Hsf1 activation by proteotoxic stress requires concurrent protein synthesis

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ABSTRACT **Heat shock factor 1 (Hsf1) activation is responsible for increasing the abundance of protein-folding chaperones and degradation machinery in response to proteotoxic conditions that give rise to misfolded or aggregated proteins. Here we systematically explored the link between concurrent protein synthesis and proteotoxic stress in the budding yeast, *Saccharomyces cerevisiae*. Consistent with prior work, inhibiting protein synthesis before inducing proteotoxic stress prevents Hsf1 activation, which we demonstrated across a broad array of stresses and validate using orthogonal means of blocking protein synthesis. However, other stress-dependent transcription pathways remained activatable under conditions of translation inhibition. Titrating the protein denaturant ethanol to a higher concentration results in Hsf1 activation in the absence of translation, suggesting extreme protein-folding stress can induce proteotoxicity independent of protein synthesis. Furthermore, we demonstrate this connection under physiological conditions where protein synthesis occurs naturally at reduced rates. We find that disrupting the assembly or subcellular localization of newly synthesized proteins is sufficient to activate Hsf1. Thus, new proteins appear to be especially sensitive to proteotoxic conditions, and we propose that their aggregation may represent the bulk of the signal that activates Hsf1 in the wake of these insults.**

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INTRODUCTION

Protein misfolding and aggregation are deleterious to cells (Stefani and Dobson, 2003; Gsponer and Babu, 2012; Holmes *et al.*, 2014). As such, cells invest heavily in protein folding and degradation machinery to prevent accumulation of these aberrant proteins. When an insult, such as heat shock or oxidative stress, results in an excess of aberrant proteins, the master eukaryotic transcription factor heat shock factor 1 (Hsf1) is activated (Akerfelt *et al.*, 2010). Subsequently, Hsf1 drives transcriptional activation of target genes consisting of more chaperone and degradation machinery (Solís *et al.*, 2016). The increase in chaperones such as Hsp70 (yeast Ssa1-4) and Hsp90 (Hsc/p82), cochaperones such as Hsp40s (Sis1, Ydj1), the disaggregase Hsp104, and the downstream accumulation of proteasomes allows cells to deal with the increase in aberrant proteins and restore proteostasis. Once chaperones again reach excess relative to protein clients, Hsf1 is turned back off through a proposed mechanism involving reassociation with Hsp70 (Mosser *et al.*, 1988; Abravaya *et al.*, 1992; Baler *et al.*, 1992; Shi *et al.*, 1998; Zheng *et al.*, 2016; Masser *et al.*, 2019)

Despite our understanding of this sophisticated homeostatic mechanism, it remains unclear what protein-folding defect in the cell necessitates Hsf1 activation in proteotoxic stress conditions. Importantly, multiple data points indicate that Hsf1 is a bona fide misfolded/aggregated protein sensor. First, overexpression of a mutant, aggregation-prone protein or treatment of cells with the strained proline analog azetidine-2-carboxylic acid (AZC), which causes nascent chain aggregation when incorporated, are sufficient to activate a highly specific Hsf1-dependent response (Trotter *et al.*, 2002; Geiler-Samerotte *et al.*, 2011). Second, its activity seems to be controlled by titration of chaperones Hsp70/90, which interact with exposed hydrophobic regions of proteins (Mayer and Bukau, 2005) as would be expected for misfolded/aggregated proteins (Beckmann *et al.*, 1992). In spite evidence of Hsf1 being activated in

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Abbreviations used: AZC, azetidine-2-carboxylic acid; CMX, cycloheximide; Hsf1, heat shock factor 1.

response to unfolded/aggregated proteins, the origin of this signal in proteotoxic conditions remains enigmatic. One possibility are nascent chains or newly synthesized proteins because stabilizing maturation steps may not have occurred, including final folding steps, post-translational modifications, formation of protein–protein interactions, and localization within the cell, rendering them more liable to misfold or aggregate in response to proteotoxic conditions, such as heat shock.

Early work on Hsf1 in mammalian tissue culture cells demonstrated that treatment with the translation elongation inhibitor cycloheximide (CHX) prior to heat shock, oxidative stress/protein disulfide altering compounds, or proteasome inhibition blocked induced DNA binding by Hsf1 that coincides with target gene activation (Amici *et al.*, 1992; Baler *et al.*, 1992; Liu *et al.*, 1996; Tanabe *et al.*, 1997; Kim *et al.*, 1999). However, it was found that if heat shock or the protein disulfide alkylating agent, iodoacetamide, were increased to high levels, the CHX-induced block on Hsf1 DNA binding could be overcome. Two intriguing hypotheses emerge from these observations. First, that in normal conditions, chaperones are occupied by the folding and maturation of newly synthesized proteins, and thus on heat shock, are unavailable to deal with aberrant proteins of any origin. By inhibiting translation before heat shock, there would be an excess of free chaperones to deal with aberrant proteins, obviating the need for Hsf1 activation. Alternatively, inhibiting translation prior to proteotoxic stress prevents Hsf1 activation because newly synthesized proteins are those proteins that unfold and aggregate. Thus, in the absence of their synthesis, cells do not experience proteotoxic stress. Consistent with the latter hypothesis, newly synthesized ribosomal proteins are competent to activate Hsf1 if their assembly is blocked (Albert *et al.*, 2019; Tye *et al.*, 2019). However, these experiments are limited as typically a single, pharmacological approach has been used to stop translation. Additionally, there has not been a systematic study across the variety of proteotoxic stressors. Thus, the connection between newly synthesized proteins and proteotoxic stress remains to be further explored.

Here, using budding yeast as a model, we demonstrate that the amount of proteotoxic strain experienced by the cell correlates with the level of protein synthesis transpiring in that cell. Using multiple pharmacological perturbations, we find that Hsf1 activation requires concurrent translation in a variety of different conditions, including oxidative stress, proteasome inhibition, and the denaturant ethanol. Conversely, cells maintain the ability to activate another general stress response program, controlled by Msn2/4, in the absence of protein synthesis, demonstrating specificity to the translation-Hsf1 axis. We demonstrate that this connection between translation and proteotoxic strain is physiological: cells that are naturally synthesizing fewer proteins experience a diminished heat shock response. Finally, we show that several perturbations that interfere with the proper assembly or localization of newly synthesized proteins activate Hsf1, providing evidence that newly synthesized proteins can underlie the proteotoxic stress leading to Hsf1 activation.

RESULTS

Translation inhibition by CHX prevents Hsf1 activation across diverse proteotoxic stressors

Treatment of mammalian tissue culture cells with the translation elongation inhibitor CHX prior to heat shock, oxidative stress, or proteasome inhibition blocks activation of Hsf1, primarily at the level of induced DNA binding (Amici *et al.*, 1992; Baler *et al.*, 1992; Liu *et al.*, 1996; Tanabe *et al.*, 1997; Kim *et al.*, 1999). We assessed whether budding yeast *Saccharomyces cerevisiae* treated with CHX are similarly blocked in their activation of Hsf1. Pretreatment with

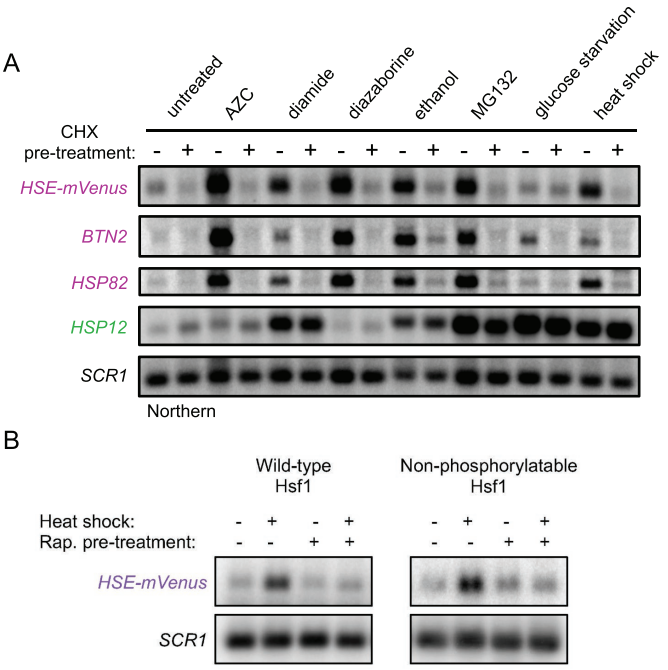


FIGURE 1: Translation inhibition prevents Hsf1 activation across diverse proteotoxic stressors. (A) Cells were grown to midlog and treated with either 0.2% vol/vol vehicle (DMSO, –) or 200 µg/ml CHX (+) for 3 min prior to treatment with the indicated condition. Shown are Northern blots of RNA from cells treated as indicated, probed for Hsf1-dependent (purple) and Msn2/4-dependent (green) transcripts. *HSE-mVenus*, Hsf1 reporter transgene of *mVenus* driven by four repeats of the heat shock element (Hsf1-binding site). Treatments: AZC (10 mM, 30 min), diamide (1.5 mM, 30 min), diazaborine (15 µg/ml, 30 min), ethanol (5%, 20 min), MG132 (50 µm, 30 min), glucose starvation (shift to 0%, 30 min), and heat shock (37°C, 20 min). Note that a *pdr5*Δ strain was used for MG132 to facilitate uptake. The experiment was replicated twice. (B) (Left) Wild-type cells grown to midlog were either treated with mock or 200 ng/ml rapamycin (rap.) for 30 min prior to instantaneous upshift to 37°C for 20 min. The abundance of the Hsf1 reporter transcript *HSE-mVenus* was assessed by Northern blot. (Right) Same as left, except using a strain that contained a nonphosphorylatable Hsf1 mutant, where all serine and threonine residues are mutated to alanine with the exception of S225, which is required for DNA binding (Zheng *et al.*, 2016). The experiment was replicated three times.

CHX for 3 min prior to exposure to many stress conditions completely blocks activation of Hsf1 as observed by lack of accumulation of three target transcripts (Figure 1A). Consistent with what is observed in mammalian cells, we found CHX treatment blocks Hsf1 activation in oxidative stress induced by diamide and proteasome inhibition by MG132. Additionally, CHX blocks Hsf1 activation by heat (Masser *et al.*, 2019), the denaturant ethanol (Herskovits *et al.*, 1970), and the ribosome assembly inhibitor diazaborine (Tye *et al.*, 2019). These stresses cover a broad range of known proteotoxic conditions known to activate Hsf1, thus showing the consistent requirement for concurrent protein synthesis for Hsf1 activation (Morano *et al.*, 2012; West *et al.*, 2012).

As Hsf1 is a stress-induced transcription factor, we asked whether the effect of CHX was specific, or had a general effect on stress-induced transcriptional responses. Many of the conditions we analyzed also activate the general environmental stress response, controlled by the transcription factors Msn2/4 (Gasch *et al.*, 2000; Sadeh *et al.*, 2011). We found that the Msn2/4 target transcript *HSP12*

accumulates normally when cells are pretreated with CHX (Figure 1A). These data suggest that translation inhibition by CHX blocks Hsf1 activation across a broad range of proteotoxic conditions in yeast, yet not through a general defect in transcriptional stress responses.

As CHX inhibits translocation of actively engaged ribosomes and thus may have anomalous consequences, we down-regulated translation using an orthogonal strategy, the TOR inhibitor rapamycin, which represses translation initiation (Barbet *et al.*, 1996; Loewith and Hall, 2011). Wild-type cells were pretreated for 30 min with rapamycin prior to heat shock, which completely blocked accumulation of an Hsf1 activity reporter (Figure 1B). Previous work has argued that TOR directly regulates Hsf1 activity via phosphorylation (Chou *et al.*, 2012; Millson and Piper, 2014). Therefore, to determine whether the block of Hsf1 activation by rapamycin pretreatment was a consequence of altered Hsf1 phosphorylation, we repeated the experiment with a mutant Hsf1 where all serine and threonine residues are mutated to the nonphosphorylatable residue alanine, except within the DNA-binding domain S225. In response to heat shock, this mutant protein shows no signs of phosphorylation, while retaining its ability to activate Hsf1-dependent genes (Zheng *et al.*, 2016). We found that rapamycin likewise blocked Hsf1 activation in this mutant strain, arguing against a role for Hsf1 phosphorylation (Figure 1B). Together, these data demonstrate that Hsf1 activation is predicated on concurrent protein synthesis.

Extreme ethanol stress rescues Hsf1 activation in the absence of protein synthesis

Next we asked whether blocking translation prevents Hsf1 activation indirectly by preventing synthesis of a specific protein product required for the Hsf1 activation mechanism, rather than new proteins being the putative source of Hsf1-activating misfolded proteins. We asked whether Hsf1 could function at all without translation. In mammalian cells, the sulfhydryl alkylating agent iodoacetamide can be titrated to a high level that rescues Hsf1-binding activity in the presence of CHX (Baler *et al.*, 1992; Liu *et al.*, 1996). However, as Hsf1 DNA binding and transcriptional activation can be uncoupled (Giardina and Lis, 1995; Zuo *et al.*, 1995; Voellmy, 2004), we searched for a condition that would rescue the transcriptional activity of Hsf1. Ethanol is a protein denaturant in vitro and in vivo (Kato *et al.*, 2019), so we treated yeast cells with increasing amounts of ethanol, in the presence or absence of CHX to block translation. In the absence of CHX, Hsf1 was robustly activated by 5% and 7.5% ethanol. At 10% ethanol no transcription occurs as the cells are likely stressed beyond recovery. CHX pretreatment for 3 min robustly blocked accumulation of Hsf1 target transcripts by 5% ethanol. However, Hsf1 was fully competent for activation by 7.5% ethanol, even with CHX pretreatment (Figure 2). This demonstrates that Hsf1 is competent for activation in the absence of translation in this more extreme unfolding condition, likely as a result of increased unfolding of mature proteins. This is likely true for additional extreme versions of proteotoxic stressors tested in Figure 1, such as heat shock, though this remains to be tested. Importantly, this result argues against models requiring the concurrent translation of a signaling protein to activate Hsf1 and rather argues in favor of newly synthesized proteins misfolding to necessitate Hsf1 activation in proteotoxic conditions.

Diminished protein aggregation in the absence of translation

Though Hsf1 is considered a sensor of aberrant proteins, we wanted to test directly whether in the absence of translation there was

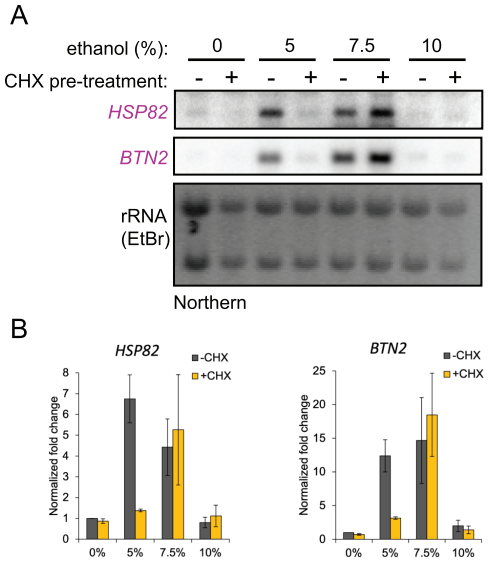


FIGURE 2: Rescue of Hsf1 activation in the absence of translation by titrating ethanol. Midlog phase cells were treated with 0.2% vol/vol DMSO (–) or 200 µg/ml CHX (+) for 3 min. The cultures were then split and treated with the indicated final concentration of ethanol for 20 min. Shown are Northern blots for Hsf1-dependent transcripts (purple). Quantification of *HSP82* and *BTN2* expression shows average (bar height) and range of two replicates (error bars), with values normalized to *25S rRNA* loading control and setting the -CHX, 0% sample to a value of 1. The experiment was replicated twice.

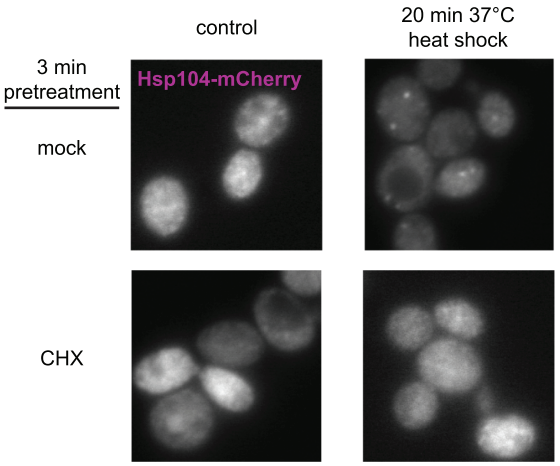
diminished protein aggregation in cells experiencing heat shock. We used the disaggregase protein Hsp104 as a marker for protein aggregate formation, as it nonspecifically binds to aggregated proteins (Glover and Lindquist, 1998; Tkach and Glover, 2004; Kaganovich *et al.*, 2008; Zhou *et al.*, 2014). In heat shock, Hsp104 foci are rapidly formed (Figure 3). Yet if cells are pretreated with CHX for 3 min, Hsp104 foci do not form, consistent with other measurements (Zhou *et al.*, 2014; Masser *et al.*, 2019) (Figure 3). These data suggest that when translation is not active, there is a reduction in protein aggregation, further supporting the model that Hsf1-activating proteotoxic stress is linked to concurrent protein synthesis.

Slow growth rate attenuates Hsf1 activation

Slowly growing yeast cells naturally experience lower protein synthesis levels (Metzl-Raz *et al.*, 2017). We asked whether slow-growing cells experience diminished proteotoxic stress compared with rapidly proliferating cells. We grew cells in glucose or glycerol containing medium, resulting in doubling times of 1.6 and 3.7 h, respectively. Cells were exposed to a variety of proteotoxic conditions. Strikingly, cells grown in glycerol showed reduced accumulation of Hsf1 target transcripts in all stress conditions tested, including in heat shock, proteasome inhibition, and oxidative stress (Figure 4A). Importantly, cells grown in glycerol did not have lower amounts of Hsf1 protein present, and the basal abundance of Hsf1 targets is not higher in glycerol (Figure 4, B and C). In sum, these data provide a physiological context that recreates the link between concurrent protein synthesis and proteotoxic load in stress conditions.

Interfering with the processing of newly synthesized proteins activates Hsf1

These results lead to the model that newly synthesized proteins elicit proteotoxic stress that activates Hsf1 activation. An expectation of this model is that Hsf1 should be activated when the



Pre-treatment:	Mock (spots/cells)	CHX (spots/cells)
Replicate A	2.5	0.1
Replicate B	2.7	0.0

FIGURE 3: Diminished heat shock-induced protein aggregation in the absence of translation. Cells expressing a C-terminal mCherry fusion of Hsp104 were treated with 0.2% DMSO (mock) or 200 µg/ml CHX for 3 min and then either maintained at 30°C or instantaneously shifted to 37°C for 20 min. (Right) Quantification from two replicate experiments for cells pretreated with mock or CHX prior to heat shock. The total number of foci in each field was counted and divided by the number of cells in the field.

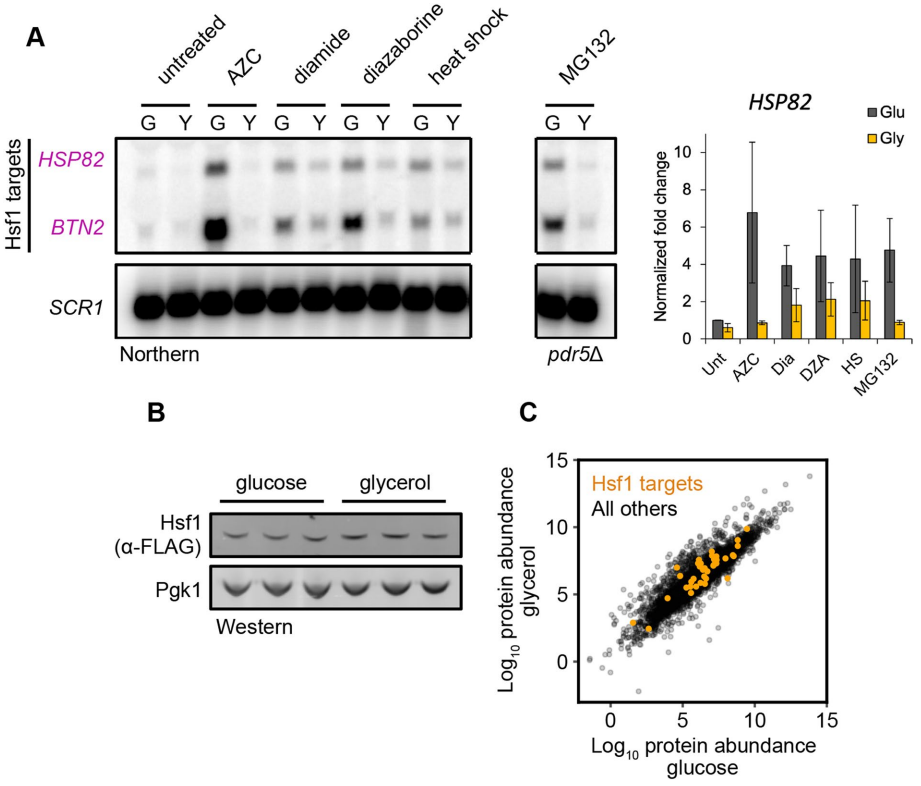


FIGURE 4: Attenuated Hsf1 activation recreated in slow- versus fast-growing cells. (A) Cells were grown to midlog in either 2% glucose (G, 1.6 h doubling time) or 2% glycerol (Y, 3.7 h doubling time) and exposed to the indicated stressors (same parameters as Figure 1) for 30 min. A *pdr5*Δ strain was used for MG132 treatment to facilitate uptake. Shown are Northern blots for Hsf1-dependent transcripts (purple). Quantification of *HSP82* expression shows average (bar height) and range of two replicates (error bars), with values normalized to *SCR1* loading control and setting the untreated, glucose-grown sample to a value of 1. The experiment was replicated twice. (B) Cells were grown to midlog in 2% glucose or glycerol and the abundance of Hsf1-FLAG-V5 was assessed by Western blot. Shown are three biological replicates for each condition. (C) Mass spectrometry-proteomics data for cells grown in glucose and glycerol, normalized to parts per million and shown in $\log_{10}$. Hsf1 targets are shown in orange. Data are from Paulo *et al.* (2016)

processing of new proteins is interfered with. In an earlier study, we showed that preventing the assembly of newly synthesized ribosomal proteins into ribosome complexes results in the aggregation of these orphan proteins and subsequent Hsf1 activation (Tye *et al.*, 2019). We analyzed the results of two other studies where the processing of new proteins was disrupted: the import of newly synthesized proteins into the endoplasmic reticulum (Costa *et al.*, 2018) or mitochondria (Weidberg and Amon, 2018). In both of these cases, a similar signature of Hsf1 activation is observed (Figure 5). The results from interfering with endogenous proteins expand on prior work showing that inducing overexpression of an exogenous mutant aggregation-prone protein is sufficient to activate Hsf1 (Geiler-Samerotte *et al.*, 2011). Together, these data demonstrate three independent contexts—ribosomal protein assembly, mitochondrial protein import, and ER protein import—where orphan newly synthesized proteins seem to be sufficient to drive proteotoxic stress.

DISCUSSION

We envision two potential nonexclusionary models to explain the link between concurrent protein synthesis and Hsf1 activation/protein aggregation in proteotoxic stress. First, nascent and newly synthesized proteins are key substrates of chaperones and the proteasome. Therefore, in the absence of concurrent protein synthesis, there may be an increased availability of chaperones and proteasomes that would obviate the

need for Hsf1 activation by a proteotoxic stressor. Alternatively, newly synthesized proteins could be those most prone to misfold/aggregate and thus elicit Hsf1 activation on stress. On extreme stress conditions, such as high concentrations of ethanol, a greater pool of proteins unfold and contribute to Hsf1 activation (Beckmann *et al.*, 1992). The second model posits that the principal signal that activates Hsf1 in a condition such as heat shock would be the aggregation of newly synthesized proteins, rather than the unfolding and subsequent aggregation of mature proteins.

A number of lines of evidence point to the prospect that newly synthesized proteins, rather than mature proteins, underlie Hsf1 activation. Using heat shock as a prototype, the energetic input required to transiently unfold mature proteins and expose hydrophobic segments that can either self-associate and form aggregates, or directly engage the activity of chaperones such as Hsp70, would be expected to be a greater barrier than for nascent or incompletely folded proteins. Consistent with this, human Hsp70 remains engaged with newly synthesized proteins for an extended period of time after heat stress (Beckmann *et al.*, 1992). As the temperature increases, the proportion of mature proteins that engage Hsp70 increases, suggesting that at lower temperatures, newly synthesized proteins are likely the dominant species associated with Hsp70. Consistent with this, newly synthesized proteins appear to be the predominant induced clients of Hsp70 during heat shock in yeast (Masser *et al.*, 2019). While we observed diminished heat-induced protein aggregation in the absence of translation, at more extreme temperatures, protein aggregation can be detected biochemically even in the absence of ongoing translation (Wallace *et al.*, 2015). Further, in yeast, newly synthesized proteins seed protein aggregate formation (Zhou *et al.*, 2014) and are the predominant target of stress-induced protein degradation by the ubiquitin-proteasome system, whereas mature proteins are spared (Medicherla and Goldberg, 2008). In sum, our findings point to newly synthesized proteins being particularly labile to various forms of stress, and the potential for such proteins to underlie Hsf1 activation is seen in three distinct scenarios (Figure 5).

Each stress analyzed that activates Hsf1 in a translation-dependent manner (Figure 1) is likely linked to newly synthesized proteins in different ways. AZC results directly in misfolding and aggregation of newly synthesized proteins when it is incorporated in lieu of proline into nascent chains. Indeed, a large increase in ubiquitination can be seen on nascent chains in AZC-treated cells (Duttler *et al.*, 2013). Diazaborine interferes with the assembly of newly synthesized ribosomal proteins (Loibl *et al.*, 2014). Heat shock and ethanol, on the other hand, may biochemically interfere with the folding of nascent chains by decreasing the enthalpic advantage of burying hydrophobic segments, allowing for interaction of exposed hydrophobic segments between incompletely folded polypeptides (Herskovits *et al.*, 1970). A key job of the proteasome is to clear out a subset of newly synthesized proteins, such as those protein complex subunits that are produced in stoichiometric excess (Duttler *et al.*, 2013; McShane *et al.*, 2016). Thus, inhibition by MG132 may result in accumulation of these orphan proteins that were destined for degradation. Diamide likely causes protein aggregation by interfering with the formation of structurally critical disulfide bonds and introducing bulky cysteine adducts during protein synthesis and maturation (Jansens *et al.*, 2002; Cumming *et al.*, 2004; Pöther *et al.*, 2009).

The link between the level of protein synthesis and the cellular experience of proteotoxic insults has important implications for cell physiology. In one regard, inhibiting translation in cancer cells leads to inactivation of constitutively activated Hsf1 (Santagata *et al.*, 2013). This may therefore be the result of cancer cells having higher than normal protein synthesis levels, which render cells reliant on increased Hsf1 activity (Puustinen and Sistonen, 2020). Indeed, imbalance in newly synthesized protein components has been proposed to underlie the proteotoxic stress and sensitivity of aneuploid cells to drugs targeting proteostasis factors (Torres *et al.*, 2007; Oromendia *et al.*, 2012; Dephoure *et al.*, 2014). The need for elevated Hsf1 activity with elevated levels of protein production suggests that protein synthesis is a liability to the integrity of the proteome.

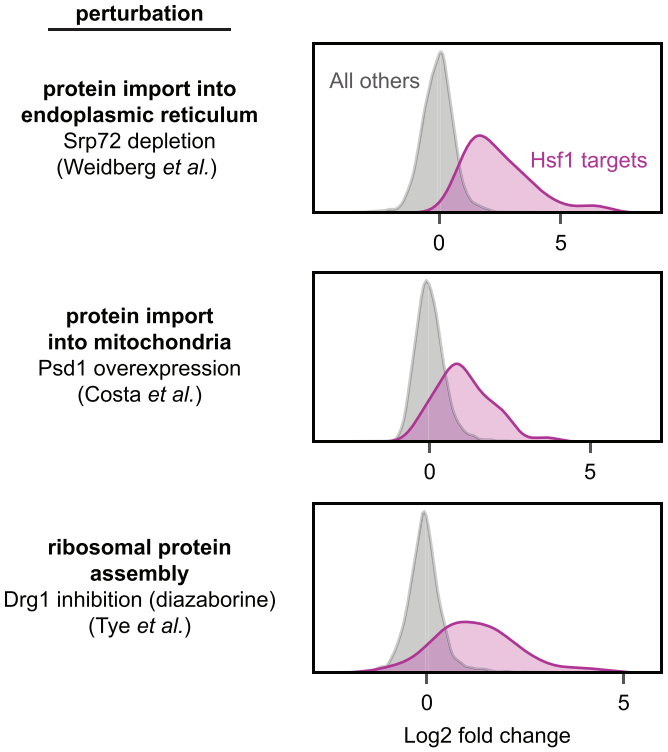


FIGURE 5: Interfering with organellar import or assembly of newly synthesized proteins activates Hsf1. Density plot of gene expression data for the indicated perturbation for Hsf1 targets ($n = 42$) and all other transcripts. "Protein import into endoplasmic reticulum:" ribosome profiling data after 30 min of depleting the signal recognition particle protein Srp72 by auxin-inducible degradation (Costa *et al.*, 2018). "Protein import into mitochondria:" RNA-seq data after 4 h of overexpressing Psd1 (Weidberg and Amon, 2018). "Ribosomal protein assembly:" RNA-seq data after 15 min of Drg1 inhibition (diazaborine) treatment (Tye *et al.*, 2019).

MATERIALS AND METHODS

Yeast cell growth

Saturated overnight cultures were grown in yeast extract-peptone-2% glucose overnight, back-diluted into fresh medium containing either 2% glucose or 2% glycerol as indicated, and grown to midlog. For CHX treatment, cultures were split, and one half was treated with 0.2% DMSO (vehicle) and the other half was treated with a final concentration of 200 µg/ml CHX (from 100 mg/ml stock) for 3 min. Cells were then exposed to stressors as indicated in the figure legends. Rapamycin was used by treating cells for 30 min with a final concentration of 200 ng/ml rapamycin freshly prepared in ethanol. Heat shock was performed by addition of an equal volume of 44°C medium and shifting to a 37°C incubator.

Yeast strains

Figures 1A, 2, and 4 used a BY4741 strain with the Hsf1 reporter *4xHSE::mVenus::LEU2* integrated at the *LEU2* locus (strain YBT256; Tye *et al.*, 2019), with the exception of the MG132 experiments performed by transforming the same reporter construct into *pdr5*Δ from the haploid deletion collection (strain YBT257). Figure 1B used previously reported W303a derivatives (Zheng *et al.*, 2016): wild-type *HSF1* with the *4xHSE::mVenus* reporter (strain DPY304, courtesy of David Pincus, University of Chicago); nonphosphorylatable *HSF1* with reporter (all serine/threonine mutated to alanine, except the DNA-binding S225, strain DPY416; David Pincus). Figure 3 used a strain that had the endogenous *HSP104* locus tagged with a C-terminal *mCherry::HIS3* cassette (strain YBT230). The Hsf1 Western blot used a strain containing a C-terminal FLAG-V5 tag (Zheng *et al.*, 2016) (David Pincus).

Total RNA extraction and Northern blotting

RNA was extracted and analyzed by Northern blot as previously described (Tye *et al.*, 2019). Quantification was performed using ImageJ.

Total protein extraction and Western blotting

Proteins were extracted and analyzed by Western blot as previously described (Tye *et al.*, 2019). Hsf1-FLAG-V5 was detected using mouse anti-FLAG (Millipore Sigma, F1804, 1:1,000). Pgk1 was detected using mouse anti-Pgk1 (Abcam, ab113687, 1:10,000).

Fluorescence microscopy

Cells expressing Hsp104-mCherry were treated with 1/10th vol of 37% formaldehyde for 10 min and washed twice in phosphate-buffered saline. Samples were mounted onto 2% agarose pads on a glass slide and covered with a glass coverslip. Images were acquired on a Nikon Ti2 microscope with a 100× objective and an ORCA-R2 cooled CCD camera (Hamamatsu).

Genomics data analysis

For Srp72 depletion, ribosome profiling data in Supplemental Table S3 from Costa *et al.* (2018) were used, and the fold change values were determined by dividing the 30 min auxin time point ("sec63BirA_srp72-AID_30mAuxin_2mCHX_2mBiotin_input_rpkm") by the 0 min auxin time point ("sec63BirA_srp72-AID_0mAuxin_2mCHX_2mBiotin_input_rpkm"). For Psd1 overexpression, RNA-seq data in Supplemental Table S1 from Weidberg and Amon (2018), column "GalPsd1.Empty.logFC," were used. "Hsf1 targets" and "All others" gene groups were defined as in Tye *et al.* (2019)

Mass spectrometry-proteomics data analysis

Data from Paulo *et al.* (2016), Supplemental Table S2, were normalized by dividing the sum of signal for each condition, dividing each protein in that condition by that sum, and multiplying by 1,000,000 to get parts per million (referred to as "protein abundance"). Hsf1 targets are from the same list as above.

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