 6.36 +++=  |
| YOR118W   | Unknown Unknown                                      | 6.32 +**   |
| YJL034W   | KAR2 Heat shock protein                               | 6.23 +=    |
| YDL020C   | RPN4 Multicatalytic endopeptidase                     | 6.12 +=    |
| YJL077C   | ICS3 Unknown                                          | 6.11 ++*   |
| NORF      |                                                      | 6.09       |
| YOL053C-A | DDR2 Unknown                                           | 5.99       |
| YAL005C   | SSA1 Heat shock protein                               | 5.93 +**   |
| YGR127W   | Unknown Unknown                                      | 5.83 +**   |
| YKL142W   | MRP8 Structural protein of ribosome                   | 5.78 +=    |
| YLR345W   | Unknown Unknown                                      | 5.74       |
| YFR003C   | Unknown Unknown                                      | 5.61 +**   |
| YJL161W   | Unknown Unknown                                      | 5.59 +++*  |
| YJR045C   | SSC1 Heat shock protein                               | 5.94/5.16 += |
| YCLX03C   | Unknown Unknown                                      | 5.95/5.12  |
| YLR455W   | Unknown Unknown                                      | 5.48 ++*   |
| YPL240C   | HSP82 Heat shock protein                              | 5.4 +**    |
| YGR146C   | Unknown Unknown                                      | 5.32 +**   |
| YDR074W   | TPS2 Trehalose phosphatase                            | 5.31 +**   |
| YOL016C   | CMK2 Calcium/calmodulin-dependent protein kinase      | 5.27 +**   |
| YFL016C   | MDJ1 Heat shock protein                               | 5.24 +++=  |
| YGL037C   | PNC1 Pyrazinamidase and nicotinamidase                | 5.24 +++*  |
| YKR011C   | Unknown Unknown                                      | 5.24 +**   |
| YOR173W   | Unknown Unknown                                      | 5.14 +++*  |
| YNL036W   | NCE103 Unknown                                       | 5.1 +++=   |
| YBR067C   | TIP1 Cell wall mannoprotein                           | 5.1        |
| YGR209C   | TRX2 Thioredoxin                                      | 5.09 -=    |
| YDL027C   | Unknown Unknown                                      | 5.03 ++*   |
| YBR100W   | Unknown Unknown                                      | 4.99 ++*   |
| YBR116C   | Unknown Unknown                                      | 4.99 +++*  |
| YOR121C   | Unknown Unknown                                      | 4.94 +**   |
| NORF      |                                                      | 4.75       |
| YGR284C   | Unknown Unknown                                      | 5.26/4.24 ++* |
| YOL032W   | Unknown Unknown                                      | 4.73 +**   |
| YOR364W   | Unknown Unknown                                      | 4.72 +**   |
| YGR043C   | Unknown Unknown                                      | 4.69 +**   |
| YPL087W   | YDC1 Dihydroceramidase                               | 4.64 +++*  |
| YLR120C   | YPS1 GPI-anchored aspartic protease                   | 4.58 +=    |
| YLR251W   | Unknown Unknown                                      | 4.58 +++*  |
| YDL107W   | MSS2 Unknown                                          | 4.57 -=    |
| YDR031W   | Unknown Unknown                                      | 4.52 +*    |
| YDL021W   | GPM2 Phosphoglycerate                                 | 4.47 +=    |
| Strain   | Gene   | Description                                | Score  |
|----------|--------|--------------------------------------------|--------|
| YLR252W  | Unknown| Unknown                                    | 4.47   |
| YNL006W  | LST8   | Unknown                                    | 4.45   |
| YER100W  | UBC6   | Ubiquitin-conjugating enzyme                | 4.4    |
| YOR050C  | Unknown| Unknown                                    | 4.39   |
| YLR312C  | Unknown| Unknown                                    | 4.38   |
| YMR181C  | Unknown| Unknown                                    | 4.76/3.99 |
| YLJ126W  | NIT2   | Unknown                                    | 4.35   |
| YLL024C  | SSA2   | Heat shock protein                         | 4.34   |
| YFR052W  | RPN12  | 26S Proteasome regulatory subunit           | 4.33   |
| YJL036W  | SNX4   | Unknown                                    | 4.33   |
| YOR020C  | HSP10  | Heat shock pro                             | 4.2    |
| YLR178C  | TFS1   | Unknown                                    | 4.19   |
| YGR292W  | MAL12  | a-glucosidase                              | 4.17   |
| YKL138C  | MRPL31 | Ribosomal protein                          | 4.13   |
| YGL010W  | Unknown| Unknown                                    | 4.13   |
| YMR170C  | ALD2   | Aldehyde dehydrogenase                     | 4.12   |
| YBR183C  | YCP1   | Alkaline ceramidase                        | 4.12   |
| YHR054C  | Unknown| Unknown                                    | 4.09   |
| YDR018C  | Unknown| Unknown                                    | 4.09   |
| YMR186W  | HSC82  | Heat shock protein                         | 4.22/3.96 |
| YDL180W  | Unknown| Unknown                                    | 4.05   |
| YHR157W  | REC104 | DS break formation complex                 | 4.04   |
| YDR043C  | NGR1   | Unknown                                    | 4.04   |
| YCL050C  | APA1   | ATP adenylyltransferase I                  | 4.09/3.98 |
| YMR022W  | QRI8   | Ubiquitin-conjugating enzyme               | 4.02   |
| YGR110W  | Unknown| Unknown                                    | 4.     |
| YMR251W-A| HOR7   | Unknown                                    | 3.99   |
| YJL057C  | IKS1   | Protein kinase                             | 3.95   |
| YIL097W  | Unknown| Unknown                                    | 3.95   |
| YOR282W  | Unknown| Unknown                                    | 3.95   |
| YPL196W  | Unknown| Unknown                                    | 3.89   |
| YEL039C  | CYC7   | Cytochrome c                               | 3.87   |
| YMR090W  | Unknown| Unknown                                    | 3.87   |
| YER189W  | Unknown| Unknown                                    | 3.86   |
| YHR049W  | Unknown| Unknown                                    | 3.85   |
| YNL077W  | Unknown| Unknown                                    | 3.84   |
| YDR262W  | Unknown| Unknown                                    | 4.20/3.47 |
| YBR284W  | Unknown| Unknown                                    | 3.83   |
| YKR103W  | Unknown| ATP-binding cassette                       | 3.82   |
| YJR129C  | Unknown| Unknown                                    | 3.8    |
| YLR064W  | Unknown| Unknown                                    | 3.78   |
| YOR053W  | Unknown| Unknown                                    | 3.78   |
| YBR214W  | SDS24  | Unknown                                    | 3.78   |
| YBR216C  | Unknown| Unknown                                    | 3.76   |
| YLR438W  | CAR2   | Ornithine aminotransferase                 | 3.72   |
| YOR054C  | Unknown| Unknown                                    | 3.71   |
| YNR069C  | Unknown| Unknown                                    | 3.7    |
| YNL012W  | SPO1   | Transcriptional regulator                  | 3.69   |
| YDR210W  | Unknown| Unknown                                    | 3.69   |
| Gene    | Protein Name           | Description                                      | Score |
|---------|------------------------|--------------------------------------------------|-------|
| YML004C | GLO1                   | Glyoxylase I                                     | 3.67  |
| YGL121C | Unknown                | Unknown                                          | 3.67  |
| YDR272W | GLO3                   | Glyoxalase II                                    | 3.66  |
| YOL083W | Unknown                | Unknown                                          | 3.65  |
| YJL088W | ARG3                   | Ornithine carbamoyltransferase                   | 3.64  |
| YBL049W | Unknown                | Unknown                                          | 3.63  |
| YDR275W | Unknown                | Unknown                                          | 3.61  |
| YOL099C | Unknown                | Unknown                                          | 3.6   |
| YOL055C | THI20                  | Unknown                                          | 3.58  |
| YGL096W | Unknown                | Unknown                                          | 3.58  |
| YLR457C | NBP1                   | Nap1p binding protein                            | 3.57  |
| YPL230W | USV1                   | Unknown                                          | 3.57  |
| YGL011C | SCL1                   | 20S Proteasome subunit                           | 3.56  |
| YHR099W | TRA1                   | Unknown                                          | 3.54  |
| YJL210W | PEX2                   | Unknown                                          | 3.51  |
| NORF    |                        |                                                  | 3.51  |
| YDR059C | UBC5                   | Ubiquitin conjugating enzyme                     | 3.5   |
| YGR028W | MSP1                   | Adenosine triphosphatase                         | 3.49  |
| YBR201W | DER1                   | Unknown                                          | 3.48  |
| YER079W | Unknown                | Unknown                                          | 3.47  |
| YER012W | PRE1                   | 20S Proteasome subunit                           | 3.46  |
| YGR111W | Unknown                | Unknown                                          | 3.46  |
| YLR119W | SRN2                   | Unknown                                          | 3.45  |
| YGL048C | RPT6                   | 26S proteasome regulatory subunit                | 3.42  |
| YLL055W | Unknown                | Unknown                                          | 3.41  |
| YJL017W | Unknown                | Unknown                                          | 3.41  |
| YMR169C | ALD3                   | Aldehyde dehydrogenase                           | 3.41  |
| YOR044W | Unknown                | Unknown                                          | 3.39  |
| YNL081C | Unknown                | Unknown                                          | 3.39  |
| YGR138C | Unknown                | Unknown                                          | 3.38  |
| YDL194W | SNF3                   | Glucose permease                                 | 3.37  |
| YGL058W | RAD6                   | Ubiquitin conjugating enzyme                     | 3.36  |
| YDR271C | Unknown                | Unknown                                          | 3.36  |
| YEL060C | PRB1                   | Vacuolar protease B                              | 3.33  |
| YDR209C | Unknown                | Unknown                                          | 3.31  |
| YPR149W | NCE102                 | Unknown                                          | 3.29  |
| YFL057C | Unknown                | Unknown                                          | 3.29  |
| YIL045W | PIG2                   | Unknown                                          | 3.26  |
| YOL071W | Unknown                | Unknown                                          | 3.26  |
| YOR007C | SGT2                   | Unknown                                          | 3.24  |
| YOR193W | Unknown                | Unknown                                          | 3.24  |
| YEL073C | Unknown                | Unknown                                          | 3.22  |
| YBR062C | Unknown                | Unknown                                          | 3.21  |
| YGR137W | Unknown                | Unknown                                          | 3.21  |
| YLL001W | DNM1                   | Mitochondrion organization and biogenesis        | 3.2   |
| YOL013C | HRD1                   | Unknown                                          | 3.2   |
| YPL060W | Unknown                | Unknown                                          | 3.2   |
| YDR391C | Unknown                | Unknown                                          | 3.33/3.06 |
| YOR031W | CRS5                   | Metallothionein-like protein                     | 3.18  |
| YDL193W | Unknown                | Unknown                                          | 3.17  |
| ORF       | Name     | Molecular Function                  | Fold Induction | Repression by |
|-----------|----------|-------------------------------------|----------------|--------------|
| YBL015W   | ACH1     | Acetyl-CoA hydrolase                | 3.16           | +=           |
| YIL046W   | MET30    | F-box transcription factor          | 3.14           | -=           |
| YDR503C   | LPP1     | Lipid phosphate phosphatase         | 3.13           | -=           |
| YKL145W   | RPT1     | Ubiquitin26S proteasome subunit     | 3.13           | -=           |
| YML100W   | TSL1     | a,a-trehalose-phosphate synthase    | 3.12           | +*           |
| YDR020C   | Unknown  | Unknown                             | 3.09           | -*           |
| YDR199W   | Unknown  | Unknown                             | 3.08           | +*           |
| YBR203W   | Unknown  | Unknown                             | 3.07           | +**          |
| YIL050W   | PCL7     | Cyclin                              | 3.06           | -=           |
| YIR024C   | GIF1     | Unknown                             | 3.05           | -=           |
| YLR311C   | Unknown  | Unknown                             | 3.04           | +*           |
| YGL091C   | Unknown  | Unknown                             | 3.04           | +*           |
| YMR004W   | MVP1     | Unknown                             | 3.04           | -=           |
| YLRO311W  | Unknown  | Unknown                             | 3.04           | +*           |
| YOR215C   | Unknown  | Unknown                             | 3.03           | +*           |
| YDL247W   | Unknown  | Unknown                             | 3.03           |             |
| YJL146W   | IDS2     | Unknown                             | 3.02           | +*           |
| YDR214W   | Unknown  | Unknown                             | 3.01           | +*           |
| YFR004W   | RPN11    | Multicatalytic endopeptidase        | 3              | -=           |

Repressed Genes:

| ORF       | Name     | Molecular Function                  | Fold Induction | Repression by |
|-----------|----------|-------------------------------------|----------------|--------------|
| YPL220W   | RPL1a    | Ribosomal protein                   | 0.05           | ++*          |
| YGR148C   | RPL24b   | Ribosomal protein                   | 0.05           | ++*          |
| YLR441C   | RPS1a    | Ribosomal protein                   | 0.05           | +++*         |
| YML063W   | RPS1b    | Ribosomal protein                   | 0.05           | +++*         |
| YJL148W   | RPA34    | RNA polymerase I subunit            | 0.05           | +*           |
| YOR394W   | Unknown  | Unknown                             | 0.05           |             |
| YJL190C   | RPS22A   | Ribosomal protein                   | 0.05/0.06      | ++*          |
| YNL069C   | RPL16b   | Ribosomal protein                   | 0.06           | ++*          |
| YGL031C   | RPL24a   | Ribosomal protein                   | 0.06           | +++*         |
| YDR382W   | RPP2b    | Ribosomal protein                   | 0.06           | +*           |
| YLR448W   | RPL6b    | Ribosomal protein                   | 0.06           | +*           |
| YOL039W   | RPP2a    | Ribosomal protein                   | 0.06           | +*           |
| YHL015W   | RPS20    | Ribosomal protein                   | 0.06           | ++*          |
| YLR197W   | SIK1     | Nucleolar protein                   | 0.06           | +++*         |
| YLR325C   | RPL38    | Ribosomal protein                   | 0.06           | ++*          |
| YMR143W   | RPS16a   | Ribosomal protein                   | 0.06           | +++=         |
| YJL136C   | RPS21b   | Ribosomal protein                   | 0.05/0.07      | ++*          |
| YOR278W   | HEM4     | Uroporphyrinogen III synthase       | 0.07           | -*           |
| YPR102C   | RPL11a   | Ribosomal protein                   | 0.07           | ++*          |
| YLR344W   | RPL26a   | Ribosomal protein                   | 0.07           | +*           |
| YDL184C   | RPL41a   | Ribosomal protein                   | 0.07           | ++*          |
| YGR027C   | RPS25a   | Ribosomal protein                   | 0.07           | ++*          |
| YLR367W   | RPS22b   | Ribosomal protein                   | 0.07           | +++*         |
| YPL197C   | Unknown  | Unknown                             | 0.07           | +++=         |
| YDR064W   | RPS13    | Ribosomal protein                   | 0.07           | +++*         |
| YKL009W   | MRT4     | Unknown                             | 0.08           | +++=         |
| YDR418W   | RPL12b   | Ribosomal protein                   | 0.08           | ++*          |
| YGL147C   | RPL9a    | Ribosomal protein                   | 0.08           | ++*          |
| Gene   | Protein          | Function                                   | Score |
|--------|------------------|--------------------------------------------|-------|
| YDR447C| RPS17b           | Ribosomal protein                          | 0.08  |
| YOL121C| RPS19a           | Ribosomal protein                          | 0.08  |
| YIL069C| RPS24b           | Ribosomal protein                          | 0.08  |
| YLR333C| RPS25b           | Ribosomal protein                          | 0.08  |
| YGL189C| RPS26a           | Ribosomal protein                          | 0.08  |
| YER131W| RPS26b           | Ribosomal protein                          | 0.08  |
| YLR388W| RPS29a           | Ribosomal protein                          | 0.08  |
| YGL102C| Unknown          | Unknown                                    | 0.08  |
| YOR261C| RPN8             | 26s proteasome regulatory subunit           | 0.09  |
| YLR075W| RPL10            | Ribosomal protein                          | 0.09  |
| YGR085C| RPL11b           | Ribosomal protein                          | 0.09  |
| YKL006W| RPL14a           | Ribosomal protein                          | 0.09  |
| YJL177W| RPL17b           | Ribosomal protein                          | 0.09  |
| YOR182C| RPS30b           | Ribosomal protein                          | 0.09  |
| YGR160W| Unknown          | Unknown                                    | 0.09  |
| YLR062C| Unknown          | Unknown                                    | 0.09  |
| YLR076C| Unknown          | Unknown                                    | 0.09  |
| YLR198C| Unknown          | Unknown                                    | 0.09  |
| YOL077C| Unknown          | Unknown                                    | 0.09  |
| YHR007C| ERG11            | Cytochrome P450 Lanosterol                 | 0.09  |
| YGL135W| RPL1b            | Ribosomal protein                          | 0.1   |
| YGL030W| RPL30            | Ribosomal protein                          | 0.1   |
| YPR043W| RPL43a           | Ribosomal protein                          | 0.1   |
| YLR185W| RPL37a           | Ribosomal protein                          | 0.1   |
| YNL067W| RPL9b            | Ribosomal protein                          | 0.1   |
| YDL083C| RPS16b           | Ribosomal protein                          | 0.1   |
| YNL302C| RPS19b           | Ribosomal protein                          | 0.1   |
| YGR118W| RPS23a           | Ribosomal protein                          | 0.1   |
| YOR167C| RPS28a           | Ribosomal protein                          | 0.1   |
| YNL178W| RPS3             | Ribosomal protein                          | 0.1   |
| YGR175C| ERG1             | Squalene monoxygenase                      | 0.1   |
| YGR103W| Unknown          | Unknown                                    | 0.1   |
| YOR262W| Unknown          | Unknown                                    | 0.1   |
| YML024W| RPS17a           | Ribosomal protein                          | 0.1   |
| NORF   | Unknown          | Unknown                                    | 0.1   |
| YIL053W| RHR2             | DL-glycerol-3-phosphatase                  | 0.11  |
| YGR159C| NSR1             | NLS-binding protein                        | 0.11  |
| YGR234W| YHB1             | Putflavohemoglobin                         | 0.11  |
| YOR095C| RKI1             | Ribose-5-phosphate ketol-isomerase         | 0.11  |
| YGL103W| RPL28            | Ribosomal protein                          | 0.11  |
| YIL052C| RPL34b           | Ribosomal protein                          | 0.11  |
| YGL076C| RPL7a            | Ribosomal protein                          | 0.11  |
| YLR264W| RPS28b           | Ribosomal protein                          | 0.11  |
| YJL079W| Unknown          | Unknown                                    | 0.11  |
| YER102W| RPS8b            | Ribosomal protein                          | 0.11  |
| YIL133C| RPL16a           | Ribosomal protein                          | 0.11  |
| YBR191W| RPL21a           | Ribosomal protein                          | 0.11  |
| YIL018W| RPL2b            | Ribosomal protein                          | 0.11  |
| YGL029W| CGR1             | Unknown                                    | 0.12  |
| YOR350C| MNE1             | Unknown                                    | 0.12  |
| Gene ID   | Gene Symbol | Description                                      | Score  |
|----------|-------------|-------------------------------------------------|--------|
| YLR150W  | STM1        | Unknown                                         | 0.12   |
| YLR175W  | CBF5        | Centromeric microtubule binding protein          | 0.13+++ |
| YOR259C  | RPT4        | 26s proteasome regulatory subunit               | 0.13-*  |
| YGR034W  | RPL26b      | Ribosomal protein                               | 0.13++* |
| YHR141C  | RPL42b      | Ribosomal protein                               | 0.13*   |
| YHL033C  | RPL8a       | Ribosomal protein                               | 0.13++* |
| YDL061C  | RPS29b      | Ribosomal protein                               | 0.13++* |
| YNL248C  | RPA49       | RNA polymerase I subunit                        | 0.13+++*|
| YNL110C  | Unknown     | Unknown                                         | 0.13+++ |
| YNL119W  | Unknown     | Unknown                                         | 0.13+++ |
| NORF     |             |                                                 | 0.13   |
| YER074W  | RPS24a      | Ribosomal protein                               | 0.13/0.14++*|
| YCR016W  | Unknown     | Unknown                                         | 0.12/0.16+++|
| YBL003C  | HTA2        | Histone 2a                                      | 0.14/0.14++*|
| YDR225W  | HTA1        | Histone 2a                                      | 0.14++* |
| YDL081C  | RPP1a       | Ribosomal protein                               | 0.14+* |
| YHR021C  | RPS27b      | Ribosomal protein                               | 0.14+* |
| YPR110C  | RPC40       | RNA polymerase III subunit                      | 0.14+++*|
| YBR154C  | RPB5        | Shared subunit of RNA polymerases               | 0.14+* |
| YMR011W  | HXT2        | Hexose permease                                 | 0.14+* |
| YBL028C  | Unknown     | Unknown                                         | 0.14+++|
| YPL142C  | Unknown     | Unknown                                         | 0.14+++|
| YDR023W  | SES1        | tRNA synthetase                                 | 0.14+* |
| YJL188C  | Unknown     | Unknown                                         | 0.13+* |
| YJL188C  | Unknown     | Unknown                                         | 0.16+* |
| YBR181C  | RPS6b       | Ribosomal protein                               | 0.15++* |
| YKL081W  | TEF4        | Translation elongation factor                    | 0.15++* |
| YGR035C  | Unknown     | Unknown                                         | 0.15+* |
| YIL127C  | Unknown     | Unknown                                         | 0.15+++|
| YLR040C  | Unknown     | Unknown                                         | 0.15+  |
| YOL109W  | ZEO1        | Unknown                                         | 0.15+* |
| YBR048W  | RPS11b      | Ribosomal protein                               | 0.15+* |
| YHR010W  | RPL27a      | Ribosomal protein                               | 0.15+++*|
| YNL192W  | CHS1        | Chitin synthase                                 | 0.15-* |
| YMR230W  | RPS10b      | Ribosomal protein                               | 0.15+  |
| YML026C  | RPS18b      | Ribosomal protein                               | 0.15+++|
| YDR496C  | Unknown     | Unknown                                         | 0.13/0.17++++=|
| YKR057W  | RPS21a      | Ribosomal protein                               | 0.16++* |
| YKL180W  | RPL17a      | Ribosomal protein                               | 0.16++* |
| YOL127W  | RPL25       | Ribosomal protein                               | 0.16++* |
| YKR094C  | RPL40b      | Ribosomal protein                               | 0.16++* |
| YKL156W  | RPS27a      | Ribosomal protein                               | 0.16++* |
| YMR015C  | ERG5        | C-22 sterol desaturase                          | 0.16-* |
| YMR307W  | GAS1        | Cell surface glycoprotein                       | 0.16+  |
| YLR276C  | DBP9        | Putative RNA helicase                           | 0.16+++=|
| YGR265W  | Unknown     | Unknown                                         | 0.16+  |
| YLL044W  | Unknown     | Unknown                                         | 0.16+++|
| YNL303W  | Unknown     | Unknown                                         | 0.16+  |
| YMR194W  | RPL36a      | Ribosomal protein                               | 0.16++++=|
| YNR015W  | SMM1        | Unknown                                         | 0.16+* |
| Gene      | Protein/Function                              | Score |
|-----------|----------------------------------------------|-------|
| NORF      |                                              | 0.16  |
| YPL079W   | RPL21B Ribosomal protein                     | 0.16  |
| YCLX02C   | Unknown Unknown                              | 0.13/0.2 |
| YDR496C   | Unknown Unknown                              | 0.17  |
| YLR300W   | EXG1 Exo-b-1,3-glucanase                    | 0.17  |
| YDL055C   | PSA1 Mannose-1-phosphate guanyltransferase   | 0.17  |
| YKL178C   | STE3 a-factor receptor                       | 0.17  |
| YOL120C   | RPL18a Ribosomal protein                     | 0.17  |
| YPL029W   | SUV3 Mitochondrial RNA helicase              | 0.17  |
| YGR151C   | Unknown Unknown                              | 0.17  |
| YGR272C   | Unknown Unknown                              | 0.17  |
| YHR052W   | Unknown Unknown                              | 0.17  |
| YLR222C   | Unknown Unknown                              | 0.17  |
| YGL028C   | SCW11 Putative glucanase                     | 0.18  |
| YJR057W   | CDC8 Thymidylate kinase                      | 0.18  |
| YBR084C-A | RPL19a Ribosomal protein                     | 0.18  |
| YPL143W   | RPL33a Ribosomal protein                     | 0.18  |
| YIL148W   | RPL40a Ribosomal protein                     | 0.18  |
| YPL198W   | RPL7b Ribosomal protein                      | 0.18  |
| YGL123W   | RPS2 Ribosomal protein                       | 0.18  |
| YDR417C   | Unknown Unknown                              | 0.18  |
| YDR091C   | RLI1 Unknown                                 | 0.18  |
| YNR045W   | PET494 Translation activator of COX3        | 0.18  |
| YJL189W   | RPL39 Ribosomal protein                      | 0.17/0.19 |
| YBL027W   | RPL19b Ribosomal protein                     | 0.19  |
| YJL138C   | TIF2 Translation initiation factor           | 0.19  |
| YGL078C   | DBP3 RNA helicase                             | 0.19  |
| YOR210W   | RPBI0 Shared subunit of RNA polymerases      | 0.19  |
| YKL120W   | OAC1 Oxaloacetate carrier                    | 0.19  |
| YLR196W   | PWP1 Unknown                                 | 0.19  |
| YOR286W   | Unknown Unknown                              | 0.19  |
| YGR128C   | Unknown Unknown                              | 0.19  |
| YMR142C   | RPL13b Ribosomal protein                     | 0.19  |
| YML123C   | PHO84 Inorganic phosphate permease           | 0.2   |
| YGL101W   | Unknown Unknown                              | 0.2   |
| YGR081C   | Unknown Unknown                              | 0.2   |
| YBL002W   | HTB2 Histone H2B                             | 0.21  |
| YGR285C   | ZJO1 Binds Z-DNA                             | 0.21  |
| YPL146C   | Unknown Unknown                              | 0.21  |
| YOL041C   | Unknown Unknown                              | 0.21  |
| YPR132W   | RPS23b Ribosomal protein                     | 0.21  |
| YFR031BC  | Unknown Unknown                              | 0.21  |
| YDR025W   | RPS11a Ribosomal protein                     | 0.20/0.22 |
| YNL030W   | HHF2 Histone H4                              | 0.22  |
| YLR061W   | RPL22a Ribosomal protein                     | 0.22  |
| YLR221C   | Unknown Unknown                              | 0.22  |
| YLR413W   | Unknown Unknown                              | 0.22  |
| YPL014W   | Unknown Unknown                              | 0.22  |
| YKL172W   | EBP2 Unknown                                 | 0.22  |
| YGR145W   | Unknown Unknown                              | 0.22  |
| Gene ID   | Description                          | Expression Level |
|-----------|--------------------------------------|------------------|
| YER006W   | Unknown                              | 0.22 ++=         |
| YAL036C   | Fun11                                | 0.22 +++=        |
| YPL160W   | Cdc60                               | 0.22 +*          |
| YER056C-A | Rpl34a                              | 0.22             |
| YFL035C-B | Rpl22b                              | 0.22             |
| YGR152C   | Rsr1                                | 0.23 +          |
| YLR286C   | Cts1                                | 0.23 +*          |
| YOL139C   | Cdc33                               | 0.23 ++*         |
| YGR264C   | Mes1                                | 0.23 +*          |
| YHR196W   | Unknown                              | 0.23 +++=        |
| YOR169C   | Unknown                              | 0.23 +=          |
| NORF      |                                     | 0.23             |
| YBR009C   | Hhf1                                | 0.24 ++*         |
| YEL026W   | Snu13                               | 0.24 ++=         |
| YLR449W   | Fpr4                                | 0.24 ++*         |
| YLL045C   | Rpl8b                               | 0.24 ++*         |
| YLR167W   | Rps31                               | 0.24 ++*         |
| YML022W   | Apt1                                | 0.24 +*          |
| YDR399W   | Hpt1                                | 0.24 ++*         |
| YNL075W   | Imp4                                | 0.24 +++=        |
| YHR143W-A | Rpc10                               | 0.24 ++=         |
| YBR089W   | Unknown                              | 0.24 ++=         |
| YDL062W   | Unknown                              | 0.24 +++=        |
| YLR068W   | Unknown                              | 0.24 ++=         |
| YCL059C   | Krr1                                | 0.24 +++=        |
| YCL054W   | Spb1                                | 0.24 ++*         |
| YPL093W   | Nog1                                | 0.24 +++=        |
| YLR287C-A | Rps30a                              | 0.22/0.26 +=     |
| YDR012W   | Rpl4b                               | 0.22/0.28 +=    |
| YCR031C   | Rps14a                              | 0.19/0.30 +=    |
| YGL055W   | Ole1                                | 0.25 ++=         |
| YMR309C   | Nip1                                | 0.25 +*          |
| YOR063W   | Rpl3                                | 0.25 ++*         |
| YHR203C   | Rps4b                               | 0.25 ++*         |
| YHR193C   | Egd2                                | 0.25 +*          |
| YPR187W   | Rpo26                               | 0.25 ++*         |
| YDR365C   | Unknown                              | 0.25 +++=        |
| YKL056C   | Unknown                              | 0.25 +++=        |
| YLR094C   | Gis3                                | 0.25 +=          |
| YKL083W   | Unknown                              | 0.25 +=          |
| NORF      |                                     | 0.25             |
| YNL060C   | Unknown                              | 0.25             |
| YOL007C   | Csi1                                | 0.26 ++*         |
| YBR189W   | Rps9b                               | 0.26 ++*         |
| YPL037C   | Egd1                                | 0.26 +*          |
| YDR083W   | Unknown                              | 0.26 +++=        |
| YDR361C   | Unknown                              | 0.26 +++=        |
| YMR014W   | Unknown                              | 0.26 +++=        |
| YMR269W   | Unknown                              | 0.26 +++=        |
| YJ R004C  | Sagg1                               | 0.26.0.27 -*     |
| Gene  | Description                      | Score |
|-------|-----------------------------------|-------|
| YLR153C | ACS2 Acetyl-CoA synthetase       | 0.27  |
| YBR078W | ECM33 Unknown                   | 0.27  |
| YDR224C | HTB1 Histone H2B                | 0.27  |
| YNL111C | CYB5 Cytochrome B5              | 0.27  |
| YGR155W | CYS4 Cystathionine b-synthase   | 0.27  |
| YER009W | NTF2 Nuclear transport factor   | 0.27  |
| YOR184W | SER1 Phosphoserine              | 0.27  |
| YBR104W | YMC2 Mitochondrial carrier      | 0.27  |
| YDR165W | Unknown Unknown                 | 0.27  |
| YMR049C | Unknown Unknown                 | 0.27  |
| YOR051C | Unknown Unknown                 | 0.27  |
| YPR069C | SPE3 Spermidine synthase        | 0.27  |
| YMR108W | ILV5 Ketal-acid reductoisomerase | 0.27  |
| YDL063C | Unknown Unknown                 | 0.27  |
| YDR012W | RPL4b Ribosomal protein         | 0.28  |
| YLR340W | RPO0 Ribosomal protein          | 0.28  |
| YDL082W | RPL13a Ribosomal protein        | 0.28  |
| YLR029C | RPL15a Ribosomal protein        | 0.28  |
| YAL003W | EFB1 Translation elongation factor | 0.28   |
| YOL097C | WRS1 tRNA ligase                | 0.28  |
| YKL216W | URA1 Dihydroorotate dehydrogenase | 0.28  |
| YPR142C | Unknown Unknown                 | 0.28  |
| YPR143W | Unknown Unknown                 | 0.28  |
| YCR024C | Unknown tRNA synthetase         | 0.28  |
| YML106W | URA5 Orotate phosphoribosyltransferase | 0.29  |
| YNL113W | RPC19 RNA polymerases I + III subunit | 0.29  |
| YDL213C | Unknown Unknown                 | 0.29  |
| YOL092W | Unknown Unknown                 | 0.29  |
| YER042W | MXR1 Peptide-methionine sulfoxide | 0.29  |
| YCL066W | HMLa1 Transcription co-activator | 0.29  |
| YBL087C | RPL23a Ribosomal protein        | 0.3   |
| YOL040C | RPS15 Ribosomal protein         | 0.3   |
| YKR059W | TIF1 Translation initiation factor | 0.3   |
| YBR092C | PHO3 Acid phosphatase           | 0.3   |
| YBR266C | Unknown Unknown                 | 0.3   |
| YDL050C | Unknown Unknown                 | 0.3   |
| YEL040W | UTR2 Unknown                    | 0.3   |
| YFL004W | Unknown Unknown                 | 0.3   |
| YJL011C | Unknown Unknown                 | 0.3   |
| YKL082C | Unknown Unknown                 | 0.3   |
| YJL191W | RPS14b Ribosomal protein        | 0.30/0.31 |
| YLR009W | RPL24b Ribosomal protein        | 0.31  |
| YOR133W | EFT1 Translation elongation factor | 0.31  |
| YMR005W | MPT1 Unknown                    | 0.31  |
| YLR110C | Unknown Unknown                 | 0.31  |
| YER072W | NRF1 Unknown                    | 0.31  |
| YCL065W | Unknown Unknown                 | 0.31  |
| YBR010W | HHT1 Histone H3                 | 0.32  |
| YAL025C | MAK16 Unknown                   | 0.32  |
| YLR048W | RPS0B Ribosomal protein         | 0.32  |
| Gene   | Protein Name | Function Description                              | Score |
|--------|--------------|---------------------------------------------------|-------|
| YGR245C| Unknown      | Unknown                                           | 0.32  |
| YOR135C| Unknown      | Unknown                                           | 0.32  |
| YGR187C| HGH1         | Unknown                                           | 0.32  |
| YDR312W| SSF2         | Unknown                                           | 0.32  |
| NORF   |              | Unknown                                           | 0.32  |
| YMR001C| CDC5         | G2/M protein kinase                               | 0.33  |
| YBR130C| SHE3         | Asymmetric HO expression                          | 0.33  |
| YDR184C| ATC1         | Member of Bud6p complex                           | 0.33  |
| YFL045C| SEC53        | Phosphomannomutase                                | 0.33  |
| YBR031W| RPL4a        | Ribosomal protein                                 | 0.33  |
| YKL004W| AUR1         | Unknown                                           | 0.33  |
| YPL273W| SAM4         | S-methyltransferase                               | 0.33  |
| YLR041W| Unknown      | Unknown                                           | 0.33  |
| YML093W| Unknown      | Unknown                                           | 0.33  |
| YJR003C| Unknown      | Unknown                                           | 0.33  |
Misfolded proteins are competent to mediate a subset of the responses to heat shock in *Saccharomyces cerevisiae*
Eleanor W. Trotter, Camilla M.F. Kao, Ludmilla Berenfeld, David Botstein, Gregory A. Petsko and Joseph V. Gray

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