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That which does not kill me makes me stronger: adapting to chronic ER stress

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Cells respond to the accumulation of unfolded proteins by activating signal transduction cascades that improve protein folding. One example of such a cascade is the unfolded protein response (UPR), which senses protein folding stress in the endoplasmic reticulum (ER) and leads to improvement in the protein folding and processing capacity of the organelle. A central paradox of the UPR, and indeed of all such stress pathways, is that the response is designed to facilitate both adaptation to stress and apoptosis, depending upon the nature and severity of the stressor. Understanding how the UPR can allow for adaptation, instead of apoptosis, is of tremendous physiological importance. Recent advances have improved our understanding of ER stress and the vertebrate UPR, which suggest possible mechanisms by which cells adapt to chronic stress.

The two-faced nature of ER stress

The term 'endoplasmic reticulum stress' (see Glossary) defines any perturbation that compromises the protein folding functionality of the ER. In addition to being the site of synthesis of all secretory proteins and resident proteins of the endomembrane system, the ER also participates in lipid metabolism, oligosaccharide synthesis, calcium storage, and in certain cell types, drug detoxification. Accordingly, any number of pathological or pharmacological insults can produce ER stress either directly or indirectly, and this perturbation can be acute or chronic in nature [1,2]. As examples of acute stress, insults such as hypoxia and/or ischemia, calcium depletion and glucose deprivation can all lead to rapid activation of the unfolded protein response (UPR). In principle, the cell needs only to tolerate such stresses for their relatively brief durations (on the order of minutes to hours) and clear the ER of whatever unfolded proteins have accumulated in that time, which demands a UPR that is relatively fast acting and also readily deactivated.

By contrast, chronic stresses require quasi-permanent changes in cellular function in general and in ER function in particular. Chronic stresses that activate the UPR can encompass: genetic mutation of either ER chaperones or chaperone substrates that lead to persistent misfolding problems; viral infection that can co-opt the secretory pathway for production of viral proteins; various neurodegenerative disorders of protein aggregation; or even the normal differentiation and maintenance of cells whose primary functions include the production and secretion of proteins, such as immune cells, endocrine and paracrine cells, and hepatocytes [3]. A commonality among these chronic ER stressors is that they demand a mechanism whereby ER stress can be persistently tolerated on a timescale ranging from days to years (Box 1). In these contexts, even if cell death occurs to a small extent, the majority of cells must ultimately survive and adapt to the stressful stimulus.

Much of our understanding of the UPR, including its activation, perpetuation and regulation, comes from studies that utilize severe pharmacological perturbation of cultured cells. However, these studies almost certainly fail to recapitulate the UPR as it is elicited under the conditions of milder, but more persistent ER stress that could be encountered physiologically. Thus, one of the fundamental questions of the UPR is how can the response allow cells to escape death and adapt to stress? We will therefore consider recent insights into how the response is regulated, which might provide clues as to how stress signals from the ER are integrated into a life-or-death response.

The four As of the UPR: activation, acute response, adaptive response and apoptosis

The mechanisms of UPR activation, the signaling pathways initiated by it, the changes in gene expression that result and the subsequent induction of apoptotic cascades have been exhaustively reviewed elsewhere, and are summarized in Figure 1. Here, we will provide a brief summary of these events as a way of introducing what might represent control points in cellular adaptive decisions.

Glossary

Acute stress: Stresses that are transient in nature (on the order of minutes to hours) and thus require only that cells tolerate a brief period of perturbation, and then recover by restoring homeostasis within the ER.
Adaptation: The ability to maintain cellular function and avoid apoptosis during chronic stress.
Chronic stress: Stresses that are persistent in nature (on the order of days to years). These can encompass either pathological or developmental processes, but in either case they require long term adjustments to cellular function.
ER stress: Any perturbation that compromises the protein folding functionality of the ER.
Preconditioning: The ability of a brief exposure to stress to protect cells from a subsequent stress, both in terms of cell survival and activation of stress response pathways.
Protein processing: Refers collectively to the ability of the ER to fold, modify, traffic, and if necessary degrade newly imported proteins.

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Box 1. Examples of chronic stress

Genetic

Insulin-producing β cells of the pancreas seem particularly sensitive to disruption of ER function. Diabetic phenotypes can be induced by loss of PERK–eIF2α signaling [14], genetic compromise of ER quality control [59], and dietary stress in a UPR-sensitized background [60]. In the *Akita* mouse model of diabetes, heterozygosity for a point mutation in the mouse *Ins2* gene (which is one of two insulin-encoding genes in the mouse genome) prevents proper insulin oxidative folding, causes ER stress, and results in progressive hyperglycemia [61]. More tellingly, loss of the proadaptive BiP cochaperone $p58^{IPK}$ (58-kDa inhibitor of the RNA-activated human and mouse protein kinase), which is upregulated by ER stress, exacerbates the Akita phenotype [62,63]. This outcome underscores the importance of improved ER protein folding in protecting against chronic stress on the β cells.

Environmental

Elevated plasma levels of homocysteine (Hcy) are a major risk factor for atherosclerosis, diabetes, neurodegenerative disorders, and other chronic pathologies. Various factors related to gender, age, genetic composition, and diet can contribute to hyperhomocysteinemia, which is particularly toxic to the liver [64]. Hcy can directly induce ER stress in cultured hepatocytes [65]. Interestingly, chronic alcohol consumption also both increases plasma Hcy and leads to ER stress [66], which raises the possibility that alcoholic liver injury might be at least partially a consequence of a gradual overwhelming of the UPR protective capacity in the liver.

Pathogenic

Many viruses have been shown to activate the UPR upon infection. One of the most notable of these is hepatitis C virus (HCV), which can result in latent infection, or progress to chronic hepatitis, cirrhosis, or hepatocellular carcinoma [67]. How persistent viral infection leads to these alternate fates is poorly understood. Although this process is certainly affected by UPR independent mechanisms such as antiviral responses and autoimmunity, one possible contributing factor would be if HCV and other viruses can hijack the UPR to elicit survival and adaptation rather than apoptosis, which would tend to favor the development of hepatocellular carcinoma.

Developmental

The best studied developmental role of the UPR is in the differentiation of B lymphocytes into antibody-secreting plasma cells. Stimulation of B cell differentiation results in massive ER expansion and upregulation of ER chaperones, which is at least partially a consequence of UPR activation [68]. Beyond merely accommodating the increase in secretory protein production in these and other secretory cells, the UPR must also maintain this capacity for the life of the cell. Therefore, there must exist mechanisms by which the upregulation of ER chaperones can be maintained in the differentiated B cell while apoptotic cascades are not executed.

Activation

ER stress is sensed in vertebrates by three ER-resident transmembrane proteins. The lumenal domains of all of these proteins bind to the abundant ER chaperone binding protein (BiP) [4]. Inositol requiring enzyme 1α (IRE1α), which is conserved across eukaryotic species, contains a lumenal stress sensing domain and a cytosolic protein kinase domain. It is thought that IRE1α is activated by dimerization, which leads to trans-autophosphorylation and activation of a cytosolic endoribonuclease activity. To date, the only known substrate of the IRE1α endoribonuclease is *X-box binding protein 1* (*Xbp1*) mRNA, from which a 26 base intron is removed by IRE1α. This splicing event alters the *Xbp1* translational reading frame to fuse a C-terminal transactivation domain to the N-terminal DNA binding domain. The pancreatic ER eIF2α kinase (PERK) protein, which consists of a lumenal domain homologous to IRE1α and an unrelated cytosolic Ser/Thr kinase domain, similarly dimerizes or oligomerizes during ER stress. Its autophosphorylation directs PERK activity toward the α subunit of the eukaryotic initiation factor 2 (eIF2) translation initiation complex. eIF2α phosphorylation inhibits delivery of the initiator methionyl-$tRNA_i$ to the ribosome and ultimately results in a general inhibition of protein translation. Finally, the activating transcription factor 6α (ATF6α) protein is activated when ER stress liberates the molecule for transit from the ER to the Golgi, where regulated intramembrane proteolysis by site-1 protease and site-2 protease releases a cytosolic fragment that is a transcriptional coactivator. Based on the fact that IRE1α, PERK and ATF6α are freed from BiP during ER stress, BiP binding seems important in regulating, either directly or indirectly, activation of each arm of the UPR.

Acute response

Acute stress is better relieved by immediate reductions in the protein load on the ER than by the slower transcriptional induction of ER protein folding, processing, and degradation pathways. Accordingly, one consequence of UPR activation is the rapid phosphorylation of eIF2α and subsequent inhibition of translation [5].

In addition to this general mechanism, two ER-selective mechanisms for reducing protein load have been proposed recently. One of these is the IRE1α-dependent degradation of a subset of ER-associated mRNAs, which might help to clear the ER membrane of polysome-associated transcripts [6]. Another mechanism is inhibition of the ER translocation of many, but not all, ER substrates in a process known as preemptive quality control (pQC) [7]. One commonality among all three of these processes is that they are not compatible over the long term with cellular function; therefore, each is readily reversible.

Adaptive response

The rapid reduction in protein load notwithstanding, the UPR is classically defined as a transcriptional upregulation of genes that augment the protein folding and processing capacity of the ER [8,9]. Each UPR pathway contributes to this transcriptional effect: ATF6α as a transcriptional coactivator itself; IRE1α through the production of the active XBP1 transcription factor; and PERK through the production of ATF4. The 5′ untranslated region of *Atf4* mRNA contains upstream open reading frames (uORFs) that normally inhibit *Atf4* translation, but stimulate its translation when eIF2α is phosphorylated [10]. Together, ATF6α, XBP1 and ATF4 regulate the expression of genes that facilitate ER function either directly or indirectly. These include genes encoding ER chaperones, including BiP; ER-associated degradation (ERAD) factors; and other ER functions such as protein translocation, protein trafficking, and lipid and carbohydrate synthesis. In addition, non-ER-specific processes such as amino acid metabolism, mitochondrial function (and thus energy production), cellular redox state, and small molecule transport are also

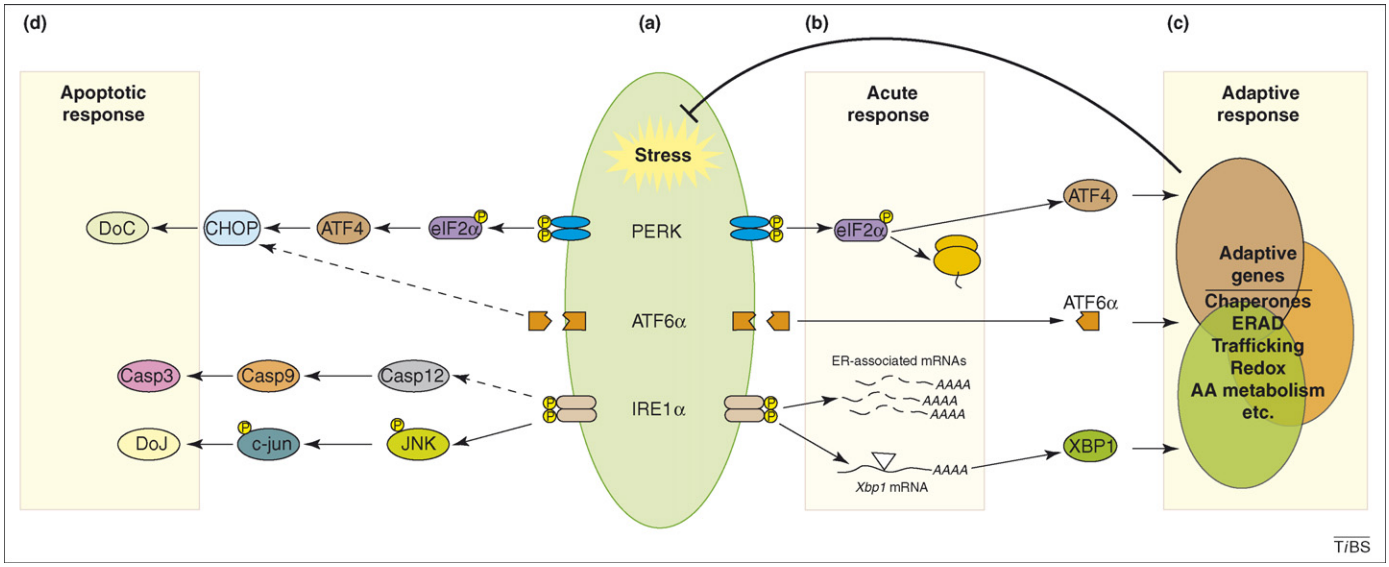

Figure 1. The UPR simultaneously initiates adaptive and apoptotic signaling cascades. **(a)** Activation of PERK (blue), ATF6α (orange), and IRE1α (pale brown) in the ER is needed for **(b)** short-term protection from acute stress by inhibition of translation through eIF2α phosphorylation, degradation of ER-associated mRNAs by IRE1α, and inhibition of protein import through the translocon. **(c)** Activation of all three pathways also results in transcriptional activation through ATF4, ATF6α, and XBP1 for longer term upregulation of genes that help cells to adapt to persistent stress. **(d)** These same sensors also initiate apoptotic signaling cascades, of which the best characterized are illustrated here. The mechanisms by which expression of CHOP and JNK initiate apoptosis are not yet definitively known, and so their hypothetical proapoptotic targets are here listed as DoC (downstream of CHOP) and DoJ (downstream of JNK and c-jun). Broken arrows indicate pathways that have been proposed but not yet definitively established. Note the multiplicity of steps involved in apoptotic cascades versus the more direct pathways from sensor activation to gene regulation, particularly for the ATF6α pathway. Caspases 12 and 9 are initiator caspases and caspase 3 is an executioner caspase. Abbreviations: *AAAA*, poly(A) tails of mRNAs; Casp3/9/12, caspase 3/9/12; P, phosphate group.

transcriptionally regulated by an activated UPR. Although the subsets of genes regulated by each pathway are at least partially distinct, there is also a considerable amount of overlap between the pathways. Collectively, UPR transcriptional activation steps ensure that exposure to ER stress, however brief or prolonged, fundamentally alters multiple subcellular processes and ideally allows a stressful stimulus to be relieved by improved efficiency within the secretory pathway. These changes in gene expression can thus be seen as the crux of any ability of the cell to adapt to chronic stress.

Apoptosis

Activation of the UPR also sets in motion pro-apoptotic signaling cascades, some of which appear to be relatively specific to ER stress and others of which feed into general apoptotic pathways [11]. Most notable in the first category is cleavage of the ER-localized caspase-12 in mouse (or the orthologous caspase-4 in humans) [12]. However, it is clear that ER stress-mediated apoptosis also leads to mitochondrial membrane depolarization and the initiation of other cascades that are well characterized elements of apoptotic signaling in response to other types of cellular insult [11]. Interestingly, whereas severe ER stress can induce mitochondrial relocalization of the pro-apoptotic proteins Bax (BCL2-associated X protein) and Bak (BCL2 antagonist of cell death), the ER localized forms of these proteins apparently potentiate IRE1α signaling [13]. Although the significance of this interaction is not yet fully understood, it highlights the potential for direct crosstalk between stress sensors and the molecules that execute the apoptotic program. One additional pro-apoptotic cascade seemingly of particular importance is the transcriptional activation of C/EBP homologous protein (Chop) by ATF4 (Box 2).

There are thus multiple apoptotic pathways emanating from UPR activation, and ablation of any one of them typically confers a degree of protection against ER stress-induced cell death. The commitment to adaptation over apoptosis requires that the signaling cascades that enhance ER and cellular function be perpetuated, whereas those that lead to apoptosis are suppressed. We discuss below the general mechanisms by which this might occur.

Dissociation of stress sensors

One potentially appealing mechanism to account for adaptation to chronic stress is the selective activation, or alternatively the selective perpetuation, of signaling through one or two of the limbs of the UPR, but not through all three (Figure 2). This model presupposes that long term activation of one or more UPR stress sensors is intrinsically apoptotic, so that its suppression would be necessary for adaptation.

Evidence for dissociation

One of the strongest arguments in favor of the dissociability of the three stress sensors is the divergent phenotypes of *Perk*$^{-/-}$ versus *Ire1*α$^{-/-}$ and *Xbp1*$^{-/-}$ mice. *Perk*$^{-/-}$ animals are viable through embryonic development, and display a progressive postnatal loss of pancreatic β-cell function [14]. By contrast, both *Ire1*α$^{-/-}$ and *Xbp1*$^{-/-}$ animals show embryonic defects in liver formation and B lymphocyte differentiation [15–17]. In addition, *Xbp1*$^{-/-}$ animals developmentally rescued by a liver-specific *Xbp1* transgene progress only to the early neonatal period, when defects in the secretory pathway of pancreatic acinar cells lead to death from digestive failure [18]. One possible explanation for these results is that the differentiation program of β cells activates the PERK pathway to a

Box 2. The connection between ER stress and oxidative stress

Whereas many of the apoptotic pathways initiated by UPR activation fall into well described mechanisms of execution, the mechanism by which upregulation of the CHOP protein promotes apoptosis is much more difficult to rationalize, although its expression correlates closely with cell death. Overexpression of CHOP can cause cell death in the absence of additional stimuli; CHOP expression is often detected during pathological activation of the UPR; and CHOP deletion protects both cells and animals against ER stress-induced cell death to a certain degree [69]. Various reports have linked CHOP to downregulation of the anti-apoptotic BCL-2 protein [70] and upregulation of death receptor 5 (DR5), but the significance of these events remains unclear [71]. More recently, CHOP was shown to regulate cell death through transcriptional induction of the proapoptotic Bim protein [72]. One target of CHOP is the growth arrest and DNA damage inducible 34 (GADD34) protein, which interacts with protein phophatase I (PPI) to mediate eIF2α dephosphorylation as part of a negative feedback loop within the UPR. Cells are protected from cell death induced during acute ER stress by deletion of either CHOP or GADD34, and upregulation of GADD34 promotes the cellular accumulation of reactive oxygen species (ROS) [73]. Therefore, one mechanism by which CHOP might promote apoptosis is by facilitating the resumption of protein synthesis in the stressed ER and attendant ROS production, although whether GADD34 expression is similarly counteradaptive during milder stress has not been tested.

Activation of the UPR enhances the expression of a host of genes involved in protecting cells against oxidative stress [25], implying that ROS contribute to cellular damage during ER stress. ROS can be produced either in mitochondria, as a consequence of oxidative phosphorylation, or apparently in the ER. The ER-derived source of ROS is not yet well understood, although one potential mechanism is maintenance of the oxidative folding capacity of the ER lumen, which ultimately derives its oxidation potential from molecular oxygen [74]. Compromising the PERK pathway of the UPR leads to enhanced ROS production during ER stress [25], as does impairment of ERAD [75]. ROS have also been implicated in the pathogenesis of diabetes, neurodegenerative diseases and viral pathologies, among others. Thus, it is possible that a major component of the adaptive response is protection against ROS and also, that ROS play a crucial role in the eventual execution of cell death in these pathologies.

greater extent than the IRE1α pathway, and conversely that the differentiation program of B lymphocytes etc. activates IRE1 but not PERK. However, an alternative explanation for the differential developmental requirements of IRE1α and PERK is that both pathways are activated, but that certain downstream targets of these pathways, rather than the pathways themselves, are important in different cell types.

If adaptation requires suppression of one or more UPR stress sensing molecules, one candidate would be the PERK pathway. PERK–eIF2α signaling is rapidly downregulated as a consequence of negative feedback loops within the UPR [19,20], which might indicate that long term activation of this signaling axis is deleterious to adaptation, most plausibly because of upregulation of CHOP and its targets. Unfortunately, the increased sensitivity to acute stresses of *Perk*$^{-/-}$ cells and cells homozygous for an allele encoding nonphosphorylatable eIF2α makes it difficult to test whether the PERK pathway is needed during longer term stress. Prolonged IRE1α signaling could also be counteradaptive, because IRE1α signaling has been linked to activation of the proapoptotic c-jun N-terminal kinase (JNK) pathway [21,22] and possibly capsase-12 activation as well.

ATF6α might be an appealing candidate for a pathway that would remain activated even during persistent stress. ATF6α is required for the full induction of genes that preserve the protein processing capacity of the ER, including ER chaperones and ER-associated degradation machinery [23,24]. This deficiency renders Atf6α-null cells particularly sensitive to long term or repeated stress, suggesting that the pathway has evolved to augment the protective functions of the UPR during chronic stress [23]. However, the PERK and IRE1α pathways are also involved in the regulation of ER chaperones, ER-associated degradation, and other protective functions [24–29]. Thus, no UPR pathway could be selectively deactivated without

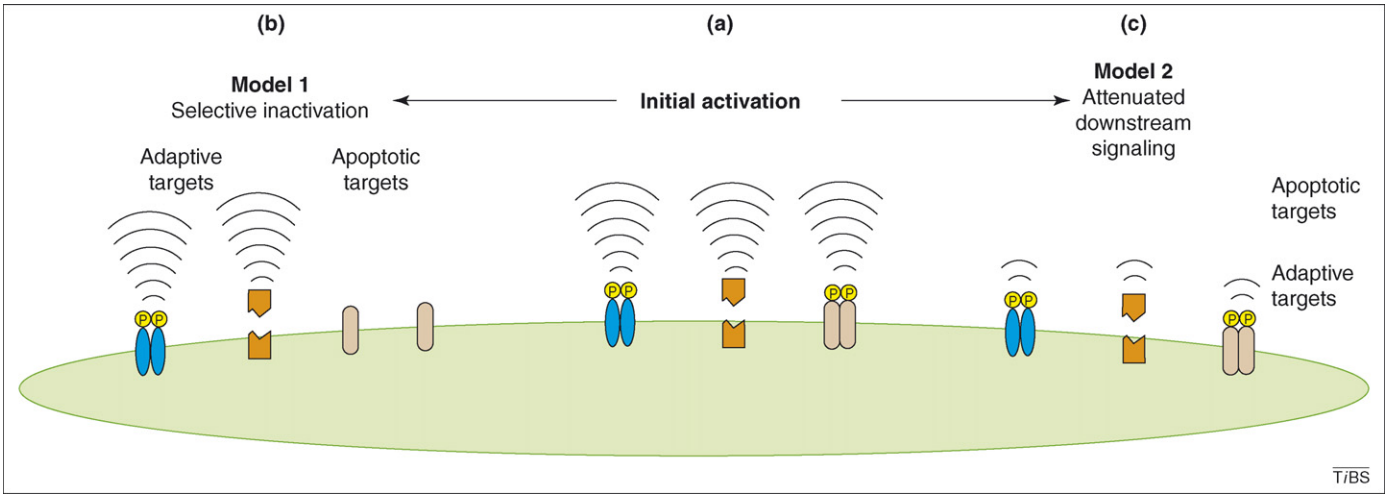

Figure 2. Two models to describe how cells could adapt to chronic stress. Initial exposure to ER stress will produce full activation of all three stress sensors, **(a)** which will initiate both adaptive and apoptotic signals. One way that cells might adapt to persistent stress is by deactivating a sensor that perpetuates preferentially proapoptotic signals, **(b)** while maintaining activation of sensors that are on balance adaptive. Although attenuated activation of the PERK pathway is suggested in this illustration, this has not been documented experimentally and thus represents but one possibility. **(c)** An alternative (not mutually exclusive) model proposes that improved protein folding suppresses signaling through all stress sensors, to an extent that remains sufficient to effect changes in the adaptive program but not to execute apoptosis. Note, the same iconography and color scheme as Figure 1 has been used. Signaling from the protein sensors and their relative strength are indicated by the waves emanating from them. Abbreviation: P, phosphate group.

sacrificing at least some of the adaptive capacity of the cell as well.

Possible mechanisms of dissociation

Rather than complete and selective inactivation of a single UPR pathway during chronic stress, long term UPR activation might allow the sensors to be partially suppressed to greater or lesser extents. In principle, any divergence in the mechanism of activation of the three sensors could account for partial dissociability. For example, during activation ATF6α appears to undergo changes in both its disulfide bonded state and its glycosylation state [30,31]. If these events are required for its activation, then both the nature of the stressor and the alterations in ER function that occur as a consequence of UPR activation could influence the extent to which ATF6α specifically is activated. Likewise, IRE1α can be activated by autophosphorylation in a way that brings about regulation of downstream genes but does not result in splicing of *Xbp1* mRNA, implying that IRE1α exists in more states than simply 'on' and 'off' [32,33]. Perhaps ER stress sensors can exist in alternate states subject to differential post-translational modification, each of which signals a unique cascade, with the preponderance of these states dependent upon the nature and persistence of the stressor.

The possibility of at least partial dissociation of the stress sensors becomes more plausible if they are not all activated by the same simple mechanism of titration from BiP. Different stressors appear to activate the three pathways with differing kinetics [34], which suggests some divergence in the mechanisms of their activation. The crystal structure of yeast Ire1p led to the proposal that Ire1p binds to unfolded proteins directly, and that BiP binding serves as, at most, a modulator of Ire1p activity [35]. However, the crystal structure of mammalian IRE1α challenges this model [33]. Biochemical characterization of the activation and stress responsiveness of various dimerization-defective mutants of both IRE1α and PERK suggests a similar mechanism of activation [33], and this possibility is supported by the observation that the lumenal domains of the two proteins are functionally interchangeable [36,37]. Ultimately, the determination of whether the UPR can exist in a selectively activated state will require more sensitive assays for directly monitoring sensor activation.

It is possible that additional limbs of the UPR alter the nature of UPR signaling and regulate cell fate decisions during chronic stress. ATF6α is but one of a rapidly emerging family of ER-localized transmembrane proteins cleaved by regulated intramembrane proteolysis during ER stress. ATF6β is loosely homologous to ATF6α, and like ATF6α, is ubiquitously expressed. It is activated under the same general conditions as is ATF6α, and can in principle bind to at least some of the same promoter elements [38]. It has been proposed that ATF6β acts as a transcriptional repressor of ATF6α-dependent genes [39]. It is conceivable that as cells adapt to stress, ATF6β activity predominates over ATF6α activity, with potential consequences for the patterns of gene expression that then result. The cyclic-AMP-responsive-element-binding protein H (CREBH) protein has been identified as a liver specific, ER stress-activated transmembrane transcription factor. *In vivo* knockdown of CREBH does not compromise upregulation of ER stress dependent genes, but does impair the systemic inflammatory response initiated by the liver in response to challenge [40], which illustrates that the UPR has a role not just in cellular protection, but in organismal protection as well. Proteins such as CREBH, ATF6β, and others could interact with the core UPR machinery to modulate its output. The potential for such modulation can be seen in the ability of XBP1 to regulate the expression of different sets of genes, through different *cis*-acting sequence motifs, in a cell-type specific manner [41]. Ultimately, the roles of these proteins in the adaptive responses of specific tissues awaits a more detailed characterization of the conditions under which they are activated and of the nature of their targets.

Divergence in downstream responses

Cells can survive and proliferate despite persistent exposure to pharmacological stress, if the stress is sufficiently mild, despite activation of all three arms of the UPR [42]. Their adaptation is marked by persistent expression of ER chaperones such as BiP, but not of CHOP and at least one of CHOP's targets. These observations raise the possibility that cells might adapt to persistent stress not by selectively regulating activation of the three limbs of the UPR, but by the manner in which the expression of downstream effector molecules of the UPR is controlled during exposure to tolerable stress (Figure 2).

Adaptation by improved protein processing

Two stages would be required for cells to adapt by a mechanism that alters the downstream signaling output of the UPR: (i) the capacity of the ER to process client proteins would have to be improved to an extent sufficient to mitigate the perturbing effects of the stressor and attenuate UPR signaling; and (ii) a new equilibrium would have to be established in which signaling through the UPR could maintain improved ER protein processing capacity, but not execute apoptotic cascades.

Functionality of ER protein processing can be improved either by the removal of misfolded proteins or by enhanced folding and transport of nascent proteins such that they avoid misfolding in the first place. The classical method for the removal of misfolded proteins is ERAD, which is stimulated as a consequence of UPR activation, and can likely be attributed to enhanced expression of ERAD-facilitating proteins such as EDEM and Derlin-2 among others [27,29,43]. In addition, there apparently exist mechanisms, at least in lower eukaryotes and possibly in vertebrates as well, for segregating large quantities of misfolded or unfolded proteins for autophagic destruction [44]. However, although such mechanisms no doubt help to alleviate the protein misfolding load during stress, it is not clear whether they actually improve the protein processing capacity of the ER, or simply mitigate more severe damage.

Unfortunately, the more direct demonstration that the ER of the UPR-activated cell moves proteins more efficiently forward through the secretory pathway has been lacking, in part because the conditions typically used to induce ER stress are sufficiently severe that even a

maximally activated UPR is unlikely to result in a net gain in ER functionality. However, indirect support for this idea comes from studies in which cells that have been briefly UPR-activated (either by transient exposure to a stressor, or by artificial activation of individual UPR pathways) become resistant to challenge with a second stressor, both in terms of cell survival and UPR activation status. This phenomenon, termed 'preconditioning', is accompanied by upregulation of UPR-dependent genes, including ER chaperones [45,46]. Therefore, preconditioning likely protects cells by improving the ER protein processing capacity.

Further support for the importance of improved protein processing in adaptation to physiological stress comes from studies that explore the peripheral insulin resistance that accompanies obesity. This resistance could be attenuated by treatment of obese mice with chemical chaperones that improved protein folding in the periphery [47]. In addition, heterozygosity for XBP1 in mice sensitized these animals to obesity-induced insulin resistance [48]. Thus, the role of XBP1 in the protection of insulin signaling in the periphery might be to improve ER protein folding, although this has not yet been demonstrated rigorously.

Suppression of downstream signaling

Improvements in the capacity of ER protein processing would have the effect of suppressing further UPR activation, yet the UPR must remain activated to an extent sufficient to maintain this improved capacity for as long as the stressful stimulus persists. How can this continuous activation be accommodated without concomitant execution of UPR dependent apoptosis? One possibility is that the placement of apoptotic events into multistep regulatory cascades suppresses apoptosis during mild stress, based on the ability of such cascades to convert a graded stimulus into a binary (in this case, life versus death) output [49] (Figure 3). Further acting to suppress apoptosis and favor adaptation under such circumstances is the stability of mRNA and protein of at least a subset of adaptive genes, including the ER chaperones *BiP* and *Grp94*, and the instability of mRNA and protein of the proapoptotic transcription factor *Chop* and at least one of its downstream targets [42]. UPR activation also elicits epigenetic modifications in the loci of UPR targets [50], and at least in yeast also has been shown to directly interface with a histone deacetylase complex [51], and so it is possible that such changes could perpetuate UPR-mediated gene regulation even as UPR signaling is attenuated.

It is worth noting that some of the key stress signaling molecules within the UPR are themselves subjected to transcriptional regulation by activation of the response, most notably ATF6α and XBP1 [52–54]. Upregulation of these mRNAs probably helps to replenish the pools of inactive ATF6α and XBP1, to restore the recovering cell to equilibrium. More broadly, regulation of the abundance of upstream components within the UPR could potentially modulate the sensitivity of the response to ongoing stress, which makes the cells either more or less sensitive to such challenge. The actual outcome of such regulation would depend highly upon many other factors. These include the nature of the control points (e.g. the rate-limiting steps) in the UPR, the mechanism by which the sensors are activated (whether directly by unfolded protein, indirectly by BiP, or by some hybrid mechanism), and relative abundance of supporting molecules (such as ER chaperones) and targets. Ultimately, a quantitative systems analysis of the

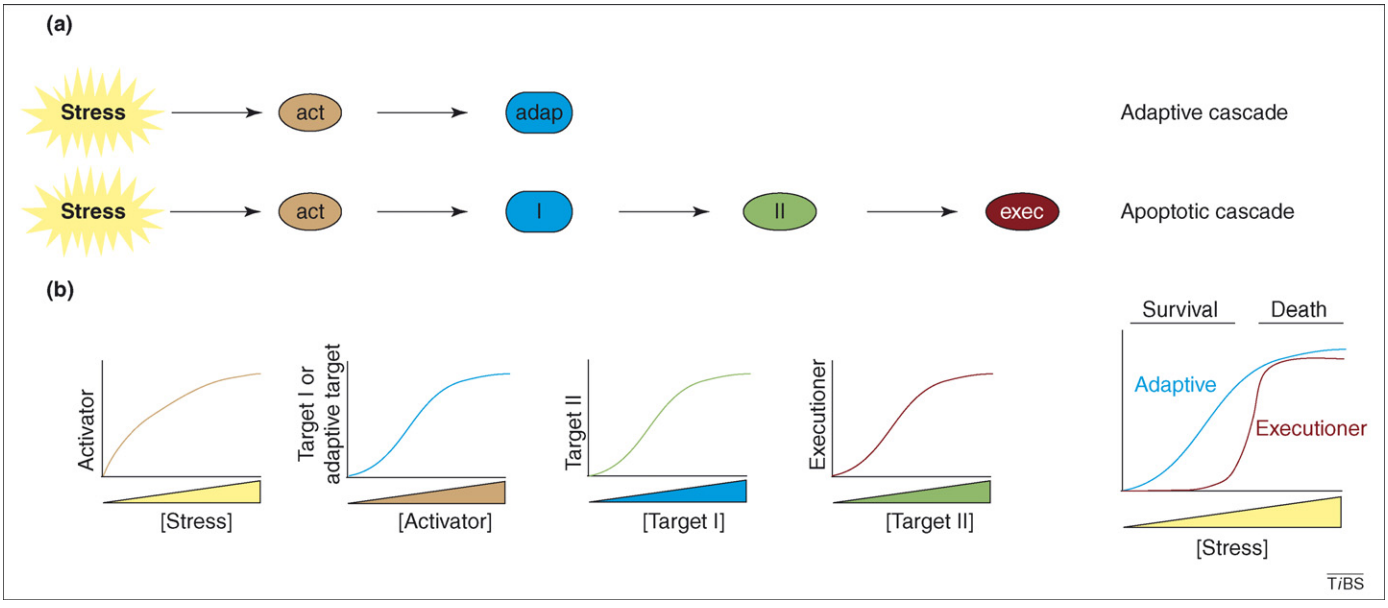

Figure 3. Multistep signaling cascades can convert graded input into binary signals. **(a)** Shown are two hypothetical cascades that progress from the stress signal to production of an activator (e.g. ATF4, cleaved ATF6α, or XBP1 = 'act'), with the responsiveness of the activator to the stressor being essentially Michaelian. The activator then either directly regulates expression of an adaptive target (e.g. genes encoding ER chaperones = 'adap') or expression of a nonadaptive target (e.g. CHOP = 'Target I') that then regulates expression of a second target (e.g. a target of CHOP = 'Target II') that then regulates cell death through a hypothetical executioner ('adap'). **(b)** All of these steps are assumed to be sigmoidal in their responsiveness, as shown in the hypothetical plots. The plots are color coded to correspond to the activators and targets shown in (a). In this paradigm, the execution of cell death becomes in effect a binary switch, based upon the multiplicative combination of each element of the apoptotic cascade, whereas production of adaptive proteins remains more linearly responsive to the strength of the stressor. A UPR structured in this manner will promote adaptation at low concentrations of stress and death at high concentrations, without requiring selective inactivation of stress sensors. This figure was adapted from concepts presented in [49].

UPR will be necessary to understand how its output can be modulated by the abundance and activities of its constitutents.

The UPR is known to interact with other cellular signaling cascades that could modulate its output, including mitogen-acitvated protein (MAP) kinase cascades [55] and nuclear factor kappa B (NF-κB) signaling [56,57]. Unfortunately, the paucity of studies that explore the consequences of long term UPR activation makes it unclear how these pathways might influence the outcome of UPR signaling. However, the apparent interface between the UPR and other cell signaling pathways is underscored by the recent discovery that chondrocytes, which are involved in bone differentiation and maturation, can adapt to the stress of production of misfolded collagen by initiating a program of reverse differentiation [58]. This process appears to temporarily interrupt endochondral bone formation while chondrocytes upregulate the UPR and suppress expression of the mutant collagen. It will be interesting to determine whether this ability to closely tie differentiation status to ER load is a general feature of the development of professional secretory cells, or is specific to chondroyctes.

Future directions

The fact that diseases such as diabetes and neurodegenerative disorders manifest over the course of decades argues that only very slight adjustments to the balance between adaptation and apoptosis would be necessary to have a significant beneficial impact on health. However, before such manipulation becomes feasible, much more needs to be understood about how the UPR responds to the types of stresses that underlie such pathologies, compared with the classical UPR that has been defined within a relatively artificial experimental context. More sensitive measures to detect subtle variations in the status of the UPR sensors and their downstream effects will be needed to identify the mechanisms of activation in response to very mild ER perturbations of different sorts. Understanding the interface between the UPR and other cellular signaling cascades will help to identify additional mechanisms whereby cells adapt. Tissue specific deletion of stress sensing molecules and their downstream effectors in the mouse, in combination with genetic or environmental challenge, will reveal critical control points in the cellular decision to adapt to both pathological and developmental stresses. Ultimately, the regulation of cellular adaptive decisions during ER stress will shed light on how cells adapt to the variety of chronic stresses that exist in diverse disease states.

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