Biochemical and Biophysical Research Communications 457 (2015) 473–478

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Sir2 links the unfolded protein response and the heat shock response in a stress response network

Esther Weindling, Shoshana Bar-Nun*

Department of Biochemistry and Molecular Biology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel

ARTICLE INFO

Article history:
Received 23 December 2014
Available online 16 January 2015

Keywords:
Hsf1
HSR
Sir2
UPR

ABSTRACT

The Heat Shock Response (HSR) in the cytosol and the Unfolded Protein Response (UPR) in the endoplasmic reticulum are major pathways of the cellular proteostasis network. In *Saccharomyces cerevisiae*, HSR is regulated by transcription factor Hsf1, and UPR Ire1 branch activates transcription factor Hac1. Here we demonstrate systemic regulation of proteostasis through a direct link between UPR and HSR. Hsf1 is activated by UPR and its HSR depends on intact UPR. This link is mediated by Sir2, which is not only essential for Hsf1 HSR but also required for Hsf1 activation by UPR. Excess Sir2 augments Hsf1 activation by UPR and can compensate for its impairment in UPR-defective strains. Sir2 is upregulated by UPR but, in turn, it also attenuates this pathway, ensuring that UPR functions only transiently.

1. Introduction

Proteostasis assists and maintains correct protein folding in cells that face physical and chemical challenges and stresses and is, therefore, crucial to cellular health and survival. It alleviates misfolded protein load by improving protein folding through induction of molecular chaperones, by attenuating protein translation, and by enhancing degradation of terminally misfolded proteins. Proteostasis is controlled in a cell-autonomous manner, and is generally accepted to be activated by compartment-specific stress response pathways. Among these are the Heat Shock Response (HSR) in the cytosol and nucleus, the Unfolded Protein Response (UPR) in the endoplasmic reticulum (ER), and the UPR in the mitochondria, all coordinated in a network [1–6].

The HSR [7–9] is regulated in *Saccharomyces cerevisiae* by a single transcription factor, heat shock factor 1 (Hsf1), encoded by the essential *HSF1* gene. Hsf1 is conserved from yeast to man and it functions by binding to heat shock elements (HSEs) in the promoter region of its many target genes. In *S. cerevisiae*, HSE is composed of at least three inverted repeats of the nGAAn/nTTCn module and it binds Hsf1 as a homo-trimer. As a major regulator of stress responses, Hsf1 orchestrates many stimuli and, in addition to heat shock, it responds to oxidative stress, glucose starvation, ethanol exposure and osmotic stress. In metazoans, Hsf1 resides in the cytosol in association with Hsp90 and its activity is repressed. In response to stress, Hsf1 dissociates from Hsp90, translocates to the nucleus, trimerizes and binds to HSEs. In *S. cerevisiae*, however, Hsf1 resides exclusively in the nucleus as a trimer that is constitutively bound to HSEs. Hence, although biochemical and genetic evidence suggest that the Hsp90 complex represses Hsf1 activity also in yeast, dissociation of Hsf1 from this chaperone is accompanied neither by translocation to the nucleus nor by homotrimerization. The Hsp70 Ssa1 is another chaperone implicated in Hsf1 regulation; two conserved cysteines in Ssa1, which represses Hsf1 in the absence of stress, are modified by thiol-reactive compounds but not by heat shock, demonstrating that Ssa1 can discriminate between two distinct environmental stresses [10]. In addition, cooperative Hsf1-HSE interactions are strengthened and stabilized by Hsf1 hyperphosphorylation, with distinct patterns and kinetics in response to either heat shock or oxidative stress [11].

The ER UPR is part of the quality control apparatus that inspects nascent proteins in the secretory pathway and scrutinizes their glycosylation, folding and maturation. UPR is activated when unfolded proteins within the ER exceed the capacity of the ER proteostasis machinery [1,4,12]. Of the three UPR branches in metazoans, characterized by the ER membrane proteins Ire1, PERK/PEK and ATF6, only the Ire1 branch is conserved in *S. cerevisiae* [13,14]. Ire1 is a kinase/endoribonuclease whose endoribo-

Abbreviations: DTT, dithiothreitol; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated degradation; HSE, heat shock element; Hsf1, heat shock factor 1; HSR, heat shock response; TM, tunicamycin; UPR, unfolded protein response; UPRE, unfolded protein response element.

* Corresponding author.
E-mail address: shoshbn@tauex.tau.ac.il (S. Bar-Nun).

http://dx.doi.org/10.1016/j.bbrc.2015.01.021

nucleolytic activity is unleashed upon Ire1 dimerization and trans-autophosphorylation. This endoribonucleolytic activity is involved in the nonconventional splicing of mRNAs in the cytoplasm, most notably of XBP1 in metazoans and of HAC1 in *S. cerevisiae*. HAC1 mRNA is constitutively expressed, but cannot be translated because of a 3′ hairpin that is removed by activated Ire1. Following ligation by the Trl1 RNA ligase, HAC1 mRNA translation can proceed, expressing Hac1 as a functional transcriptional activator. Hac1 translocates to the nucleus, binds to UPR elements (UPREs; CAGNGTG) found in the promoter region of its many target genes, activating the large UPR transcriptional program. Among the Hac1-induced genes are ER chaperones and components of the ER-associated degradation (ERAD) machinery, which dispose of the accumulated misfolded proteins, thus alleviating ER stress.

Here we demonstrate that proteostasis is systemically regulated by showing that UPR and HSR are intimately linked through Sir2. In fact, intact UPR as well as Sir2 are required for Hsf1 HSR but not for Hsf1 activation by oxidative stress. Sir2 expression is upregulated by UPR and excess Sir2 augments Hsf1 activation by UPR and compensates for its impairment in UPR-defective strains. Yet, Sir2 ensures that UPR functions only transiently by attenuating this pathway. Thus, by connecting UPR and HSR, Sir2 establishes an inter-organellar stress response regulatory network.

2. Materials and methods

2.1. Strains and plasmids

The *S. cerevisiae* wild-type strains used in this study were BY4741 (*MAT*a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and W303−1b (*MATα ura3-52 trp1D2 leu2−3112 his3−11 ade2−1 can1−100*). Mutants deleted in individual non-essential genes generated from BY4741 [15] included *ire1*Δ, *hac1*Δ and *sir2*Δ. A plasmid for expressing excess *SIR2* (pCLW21) [16] was a kind gift from Rolf Sternglanz (Stony Brook University, USA), and a plasmid for expressing excess *HSF1* (pAKS80) was generously provided by Dennis Winge (University of Utah, USA). Endogenously expressed GFP-tagged proteins library [17] was used to measure GFP as readout of proteins levels. HSR was monitored with the HSE2-*lacZ* construct (GA1695) [18], containing synthetic HSE2 (ctaGAAgcTTCtaGAAgcTTCtagaggatccccg), generously provided by Ian Dawes (University of New South Wales, Australia). UPR was monitored with the UPRE-*lacZ* construct (tcgaGGAACTGGACAGCGTGTCGAAA) [13] generously provided by Randy Schekman (Berkeley University, USA).

2.2. HSR and UPR conditions

Yeast was grown in synthetic complete media containing 2% (w/v) glucose, and drop-out media were used for selecting transformants. Cells were grown at 30 °C in 20 ml medium in 100 ml loosely-capped bottles with constant shaking (200 rpm). Overnight grown cells were used to inoculate fresh cultures to logarithmic phase (0.2−0.8 A_{600}; 1 $A_{600} = 1.5x10^7$cells/ml). For HSR, cells were incubated for 20 min at 42 °C; for UPR, cells were incubated for 1 h with either 5 μg/ml TM or 6 mM DTT; for oxidative stress, cells were incubated for 30 min with 3 mM H_2O_2.

2.3. β-galactosidase assay

In this assay [19], *ortho*-nitrophenol produced in cell lysate from *ortho*-nitrophenyl-β-galactoside was measured as absorbance at 420 nm ($\varepsilon_{420} = 4500\ M^{-1}\ cm^{-1}$; Genesys 10UV spectrophotometer), protein concentration was determined with Bradford reagent, and β-galactosidase specific activity was calculated as nmol *ortho*-nitrophenol/min/mg protein.

2.4. Levels of GFP-tagged proteins

The levels of GFP-tagged proteins [17] were estimated by fluorescence measurements. Cell pellets were resuspended in water, their concentration was determined as A_{600}, and 0.5 ml aliquots were added to 48-wells plate. GFP fluorescence was measured in a Biotek Synergy plate reader, with excitation at 485 nm and emission at 528 nm. Samples were shaken for 20 s prior to reading and measurements were obtained at gain 100. Values were normalized to the cells concentration, and fluorescence of parental cells not expressing any GFP was subtracted.

3. Results

3.1. Hsf1 is activated by UPR and requires intact UPR for its HSR

Our interest in a possible link between HSR and UPR was initiated by our findings that HSR [20] as well as UPR (data not shown) deteriorated upon yeast aging. Proteostasis, and stress responses in particular, decline with aging, and are thought to be a major cause for the accumulation of damaged, misfolded and aggregated proteins [5,21–23]. Hence, although sensing of misfolded proteins is compartmentalized, we hypothesized that HSR and UPR may be linked in a systemically coordinated stress response network. To monitor either HSR or UPR, yeast was transformed with reporter constructs specific for a single response, either HSE2-*lacZ* [18] or UPRE-*lacZ* [13], respectively, and expression of the reporters was measured by their β-galactosidase activity. We deliberately did not follow the expression of endogenous targets because many such genes appear to be regulated by both HSR and UPR [24], as initially reported for the *KAR2/BIP* gene, an established UPR target that contains both UPRE and HSE in its promoter [13]. To elicit HSR, yeast grown at 30 °C was heat-shocked at 42 °C for 20 min. To elicit UPR, cells were exposed to either tunicamycin (TM), which activates UPR by inhibiting N-linked glycosylation occurring only within the ER lumen [13], or to dithiothreitol (DTT), which prevents disulfide bonds formation prevalent within the ER lumen [25]. In yeast harboring UPRE-*lacZ*, similar activities of β-galactosidase were measured upon incubation with TM for either 30 min or 4 h, and slightly lower activation was achieved by DTT, with no difference between 1 or 2 h incubation (data not shown). In subsequent experiments, cells were incubated with TM or DTT for 1 h.

To address a possible link between HSR and UPR, we first showed that UPRE-*lacZ* responded to HS, as expected from heat-induced accumulation of misfolded proteins throughout the cell, including within the ER. This response depended on intact UPR because it was abolished in strains lacking *IRE1* or *HAC1* (Fig. 1A). We next examined the less obvious possibility and showed that HSE2-*lacZ* responded to UPR elicitors (Fig. 1C). Since neither TM nor DTT are known to affect protein folding outside the secretory pathway, we asked whether these compounds activated Hsf1 via UPR or affected this transcription factor directly. Therefore, we followed the activation of Hsf1 by TM or DTT in the UPR-defective strains. Clearly, both *ire1*Δ and *hac1*Δ strains were defective in UPR, as UPRE-*lacZ* did not respond to DTT (Fig. 1B). More importantly, TM hardly activated Hsf1 in either UPR-defective mutant, although it enhanced HSE2-*LacZ* response >2-fold in wild-type cells (Fig. 1D). These results indicate that TM activates Hsf1 via UPR and does not affect this transcription factor directly. Similar data were obtained with DTT, although its effect on Hsf1 activation was strongly attenuated, but not totally abolished, in the UPR-defective strains (Fig. 1D). It should be noted that unlike TM, which generates

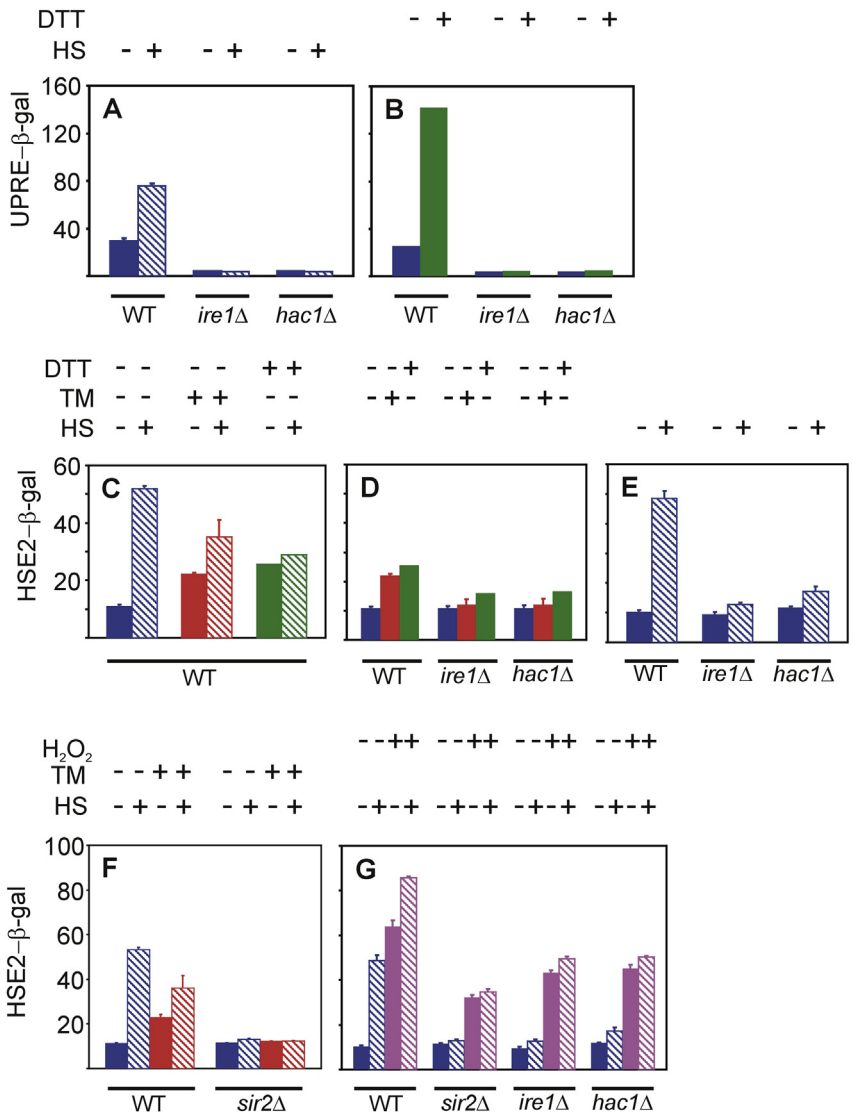

Fig. 1. Hsf1 activation by either HS or UPR is impaired in UPR-defective cells, mediated by Sir2 and restricted to HSR. Wild-type, *ire1*Δ, *hac1*Δ or *sir2Δ* BY4741-derived cells, harboring the UPRE-*LacZ* plasmid (A,B) or the HSE2-*LacZ* plasmid (C–G) were incubated for 1 h with (+) or without (−) TM (C,D,F) or DTT (B–D), or for 30 min with (+) or without (−) H_2O_2 (G). Then, cells were further incubated for 20 min either at 30 °C (−) or heat shocked (HS) at 42 °C (+) (A,C,E-G). UPR (A,B) and Hsf1 activation (C–G) were monitored as β-galactosidase specific activity. Results are mean + SEM of 3 independent experiments.

misfolded proteins only within the ER lumen and therefore, TM signaling depends entirely on the Ire1/Hac1 axis, DTT likely causes some protein misfolding also in the cytosol and nucleus, thus may activate Hsf1 in a fashion requiring neither Ire1 nor Hac1. Moreover, DTT may also activate Hsf1 directly by altering the redox state of cysteines, either within Hsf1 itself, as shown for mammalian Hsf1 [26], or within its associated chaperone Ssa1 [10]. Even more surprising was our observation that Hsf1 activation by heat shock was nearly obliterated in the *ire1*Δ or *hac1*Δ mutants (Fig. 1E; ~5-fold induction by heat shock in wild-type cells vs. ~1.4-fold change in the UPR-defective strains). This result demonstrates that also the HSR of Hsf1 requires intact UPR.

3.2. Sir2 links UPR and HSR

Inasmuch as TM does not activate Hsf1 directly but via UPR, and HSR of Hsf1 also requires intact UPR, we hypothesize that component(s) essential for HSR are regulated by UPR. Sir2 came to mind because TM and excess Sir2 exerted similar effects on Hsf1. We have recently shown that Sir2 is essential for HSR, and overexpression of Sir2 mimics HSR, while Hsf1 is hardly activated further when excess Sir2 and heat shock are combined (Fig. 1F and Fig. 2A,B; [20]). Likewise, the UPR elicitors activated Hsf1 but attenuated the effect of heat shock on Hsf1 activity (Fig. 1C, and wild-type cells in Fig. 1F). This led us to examine the potential role of Sir2 in the UPR-dependent activation of Hsf1. Indeed, Hsf1 activation by TM was abolished in *sir2*Δ cells, whether or not heat shock was also applied (Fig. 1F). To corroborate the involvement of Sir2 in Hsf1 activation by UPR, we tested another Sir2-independent mode of Hsf1 activation. We previously showed that Hsf1 activation by oxidative stress is maintained in *sir2*Δ strain (Fig. 1G; [20]). Here, Hsf1 activation by H_2O_2 in the UPR-defective *ire1*Δ or *hac1*Δ mutants was as effective as in wild-type yeast or in the *sir2*Δ strain (Fig. 1G). Moreover, the persistence of Hsf1 activation by oxidative stress and lack of HSR in the UPR-defective mutants was phenocopied in the *sir2*Δ cells (Fig. 1G), lending further support to the notion that Sir2 links the UPR and HSR.

It is plausible that Sir2 expression is regulated by UPR and when this pathway is defective, Sir2 declines to limiting levels that impair HSR. To test this hypothesis, excess *SIR2*, driven by its authentic

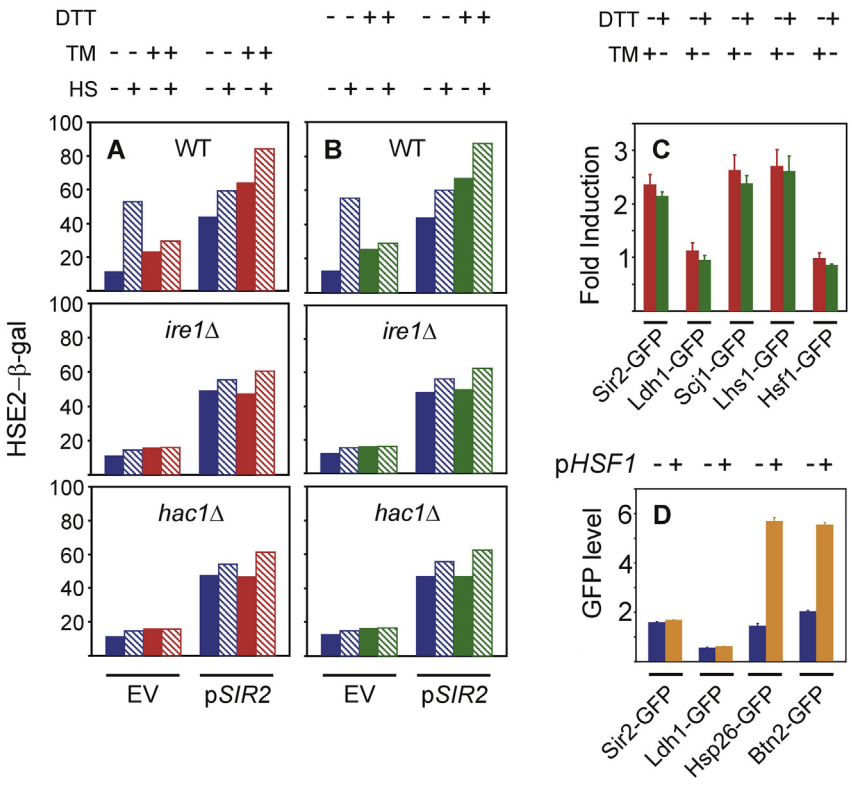

Fig. 2. *SIR2* is upregulated by UPR, while excess *SIR2* compensates for impaired HSR in UPR-defective strains and further enhances HSR upon UPR activation. Wild-type, *ire1*Δ or *hac1*Δ BY4741-derived cells, harboring the HSE2-*LacZ* plasmid, were transformed with an empty vector (EV) or with a p*SIR2* plasmid. Cells were incubated for 1 h with (+) or without (−) TM (A) or DTT (B). Then, cells were further incubated for 20 min either at 30 °C (−) or heat shocked (HS) at 42 °C (+). Hsf1 activation was monitored as β-galactosidase specific activity. BY4741-derived cells expressing the indicated GFP-tagged proteins were either incubated for 1 h with (+) or without (−) TM or DTT (C) or transformed with an empty vector (−) or with a p*HSF1* plasmid (pAKS80 (+) (D)). Levels of GFP-tagged proteins were measured by GFP fluorescence, as described in Materials and methods. Results, given as fold-induction over untreated cells (set as 1 (C)) or as GFP fluorescence ($\times 10^{-3}$; (D)), are mean + SEM of 4 independent experiments.

promoter regions [16], was expressed from a plasmid in wild-type and in the UPR-defective *ire1*Δ or *hac1*Δ strains. Excess *SIR2* mimicked HSR, as shown in wild-type cells that responded to heat shock, but strikingly it also compensated for the impaired HSR in the UPR-defective mutants (Fig. 2A,B). The link between Sir2 and UPR was further supported by the effect of TM or DTT on cells overexpressing *SIR2*. Clearly, only in wild-type cells was the effect of excess *SIR2* on Hsf1 activation further augmented by TM or DTT, indicating that this effect was absolutely dependent on UPR. In the UPR-defective *ire1*Δ or *hac1*Δ strains, excess *SIR2* could mimic HSR, but Hsf1 activation was not further augmented by TM or DTT (Fig. 2A,B). Taken together, these results establish a direct link between UPR and HSR via Sir2.

3.3. Sir2 is upregulated by UPR and attenuates the UPR pathway

To examine directly whether Sir2 expression was regulated by UPR, we exposed to TM or DTT cells from the library of endogenously expressed GFP-tagged proteins [17]. These included the tested Sir2, the Hsp70 Lhs1 and Hsp40 Scj1 as known ER UPR targets, Hsf1 for comparison, and Ldh1 as a negative control. We measured GFP fluorescence as readout for the expression of these GFP-tagged proteins. Relative to unchallenged cells, TM or DTT treatment increased Sir2-GFP levels 2–2.5-fold, to the same extent as the known UPR targets Lhs1-GFP or Scj1-GFP (Fig. 2C). Neither Hsf1-GFP nor Ldh1-GFP was affected by these elicitors (Fig. 2C). While UPR upregulated Sir2 expression, which, in turn, activated Hsf1, this regulation was unidirectional. Overexpressing *HSF1* from a plasmid, which based on GFP fluorescence upregulated the expression of its established GFP-tagged targets Hsp26 and Btn2, had no effect on the levels of Sir2-GFP or Ldh1-GFP (Fig. 2D).

Recently a connection between sirtuins and UPR has been reported in mammalian models, where Sirt1, the closest homolog of yeast Sir2, has been shown to deacatylate, hence deactivate Xbp1, the mammalian homolog of yeast Hac1 [27,28]. To examine whether Sir2 attenuated UPR also in *S. cerevisiae*, we monitored UPR in cells lacking *SIR2* (Fig. 3A) or in cells overexpressing *SIR2* from a plasmid (Fig. 3B). Clearly, in *sir2*Δ cells UPR was activated >3-fold, even in the absence of UPR elicitors, and UPR overshot 13–14-fold by TM or DTT (Fig. 3A). Consistently, neither TM nor DTT elicited UPR in cells overexpressing *SIR2* (Fig. 3B). These results indicate that Sir2, which is upregulated by UPR, attenuates this pathway by acting as a negative regulator of UPR.

4. Discussion

Cells are constantly challenged by physico-chemical insults on their proteome. Thus, stress response mechanisms that sense and initiate programs to correct these insults are fundamental for cell viability. Indeed, in eukaryotes, distinct stress response pathways exist in the cytosol/nucleus (HSR), the ER/secretory pathway and mitochondria (UPR). Although it is generally held that these stress response pathways are activated in a compartment-specific manner, robust proteostasis must connect them into a coordinated inter-organellar network. Previously, a link between HSR and UPR in *S. cerevisiae* was suggested in a report where ER-stressed *ire1*Δ cells were rescued by the constitutively active R206S Hsf1 [24]. The HSR-induced proteins implicated in this relief were

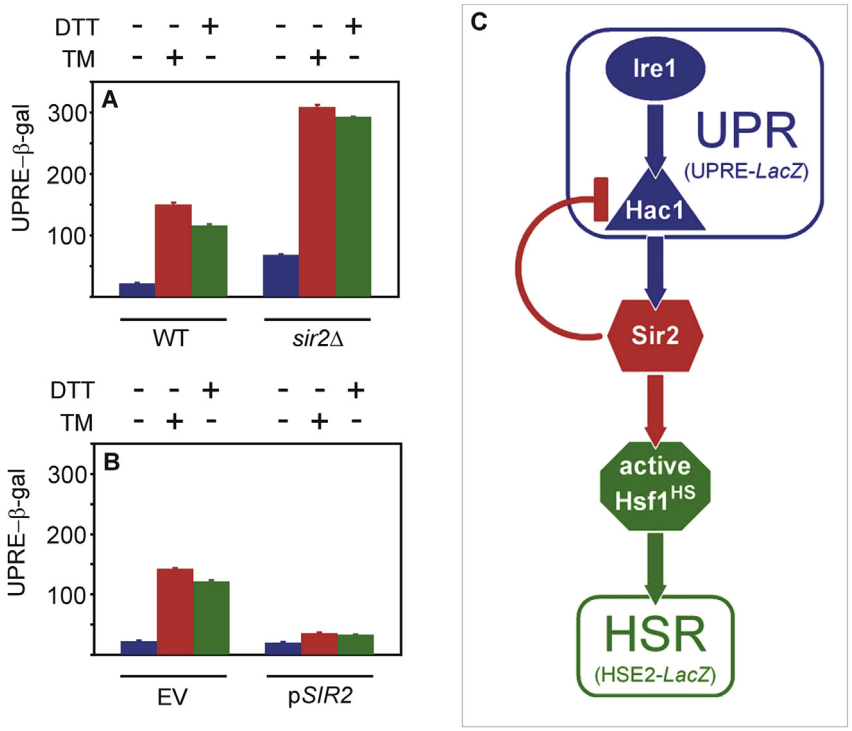

Fig. 3. Sir2 attenuates the UPR. UPRE-*LacZ* harboring wild-type (A,B) or *sir2*Δ (A) BY4741-derived cells were transformed with an empty vector (EV) or p*SIR2* plasmid (B). Cells were incubated for 1 h with (+) or without (−) TM or DTT. UPR was monitored as β-galactosidase specific activity. Results are mean + SEM of 3 independent experiments. (C) Model for Sir2-mediated UPR-HSR link and UPR regulatory loop. Sir2 is upregulated by UPR, it is essential for Hsf1 activation by UPR and HSR, and it negatively regulates UPR.

vesicle-associated Erv29 and ER Kar2/BiP. That study also reported that ER stress activated HSR, but restricted this phenomenon only to UPR-defective cells [24], in contrast to our current findings. In a more recent paper, constitutive HSR activation resulted in increased ER resistance and decreased UPR activation [29]. This phenomenon occurred in wild-type but also in UPR-defective cells, it was only partially dependent on Kar2/BiP overexpression, it did not involve increased proteasomal degradation, and was related to the oxidative stress response. Transcription data suggested that HSR enhanced ER stress resistance mainly through facilitating protein folding and secretion [29].

Here, we demonstrate a regulatory link in the opposite direction by showing that UPR in the ER regulates HSR in the cytosol/nucleus. This inter-organellar cell-autonomous stress response network is reminiscent of the looming cell-nonautonomous transcellular signaling that coordinates stress responses between tissues [30,31]. This systemic cellular regulation of stress responses is supported by a genome-wide comparison of 383 UPR- and 165 HSR-regulated genes [25,32], where at least nine genes, common to both data sets, appear to be regulated by both UPR and HSR [24]. Such dual regulation was initially reported for the *KAR2/BIP* gene, an established UPR target that contains UPRE as well as HSE in its promoter region [13]. A recent paper adds to Kar2/BiP in *S. cerevisiae* a third mode of stress sensing, since this molecular chaperone acts as a sensor for ER redox imbalance [33].

In addition to showing activation of HSR by UPR, we demonstrate that intact UPR is required for HSR and implicate Sir2 in this link (Fig. 3C). Sir2 is essential for Hsf1 activation by HSR, but it is also required for Hsf1 activation by UPR. The role of Sir2 in mediating Hsf1 activation by UPR is restricted to HSR, whereas Hsf1 activation by oxidative stress requires neither Sir2 nor intact UPR. We show that Sir2 is upregulated by UPR and that excess Sir2 augments Hsf1 activation by UPR and can compensate for its impairment in UPR-defective strains. Sir2 as the link between UPR and HSR, and particularly Sir2's upregulation by UPR, were entirely unexpected since Sir2 never came up as a target in UPR transcriptional [25,34,35] or translational [36] screens, suggesting that Sir2 upregulation is post-transcriptional. Interestingly, a hint for Sir2 upregulation by UPR was recently documented in a paper addressing *S. cerevisiae* lifespan extension [35]. In that study, UPR activation by deletion of *ALG12* or *BST1* extended lifespan, a phenomenon that depended on intact UPR. However, overexpression of *SIR2* from a plasmid restored lifespan extension in the UPR-defective *ire1*Δ or *hac1*Δ strains [35]. These findings can be explained if Sir2, a known lifespan effector, is upregulated by the UPR pathway.

Sirtuins have been associated with UPR in mammalian models, where Sirt1, the closest homolog of Sir2, deacatylates hence deactivates Xbp1 [27,28]. Here we show that also in *S. cerevisiae* Sir2 curtails UPR. Being upregulated by UPR, Sir2, in turn, shuts off this pathway (Fig. 3C), possibly by deacetylating Hac1, the yeast homolog of mammalian Xbp1. This Sir2-centered regulatory loop ensures that UPR functions only transiently, preventing deleterious consequences of prolonged UPR. Thus, by linking UPR in the ER with HSR in cytosol/nucleus, Sir2 coordinates a cellular network of stress responses.

Conflict of interest

None.

Acknowledgments

This study was supported by a grant from The Israel Science Foundation (grant no 394/11 to SB). We would like to thank Rolf Sternglanz (Stony Brook University), Ian Dawes (University of New

South Wales), Randy Schekman (Berkeley University) and Dennis Winge (University of Utah) for generously providing strains and plasmids. We would like to thank Joseph Roitelman (Sheba Medical Center) for insightful discussions and challenging suggestions, and for critical reading of the manuscript, Kevin Morano (University of Texas Medical School at Houston) for sharing with us his vast knowledge on Hsf1 and chaperones, and members of our group for helpful suggestions and critical reading of the manuscript.

References

[1] D. Ron, P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response, Nat. Rev. Mol. Cell. Biol. 8 (2007) 519–529.
[2] W.E. Balch, R.I. Morimoto, A. Dillin, J.W. Kelly, Adapting proteostasis for disease intervention, Science 319 (2008) 916–919.
[3] M.A. kerfelt, R.I. Morimoto, L. Sistonen, Heat shock factors: integrators of cell stress, development and lifespan, Nat. Rev. Mol. Cell. Biol. 11 (2010) 545–555.
[4] P. Walter, D. Ron, The unfolded protein response: from stress pathway to homeostatic regulation, Science 334 (2011) 1081–1086.
[5] R.C. Taylor, A. Dillin, Aging as an event of proteostasis collapse, Cold Spring Harb. Perspect. Biol. 3 (2011) a004440.
[6] E.T. Powers, W.E. Balch, Diversity in the origins of proteostasis networks–a driver for protein function in evolution, Nat. Rev. Mol. Cell. Biol. 14 (2013) 237–248.
[7] K.A. Morano, C.M. Grant, W.S. Moye-Rowley, The response to heat shock and oxidative stress in *Saccharomyces cerevisiae*, Genetics 190 (2012) 1157–1195.
[8] J. Verghese, J. Abrams, Y. Wang, K.A. Morano, Biology of the heat shock response and protein chaperones: budding yeast (Saccharomyces cerevisiae) as a model system, Microbiol. Mol. Biol. Rev. 76 (2012) 115–158.
[9] R. Voellmy, F. Boellmann, Chaperone regulation of the heat shock protein response, Adv. Exp. Med. Biol. 594 (2007) 89–99.
[10] Y. Wang, P.A. Gibney, J.D. West, K.A. Morano, The yeast Hsp70 Ssa1 is a sensor for activation of the heat shock response by thiol-reactive compounds, Mol. Biol. Cell. 23 (2012) 3290–3298.
[11] X.D. Liu, D.J. Thiele, Oxidative stress induced heat shock factor phosphorylation and HSF-dependent activation of yeast metallothionein gene transcription, Genes. Dev. 10 (1996) 592–603.
[12] B.M. Gardner, D. Pincus, K. Gotthardt, C.M. Gallagher, P. Walter, Endoplasmic reticulum stress sensing in the unfolded protein response, Cold Spring Harb. Perspect. Biol. 5 (2013) a013169.
[13] K. Mori, A. Sant, K. Kohno, K. Normington, M.J. Gething, J.F. Sambrook, A 22 bp cis-acting element is necessary and sufficient for the induction of the yeast KAR2 (BiP) gene by unfolded proteins, EMBO J. 11 (1992) 2583–2593.
[14] J.S. Cox, C.E. Shamu, P. Walter, Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase, Cell 73 (1993) 1197–1206.
[15] G. Giaever, A.M. Chu, L. Ni, C. Connelly, L. Riles, S. Veronneau, et al., Functional profiling of the *Saccharomyces cerevisiae* genome, Nature 418 (2002) 387–391.
[16] C.L. Wang, J. Landry, R. Sternglanz, A yeast sir2 mutant temperature sensitive for silencing, Genetics 180 (2008) 1955–1962.
[17] W.K. Huh, J.V. Falvo, L.C. Gerke, A.S. Carroll, R.W. Howson, J.S. Weissman, E.K. O'Shea, Global analysis of protein localization in budding yeast, Nature 425 (2003) 686–691.
[18] P.K. Sorger, H.R. Pelham, Purification and characterization of a heat-shock element binding protein from yeast, EMBO J. 6 (1987) 3035–3041.
[19] L. Guarente, Yeast promoters and lacZ fusions designed to study expression of cloned genes in yeast, Methods Enzymol. 101 (1983) 181–191.
[20] I. Nussbaum, E. Weindling, R. Jubran, A. Cohen, S. Bar-Nun, Deteriorated stress response in stationary-phase yeast: sir2 and yap1 are essential for hsf1 activation by heat shock and oxidative stress, respectively, PLoS One 9 (2014) e111505.
[21] J. Labbadia, R.I. Morimoto, F1000Prime.Rep., Proteostasis and Longevity: When Does Aging Really Begin?, 6, 2014, p. 7
[22] R.I. Morimoto, A.M. Cuervo, Proteostasis and the aging proteome in health and disease, J. Gerontol. A Biol. Sci. Med. Sci 69 (Suppl. 1) (2014) S33–S38.
[23] M.S. Hipp, S.H. Park, F.U. Hartl, Proteostasis impairment in protein-misfolding and -aggregation diseases, Trends Cell. Biol. 24 (2014) 506–514.
[24] Y. Liu, A. Chang, Heat shock response relieves ER stress, EMBO J. 27 (2008) 1049–1059.
[25] K.J. Travers, C.K. Patil, L. Wodicka, D.J. Lockhart, J.S. Weissman, P. Walter, Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation, Cell 101 (2000) 249–258.
[26] S.G. Ahn, D.J. Thiele, Redox regulation of mammalian heat shock factor 1 is essential for Hsp gene activation and protection from stress, Genes. Dev. 17 (2003) 516–528.
[27] F.M. Wang, Y.J. Chen, H.J. Ouyang, Regulation of unfolded protein response modulator XBP1s by acetylation and deacetylation, Biochem. J. 433 (2011) 245–252.
[28] Y. Li, S. Xu, A. Giles, K. Nakamura, J.W. Lee, X. Hou, G. Donmez, J. Li, Z. Luo, K. Walsh, L. Guarente, M. Zang, Hepatic overexpression of SIRT1 in mice attenuates endoplasmic reticulum stress and insulin resistance in the liver, FASEB J. 25 (2011) 1664–1679.
[29] J. Hou, H. Tang, Z. Liu, T. Osterlund, J. Nielsen, D. Petranovic, Management of the endoplasmic reticulum stress by activation of the heat shock response in yeast, FEMS Yeast Res. 14 (2014) 481–494.
[30] R.C. Taylor, K.M. Berendzen, A. Dillin, Systemic stress signalling: understanding the cell non-autonomous control of proteostasis, Nat. Rev. Mol. Cell. Biol. 15 (2014) 211–217.
[31] P. Oosten-Hawle, R.I. Morimoto, Organismal proteostasis: role of cell-nonautonomous regulation and transcellular chaperone signaling, Genes. Dev. 28 (2014) 1533–1543.
[32] J.S. Hahn, Z. Hu, D.J. Thiele, V.R. Iyer, Genome-wide analysis of the biology of stress responses through heat shock transcription factor, Mol. Cell. Biol. 24 (2004) 5249–5256.
[33] J. Wang, K.A. Pareja, C.A. Kaiser, C.S. Sevier, Redox signaling via the molecular chaperone BiP protects cells against endoplasmic reticulum-derived oxidative stress, Elife 3 (2014) e03496.
[34] R. Casagrande, P. Stern, M. Diehn, C. Shamu, M. Osario, M. Zuniga, P.O. Brown, H. Ploegh, Degradation of proteins from the ER of *S. cerevisiae* requires an intact unfolded protein response pathway, Mol. Cell. 5 (2000) 729–735.
[35] V.M. Labunskyy, M.V. Gerashchenko, J.R. Delaney, A. Kaya, B.K. Kennedy, M. Kaeberlein, V.N. Gladyshev, Lifespan extension conferred by endoplasmic reticulum secretory pathway deficiency requires induction of the unfolded protein response, PLoS. Genet. 10 (2014) e1004019.
[36] T. Payne, C. Hanfrey, A.L. Bishop, A.J. Michael, S.V. Avery, D.B. Archer, Transcript-specific translational regulation in the unfolded protein response of Saccharomyces cerevisiae, FEBS Lett. 582 (2008) 503–509.