The unpredictability of prolonged activation of stress response pathways

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In response to stress, cellular compartments activate signaling pathways that mediate transcriptional programs to promote survival and reestablish homeostasis. Manipulation of the magnitude and duration of the activation of stress responses has been proposed as a strategy to prevent or repair the damage associated with aging or degenerative diseases. However, as these pathways likely evolved to respond specifically to transient perturbations, the unpredictability of prolonged activation should be considered.

In response to stress, cellular compartments activate signaling pathways that mediate transcriptional programs to promote survival and reestablish homeostasis. Manipulation of the magnitude and duration of the activation of stress responses has been proposed as a strategy to prevent or repair the damage associated with aging or degenerative diseases. However, as these pathways likely evolved to respond specifically to transient perturbations, the unpredictability of prolonged activation should be considered.

Cellular stresses, such as unfolded or misfolded protein accumulation and organelle deterioration, are associated with numerous diseases as well as the aging process. Thus, enhanced activation of pathways that have evolved to protect against these defects may protect against degenerative diseases such as Parkinson’s and Alzheimer’s or the ill effects of normal aging (Powers et al., 2009; Bratic and Larsson, 2013). Stress response pathways are typically maintained in the off state or at a baseline level. Upon organelle perturbation, they are activated to the appropriate magnitude and duration to efficiently promote cellular survival and organelle recovery. Once homeostasis is reestablished, the pathway is down-regulated so that cells can properly respond to future stress (Fig. 1 A).

Manipulations of these pathways can mitigate the intracellular damage that occurs during aging or in degenerative diseases. However, these pathways did not likely evolve to deal with prolonged stress or to be activated for extended periods of time (Fig. 1 B). If continued activation were entirely beneficial, these pathways would likely have evolved to be hard-wired into developmental or cell-specific programs rather than to be stress inducible. We hypothesize that prolonged stress response activation has not been subject to evolution, as conditions that cause perpetual activation, such as deleterious gene mutations, result in cellular damage and would be selected against. Thus, the potential outcomes of prolonged stress response activation are difficult to predict. Here, we review the evidence suggesting that stress response pathways evolved to be transiently activated to a precise magnitude to match the level of dysfunction and allow the most efficient recovery, and consider the positive and negative effects of enhanced stress response activation. We also consider approaches to therapeutically engage stress response signaling.

Stress detection and matched transcriptional activation

Several organelle or stress-specific stress responses have been identified and are described in more detail elsewhere (Åkerfelt et al., 2010; Walter and Ron, 2011; Jensen and Jasper, 2014). Here, we focus on specific responses that are activated by cytosolic, ER, or mitochondrial stress or dysfunction.

The heat shock response (HSR). The HSR is mediated by the transcription factor HSF1 and occurs during conditions that cause an increase in unfolded or misfolded proteins primarily in the cytosol and nucleus, such as increased temperature, oxidative stress, and exposure to heavy metals (Ananthan et al., 1986; Åkerfelt et al., 2010). However, it can also be activated independently of misfolded proteins, as stalled ribosome complexes also activate the response (Brandman et al., 2012). The HSR is a transcriptional program that involves the induction of ∼500 genes, including cytosolic and nuclear-localized protein homeostasis (proteostasis) machinery such as molecular chaperones and genes involved in protein synthesis, the cell cycle, and the regulation of cell death (Mendillo et al., 2012; Ryno et al., 2014). While the induction of chaperones and proteases garner much of the attention, the HSR also includes the repression of ∼1,000 genes including developmental, immune, apoptotic (Mendillo et al., 2012; Ryno et al., 2014), and cytoskeletal maintenance components (Baird et al., 2014; Fig. 2 A). Normally, HSF1 is repressed by the cytosolic and nuclear-localized molecular chaperone Hsp90, which binds and maintains the transcription factor at a baseline level (Morimoto, 1998; Zou et al., 1998). As unfolded proteins increase, HSF1 is released, allowing it to bind the heat shock promoter element and regulate transcription (Fig. 2 A; Topol et al., 1985; Morgan et al., 1987). In addition to direct regulation by chaperones, HSF1 is also subject to multiple posttranslational modifications (Anckar and Sistonen, 2011). For example, the magnitude and duration of HSF1 activation are further regulated by the acetyltransferase EP300 and the deacetylase SIRT1. HSF1 acetylation by EP300 controls the quantity of HSF1 available for activation by preventing proteasome-dependent degradation (Raychaudhuri et al., 2014). Conversely, deacetylation of HSF1 by SIRT1 promotes activation of HSF1 during stress (Westerheide et al., 2009), but will eventually lead to HSF1 turnover to down-regulate the response (Raychaudhuri et al., 2014). Further regulation of the HSR occurs at the organismal level via thermosensory
neurons and neuroendocrine signaling. Non–cell-autonomous HSF1 activation presumably allows for more precise matching of the HSR to the behavioral and metabolic status of the organism (Pralhad et al., 2008; Prahlad and Morimoto, 2011).

![Diagram](image)

**Figure 1.** Transient versus perpetual or prolonged stress response activation. (A) The HSR, UPR, and the UPR mt remain in an “off” or “low-activation” state until they are activated by compartment-specific stress. As these pathways are not constitutively active, these stress responses likely evolved to promote survival during temporary stressful conditions and ultimately recover once the condition causing the cellular dysfunction is alleviated. Transient stresses include environmental effects such as temperature shifts, exposure to toxins, or altered nutritional status. (B) Genetic mutations that perturb cytosolic, ER, or mitochondrial proteostasis are typically selected against evolutionarily as they cause cellular dysfunction. However, genotoxic perturbations, or damage that has accrued over long periods of time during aging or disease, may cause prolonged or perpetual activation of the HSR, UPR, and UPR mt, as the mutation cannot be rectified. Prolonged or perpetual stress response activation is potentially very different than transient activation as there is never a recovery. As evolution did not select for prolonged stress response activation, we hypothesize that it is difficult to predict the outcome of prolonged stress response activation.

The unfolded protein response (UPR). The ER is the site of protein synthesis and folding for the vast majority proteins that are secreted or localized within the secretory pathway. In response to increased protein flux through the ER or to conditions that perturb ER protein folding, UPR activation limits the load on the stressed organelle by reducing localized protein synthesis and activating protective ER-specific transcriptional programs to reestablish organelle homeostasis (Walter and Ron, 2011; Fig. 2 B). The most conserved branch of the UPR is regulated by the ER–membrane localized kinase IRE1 and the transcription factor XBP1 (Hac1 in yeast). The UPR is activated when accumulating unfolded proteins directly interact with the luminal domain of IRE1 (Gardner and Walter, 2011), causing it to oligomerize, activating the cytosolic RNase domain (Korenykh et al., 2009). IRE1 cleaves several ER-localized mRNAs, resulting in their degradation, and thus reducing their translation and the burden on the ER protein-folding environment (Han et al., 2009; Hollen et al., 2009). Concomitantly, IRE1 also cleaves an inhibitory intron from the transcript encoding XBPI, which upon ligation allows the translation of the active transcription factor (Cox and Walter, 1996; Yoshida et al., 2001; Calfon et al., 2002). Once translated, XBPI activates a broad transcriptional response that includes ER-localized components that promote protein folding and quality control, compartmental expansion, and increased ER–Golgi trafficking (Travers et al., 2000; Shoulders et al., 2013; Fig. 2 B). However, if ER stress cannot be rectified, an apoptotic program is engaged to eliminate the unsalvageable cell (Tabas and Ron, 2011; Upton et al., 2012; Lu et al., 2014).

The mitochondrial UPR (UPR mt). The UPR mt is a transcriptional response that occurs specifically during mitochondrial dysfunction to promote survival and recovery of mitochondrial activity. The UPR mt in *Caenorhabditis elegans* is regulated by the transcription factor ATFS-1, which is normally imported into mitochondria and degraded (Nargund et al., 2012; Haynes et al., 2013). However, during conditions that impair mitochondrial protein import such as respiratory chain dysfunction, mitochondrial unfolded protein accumulation, or high levels of reactive oxygen species (ROS), general mitochondrial import efficiency is reduced, causing mitochondrial proteins to accumulate in the cytosol (Wright et al., 2001; Nargund et al., 2012; Harbauer et al., 2014). As ATFS-1 has a nuclear localization sequence, some of the cytosolic ATF5-3 pool then traffics to the nucleus to mediate UPR mt activation (Nargund et al., 2012; Fig. 2 C). Similar to the HSR and UPR, the UPR mt receives non–cell-autonomous regulatory inputs (Durieux et al., 2011; Owusu-Ansah et al., 2013; Taylor and Dillin, 2013).

ATFS-1 activation increases mitochondrial protein folding capacity and promotes mitochondrial recovery by increasing mitochondrial chaperones, proteases, respiratory chain complex assembly factors, import, and fission components (Nargund et al., 2012, 2015). Concomitantly, ATFS-1 limits expression of the tricarboxylic acid (TCA) cycle and respiratory chain components, suggesting that ATFS-1 promotes mitochondrial recovery by increasing protein folding and complex assembly capacity while slowing the rate of respiratory complex biogenesis to match the stressed organelle’s capacity (Nargund et al., 2015; Fig. 2 C). To facilitate organelle repair, ATFS-1 increases expression of all glycolysis components in order to maintain energy levels. Thus, the UPR mt includes a shift in cellular metabolism to promote survival during mitochondrial dysfunction that is reminiscent of the metabolism in rapidly dividing cells (Vander Heiden et al., 2009), and which presumably must be down-regulated upon return to homeostasis.

**Regulation of response duration and recovery**

In addition to the magnitude of a specific stress response, which is largely governed by activating mechanisms, the duration of the response must be tightly regulated to match cell physiology and promote efficient recovery. Consistent with the idea that prolonged activation of each pathway is potentially detrimental, multiple mechanisms exist to limit and down-regulate stress response activation. Included in the HSR, UPR and UPR mt are...
components that down-regulate HSF1, XBP1, and ATFS-1, respectively, via negative feedback loops. For example, HSF1 induces expression of Hsp70 and Hsp90, which in addition to promoting efficient protein folding also associates with active HSF1 to dampen the response (Shi et al., 1998). Similarly, XBP1 induces expression of ER-localized chaperones, which associate with IRE1 to attenuate signaling upon proteostasis recovery (Todd-Corlett et al., 2007; Eletto et al., 2014). ATFS-1 also induces multiple components that promote mitochondrial import efficiency, which serves to reduce cytosolic and ultimately nuclear accumulation of ATFS-1 (Nargund et al., 2012). Furthermore, all three pathways increase components that target the active transcription factor for degradation, including ubiquitin ligases. In addition to negative regulation of the response regulators, the outputs of the responses are also down-regulated once proteostasis is reestablished. For example, as misfolded or unfolded proteins are depleted, the HSF1-induced chaperone Hsp70 is ubiquitinated by the HSF1-induced ubiquitin ligase CHIP and is degraded by proteasomes, returning the chaperone capacity to baseline levels (Qian et al., 2006).

**Effects of prolonged activation**

HSF1, XBP1, and ATFS-1 have all been shown to be protective during organelle-specific stress, promoting survival and cellular proliferation during conditions that perturb cytosolic and nuclear (Morano et al., 1999; Xiao et al., 1999; Hsu et al., 2003; Morley and Morimoto, 2004), ER (Cox et al., 1993; Shen et al., 2001; Lin et al., 2009; Richardson et al., 2010), or mitochondrial proteostasis (Baker et al., 2012; Nargund et al., 2012), respectively. Interestingly, cellular damage that accrues in aging animals activates each pathway when it occurs in young animals. However, all three pathways (Yoneda et al., 2004; Ben-Zvi et al., 2009; Durieux et al., 2011; Owusu-Ansah et al., 2013; Schieber and Chandel, 2014) are attenuated and less effective in aging animals, which coincides with a proteostatic collapse (Ben-Zvi et al., 2009), further suggesting that enhanced activation may be beneficial.

Several interesting observations reveal their protective effects against age-associated cellular damage. Impaired insulin signaling, which extends worm lifespan considerably, requires multiple transcription factors including HSF1 and XBP1 (Kimura et al., 1997; Lin et al., 1997; Hsu et al., 2003; Henis-Korenblit et al., 2010). Similarly, modest mitochondrial dysfunction that activates the UPR also extends lifespan in multiple species including mice, flies, and worms (Dillin et al., 2002b; Durieux et al., 2011; Houtkooper et al., 2013; Owusu-Ansah et al., 2013; Schieber and Chandel, 2014). Thus, pathway activation can mitigate age-associated damage; however, it should be noted that this often comes at the expense of fecundity and normal development (Dillin et al., 2002a).

**HSR.** Impressively, HSF1 activation is sufficient to extend the lifespan of normal worms, indicating that the HSR can be protective over longer periods of time (Hsu et al., 2003; Westerheide et al., 2009). HSF1 activity positively affects proteostasis and reduces aggregation of disease-associated proteins in multiple organisms such as those containing polyglutamine stretches (Calamini et al., 2012; Brunquell et al., 2014), α-syn-
In addition to these protective effects, accumulating evidence indicates that HSF1 activation can also negatively affect proteostasis. Defects in folding and trafficking of the CFTR protein caused by an amino acid deletion result in cystic fibrosis. While expression of mutated CFTR activates HSF1, it was recently shown that HSF1 inhibition increases CFTR trafficking and function, suggesting that prolonged HSF1 activation creates a maladaptive state (Wang et al., 2006; Roth et al., 2014). Similarly, HSF1 overexpression has been shown to exacerbate aggregation of the polyglutamine protein Huntingtin (Bersuker et al., 2013). Lastly, the HSF1 expression level is associated with poor prognoses in breast cancers, which is consistent with many cancer types requiring HSF1 activity to promote proliferation (et al., 2013). These results indicate that enhanced UPR activation can be protective, but the magnitude and duration of the response should be considered as well as other factors that potentially coordinate with the UPR.

Conclusions

Manipulations to enhance stress response activation hold promise therapeutically to mitigate the cellular damage that accrues during aging and disease (Calamini et al., 2012; Mouchiroud et al., 2013). Response activation in principle can be achieved by (1) perturbing the protein folding environment, (2) activating the transcription factor directly, or (3) impairing the turnover of the active transcription factors. But the evidence reviewed here suggests that it is difficult to predict the outcome of prolonged activation, as these responses likely evolved to resolve transient proteotoxic stress (Fig. 1 A). Therefore, manipulation of these stress response pathways as a therapeutic measure will require careful consideration of the effects of prolonged activation (Fig. 1 B). Considering the number of transcripts XBP1, ATFS-1, and HSF1 affect in addition to those promoting proteostasis, prolonged activation may alter fundamental aspects of a particular cell. For example, to promote mitochondrial recovery, ATFS-1 activation shifts metabolism to that typically observed in rapidly proliferating cells, which may be detrimental to postmitotic cells if activation is prolonged (Fig. 2 C).

Despite the challenges in manipulating these pathways to promote organelle recovery and cell survival, we are optimistic that as more knowledge is gained regarding pathway regulation and outputs, therapeutic manipulations can be tailored to limit cellular damage so as to avoid unintended effects of prolonged alterations. A particularly exciting example has been the development of phosphatase inhibitors that partially prolong the effects of the UPR branch that attenuates translation during ER stress (Novoa et al., 2001; Boyce et al., 2005). Impaired activation, or extreme prolonged activation, of the translational control branch of the UPR results in cell death and developmental arrest (Harding et al., 2000, 2009). However, guanabenz inhibits only one of the two phosphatases that attenuate the pathway (Tsaytler et al., 2011). Impressively, guanabenz and, more recently, a related compound have been shown to be protective in a variety of cultured cell lines as well as in mouse models of neurodegeneration (Das et al., 2015; Way et al., 2015).

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Lapierre, L.R., C.D. De Magalhaes Filho, P.R. McQuary, C.C. Chu, O. Visvikis, J.T. Chang, S. Gelinio, B. Ong, A.E. Davis, J.E. Irazoqui, et al. 2013. The TFE3 orthologue HLH-30 regulates autophagy and modulates lon-
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