Intracellular signaling from the endoplasmic reticulum to the nucleus: the unfolded protein response in yeast and mammals

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Cellular survival of endoplasmic reticulum stress requires the unfolded protein response (UPR), a stress response first elucidated genetically in yeast. While we continue to refine our knowledge of the yeast system, especially the breadth and significance of the transcriptional response, conservation of the system's elements has allowed identification of corresponding and additional components of the mammalian UPR. Recent results reveal that the output of the mammalian UPR reaches beyond transcriptional regulation of secretory pathway components to control of general translation, the cell cycle and programmed cell death.

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Abbreviations
ERAD	ER-associated protein degradation
PS1	presenilin-1
UPR	unfolded protein response
UPRE	UPR element

Introduction

When unfolded proteins accumulate in the endoplasmic reticulum (ER), a signal is sent across the ER membrane into the nuclear and cytoplasmic compartments. There, effector proteins respond by upregulating the transcription of a characteristic set of target genes and slowing general translation, and the cell is enabled to tolerate and survive conditions which compromise protein folding in the ER. This reaction to ER stress is known as the unfolded protein response (UPR).

Given the importance of ER protein folding to normal cellular function, the benefit of the UPR appears self-evident. The ER contains an environment optimized for protein folding, and it is there that proteins translocated into the secretory pathway undergo modifications and interactions with chaperones that are essential for maturation into their final conformations. Failure to fold proteins efficiently not only robs the cell of the intended function of new proteins, but also exposes the cell to the potentially toxic effects of unfolded proteins per se. Therefore, it is not surprising that a mechanism exists in eukaryotic cells to monitor the folding state of the ER and respond actively to signs of trouble.

A UPR is present in all eukaryotes studied to date. Because many molecular details of the response have been conserved, it is likely that we can, with appropriate caution, extrapolate results garnered in yeast to the mammalian UPR. Several themes have emerged, revealing conservation at all levels of the pathway: information about ER stress is communicated to the cytosol by transmembrane kinases that are activated by *trans*-autophosphorylation and oligomerization. The endonuclease activity of these transmembrane kinase components, first identified in yeast, also exists in the mammalian system, although the specific mammalian target(s) of the activity are not known. The transcriptional effector functions of the response are carried out by soluble factors in the cytoplasmic and nuclear compartments, in particular, by members of the ATF/CREB family of basic leucine zipper proteins. The list of known targets of this transcriptional upregulation has grown, from a small group of ER-resident chaperones to a long roster of genes representing functions at every stage of the secretory pathway.

In addition to these similarities, studies focusing on the mammalian pathway have also revealed new complexities and divergences. Several new UPR components have been discovered, and we now know that the functions of homologous genes, as well as the linear connections between 'circuit elements', may not be strictly conserved throughout evolution. It is on these recent findings, both with respect to similarities and divergences between the yeast and mammalian systems, that we focus attention in this review.

The unfolded protein response in yeast

The initial characterization of the UPR's effector molecules was performed in the budding yeast *Saccharomyces cerevisiae*, where genetic screens revealed that three proteins are required for signal tranduction from the ER to the nucleus. These are Ire1p, which senses unfolded protein accumulation in the ER lumen and communicates this information across the ER membrane; Hac1p, which directly activates transcription of UPR target genes; and Rlg1p (tRNA ligase), which plays a critical role in bridging activation of Ire1 and production of Hac1p. The pathway is schematized in Figure 1.

The most upstream component of the pathway, Ire1p, is a transmembrane serine/threonine kinase with three functional domains. The most amino-terminal domain resides in the ER lumen and is thought to sense abnormally high levels of unfolded ER proteins [1]. Accumulation of unfolded proteins in the ER lumen (experimentally induced by agents such as tunicamycin, which blocks protein glycosylation, or dithiothreitol, which impairs disulfide bond formation) causes Ire1p to oligomerize and *trans*-autophosphorylate via its cytosolic kinase domain [2,3]. The activated kinase then stimulates the activity of Ire1p's most carboxy-terminal domain, which is a site-specific endoribonuclease.

Figure 1
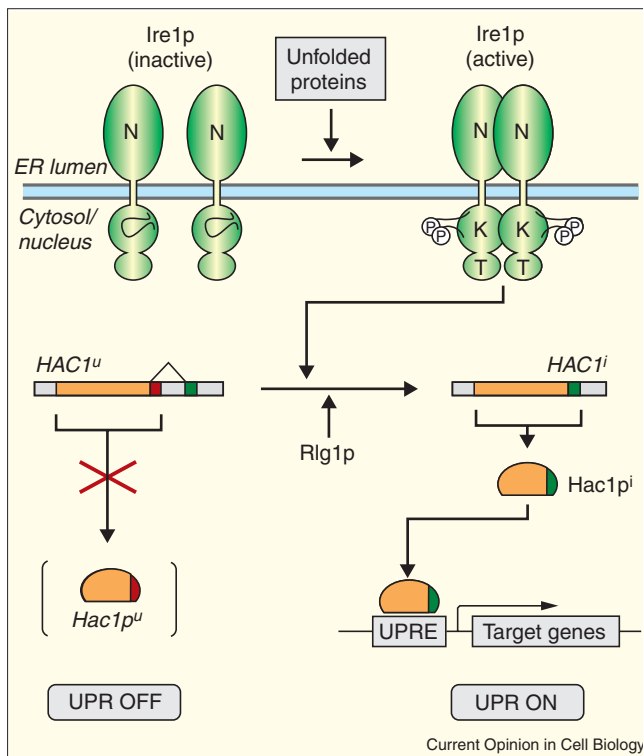
A schematic of the unfolded protein response in yeast. Ire1p is a transmembrane serine-threonine kinase, oriented with the amino terminus (N) in the ER lumen and the carboxyl terminus in the cytosol. When unfolded proteins accumulate in the ER, Ire1p oligomerizes, *trans*-autophosphorylates via the cytosolic kinase domain (K) and activates the endonuclease in the tail domain (T). The endonuclease Ire1p cuts *HAC1* mRNA at two sites, removing a nonclassical intron; the two exons are rejoined by Rlg1p (tRNA ligase). *HAC1*u ('uninduced') is not translated owing to the presence of the intron, and Hac1pu is not produced (brackets). After Ire1-mediated splicing, *HAC1*i mRNA is efficiently translated into Hac1pi, a transcriptional activator that upregulates expression of UPR target genes after binding to the unfolded protein response element (UPRE) in the promoters of genes encoding ER-resident chaperones and other proteins.

The only known substrate of the Ire1p endonuclease is the *HAC1* mRNA, which encodes the basic leucine zipper (bZIP) transcription factor that ultimately activates transcription of the UPR target genes. *HAC1*u ('uninduced') mRNA is constitutively transcribed, but the encoded protein (Hac1p) is not detectable under normal conditions [4]. *HAC1*u mRNA contains a non-classical intron near the 3′ end of the open reading frame, including the carboxy-terminal 10 amino acids and stop codon of the predicted protein [4]; when this intron is present, the mRNA is not translated [5,6]. Upon activation of the UPR, the intron is removed by two site-specific cleavages, shown directly by *in vitro* studies to be executed by Ire1p [7]. The 5′ and 3′ portions of the mRNA are rejoined by tRNA ligase [8], generating a new mRNA (*HAC1*i, 'induced') encoding a different protein in which a new 18 amino acid tail is appended. The new mRNA, free of the inhibitory intron, is efficiently translated to produce the transcription activator

Hac1p. *HAC1* mRNA splicing thus provides the key regulatory step in the UPR pathway in yeast.

The removal of the *HAC1* intron and the subsequent rejoining of the two exons is mechanistically distinct from spliceosome-mediated mRNA splicing, and it can occur even when spliceosomal function is conditionally blocked [8]. As might be expected from the role played by Rlg1p (tRNA ligase) in the rejoining of the *HAC1* exons, the chemistry of the overall reaction is more reminiscent of tRNA splicing than conventional mRNA splicing. In contrast to the spliceosomal case, where the 5′ splice site must be cleaved before the 3′ site, the *HAC1* junctions may be cleaved in either order [7,9], as is the case for tRNA splicing, and the cyclic phosphate intermediates of the *HAC1* splicing reaction are identical to those generated during tRNA splicing [10]. Despite the similarity in the chemistries, however, requirements for substrate recognition by the endonuclease in *HAC1* splicing differs substantially from those in tRNA splicing: a stem–loop structure of specific size and sequence, present at both of the splice sites in the *HAC1* mRNA (and to which there is no analog at pre-tRNA splice junctions), is necessary for accurate cleavage by Ire1p [9,10].

Once Ire1p cleavage has taken place and *HAC1*i is successfully translated, Hac1p translocates to the nucleus and activates target gene transcription by binding a UPR-specific upstream activating sequence, the unfolded protein response element (UPRE) [4,6,11]. The UPRE is found in the promoters of several UPR target genes, including those of the ER-resident chaperones *KAR2*, *PDI1* and *FKB2* [12], and is necessary and sufficient for transcriptional upregulation of a given gene by the UPR [13,14]. Like *IRE1*, *HAC1* is required for stimulation of target gene transcription in response to ER stress [4,11,15]; as production of Hac1p (e.g., from a gene expressing a 'pre-spliced' version of the mRNA) is sufficient to activate transcription of target genes, we view *IRE1* and *HAC1* as members of a linear pathway wherein activation of Ire1p serves primarily to remove the *HAC1* intron and allow translation of Hac1p.

Removal of the *HAC1* intron not only allows translation of the mRNA but also changes the sequence and properties of the encoded protein. The DNA-binding domain, located in the amino-terminal 220 amino acids common to Hac1pi and Hac1pu, is undisturbed by the splicing reaction, but the *trans*-activation domain includes the carboxy-terminal tail. When either carboxy-terminal tail was fused to an unrelated DNA-binding domain, the tail of Hac1pi served as a highly active transcriptional activation domain, whereas the Hac1pu tail was essentially inactive [6,16•]. Thus, the intron of *HAC1* mRNA not only inhibits translation but also guarantees that the sequence encoding the DNA-binding domain is separated from the transcriptional activation domain, so that even if the translational attenuation provided by the intron were circumvented, translation products will contain a less efficient activation

domain. Constitutive expression of Hac1p[i] slows growth considerably ([6]; C Patil, P Walter, unpublished data); hence, it is not surprising that mechanisms have evolved to prevent inappropriate translation.

It is less obvious, however, why such an unusual apparatus is employed to regulate Hac1p production. If Hac1p expression slows growth, why is the mRNA robustly expressed under unstressed conditions? Perhaps, regulation at the level of translation allows for suitable quantities of Hac1p to be produced rapidly from a stable pool of *HAC1* mRNA large enough to meet any conceivable (survivable) insult but which does not encode functional protein unless called upon to do so.

Although Hac1p[i] expression is sufficient for induction of several ER target genes, it may not act alone. Hac1p may recruit some or all of the components of the SAGA complex, a multiprotein assembly involved in histone acetylation during transcriptional activation, to the promoters of UPR target genes; this interaction is necessary for full induction of a subset of targets [17]. Curiously, several members of the SAGA complex also interact with the cytosolic domain of Ire1p; one of the components, Ada5p, appears to be required *in vivo* for splicing of *HAC1* mRNA [18].

The unfolded protein response in mammals
Mammalian homologs of Ire1p
The search for a mammalian homolog of Ire1p revealed a diverse family of transmembrane kinases that play roles in responding to ER stress. It also showed that many of the details of the UPR have been conserved during evolution. The mammalian Ire1 family includes two ER-resident proteins that are homologous to Ire1p over their entire length: Ire1α and Ire1β [19••,20,21]. Ire1α expression has been observed in all cell types, and the gene is essential for normal development in mice; Ire1β expression is largely limited to the epithelia of the gut [22•]. A divergent family member, PERK/PEK [23•,24], shares homology with the amino-terminal lumenal domain of Ire1p, but lacks an endonuclease domain and contains a kinase domain that is more closely related to the eIF2α kinase Gcn2p than to Ire1p.

As predicted from their sequence homology, Ire1α and Ire1β are in many regards functional homologs of Ire1p. Both proteins colocalize with ER marker proteins [19••,20,21], placing them in the proper subcellular location to transduce a signal across the ER membrane. Both proteins have intrinsic kinase activity and are capable of *trans*-autophosphorylation [19••,20]. As in the yeast protein, this kinase activity is relevant to downstream signaling: overexpression of Ire1α increases transcription from the promoter of the ER chaperone BiP, and overexpression of Ire1β increases transcription of both BiP and another UPR target, the transcription factor CHOP [21]. Conversely, overexpression of a kinase-dead mutant of Ire1α not only fails to upregulate BiP promoter activity but

also abolishes the tunicamycin-dependent inducibility of BiP present in untransfected cells [20]. The function of the lumenal domain is also conserved: in chimeric proteins, the amino termini of the mammalian proteins can substitute for the yeast amino terminus in *in vivo* assays of UPR signaling [25].

The mechanism by which the lumenal domain oligomerizes in response to unfolded proteins may also be conserved. It has been suggested that the lumenal domain of Ire1 does not sense unfolded proteins directly but rather is prevented from oligomerizing by an association with free molecules of the ER-resident chaperone BiP: when unfolded proteins accumulate, BiP dissociates from Ire1p (to bind the unfolded proteins), allowing association of the Ire1 lumenal domains and activating signaling [26]. Consistent with this, in mammalian cells BiP and Ire1 form a complex, which is disrupted under conditions of ER stress and Ire1 activation [27•]. In mammals as well as yeast, overexpression of BiP diminishes activation of the UPR, as would be predicted if the degree of Ire1 activation is negatively influenced by the concentration of free BiP [13]. Dimerization of the amino terminus appears to be the sole requirement for Ire1 activation: when the lumenal domain of yeast Ire1p is replaced by an unrelated leucine zipper dimerization domain, the chimeric protein is constitutively active [25].

Whether or not the mammalian Ire1 proteins exert their downstream effects on gene regulation by splicing a substrate mRNA or by other means is not known, but several lines of evidence argue that the endonuclease activity has been conserved. Recombinant Ire1α and Ire1β cytosolic domains cleave yeast *HAC1* mRNA *in vitro*, at precisely the same positions as the yeast protein and with indistinguishable structural requirements for substrate recognition [19••], an activity that requires a functional kinase domain [20]. HeLa cells transiently expressing yeast *HAC1* mRNA can splice the yeast message at the correct junctions in an ER stress-dependent manner [19••]. The conservation of this unique biochemical activity strongly predicts that mammals express mRNA(s) suitable for cleavage by Ire1, and the search for the mammalian Ire1 substrate RNAs is ongoing.

One possible substrate of the endonuclease might be the *IRE1* mRNA itself. Cells expressing mutants of Ire1α that lack *in vitro* endonuclease activity contain much higher levels of *IRE1* mRNA [28•], and cleaved fragments of *IRE1* mRNA can be observed in cells expressing wild-type but not kinase-dead Ire1α. Curiously, the cleavage sites inferred from primer-extension assays bear no resemblance to the well characterized cleavage sites in yeast *HAC1* mRNA, suggesting that the Ire1 endonuclease might recognize a wider spectrum of sequences than previously suspected. A key prediction of this model — that the steady-state level of *IRE1* mRNA will decrease when the UPR is activated by ER protein unfolding — remains to be tested.

Control of transcription: a role for regulated proteolysis

An as-yet undiscovered mammalian Ire1 substrate mRNA may encode a transcription factor analogous to Hac1p in yeast, but it is also conceivable that Hac1p's position in the pathway as proximal activator of transcription is filled by another protein whose activation is not regulated by splicing. Mammalian UPR-responsive promoters contain a tripartite response element, the ER stress element (ERSE), which is necessary and sufficient for UPR inducibility of a promoter [29,30]. The ERSE contains binding sites for the ubiquitous transcription factors CBF/NF-Y and YY1 [29,31], but it is specifically activated as a result of binding the bZIP transcription factor ATF6 [32], which, like Hac1p, is member of the ATF/CREB protein family. Rather than being regulated by splicing, however, ATF6 is constitutively synthesized as an ER membrane protein. It is inactive as a transcription factor while bound to the membrane; under ER stress, the cytosolic domain (containing the DNA-binding and transcriptional activation domains) is proteolytically cleaved from the membrane and then translocates to the nucleus to directly activate transcription of target genes such as *BiP* [33]. The cleavage of ATF6 is dependent on the site 2 protease (S2P), which is required for cleavage of active sterol-responsive element binding protein (SREBP) from its membrane-bound inactive form in response to cholesterol starvation [34••]. ATF6 cleavage does not, however, occur when cells are deprived of sterols, nor is cleavage dependent on the SREBP cleavage activating protein (SCAP), which escorts SREBP to the Golgi for proteolysis. It remains unknown how ER stress leads to the selective, activating proteolysis of ATF6, and whether other components such as Ire1 play any role in this process.

Regulated proteolysis may play a role at another stage of the mammalian UPR: Ire1α and Ire1β are also severed from the membrane upon UPR activation and translocate in soluble form to the nucleus [19••]. This nuclear localization requires presenilin-1 (PS1), a protein identical to or intimately linked with the activity of γ-secretase, which cleaves the developmentally regulated transcription factor Notch and amyloid precursor protein (APP) to generate amyloid plaques in the pathogenesis of Alzheimer's disease. The redistribution of Ire1 immunoreactivity from the cytosol to the nucleus upon activation is apparent when Ire1 is expressed at native levels [19••], but not when it is overexpressed [20,21,35], suggesting that the capacity of the protease for Ire1 cleavage is limiting. Cells lacking PS1 fail to cleave and relocalize Ire1 upon ER stress [19••] and may suffer from compromised induction of UPR target genes: two laboratories have shown that PS1 activity is required for full induction of BiP in response to ER stress [19••,35], but a third group observed no effect of presenilin expression on induction of UPR target genes [36]. The experimental conditions employed in these studies were not strictly commensurable, and the role of presenilin in UPR activation remains controversial.

Despite their sequence homologies to yeast Ire1p and Hac1p, mammalian Ire1 and ATF6 are activated in a quite different manner from their yeast counterparts, and it remains uncertain whether mammalian Ire1 functions genetically upstream of ATF6. On the one hand, certain data suggest that Ire1 is directly upstream of ATF6: overexpression of Ire1 in mammalian cells results in transcriptional induction of UPR target genes such as *BiP* [20,21] as well as of an artificial promoter containing a consensus ATF6-binding site [37••]. On the other hand, we do not yet know whether Ire1 is required for proteolytic cleavage of ATF6 upon UPR activation nor whether ATF6 cleavage is induced upon overexpression of Ire1. Indeed, cells lacking Ire1α altogether have no obvious defect in BiP induction [22•], and the largest observed effect of PS1 mutation or gene disruption on BiP induction is less than three-fold [19••,35]. In contrast, cells that lack S2P (and are therefore unable to cleave ATF6) are largely deficient in BiP induction [34••]. These genetic data imply that Ire1 and ATF6 respond to unfolded proteins independently of each other, and (unless S2P is also required for Ire1 activation) would appear to ascribe primary responsibility for target gene induction to ATF6. If ATF6 can respond to unfolded proteins independently of Ire1 it raises the possibility that the mammalian UPR bifurcates at the membrane. If this is the case then Ire1 activation may serve a primary function distinct from regulation of genes encoding ER-resident chaperones.

Stimulation of apoptosis by the unfolded protein response

A growing body of evidence suggests that Ire1 serves a pro-apoptotic function in response to ER stress. Prolonged tunicamycin treatment stimulates activation of the ER-membrane caspase-12, resulting in apoptosis [38]. This cell death is likely to result from Ire1 activation: overexpression of Ire1β stimulates apoptosis, possibly mediated by the pro-apoptotic transcription factor CHOP, which together with BiP is transcriptionally induced [21]. Ire1 may also activate apoptotic pathways by activating phosphorylation of c-Jun, which is thought to mediate apoptosis in response to ER stress [39]. Upon activation, both Ire1α and Ire1β recruit the cytosolic adaptor protein TRAF2, which is thought to recruit and activate proximal components of the c-Jun N-terminal kinase (JNK) pathway; Ire1α is required for stimulation of c-Jun phosphorylation in response to ER stress [22].

The UPR in mammals has both cytotoxic (pro-apoptotic) functions as well as cytoprotective ones. UPR activation can therefore result in one of two outcomes: either regulated cell death triggered by apoptotic effectors or survival of the stress facilitated by beneficial UPR target genes such as chaperones. The decision between these outcomes is presumably made during a cell cycle arrest also generated in response to ER stress. Prolonged tunicamycin treatment results in decreased translation of cyclin D1, causing a cell cycle arrest in G_1 phase and preventing cells from progressing through the cell cycle before ER homeostasis is re-established [40,41•]. This delay may allow a cell to pause in the cell cycle to determine whether adaptation to stressful conditions, mediated by chaperones

and other target genes, will be possible, and if not, to continue on toward apoptosis [42].

PERK and translational control

Translational inhibition during the UPR is not limited to cyclin D1, but rather is a general phenomenon mediated by the third ER kinase, PERK, which combines the ER-lumenal features of Ire1p with the cytosolic features of a Gcn2 kinase [23•,24]. Like Ire1p, PERK oligomerizes and *trans*-autophosphorylates in response to ER folding stress. When active, like other Gcn2 kinases, PERK phosphorylates the general translation initiation factor eIF2α, resulting in downregulation of overall protein synthesis. This translational slowdown appears to protect cells from the toxic effects of unfolded protein accumulation: when the UPR is induced, PERK knockout cells experience greater ER stress as evidenced by increased levels of Ire1p phosphorylation, activation of caspase-12 and apoptosis. Such cells are partially protected, however, by cycloheximide treatment [38], indicating that PERK's primary protective effect is to decrease overall protein synthesis, preventing additional synthesis of protein under conditions that do not allow proper folding. Translational repression by PERK is temporally selective: while general translation slows within 30 minutes of tunicamycin treatment [38], cyclin D1 protein levels remain unchanged for four to eight hours [41•] — by which time translation of a subset of mRNAs (including UPR targets such as BiP and CHOP) has recovered to normal levels [40]. The mechanism by which translational regulation exhibits such kinetic and gene-specific selectivity remains to be elucidated.

The ER-resident kinases (Ire1α, Ire1β and PERK), along with the transcription factor ATF6, trigger multiple downstream effects upon UPR activation (Figure 2). These divergent outputs may allow the cell to prepare for the worst while still trying to correct the ER protein folding defects. Upon UPR induction, after some insult that compromises protein folding in the ER, signaling from PERK slows down overall translation, preventing further insult to the secretory pathway. Somewhat later, as general translational repression is relaxed or specific mechanisms allowing efficient translation of UPR target genes are activated, transcriptional targets of ATF6 and Ire1 are induced and brought to bear on the task of eliminating the source of the problem, by refolding of wayward proteins and preventing formation of toxic aggregates. Concomitantly, depression of cyclin D1 levels slows progress through the cell cycle. With luck, these measures will succeed and the cell will survive the insult. Not all stresses, however, are survivable: if the situation cannot be corrected (perhaps indicated by the cell cycle arrest lasting beyond some predetermined interval), the apoptotic effectors of Ire1 signaling (via JNKs, CHOP and caspase-12) would kill the cell in an orderly fashion.

Transcriptional output of the unfolded protein response

Clearly, the scope of UPR outputs in the mammalian cell, ranging from translation inhibition to regulation of cellular

Figure 2
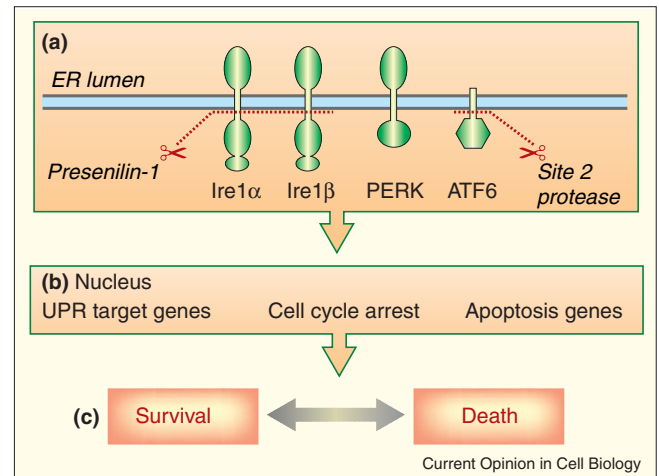
Diversity and parallelism in the mammalian unfolded protein response. **(a)** Response to ER stress in mammalian cells is mediated by multiple transmembrane proteins, including kinases (Ire1α, Ire1β and PERK) and basic leucine zipper transcription factors of the ATF/CREB family (ATF6). In the cases of the Ire1 proteins and ATF6, activation by unfolded proteins is concomitant with cleavage of the cytosolic effector domains from the ER membrane (by the protease indicated) and their subsequent translocation to the nucleus. **(b)** The diversity of UPR membrane components is reflected by the diversity of UPR outputs: under ER stress, mammalian cells upregulate beneficial target genes such as ER-resident chaperones but also activate elements of the apoptotic machinery. **(c)** The decision between survival and death is presumably made during a cell cycle arrest also triggered by UPR activation.

signaling pathways, is complex. It has recently come to light that UPR transcriptional regulation itself is more extensive than previously believed, in yeast as well as in the mammalian system. Whole-genome expression profiling, using DNA microarrays, has shown that over 5% of the yeast genome (more than 350 genes out of 6300) is regulated by the UPR [43••]. Strikingly, of the genes with a known function, more than half play roles throughout the secretory pathway in the ER and beyond; these include factors implicated in protein translocation, lipid metabolism, glycosylation, ER-associated protein degradation, ER to Golgi traffic and protein targeting to the vacuole and to the cell surface. The UPR may also play a role in sensing and responding to nitrogen starvation: in the diploid stage of the yeast life cycle, activation of the UPR inhibits two distinct nitrogen starvation responses, pseudohyphal growth and sporulation. Furthermore, the UPR is itself activated by a nitrogen-rich environment, possibly because rapid translation in a nitrogen-rich environment results in accumulation of unfolded proteins [44••].

Although the mammalian UPR still awaits comprehensive genomic analysis, recent identification of novel target genes suggests that similarly broad patterns may well exist in metazoans: the UPR positively regulates several secretory genes and processes, for example, the dolichol pathway essential for protein glycosylation [45], the ER calcium pump required for maintenance of calcium homeostasis [46] and SERP1/RAMP4, which stabilizes membrane proteins

and facilitates their glycosylation [47]. Interestingly, mammalian cells upregulate asparagine synthetase in response to ER stress [48], suggesting that the mammalian UPR might also be involved in regulation of nitrogen metabolism.

In yeast, genome-wide expression data are complemented by a genetic screen for mutants that are synthetically lethal when *IRE1* is disrupted; *IRE1* is dispensable for growth under normal conditions but not under ER stress [49•]. The screen, which is still far from saturation, revealed more than a dozen genes involved in multiple secretory events. These genes are essential in the absence of UPR function and a subset of these proved to be UPR targets in their own right. Their synthetic-lethal relationship to *IRE1* suggests that these genes' functions contribute to the physiological output of the UPR, which is essential under ER stress.

The regulation of so many classes of target genes suggests that the UPR may remodel the entire secretory pathway in response to folding stress in the ER, upregulating functions that conspire to decrease the concentration of unfolded ER proteins by increasing the rates of folding, post-translational processing, export and (if necessary) degradation, and thereby diminish the load on the folding capacity of the cell. Alternatively, the UPR may monitor not only the ER but also the entire secretory pathway, perhaps detecting an ER event that is a common consequence of multiple types of secretory defect. The breadth of the response could then be understood as an upregulation of factors that might be rate-limiting at any one of a number of steps along the secretory pathway. In either case, the functions of UPR target genes should encompass those physiological outputs that make the UPR essential for survival of ER stress. This has been demonstrated most extensively for one novel class of target genes — those involved in ER-associated protein degradation or ERAD.

ERAD is a process by which misfolded ER proteins are detected and prevented from progressing along the secretory pathway by ER-resident factors and directed to the translocon for retrotranslocation into the cytosol, where they undergo ubiquitin- and proteasome-dependent degradation (for reviews see [50,51]). Many specific components of the ERAD pathway are induced by the UPR [43••,52•,53•]. Indeed, the UPR is required for efficient ERAD, as misfolded protein substrates of the pathway are stabilized in an Δ*ire1* mutant [43••,49•,52•,53•]. Activation of the UPR accelerates the rate of degradation of misfolded proteins, even in the absence of folding stress [43••,52•], as would be expected if the consequence of upregulation of ERAD genes were to increase the capacity of the pathway: when unfolded proteins are detected at higher than normal levels, the UPR upregulates components of the ERAD machinery to more efficiently deal with the task of eliminating these unfolded proteins.

The enhancement of ERAD function upon UPR induction is of profound physiological importance to the cell. Loss of any one of several ERAD genes makes cells dependent on the UPR for normal growth [43••,49•] and even under conditions of mild stress [53•]. Cells unable to perform ERAD are under constant folding stress, as indicated by a constitutive activation of the UPR [43••,49•], and the degree of activation of the UPR is correlated with the severity of the ERAD defect [53•]. A UPR-activated reporter gene was used to exploit this phenomenon to identify novel ERAD-deficient alleles of the translocon component gene *SEC61* [54•]. These genetic interactions are evident even in the absence of exogenous stress, indicating that misfolded proteins are generated consequently over the course of normal growth and that the cell must prevent the accumulation of these misfolded proteins or fail to thrive. Rather than being individually dispensable, then, the UPR and ERAD are intimately coordinated, complementary mechanisms serving the essential function of preventing unfolded protein accumulation and mitigating its toxic consequences when it occurs.

Conclusions

Many aspects of the UPR are conserved across evolution. In both yeast and mammals, ER stress is sensed by transmembrane kinases of the Ire1 family, which oligomerize and activate cytosolic effector domains in response to unfolded protein accumulation. The site-specific endonuclease activity of yeast Ire1p is shared by the mammalian homologs, although the mammalian substrate has yet to be identified. Transcription is regulated (in whole or in part) by bZIP proteins of the ATF/CREB family, and the scope of induced target genes is broad: in yeast, functions throughout the secretory pathway are upregulated by the UPR, and early evidence from mammals suggests the same diversity of output will be observed in higher eukaryotes.

Despite these similarities, the mammalian UPR is both divergent from and more complex than the yeast pathway. The activation of the ATF/CREB transcription factor ATF6 in mammals is controlled by regulated intramembrane proteolysis, and protease activity may play a role in the activation of mammalian Ire1 as well. The functional connection (if any) between the upstream sensors of ER stress and the transcriptional effectors of the response remains to be determined. In addition to transcriptional activation, the mammalian UPR's functional output includes downregulation of overall translation, cell cycle arrest and the activation of apoptosis. The UPR, therefore, appears to simultaneously prepare the cell for both survival and annihilation — a conflict possibly resolved by the nature of the insult as well as its severity and duration.

In yeast the UPR is a linear, relatively well understood pathway that generates a broad range of outputs at the transcriptional level. In contrast, in mammalian cells the UPR is an arborized, parallel response (or suite of responses) with an even more diverse output. Future work must aim to clarify both the functional connections between the elements of the mammalian pathway as well as the mechanisms by which the multitude of outputs are integrated to maximize cellular and organismal survival in response to protein unfolding and stress in the ER.

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