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THE MAMMALIAN UNFOLDED PROTEIN RESPONSE

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■ **Abstract** In the endoplasmic reticulum (ER), secretory and transmembrane proteins fold into their native conformation and undergo posttranslational modifications important for their activity and structure. When protein folding in the ER is inhibited, signal transduction pathways, which increase the biosynthetic capacity and decrease the biosynthetic burden of the ER to maintain the homeostasis of this organelle, are activated. These pathways are called the unfolded protein response (UPR). In this review, we briefly summarize principles of protein folding and molecular chaperone function important for a mechanistic understanding of UPR-signaling events. We then discuss mechanisms of signal transduction employed by the UPR in mammals and our current understanding of the remodeling of cellular processes by the UPR. Finally, we summarize data that demonstrate that UPR signaling feeds into decision making in other processes previously thought to be unrelated to ER function, e.g., eukaryotic starvation responses and differentiation programs.

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PERSPECTIVE AND OVERVIEW

Aberrant protein conformations are a major cause for disease. Many of these diseases originate within the endoplasmic reticulum (ER). Recent work has shown that the ER is not a passive transport organelle traversed by proteins on their way to their destination in the cell or the extracellular space. It has now been established that the ER actively monitors the folding status of its cargo. Signal transduction pathways originating in the ER are activated to increase the folding capacity of the ER or induce cell death when protein folding in the ER is inhibited. In this review, we summarize our current insight into these processes, which make the ER a very interesting organelle for further investigation.

Definition of ER Stress

The ER is the first compartment in an ordered membranous network called the secretory pathway. This pathway is responsible for the synthesis, modification, and delivery of biologically active proteins to their proper target sites within the cell and the extracellular milieu. As with many other biochemical pathways, flux through the secretory pathway is controlled at its early steps. Transit from the ER to the Golgi complex is the rate-limiting step in secretion for many glycoproteins. The ER is the entry site for the vast majority of proteins processed in the secretory pathway. Early steps in the maturation of secretory proteins take place in the ER, e.g., the folding of the nascent polypeptide chains and posttranslational modifications important for proper folding and function of the protein. If the influx of nascent, unfolded polypeptides exceeds the folding and/or processing capacity of the ER, the normal physiological state of the ER is perturbed. Under these conditions, signaling pathways, termed the unfolded protein response (UPR), are activated to

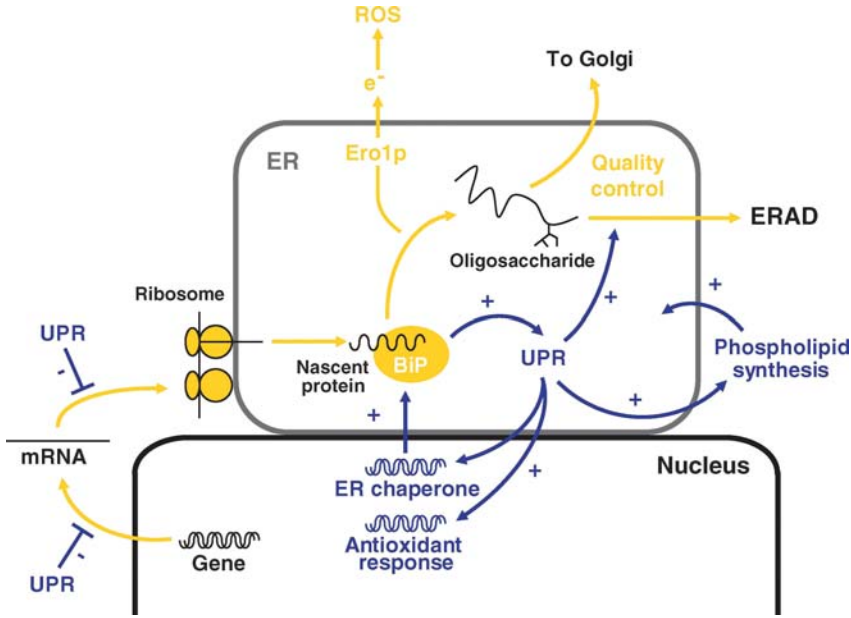


Figure 1 Protein transport through the ER (*yellow*) and activities of the UPR to couple the ER protein-folding capacity with its protein-folding burden (*purple*). Abbreviations are ER, endoplasmic reticulum, and ROS, reactive oxygen species. Modified and reprinted from *Mutation Research* (1c), copyright 2005, with permission from Elsevier.

return the ER to its normal physiological state (Figure 1). This response situation is an example of ER stress (1). The ER is also the site of the synthesis of sterols and lipids. Although recent studies have shown that perturbations in lipid metabolism cause an ER stress response, very little is known about the mechanism of UPR activation by perturbations in lipid metabolism (1a,1b).

Perturbation of ER Function

Proper function of the ER is perturbed when the influx of nascent, unfolded polypeptide chain exceeds the folding capacity of the ER. This can be achieved by overexpressing large and heavily modified proteins, e.g., blood coagulation factor VIII (2, 3) or small and structurally simple proteins, such as antithrombin III (4). These proteins were found in a complex with ER resident molecular chaperones (2) or in high-molecular-weight aggregates (4). Expression of mutant, folding-incompetent proteins induced expression of ER resident molecular chaperones (5). This observation is the biochemical basis for a wide variety of diseases termed ER storage or conformational diseases (6, 7). Recent work showed that normal biological processes, such as differentiation of B cells into plasma cells (8), viral

infection (9), and, in plants, the host's response to a microbial infection (10, 11), are associated with increases in secretory activity that, at least in the early stages, exceed the folding capacity of the ER and cause a UPR.

Remedies of Perturbed ER Function

To bring the folding capacity of the ER in line with the folding demand placed on the ER, the folding demand is decreased, and the folding capacity of the ER increased (Figure 1). To decrease the folding demand, transcription of genes, encoding secretory proteins and translation (12), are downregulated; and the clearance of slowly folding or misfolded proteins through ER-associated degradation (ERAD) is increased (13). To increase the folding capacity of the ER, the synthesis of ER resident molecular chaperones and foldases is increased (5), and the ER increases in size (3) to dilute the increased unfolded protein load.

Consequences of Perturbed ER Function

Perturbations of ER function do not remain localized to the ER but spread through the secretory pathway, the cell, and the organism. In lower eukaryotes, assembly of the cell wall and function of the plasma and vacuolar membranes are perturbed by ER stress. Loss of secretion of a particular secretory protein can cause severe diseases in humans, e.g., hemophilia through combined loss of secretion of blood coagulation factors V and VIII (14). Accumulation of folding-incompetent proteins resistant to proteasomal degradation in the ER can completely disrupt ER and cellular function, activate apoptotic-signaling pathways, and is the basis for many neurodegenerative diseases (9, 15, 16). Loss of UPR signaling, itself, can abrogate secretion and cause diseases such as diabetes (17, 18). Perturbations of cellular physiology outside of the ER can be propagated to the ER to cause ER stress and activate the UPR (19). Abrogation of mechanisms to adjust the protein flux through the ER to the protein-folding capacity of the ER, such as inhibition of the proteasome and subsequently ERAD by cytosolic polyglutamine repeats, induces ER stress, cell death, and disease. Mutations in the cytosolic portion of membrane proteins can disturb folding of the ER-luminal part of the protein and result in ER retention that causes ER stress, as in the case of the ΔF508 mutation in the cystic fibrosis transmembrane conductance regulator (20). Because many of these diseases are associated with retention of the misfolded protein in the ER, they were originally called ER storage diseases. As it became clear that abnormal protein conformations are causative for these diseases, the term "conformational diseases" was coined.

The Informative Content of UPR Signaling

The endoplasmic reticulum has evolved elaborate mechanisms to ensure that only properly folded and assembled proteins exit the ER, a process termed "quality control." An important aspect of quality control is the ability to discriminate

between folded and misfolded or unfolded polypeptides. The protein-folding status in the ER is relayed to the cytosol and nucleus by the UPR. The major ER resident molecular chaperones were identified through their induction by glucose deprivation (21). UPR-signaling pathways respond to the nutritional state of a cell (22, 23) and control well-established regulatory gene clusters involved in metabolism and starvation responses (24, 25). Disruption of UPR signaling in yeast causes inositol auxotrophy (26). Recent work has shown that the UPR is involved in controlling differentiation programs in yeast (22, 25) and mammals (8). Lastly, viral infections activate the UPR (9). The unfolded protein load of the ER may be tightly linked to the nutritional, differentiation, or infection status of the cell, and it is therefore not surprising that UPR signaling may extend beyond simply maintaining the homeostasis of the ER and that it contributes to decision making in these important cellular events.

PROTEIN FOLDING IN THE ER

The ER is a major protein-folding compartment in a eukaryotic cell and is second only to the cytosol. Many principles governing protein folding in the cytosol apply to the ER. However, protein folding in the ER is more complex than protein folding in the cytosol because proteins are posttranslationally modified, e.g., by N-linked glycosylation and disulfide bond formation. Furthermore, important cytosolic chaperones, for example an ER homologue for GroEL/GroES, have to date escaped detection.

Basics of Protein Folding

To understand why protein folding in the ER is easily disrupted, we briefly summarize the generally applicable principles of protein folding. As in any chemical reaction, a protein-folding reaction has to fulfill thermodynamic and kinetic requirements (27).

THE THERMODYNAMIC REQUIREMENT The number of all possible conformations for any given protein, defined by the number of native and total interactions of its residues (27), is determined by its amino acid sequence. The free energy of each conformation is largely determined by the contacts of its nonpolar groups. Exposure of these groups at the surface of the protein and contact with the surrounding solvent, usually ~150 mM salt in water for biological systems, increases the free-surface energy of the protein-water system. Burial of a nonpolar side chain in the core of the protein minimizes the contacts between hydrophobic side chains and water and the free surface energy of the protein-water system. In contrast, exposure of polar groups at the protein surface does result in a much less pronounced increase in the free surface energy of the protein-water system. When the free energies are plotted versus their corresponding conformations, an energy surface or

landscape is obtained. On this energy landscape, the protein folds on several competing pathways, leading to ever-decreasing free energies until a transition state is crossed and the conformation with the lowest free energy is reached. Theoretical calculations showed that this conformation usually represents the native conformation of the protein. In summary, the primary structure of the protein determines its folding energy landscape, folding pathways, and the native state. This principle was first summarized in Anfinsen's dogma (28).

THE KINETIC REQUIREMENT Protein folding is initiated by a hydrophobic collapse, in which several hydrophobic side chains shield each other from surrounding water and form the core of the protein (29). The free energies of all possible conformations resulting from a hydrophobic collapse would be very similar. Further folding through sampling different conformations with nearly equal free energies would be very slow. Another folding determinant is the burial of electrostatic interactions, such as salt bridges, hydrogen bonds, or disulfide bonds, in the hydrophobic core. These hydrophilic structures provide an energy signature to conformations formed in a hydrophobic collapse and limit its further folding choices (29). Energy landscapes, the hydrophobic collapse, and the formation of hydrophilic interactions that limit the conformational choices of the hydrophobic core of the protein contribute to nature's solution to Levinthal's paradox, which states that there are far too many possible conformations for any given protein and that the proteins cannot be expected to productively fold in a biologically relevant time span.

Individual structures in proteins fold very rapidly. α-Helices or β-turns are formed within 0.1–10 microseconds (μs) (27). Structurally simple small proteins fold in less than 50 μs (27), whereas more complex structures, e.g., β-sheets, fold more slowly (27). Modules or domains of larger proteins fold independently of each other into near native structures (27). In a final cooperative folding event, water is excluded from the protein core, and the native structure is formed (27).

Protein Folding in the Cell

In the cell, protein folding occurs cotranslationally, which is no surprise, when the rate of $\approx$2–8 amino acid residues per second for polypeptide chain synthesis by the ribosome is considered (30). Furthermore, not all proteins are synthesized in a cell at their final destination, and many have to traverse hydrophobic phospholipid bilayers in a denatured state to reach their final destination, the secretory pathway, the mitochondrion, or the chloroplast. In addition, the cell is a crowded environment. Protein concentrations in the ER reach $\approx$100 g/liter ($\approx$2 mM) (29). Even at concentrations of $\approx$4–6 μM, association of proteins can be a diffusion-controlled process (29). Through molecular chaperones, the cell provides a means that prevents hydrophobic amino acid stretches displayed on the surface of folding proteins from interacting with each other, which would result in a nonproductive aggregation of newly synthesized proteins. Major cytosolic chaperone classes

are the small heat shock proteins (HSPs), HSP60s (GroEL-GroES, CCT/TRiC), HSP70s, HSP90s, and HSP100s. One function of HSPs is to prevent protein aggregation that occurs upon thermal denaturation. Finally, the rates for spontaneous *cis-trans* isomerization of peptidyl-prolyl bonds in proteins are too low for productive protein folding in the cell (29). These reactions are catalyzed by a class of foldases called peptidyl-prolyl *cis-trans* isomerases (PPI). On the basis of their sensitivity to either cyclosporine A or FK506, this enzyme family is further divided into cyclophilins and immunophilins, respectively.

Specific Constraints on Protein Folding in the ER

Protein folding in the ER is based on the same principles that govern protein folding in the cytosol. Unique physicochemical, chemical, and biochemical features of the ER require ER-specific solutions to the folding problem.

TOPOLOGY The ER is a membrane-surrounded compartment, and its luminal space is topologically equivalent to the extracellular space. Proteins destined for the ER are directed to the ER through a predominantly hydrophobic signal sequence and have to, either co- or posttranslationally, traverse the ER membrane through an aqueous channel, the Sec61p complex. Signal peptidase cotranslationally cleaves off the signal peptide. Bacterial signal peptidases process their substrates after translation of ~80% of the polypeptide chain (29), suggesting that the signal peptide is present during initial folding steps of the protein. Indeed, the signal sequence influences the timing of N-linked glycosylation and signal sequence cleavage (31). Inefficient cleavage can result in prolonged interaction of the protein with ER chaperones (29).

CHEMICAL COMPOSITION The pH in the ER is near neutral and comparable to that of the cytosol (29). In mammalian cells the ER is the major site for Ca^{2+} storage. ER-luminal Ca^{2+} concentrations reach 5 mM, compared to 0.1 μM in the cytosol. ER-luminal Ca^{2+} concentrations rapidly and frequently fluctuate as the ER Ca^{2+} pool is mobilized during intracellular signaling. Ca^{2+} participates in electrostatic interactions in proteins and, through these, alters hydrophobic interactions. Thus, the effect of fluctuations in the ER Ca^{2+} pool on protein folding depends on the individual protein. The folding of certain proteins, such as apo-α-lactalbumin, is dependent on the presence of Ca^{2+} (32). More importantly, the majority of the ER resident molecular chaperones and foldases are low-affinity, high-capacity Ca^{2+}-binding proteins, and the majority of the ER-luminal Ca^{2+} is stored bound to ER-luminal proteins. Perturbation of ER-luminal Ca^{2+} inhibits chaperone function. For example, depletion of Ca^{2+}-dissociated heavy-chain binding protein (BiP) from T-cell antigen receptor α-chain (33), induced a conformational change and oligomerization in calnexin (CNX) (34) and induced interaction between protein disulfide isomerase (PDI) and calreticulin (CRT) (35).

POSTTRANSLATIONAL MODIFICATIONS A nascent chain undergoes numerous post-translational modifications in the ER. Inhibition of each modification inhibits protein folding. However, only disulfide bond formation and N-linked glycosylation have been linked to UPR signaling to date. These are briefly discussed.

DISULFIDE BOND FORMATION The major redox buffer in the cell is glutathione. In the cytoplasm, the ratio of reduced glutathione to oxidized glutathione is >50:1 (36). In contrast, in the ER, this ratio is 1:1 to 3:1. Disulfide bond formation in the ER is catalyzed by protein disulfide isomerases, and their disulfide bonds are recycled by the FAD-dependent oxidases Ero1p and Erv2p. The final electron acceptor for Ero1p and Erv2p is O_2. Peroxide and superoxide are minor electron acceptors for Ero1p. Further, Ero1p is essential under anaerobic conditions in yeast, suggesting that an alternative electron acceptor for Ero1p exists. Thus, uncoupling of Ero1p from its physiologic electron acceptor, e.g., during ER stress, may result in generation of reactive oxygen species (Figure 1). Glutathione contributes net reducing equivalents to disulfide bond formation in the ER. Because disulfide bond formation liberates reducing equivalents in the ER, this process generates oxidative stress.

N-LINKED GLYCOSYLATION The transfer of a core oligosaccharide structure from a membrane-bound dolichol phosphate anchor to consensus Asn-X-Ser/Thr residues in the polypeptide chain initiates the process of N-linked glycosylation. Glycosylation serves several purposes in protein folding. First, owing to the hydrophilic nature of carbohydrates, glycosylation increases the solubility of glycoproteins, and the attachment sites define the surface areas of the protein. Second, because of their large hydrated volume, oligosaccharides shield the attachment area from surrounding proteins and thus act as a chaperone. Third, oligosaccharides interact with the peptide backbone and stabilize its conformation (37). Fourth, sequential trimming of sugar residues is monitored by a lectin machinery to report on the folding status of the protein (Figure 2*a*) (37). This CNX/CRT cycle is one arm of the quality control machinery in the ER that monitors protein conformations and dictates whether a molecule is exported to the Golgi or targeted for ERAD. The monoglucosylated form of a folding protein shuttles through cycles of de- and reglucosylation by α-glucosidase II and uridine diphosphate (UDP)-glucose: glycoprotein glucosyl transferase (UGGT) (Figure 2*a*) (37). The monoglucosylated form is retained in the ER via interaction with the lectins CNX and CRT. UGGT preferentially recognizes and glucosylates partially unfolded glycoproteins. Proteins are extracted from this cycle by demannosylation by α-(1, 2)-mannosidase I (Figure 2*a*). If improperly folded, reglucosylation by UGGT initiates interaction with CNX, transfer to the lectin Mnl1p/Htm1p/ER degradation-enhancing α-mannosidase-like protein (EDEM) (38, 39), and retrograde translocation to the cytoplasm for degradation by the proteasome (Figure 2*a*). α-(1, 2)-mannosidase I may also act directly on CNX-bound $GlcMan_9GlcNAc_2$ structures. In general, glucosidase II, UGGT, and α-(1, 2)-mannosidase I accept several

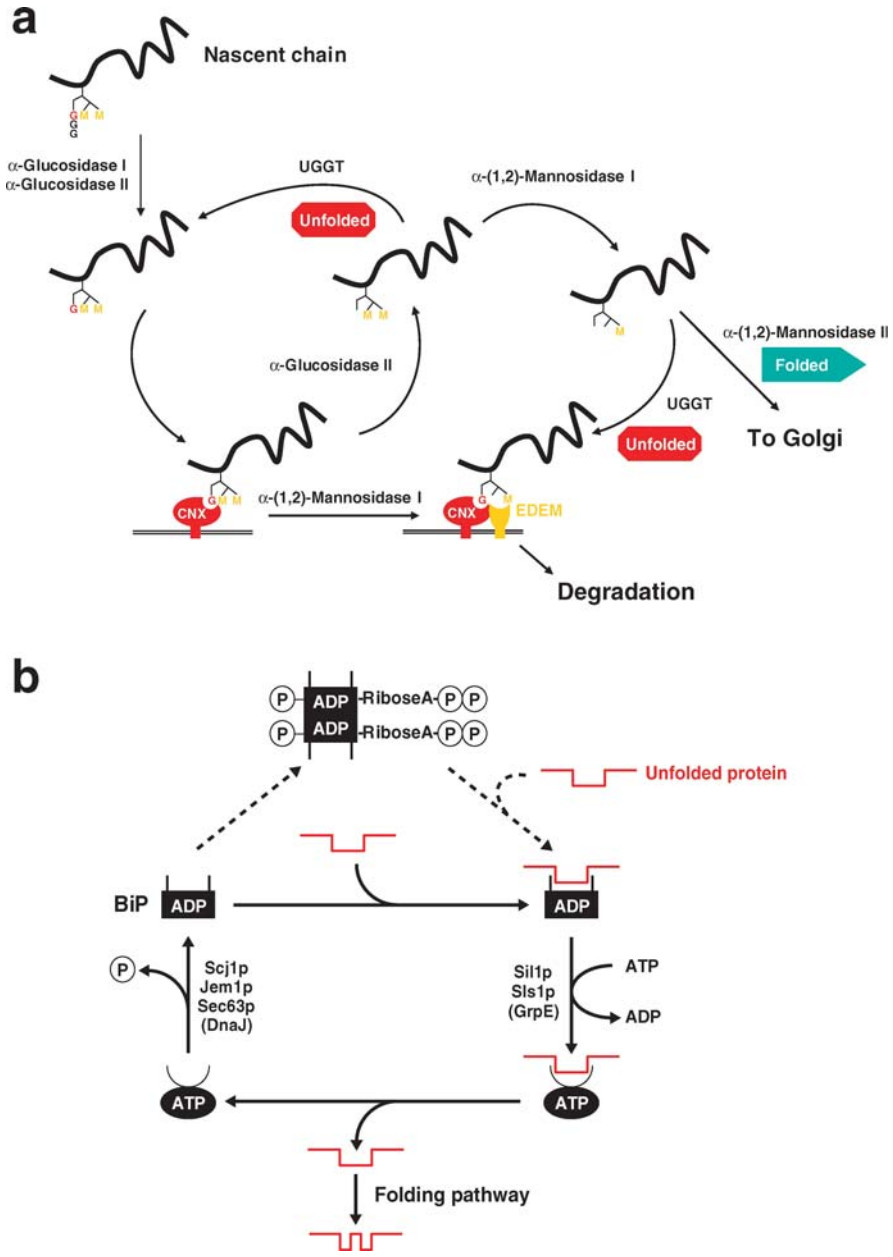


Figure 2 ER quality control mechanisms. (*a*) The calnexin/calreticulin cycle. Abbreviations are CNX, calnexin; EDEM, ER degradation-enhancing α-mannosidase-like protein; G, glucose; M, mannose; and UGGT, uridine diphosphate (UDP)-glucose:glycoprotein glucosyl transferase. (*b*) The BiP ADP-ATP cycle.

glycostructures with varying efficiencies (40, 41). The contribution of each of these enzymatic reactions and which intermediates accumulate in this quality control mechanism are not clear. The levels of UGGT and α-(1, 2)-mannosidase I activities are comparable (41, 42), indicating that a folding protein may require only a small number of deglucosylation-reglucosylation cycles to obtain its native conformation.

PROTEIN-FOLDING MACHINERY The protein-folding machinery of the ER consists of three classes of proteins, foldases, molecular chaperones, and the lectins CNX, CRT, and EDEM, as well as N-linked oligosaccharide processing enzymes (Table 1). Foldases catalyze steps in protein folding. Prominent examples are peptidyl-prolyl *cis-trans* isomerases and PDIs (see above). Molecular chaperones facilitate protein folding by shielding unfolded regions from surrounding proteins. In the ER, the HSP70 chaperones BiP/GRP78/KAR2, LHS1/GRP170 (CER1/SSI1) (43), their cochaperones of the DnaJ (44) and GrpE families (45), the HSP90 chaperone GRP94 (46), and the chaperones CNX and CRT (see above) are present. Many of these chaperones function in higher-order complexes and coordinate their activity (47). Owing to the specificity of UGGT, the CNX/CRT cycle preferentially recognizes partially folded intermediates (48). CNX and CRT interact with the oxidoreductase ERp57 to promote disulfide bond isomerization in bound unfolded glycoproteins (49). GRP94 recognizes partially folded structures on a subset of proteins, which are recognized as being unfolded by BiP or CNX/CRT (46). BiP is required for translocation across the ER membrane and interacts with early folding intermediates. The ER-luminal oxidoreductases Eps1p (50) and Pdi1p (51) are involved in the recognition of slowly folding or folding-incompetent proteins and the targeting of these proteins to the proteasome. Preferential interaction of unfolded proteins with ER-resident molecular chaperones and retention of these proteins in the ER through such interactions constitutes the second arm of the quality control machinery in the ER. In addition to these general chaperones and foldases with broad substrate specificity, many specific chaperone client protein pairs have evolved (Table 2).

RECOGNITION OF UNFOLDED PROTEINS

The chaperone machinery recognizes a protein as folded or unfolded. How one protein recognizes another protein as being unfolded is only understood for BiP and, partially, for UGGT.

Recognition of Unfolded Proteins by BiP

BiP binding to an unfolded protein does not facilitate protein folding but rather maintains the protein in a folding-competent state. BiP consists of an N-terminal ATPase and a C-terminal substrate-binding domain. In the ATP-bound form, BiP binds substrates with low affinity. Substrate binding stimulates the ATPase

TABLE 1 ER-resident molecular chaperones, foldases, lectins, and N-linked oligosaccharide-modifying enzymes

Class and name	Function
Chaperones, HSP70 class	
BiP/GRP78/Kar2p	Chaperone, translocation, folding sensor
Lhs1p/Cer1p/Ssi1p/GRP170	Chaperone
Cochaperones, DnaJ-like, HSP40 class (BiP ATPase stimulation)	
ERdj1/MTJ1	
ERdj3/HEDJ/Scj1p	
ERdj4	
ERdj5	
Jem1p	
Sec63p	Translocation
Chaperones, GrpE-like (nucleotide exchange factor for BiP)	
BAP	
Sil1p/Sls1p	
Chaperones, HSP90 class	
GRP94/endoplasmin/tumor rejection antigen gp96/ERp99	Chaperone
Lectins	
Calnexin (CNX)	Glycoprotein quality control
Calreticulin (CRT)	Glycoprotein quality control
Mnl1p/Htm1p/EDEM	Glycoprotein degradation
Carbohydrate processing enzymes	
UGGT	Folding sensor
α-glucosidase I	Removal of terminal glucose residues from glycoproteins
α-glucosidase II	Removal of terminal glucose residues from glycoproteins, release of glycoproteins from CNX
α-mannosidase I	Removal of terminal mannose residues, extraction of glycoproteins from the CNX cycle
α-mannosidase II	Removal of terminal mannose residues, extraction of glycoproteins from the CNX cycle
Foldases, subclass disulfide isomerases	
Ero1p/Ero1-Lα, Ero1-Lβ	Oxidoreductase for PDI
Erv2p	Oxidoreductase for PDI
Pdi1p/PDI/ERp59/GSBP/ER calcistorin PDI	
PDIp	Pancreas-specific

(*Continued*)

TABLE 1 (*Continued*)

Class and name	Function
P5/CaBP1/Mpd1p	
ERp72/CaBP2	
ERp61	
ERp60/Eug1p	
ERp57/GRP58	
PDIR	
ERp46	
ERp44	Retention of Ero1α in ER
ERp19	
TMX	
Mpd2p	
Eps2p	
Thioredoxin homology domain-containing chaperones	
ERp29/ERp28/Windbeutel/PDI-Dβ	
Foldases, subclass FAD-dependent oxidases	
Fmo1p	FAD-dependent oxidase
Foldases, peptidyl-prolyl *cis-trans* isomerases, family: cyclophilins (inhibited by cyclosporine A)	
S-cyclophilin	
SCYLP	
Cyclophilin B	
Drosophila ninaA	Opsin folding
Foldases, peptidyl-prolyl *cis-trans* isomerases, family: immunophilins (inhibited by FK506)	
FKBP13	
FKBP65	
Other	
ERp49	

activity of BiP to generate the ADP-bound form that has a high affinity for the bound peptide (52) (Figure 2*b*). Binding assays with random peptide libraries and affinity panning of peptides displayed on phages showed that short hydrophobic peptides, such as those forming β-strands deeply buried in the protein core, are preferentially bound by BiP (52). Thus, exposure of hydrophobic regions on its surface, the thermodynamic hallmark of an unfolded protein, is recognized by BiP. The affinity for these peptides is low (1–100 mM) (52), allowing for a wide substrate spectrum. Exchange of ADP with ATP releases the substrate from

TABLE 2 Specialized client protein chaperone pairs in the ER

Factor	Function	Client protein	Organism/cell type
BAP31	Promotes ER secretion competence	Cellubrevin	Mammalian cells
BOCA	Assembly and transport	Low-density lipoprotein (LDL) receptor	*Drosophila*
Carboxylesterase	Mediates ER retention of target molecule through KDEL-like ER retention signal	C-reactive protein	Hepatocytes
β-catenin	Targeting to the basal-lateral membrane	E-cadherin	Epithelial cells
Chs7p	Promotes ER secretion competence	Chs3p, catalytic subunit of chitin synthetase III	*Saccharomyces cerevisiae*
Egasyn	Mediates ER retention of target molecule through KDEL-like ER retention signal	β-glucuronidase	Mammalian cells
Erv14p	Potential cargo receptor	Plasma membrane protein Axl2p	*S. cerevisiae*
Gsf2p	Promotes ER secretion competence	Hexose transporters Hxt1p and Gal2p	*S. cerevisiae*
HSP47	Chaperone	Procollagen	Mammalian collagen-producing cells
Invariant chain	Escort to prevent aggregation and premature ligand binding as well as to direct endosomal targeting	Major histocompatibility complex (MHC) class II	Mammalian antigen-presenting cells
Lag1p and Dgt1p	Promotes ER secretion of GPI-anchored proteins	GPI-anchored proteins Gas1p and Yap3p	*S. cerevisiae*
LMAN1/ERGIC-53	Potential cargo receptor for glycoproteins	Cathepsin C, blood clotting-factors V and VIII	Mammalian cells
Lst1p	Potential cargo receptor	Plasma membrane H^+-ATPase Pma1p	*S. cerevisiae*
MESD	Assembly and transport	LDL-receptor-related protein 5 (LRP5) and LRP6 receptor, LDL receptor	Mammalian cells

(*Continued*)

TABLE 2 (*Continued*)

Factor	Function	Client protein	Organism/cell type
Microsomal triglyceride transfer protein	Assists translocation, assembly, and secretion	Apolipoprotein B	Primarily liver cells and intestinal cells
Neurophysin	Escort	Arginine vasopressin	Magnocellular neurons of the hypothalamus
ODR-4 and ODR-8	Promotes ER secretion competence	Odorant receptors ODR-10 and STR-2	*Caenorhabditis elegans* olfactory neurons
NinaA	Peptidyl-prolyl isomerase	Opsin	Visual response in *Drosophila*
p24 Family	Potential cargo receptors	Invertase, Gas1p (*S. cerevisiae*), many others	*S. cerevisiae*, *C. elegans*, and mammalian cells
Prolyl 4-hydroxylase	Enzyme, chaperone	Procollagen	Mammalian cells
Protective protein/ cathepsin A	Promotes ER secretion competence through direct interaction and directs lysosomal targeting	Neuraminidase and β-galactosidase	Mammalian cells
RAP	Escort to prevent aggregation and premature ligand binding	LDL receptor family	Mammalian cells
SCAP	Retention of SREBP	SREBP	Mammalian cells
Shr3p	Promotes ER secretion competence	Amino acid permeases, e.g., Hip1p and Gap1	*S. cerevisiae*
Tapasin	Prevents ER exit of MHC class I without bound antigenic peptide	MHC class I	Mammalian cells
Vma12p–Vma22p complex	Promotes complex assembly	Vacuolar H^+-ATPase subunit Vph1p	*S. cerevisiae*

BiP, which then progresses on its folding pathway. The ATPase domain of BiP hydrolyzes ATP, and BiP returns into the ADP high-affinity state (Figure 2*b*). Cycling of an unfolded protein through the BiP ADP-ATP cycle consumes energy. The folding of many secretory proteins is inhibited by depleting cellular ATP levels (52). Nucleotide exchange and ATP hydrolysis are regulated by cochaperones. The DnaJ-like proteins MTJ1/ERdj1, ERdj3/HEDJ /Scj1p, Erdj4, Erdj5, Sec63p, and Jem1p stimulate the ATPase activity of BiP. Because the affinity of HSP70s for ADP is approximately sixfold higher than for ATP, nucleotide exchange factors are required to catalyze the ADP/ATP exchange reaction. For BiP, these are the GrpE-like proteins, BiP-associated protein (BAP), and Sil1/Sls1p (53). In vitro the K_m for ATP binding by bovine HSP70 is 1–2 μM in the presence and absence of unfolded proteins (54). The cytosolic ATP concentration is in the mM range. Thus, nucleotide binding is not rate limiting for the function of cytosolic HSP70s. ATP is imported into the ER via antiport with ADP and AMP (55). ATP import may be limiting for the function of ER-luminal HSP70 chaperones. Conflicting data for the rates of the nucleotide release and ATP hydrolysis reaction have been reported (54). Therefore, we assume that differential regulation of nucleotide exchange and ATP hydrolysis by cochaperones in vivo may be important for the regulation of BiP function. Although it is generally accepted that BiP cycles between bound and unbound states on a substrate polypeptide, there is no direct evidence for BiP cycling in vivo.

BiP, as other HSP70s (54), cycles between monomeric and oligomeric states (Figure 2*b*). In the oligomeric state, BiP is posttranslationally modified by phosphorylation in its peptide-binding domain (52) and ADP ribosylation (52). Only monomeric, unmodified BiP associates with unfolded proteins (52). Therefore, it was suggested that modified oligomeric BiP constitutes a storage pool from which BiP is recruited to the monomeric pool by interaction with unfolded proteins (52). These events may be the first events in signal transduction in response to the accumulation of unfolded proteins in the ER lumen. However, it is currently unknown if these posttranslational modifications are required for BiP function or simply occur at the same time. Autophosphorylation of purified BiP preparations was suggested (52), but phosphorylation due to a contaminating kinase was not convincingly ruled out as a source for phosphate incorporation into BiP.

Recognition of Unfolded Proteins by UGGT

UGGT simultaneously recognizes two features in an unfolded protein: exposed hydrophobic sequences and the oligosaccharide moiety (40). It was proposed that UGGT recognizes the innermost N-acetylglucosamine residue of an asparagine-linked oligosaccaride, which may only be accessible in a denatured conformation (37). This residue interacts extensively with the polypeptide backbone of the protein (37). The structural flexibility of this residue and neighboring amino acids may be a key determinant in recognition of unfolded proteins by UGGT (37). However, the exact nature of protein determinants recognized by UGGT is still elusive.

UGGT binds to hydrophobic nonapeptides linked to sepharose 4B or to phenyl-superose (42). In this respect, substrate recognition by UGGT is similar to that by BiP. In contrast, completely denatured proteins are poor substrates for UGGT in vivo (48), suggesting that partially structured substrates are recognized by UGGT and that UGGT acts, on average, after BiP. Studies using N-terminal fragments of a 64-amino acid long, chemically glycosylated protein suggested that fragments possessing some, but not all, structural elements of the full-length protein were most efficiently glucosylated by UGGT (56). Alternatively, shorter fragments may simply not have provided the minimum distance required between the glucosyl acceptor site and the protein determinant recognized by UGGT or did not contain an UGGT recognition determinant. The distance between these two contact points for UGGT in a partially folded protein is controversial. Depending on the model protein studied, acceptor and recognition sites can be part of a local domain (37) or up to 4 nm apart (57).

TRANSDUCTION OF UNFOLDED PROTEIN SIGNALS ACROSS THE PHOSPHOLIPID BILAYER OF THE ER

Prolonged interaction of a folding protein with the chaperone machinery activates three ER resident transmembrane proteins: activating transcription factor 6 (ATF6), the inositol requiring kinase 1 (IRE1), and double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK), also called pancreatic eukaryotic initiation factor 2α (eIF2α) kinase (PEK), which then transduce an unfolded protein signal across the ER membrane (Figure 3). Ca^{2+} is released from the ER to activate apoptotic-signaling pathways.

Activation of ATF6

ATF6 is a type II transmembrane domain protein encoding a basic leucine zipper (bZIP) transcription factor in its cytosolic domain (58). Two homologous proteins, ATF6α and ATF6β/cAMP-response-element-binding protein (CREB)-related protein (CREB-RP)/G13 exist in mammals (Figure 3). When protein folding in the ER is inhibited, ATF6 translocates to the Golgi complex (59). Site-1 protease (S1P), a serine protease, cleaves ATF6 in the luminal domain. The N-terminal membrane-anchored half is cleaved by the metalloprotease site-2 protease (S2P) within the phospholipid bilayer (58, 60). These proteolytic reactions release the cytosolic bZIP domain of ATF6, which then translocates into the nucleus to activate transcription. ATF6 binds to the ATF/cAMP response element (CRE) (61) and to the ER stress response elements (ERSE-I and -II) [ERSE-I, CCAAT-N_9-CCACG, and ERSE-II, ATTGG-N-CCACG (62)]. Binding of ATF6 to ERSE-I and ERSE II requires nuclear factor Y (NF-Y)/CCAAT-binding factor (CBF) (62, 63).

The BiP ADP-ATP and the CNX/CRT cycle have been implicated in retention of ATF6 in the ER. Two independent and redundant Golgi localization sequences

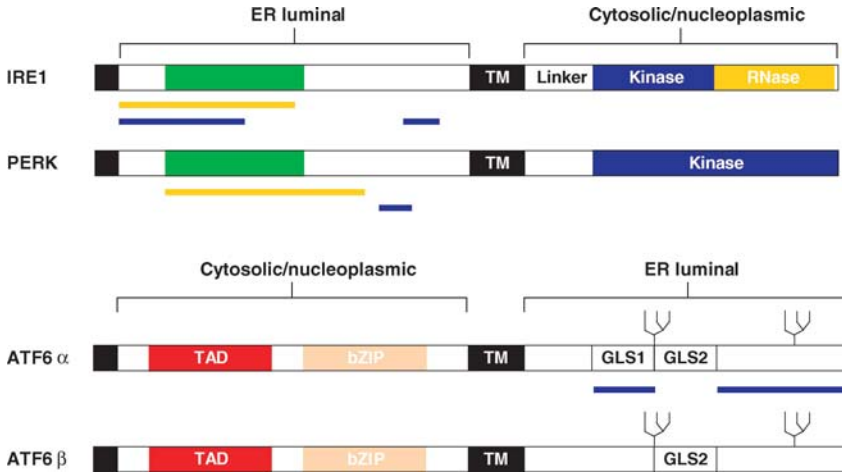


Figure 3 Primary structure of the ER stress sensors: inositol requiring kinase 1 (IRE1), protein kinase-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6). Yellow bars represent regions sufficient for signal transduction or oligomerization. Purple bars represent regions interacting with BiP. The black boxes represent the signal peptides, and the green boxes depict the region of limited homology between IRE1 and PERK. The other abbreviations are bZIP, basic leucine zipper; GLS1 and GLS2, Golgi localization sequences 1 and 2; TAD, transcriptional activation domain; and TM, transmembrane domain. Drawings are not to scale.

GLS1 and GLS2 were identified in the ER-luminal domain of ATF6 (64). BiP binds to GLS1 but not to GLS2. In the absence of BiP binding, GLS2 is dominant, resulting in constitutive translocation of ATF6 to the Golgi and ATF6 activation (64). Thus, through interaction with BiP, ATF6 is localized in the ER. Unfolded proteins sequester BiP from GLS1, and ATF6 translocates into the Golgi complex. ATF6 is also retained in the ER by interaction with the lectin CRT (65). To escape this retention mechanism under ER stress conditions, newly synthesized ATF6 is underglycosylated, which abrogates its interaction with CRT. Heterodimerization of completely and underglycosylated ATF6 via their bZIP domains would retain underglycosylated ATF6 in the ER. However, completely glycosylated ATF6 is rapidly degraded by the proteasome during ER stress, which abolishes this retention mechanism for underglycosylated ATF6 (66). Expression of ATF6α mutants in which some of their three glycosylation sites were destroyed resulted in moderate S2P-dependent activation of *GRP78* promoter-driven luciferase reporters (65). This observation is consistent with the idea that ATF6 is retained in the ER via interaction of its N-linked oligosaccharides with CRT. More definitive experiments are required to elucidate if these proposed mechanisms actually reflect the mechanism of ATF6 activation and if they possibly act in concert with each other.

Activation of IRE1 and PERK

The ER-luminal domains of the type I transmembrane proteins IRE1 and PERK are ER stress–regulated di- and oligomerization domains (67). The cytoplasmic domain of IRE1 also possesses, albeit weaker, homodimerization potential. The luminal domains of IRE1 and PERK show a small degree of homology conserved throughout all eukaryotes (Figure 3). However, no homology exists with the luminal domain of ATF6. Genetic studies in yeast revealed that the ER-luminal domains of IRE1 and PERK are interchangeable and that their function is evolutionarily conserved (68). The function of the ER-luminal domain was completely replaced by an heterologous dimerization motif, the bZIP domain of the transcription factors MafL and JunL (68). This observation suggests that these dimerization domains do not regulate dimerization but only contribute to the strength of the formed dimers. However, interactions of these bZIP proteins with ER-luminal proteins were not ruled out in this study. Biochemical evidence supports that in an inactive state the luminal domains of IRE1 and PERK are associated with BiP (69, 70) and that upon accumulation of unfolded proteins in the ER, BiP is competitively titrated from the luminal domains of IRE1 and PERK by the huge excess of unfolded proteins in the ER lumen (69). Consistent with this model is the finding that interactions of BiP with its substrates are transient. Further, the huge excess of BiP over IRE1 and PERK is set off by the low affinity of BiP for its substrates. Thus, only small fluctuations in the free BiP pool are required for its release from IRE1 and PERK. As in the regulation of ATF6 activation by BiP and CRT, these observations are consistent with normal chaperone and client protein interactions. It remains unclear how unmasking of hydrophobic BiP-binding sites in low-abundance proteins can result in efficient homooligomerization of these proteins in an environment where other hydrophobic regions are displayed in huge excess by unfolded proteins. Recent work has shown that BiP-binding sites and regions required for signaling or oligomerization in the ER-luminal domains of IRE1 and PERK can be separated. In IRE1α, the domains required for signaling, oligomerization, and BiP binding partially overlap (Figure 3) (70). Here, BiP may actually mask an important dimerization motif in IRE1α to keep it in its monomeric, inactive state. However, in PERK, the domains required for oligomerization and BiP binding are distinct (Figure 3) (71). BiP indirectly interferes with oligomerization either sterically or through induction of a conformational change in the luminal domain of PERK that inactivates the oligomerization domain. Thus, the oligomerization domains in IRE1 and PERK, masked by BiP, should possess a higher affinity for each other than for other hydrophobic surfaces on unfolded proteins to promote homooligomerization of IRE1 and PERK. This appears to be true for the luminal domain of IRE1, which forms a tight homodimer that cannot be dissociated without denaturation of the protein (70).

Two mammalian homologues, IRE1α (72) and IRE1β (73), were identified. Conservation of IRE1 between yeast and humans allows for a reliable phylogenetic evaluation of the function of its N-linked oligosaccharides. Between yeast and humans only one glycosylation site is conserved. In yeast, this site was completely

dispensable for Ire1p function (68), arguing against an involvement of CNX/CRT in regulation of the oligomerization status of IRE1.

Transduction of Apoptotic Signals

Proximal events in transduction of apoptotic signals across the ER membrane are not understood. The mechanism of Ca^{2+} release from the ER lumen during ER stress is analogous to the action of Bcl-2 protein family members at the mitochondrial membrane. The antiapoptotic Bcl-3 protein family member Bcl-2 (74), Bax inhibitor protein 1 (75, 76), the proapoptotic Bcl-2 homology 3 (BH3) domain-only proteins [Bak (77), Bax (77), Bid (78), and Spike (79)] localize to the ER membrane. Pro- and antiapoptotic Bcl-2 family members homo- and heterodimerize and thus either promote or neutralize their apoptotic activity (80). Upon apoptotic stimuli, Bid induces a conformational change in Bak and Bax, exposing their N terminus and promoting the oligomerization of these proteins (81). Oligomeric Bax forms an ion pore in the outer mitochondrial membrane, resulting in Ca^{2+} influx (81, 82). Similarly, Bak and Bax oligomerize at the ER membrane and insert themselves into the ER membrane, resulting in Ca^{2+} efflux from the ER during ER stress (77). It is likely that future work will identify additional Bcl-2 family members associated with the ER membrane. Additional apoptotic signals are generated at the ER membrane, e.g., through the integral membrane protein BAP31 (79) and through conventional signal transducers such as IRE1. How these signals are generated and how signaling specificity of IRE1 is regulated is not known.

PRIMARY MECHANISMS OF CYTOSOLIC AND NUCLEOPLASMIC SIGNAL TRANSDUCTION

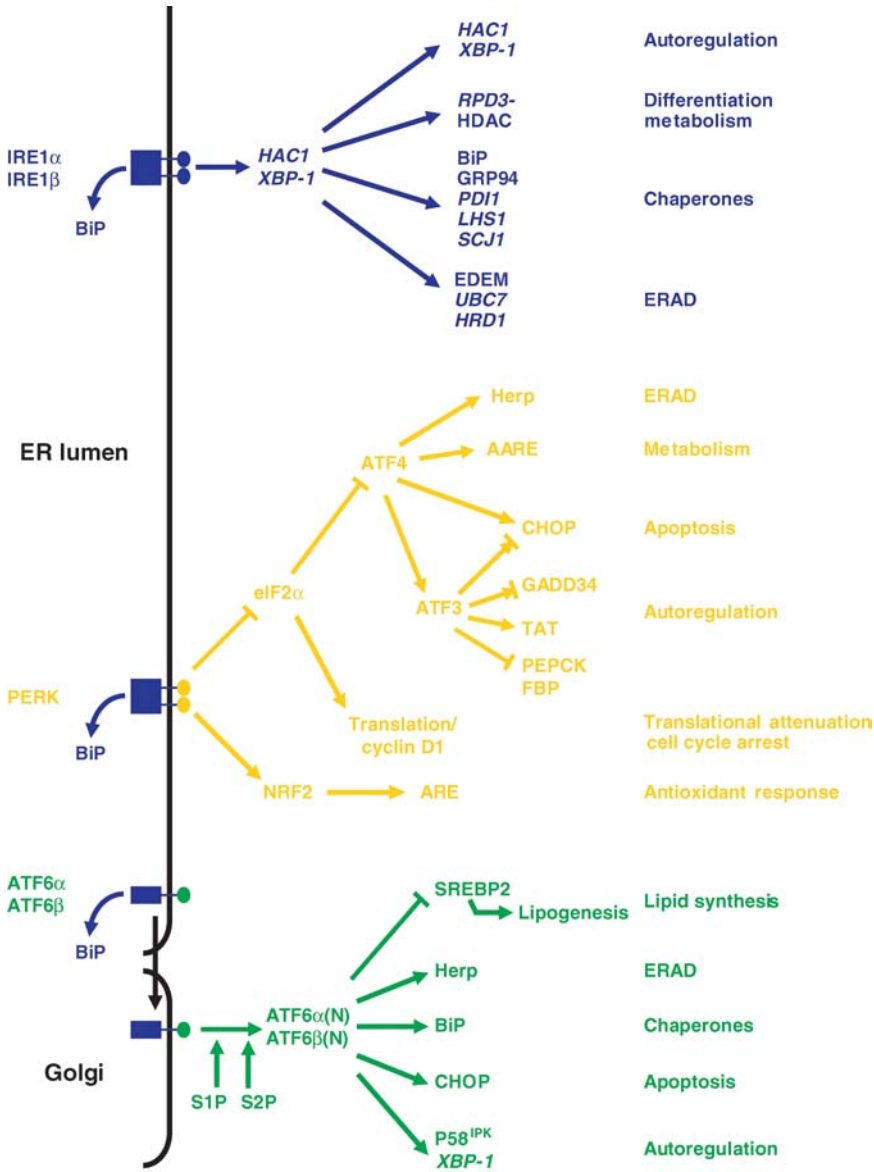
Signal transduction mechanisms diverge downstream of IRE1 and PERK. The following summarizes our current understanding of these signaling pathways.

Signal Transduction by IRE1

IRE1 is an atypical type I transmembrane protein kinase endoribonuclease (83, 84), consisting of an ER-luminal dimerization and cytosolic kinase and endoribonuclease domains. Dimeric IRE1 autophosphorylates and activates its RNase domain. The RNase domain of IRE1 shows strongest homology to RNase L (85). The requirement for autophosphorylation to activate the RNase domain is bypassed when the ATP-binding pocket of IRE1 is occupied by ADP (86). Mutations in the RNase domain of Ire1p abolished activation of an *ERp72 CAT* reporter construct (87). Transient transfection experiments with kinase- and RNase-defective Ire1p indicate that two functional RNase domains are required for signaling by Ire1p (87). This observation is in agreement with biochemical data in which activated

IRE1 behaves as a dimer during glycerol gradient sedimentation (69) and during gel filtration (67).

The substrate for the Ire1p endoribonuclease was first identified in yeast as the mRNA encoding the bZIP transcription factor Hac1p (88). The functional homolog for Hac1p in mammals is XBP-1 (8, 89, 90) (Figure 4). Activated Ire1p



cleaves both 5′- and 3′-exon-intron junctions in *HAC1* and *XBP-1* mRNA (8, 85, 90) and generates 5′-OH and 2′,3′-cyclic phosphate ends (Figure 5) (91). tRNA ligase (*RLG1*/*TRL1*) joins both exons (92). The ligase leaves a 2′-phosphate derived from the splice junction phosphate on the 5′-end of the joined junction (91). This 2′-phosphate is removed by the NAD^+-dependent phosphatase Tpt1p (93). NAD^+ serves as phosphate acceptor in a reaction that generates nicotinamide and ADP-ribose 1″-2″-cyclic phosphate (App-ribose >P) (94). The presence of RNA ligases with similar activities has been demonstrated in wheat germ, *Chlamydomonas*, and mammalian cells (95). Interestingly, mammals have an additional ligase activity, which incorporates the junction phosphate into the spliced mRNA (96). It is not known which of these ligases joins the *XBP-1* exons. An NAD^+-dependent 2′-phosphatase is conserved in bacteria (97), yeast (93), plants (97), and mammals (97). The splicing mechanism used by IRE1 is identical to pre-tRNA splicing (98). This mechanism does not provide an explanation of how the ligase distinguishes between exons and introns, in contrast to mRNA splicing or the self-splicing of group I and II introns. In vitro, the HAC1 exons remained associated after cleavage of both exon-intron junctions by Ire1p (91). The cellular localization of the splicing reaction is likely to be cytosolic. *HAC1* mRNA is spliced in polysomes (99), but association with polysomes is not a prerequisite for splicing (23). *HAC1* mRNA is located in the cytoplasm (99), and this cytoplasmic pool can be spliced and is not a dead-end product (99). In contrast, tRNA splicing is nuclear (100), and at least in mammals, IRE1 was convincingly located to the inner nuclear envelope (89). Thus, it is still controversial in which compartment *HAC1* and *XBP-1* mRNA are spliced.

The splicing reaction introduces an alternative C terminus with increased transcriptional activation potential into Hac1p (101) and XBP-1 (8, 89, 90). In yeast, splicing also removes a translational attenuator from *HAC1* mRNA (99). Translational attenuation is mediated in part, but not completely, through base pairing between the 5′-UTR and the intron of unspliced *HAC1* mRNA (99). To explain that unspliced *HAC1* mRNA is found in polysomes (99), it was proposed that loading of *HAC1* mRNA with polysomes occurs during exit of the mRNA from the nucleus with its 5′-end first and that these polysomes are then trapped on *HAC1* mRNA.

←

Figure 4 Protective ER stress-signaling pathways. Abbreviations: AARE, amino acid response element; ARE, antioxidant response element; CHOP, CCAAT/enhance-binding protein (C/EBP) homologous protein; FBP, fructose-1,6-bisphosphatase; GADD34, growth arrest and DNA damage gene 34; HAC1, homologous to ATF/CREB 1; HDAC, histone deacetylase complex; HRD1, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase degradation 1; NRF2, nuclear factor erythroid 2 (NF-E2) related factor; P58IPK, 58 kDa PKR inhibitor; PEPCK, phosphoenolpyruvate carboxykinase; RPD3, reduced potassium dependency 3; SCJ1, *Saccharomyces cerevisiae* DNA J homolog; SREBP, sterol response element binding protein; TAT, tyrosine aminotransferase; UBC7, ubiquitin conjugating enzyme 7; XBP-1, X-box binding protein; and HERP, homocysteine-induced ER protein.

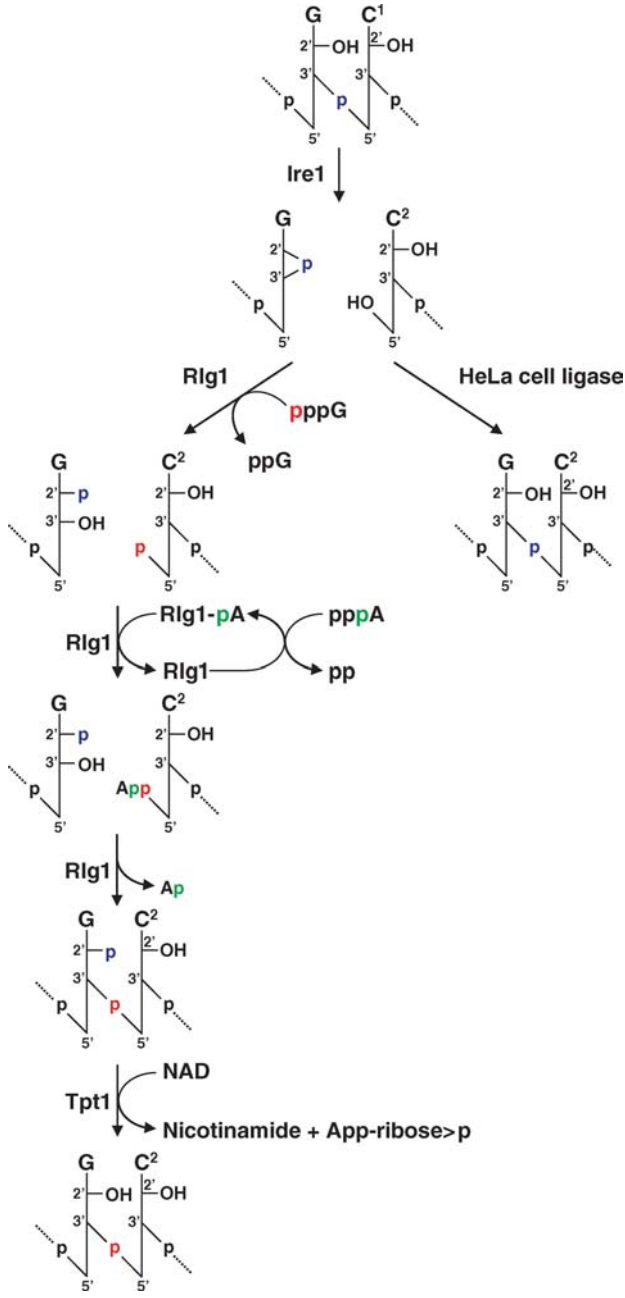


Figure 5 Mechanism of *HAC1* mRNA and tRNA splicing in yeast and mammals.

However, the quantitative contribution of the *HAC1* intron to attenuation of *HAC1* mRNA is small (99) but more pronounced in ER-stressed cells (99), suggesting the existence of a stress-regulated component that contributes to translational control. Further, unspliced *HAC1* is routinely isolated in multicopy suppressor screens in yeast, indicating that translational attenuation is leaky or bypassed at high *HAC1* mRNA levels. An association between Lhp1p, the yeast gene encoding the eukaryotic RNA-binding protein implicated in the metabolism, and translation of RNA polymerase III transcripts, such as tRNAs, and *HAC1* mRNA was recently reported, hinting that other RNA polymerase III chaperones may be involved in vivo in maturation of *HAC1* mRNA (102), for example in making the splice junctions in polysomal *HAC1* mRNA accessible for IRE1. In contrast to yeast, there is no differential translational regulation for unspliced *XBP-1* mRNAs, *XBP-1^u*, and *XBP-1^s*. Thus, competition for binding sites and dimerization partners between XBP-1^u and XBP-1^s suppresses transcriptional activation by XBP-1^s. Rapid degradation of XBP-1^u by the proteasome is required for efficient activation of the UPR in mammalian cells (103). However, this observation is controversial. When expressed in HeLa cells, XBP-1^u and XBP-1^s displayed identical decay kinetics (90).

Three promoter elements through which Hac1p regulates transcription have been identified: (*a*) the unfolded protein response element (UPRE, CAGCGTG) (104), found in the promoters of ER chaperone genes (Figure 4); (*b*) the upstream repressing sequence 1 (URS1, TCGGCGGCT) (25), found in the promoters of early meiotic genes and many genes involved in carbon and nitrogen utilization; and (*c*) a subtelomeric ATF/CREB GTA variant element (SACE, ATGGTATCAT) (105). On UPRE and SACE, Hac1p is a classical activating bZIP transcription factor. Spliced Hac1p interacted in vitro with components of the Spt-Ada-Gcn5 acetyltransferase (SAGA) histone acetyltransferase complex (106). Activation of *KAR2* and *PDI1* by ER stress, but not heat shock, was partially dependent on a functional SAGA in vivo (107). These observations suggest that spliced Hac1p recruits SAGA to UPRE to acetylate the N-terminal tails of the nucleosomal core histones to promote transcription (108). However, the interaction between Hac1p and SAGA has not been demonstrated in vivo, and increased acetylation of ER chaperone promoters in response to ER stress has also not been shown. In addition, Hac1p autostimulates its own transcription through a UPRE in its promoter (109). SACE is found in the promoters of *COS* genes of unknown function and in the promoters of ER chaperone genes (105). However, the conclusion that SACE is a Hac1p target site is based on indirect data comparing expression of SACE-*lacZ* reporters in strains deleted for several bZIP transcription factors (105), and a more direct approach is required to substantiate this claim. On URS1, Hac1p represses transcription (25) (Figure 4), making Hac1p the first bZIP transcription factor in yeast that both activates and represses transcription. URS1 is the DNA-binding site for the transcriptional regulator Ume6p, a Zn_2Cys_6 cluster protein (110). *ume6*Δ yeast were completely defective in repression by Hac1p, showing that all negative regulation of Hac1p on URS1 is transduced through Ume6p (25). Ume6p constitutively

recruits two repression complexes to URS1, the ISW2 chromatin-remodeling complex (111) and the *RPD3-SIN3* histone deacetylase complex (HDAC) (112). Genetic experimentation showed that the chromatin-remodeling complex was dispensable for repression by Hac1p. In contrast, repression by Hac1p depended completely on the catalytic activity of the *RPD3-SIN3* HDAC (25). Hac1p was also found to interact with the *RPD3-SIN3* HDAC in coimmunoprecipitation experiments. However, repression by the HDAC was largely intact in *hac1*Δ yeast, arguing that Hac1p is not an integral component of the HDAC (25). Because repression by Hac1p was abolished specifically on genomic promoters in which URS1 was mutated, the most likely interpretation of this data is that association of Hac1p with the HDAC enhances repression by the HDAC. These data suggest that all genes regulated by Ume6p may be subject to regulation by Hac1p and the UPR. Ume6p represses transcription of ∼10% to 20% of all yeast genes. Many of these genes are required for carbon and nitrogen metabolism as well as meiosis (24), indicating that repression of metabolic genes and subsequent decreased metabolic activity may be an additional mechanism for adaptation to ER stress.

XBP-1 is a bZIP transcription factor of the ATF/CREB family and controls genes containing an X-box element. Binding of XBP-1 to ERSE requires NF-Y (90). Binding of XBP-1 to ERSE-II has not been investigated. In addition, XBP-1 and ATF6 are required for transcription of *XBP-1* (89). Heterodimerization of XBP-1 and ATF6 has been demonstrated in a coiled-coil protein array (113) but still remains to be demonstrated in vivo.

REGULATION OF IRE1 SIGNALING Hac1p and XBP-1 autostimulate their transcription (89, 109). Sustained high levels of *HAC1* mRNA depended on autostimulation and were required for survival of prolonged ER stress (109). Disruption of this positive-feedback loop on the *XBP-1* promoter is associated with bipolar mood disorder in humans (114).

In yeast, Ire1p is negatively regulated by the phosphatase Ptc2p (115). Whether this negative regulation is a constitutive or ER stress-responsive activity is not known. In mammals, the Src homology 2/3 (SH2/SH3) domain-containing protein Nck-1 was implicated in interactions with IRE1 to attenuate IRE1α signaling and in IRE1α-dependent activation of ERK (116). A direct involvement of Nck-1 in ER stress signaling remains questionable. Jun activation domain-binding protein (JAB-1) has been shown to interact with the linker region of IRE1 situated between the transmembrane domain and the kinase domain of IRE1α (117) (Figure 3). JAB-1 dissociates from IRE1α during ER stress, and constitutive JAB-1 mutants attenuated activation of *GRP78* transcription and *XBP-1* splicing (117), suggesting that JAB-1 is a negative regulator of IRE1α.

Signal Transduction by PERK

Activation of PERK has two major consequences: phosphorylation of the α subunit of eukaryotic translation initiation factor 2 (eIF2α) (12) and phosphorylation of the bZIP Cap'n'Collar transcription factor Nrf2 (118) (Figure 4).

CONSEQUENCES OF eIF2α PHOSPHORYLATION Phosphorylation of eIF2α by PERK shuts off general translation (12). *perk*$^{-/-}$ cells are sensitive to ER stress and are partially rescued by translation inhibitors, e.g., cycloheximide (119). Short-lived proteins are cleared from the cell during inhibition of translation. An important example is cyclin D1, which controls exit from G_1 and entry into the S phase of the cell cycle through phosphorylation of the retinoblastoma tumor suppressor protein. Cyclin D1 activity is controlled through stimulation of its synthesis by mitogens and, thus, by its abundance in the cell. Loss of cyclin D1 during ER stress arrests mammalian cells in G_1 (120). This explains the well-documented observation that depletion of dolichol phosphate and inhibition of N-linked glycosylation cause a G_1 arrest in mammalian cells.

Phosphorylation of eIF2α activates translation of mRNAs encoding several short upstream open reading frames (uORFs) (121). eIF2α is part of the heterotrimeric complex eIF2, which in a GTP-bound form binds methionyl-initiator tRNA(Met-tRNA$_i^{Met}$). The eIF2-GTP-Met-tRNA$_i^{Met}$ complex associates with the 40 S ribosomal subunit to form the 43 S preinitiation complex. The 43 S preinitiation complex binds to the 5′-end of an mRNA and scans the mRNA in 5′ to 3′ direction until the first start codon (AUG) is reached. There the 60 S subunit joins to initiate translation (Figure 6). After termination of translation, the ribosome dissociates into the 60 S and 40 S subunits. The 40 S subunit can remain bound to the mRNA, and after loading with new eIF2- Met-tRNA$_i^{Met}$, it resumes scanning for additional AUGs to reinitiate translation. Phosphorylation of eIF2α inhibits exchange of GDP for GTP in eIF2 and decreases the concentration of the 43 S preinitiation complex. At low levels of eIF2α phosphorylation, uORFs are efficiently translated, resulting in repression of translation of the downstream ORF. When phosphorylation of eIF2α is high, the small ribosomal subunit scans through several uORFs before being loaded again with the ternary eIF2-GTP-Met-tRNA$_i^{Met}$ complex, which then allows for translation of the downstream ORF. This scanning and reinitiation mechanism has been shown to operate in translational control of *GCN4* mRNA in yeast, where translation of the last fourth uORF inhibits reinitiation at the *GCN4* ORF. Translational control in response to ER stress has been reported for only *ATF4* (18, 122) and *Cat-1* mRNAs (123). ATF4 is a transcription factor that induces expression of genes that function in amino acid metabolism, the antioxidant response, and apoptosis. *ATF4* mRNA contains two uORFs conserved in vertebrates (123a). The second uORF overlaps with the *ATF4* ORF. Mutation of the AUG in uORF1 repressed and mutation of the AUG in uORF2 derepressed translation of the *ATF4* ORF (123a, 123b). These data make *ATF4* mRNA the first vertebrate mRNA whose translation is regulated in a similar manner as yeast *GCN4*. The mRNAs for the UPR targets CHOP and GADD34 also contain several uORFs (123b). However, no translational activation of these mRNAs was observed when phosphorylation of eIF2α was induced (123c). Differences in positioning of the uORFs in relation to the *ATF4*, *CHOP*, or *GADD34* ORF may account for these contradicting observations. In *Cat-1* mRNA, an internal ribosomal entry site buried in the secondary structure in the 5′-UTR of the mRNA is unmasked through unwinding of the 5′-UTR during translation of a

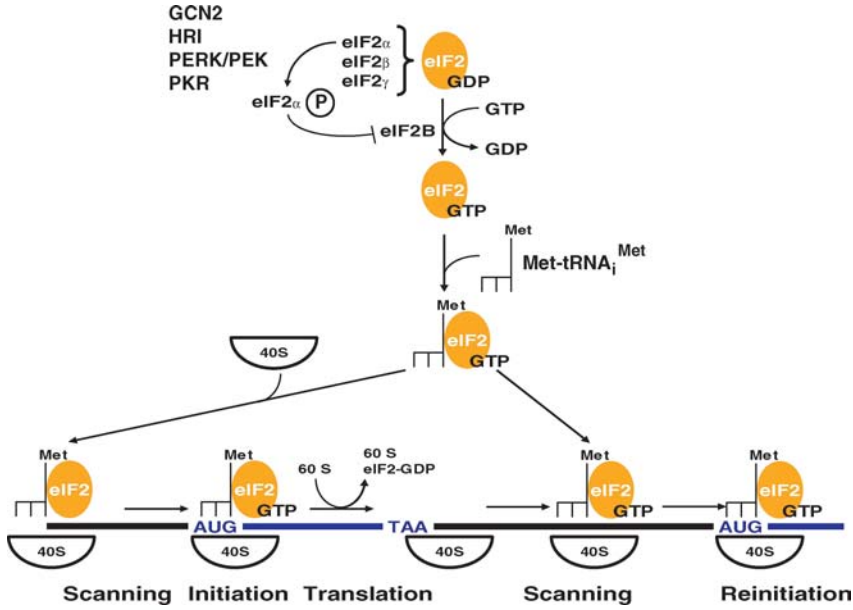


Figure 6 Translational control through reinitiation. After translation of a short uORF, the 40 S ribosomal subunit remains associated with the mRNA, resumes scanning, and is loaded with new Met-tRNA$_i^{Met}$-eIF2-GTP. Phosphorylation of eIF2α inhibits the GDP-GTP exchange reaction, formation of the ternary Met-tRNA$_i^{Met}$-eIF2-GTP complex, and reinitiation at downstream ORFs. Longer intercistronic regions promote reinitiation, and when eIF2α is phosphorylated, even longer intercistronic regions are required for reinitiation, resulting in the skipping of uORFs proximal to the first ORF and translation of distal ORFs. For simplicity only relevant events are shown.

uORF mRNA (123). However, it remains unclear why recognition of the uORF by the scanning ribosome is stimulated in cells with presumably decreased levels of the 43 S preinitiation complex.

CONSEQUENCES OF Nrf2 PHOSPHORYLATION In unstressed cells, Nrf2 is found in an inactive cytoplasmic complex with the cytoskeletal anchor Keap1 (118). When there is ER stress, PERK phosphorylates Nrf2, resulting in dissociation of the Nrf2-Keap1 complex, nuclear localization of Nrf2, and activation of transcription by Nrf2 through the antioxidant response element (ARE) (124). Nrf2 activates transcription on ARE as a heterodimer with other bZIP proteins: ATF4 (124), c-Jun, Jun-B, and Jun-D (124), but it is inhibited by heterodimerization with MafK (124). The ARE controls expression of genes involved in the phase II metabolism of xenobiotics, e.g., electrophilic thiol-reactive substances that mimic an oxidative

insult. Genes regulated by the ARE include the A1 and A2 subunits of glutathione S-transferase, NAD(P)H:quinone oxidoreductase, γ-glutamylcysteine synthetase, heme oxygenase 1, and UDP-glucuronosyl transferase (124). *perk*$^{-/-}$ cells accumulated reactive oxygen species when exposed to ER stress (125). Thus, the sensitivity of *nrf2*$^{-/-}$ cells to ER stress results from their impaired ability to respond to an oxidative insult (118). The idea that an imbalance in the cell's redox status is caused by ER stress is further supported by the observation that the redox-sensitive transcription factor NF-κB is activated in response to ER stress and that this activation was inhibited by antioxidants (126). NF-κB activation is mediated by regulated degradation of its inhibitor IκB. In addition, translational attenuation in response to eIF2α phosphorylation potentiates NF-κB activation by preventing the synthesis of IκB (127). This redox imbalance may be caused by uncoupling of the disulfide isomerase Ero1p from its, yet to be identified, physiological substrate by an elevated unfolded protein load of the ER.

REGULATION OF PERK SIGNALING To allow recovery from ER stress and to permit an efficient response to prolonged ER stress, translational inhibition by PERK is transient. Several eIF2α phosphatases have recently been characterized. GADD34 and *CreP* regulate the phosphatase activity of protein phosphatase 1 (PP1) through their homologous C-terminal domains. PP1 dephosphorylates eIF2α. *CreP* is a constitutive regulator of PP1 (128). Expression of GADD34 is induced by ATF4 late in ER stress (129) (Figure 4). The N-terminal 180 residues of GADD34 target the α isoform of PP1 to the ER (130), thus limiting the time window in which eIF2α phosphorylation attenuates translation. In addition, PERK is inhibited by binding to the HSP40 cochaperone P58^{IPK}, whose expression is induced by IRE1/XBP1 during ER stress (131) (Figure 4). Thus, activation of P58^{IPK} and GADD34 late in ER stress is a negative-feedback mechanism that limits shutoff of translation through phosphorylation of eIF2α by PERK in the early phase of ER stress. Recently, the SH2/SH3 domain-containing protein Nck-1 was implicated in attenuation of PERK signaling (132) and phosphorylation of eIF2α, presumably through interaction with eIF2β (133). However, whether the effects of Nck-1 on PERK or on eIF2α phosphorylation are direct is not known. It still has to be determined how temporal regulation of different promoters by ATF4 and ATF6 is achieved.

REPROGRAMMING OF THE CELL DURING ER STRESS

All signaling arms of the UPR activate bZIP transcription factors: ATF6, XBP-1, ATF4, CHOP, and Nrf2. Expression of ATF3 is induced by ATF4 and regulates CHOP expression (134). All these bZIP proteins can form homo- and heterodimers with other bZIP transcription factors (113) (Table 3). These dimers can be either activators or competitive repressors, or they interact with transcriptional repression

machineries. Their function is, in many cases, influenced by the context of their target promoter. How the UPR feeds into this complex network of bZIP proteins and how, for example, cell type–specific bZIP proteins modulate UPR signaling to meet the particular need of an individual cell type are not well understood. The physiological responses ultimately regulated by the UPR are induction of molecular chaperones, upregulation of ERAD and phospholipid synthesis, and apoptosis.

Induction of Molecular Chaperones

Global transcriptional profiling studies characterized the transcriptional scope of the UPR in many eukaryotes, including yeast (13) and mammals (18, 125, 135, 136). In mammals, the contribution of each of the three signaling arms of the UPR to induction of specific genes has been determined. All transcriptional profiling studies identified ER chaperones, foldases, and genes with functions in the secretory pathway as the major targets of the UPR. Especially, ATF6 (135) and XBP-1 (136) contribute to induction of these genes (Figure 4). Identification of ATF6 targets relied on overexpression of its cytosolic portion, ATF6α (1–373) (135). Silencing of ATF6α or ATF6β by RNAi did not identify any specific targets for these two proteins. Silencing of ATF6α in *xbp1*$^{-/-}$ cells abolished induction of GRP94 in response to ER stress, but it had no effect on induction of GRP78 or CHOP (136), suggesting that XBP-1 and ATF6 partially overlap in their function and that one arm of the UPR, e.g., PERK, may be sufficient for effective induction of some target genes.

Induction of ERAD

Genes involved in ERAD (reviewed in 137) were identified as a second class of UPR targets in yeast (13) (Figure 4). Induction of these genes enhances degradation of slowly folding proteins in the ER and thus decreases the folding load of the ER during stress. In mammals, ATF4 and ATF6 have been implicated in induction of Herp/Mif1 (138), a protein that is suspected to recruit the 26 S proteasome to the ER membrane during stress (137). *herp*$^{-/-}$ cells were susceptible to ER stress; they displayed enhanced UPR signaling and stabilization of an ERAD substrate (139). Several genes, involved in ERAD uniquely, require the IRE1/XBP1 pathway for induction, including EDEM, HRD1, and UGGT. EDEM, which is directly involved in recognition and targeting of unfolded proteins for degradation, is also induced by the UPR (38). Induction of EDEM is dependent on XBP-1 and stimulated by ATF6 (140). Because activation of ATF6 precedes XBP-1 splicing, it was proposed that the UPR can be separated into two phases, an early phase in which, through increased induction of ER chaperones, cells attempt to fold unfolded proteins and a late phase in which XBP-1-dependent induction of ERAD degrades unfolded proteins while folding attempts for these proteins are still ongoing (140). However, in yeast, synthetic lethalities in strains defective in the UPR and in ERAD have

TABLE 3 bZIP transcription factors in the mammalian UPR[a]

bZIP protein[b]	Alias	Interaction partner	Modifications	DNA-binding site[c]	Target
ATF3	LRF-1	ATF3	Enhanced by PKA	Enk-2 ↑[d]	Proenkephalin
	LRG-21				
	CRG-5	ATF3, other?		ATF/CRE ↓	E-selectin ↓
	TI-241	ATF3		ATF/CRE ↓	ATF3 ↓
		ATF3		ATF/CRE variant (TGATGCAAC) ↓	ATF3 ↓
		ATF3, other?		SP-1 ↑	
		ATF3		AP-1 ↓	CHOP ↓
		ATF3		C/EBP/ATF (TTGCATCA)	CHOP ↓
		ATF2/CRE-BP1			
		ATF4			
		ATF7			
		C/EBPγ			
		CHOP ↓	Enhanced by p38 Map kinase		
		CREBPA			
		c-Jun		ATF/CRE ↑	Proenkephalin
		c-Jun		AP-1 ↑	
		c-Jun		Enk-2	
		JunB		ATF/CRE ↑	
		JunB		Enk-2 ↑	
		JunB		ATF/CRE ↓	
		Jun-B		AP-1 ↓	
		JunD	Enhanced by PKA	Enk-2 ↑	Proenkephalin ↑
		p21SNFT			
		Hepatitis B virus X protein		ATF/CRE ↓	
		HTLV-1 Tax protein	Enhanced by PKA	ATF/CRE ↑	Proenkephalin ↑
		NF-κBp50			
					CHOP ↑
					GADD34 ↑
				ATF/CRE	FBP ↓
				ATF/CRE	PEPCK ↓
					TAT ↑
ATF3ΔZIP (splice variant)				ATF/CRE ↑ SP-1 ↑	

(*Continued*)

TABLE 3 (*Continued*)

bZIP protein[b]	Alias	Interaction partner	Modifications	DNA-binding site[c]	Target
ATF4	C/ATF	ATF4		ATF/CRE	Somatostatin
	CREBP2	ATF4, other?		ATF/CRE ↓	Proenkephalin ↓
	mATF4	ATF4, other?		ATF/CRE ↑	CHOP ↑
	mTR67	ATF4, other?		ATF/CRE ↑	E-selectin ↑
	TAXREB67	ATF-1		CD38RE-TRE composite element ↑	Interleukin 2 ↑
		ATF-3			
		ATF-7			
		B-ATF			
		C/EBPα			
		C/EBPβ		ATF/CRE ↑	*COL-8* ↑
		C/EBPβ		Asymmetric ATF/CRE (TGACGCAG) ↑	PEPCK ↑
		C/EBPβ		Asymmetric ATF/CRE (TGACGTAA) ↑	Proenkephalin ↑
		C/EBPβ		C/EBP-ATF composite site (TTGCATCA) ↑	CHOP ↑
		C/EBPβ		C/EBP-ATF composite site (TTGCATCA) ↑	Herp ↑
		C/EBPγ/IgEBP/ GPE1-BP			
		C/EBPδ			
		C/EBPε/CRP1			
		CHOP			
		CREBPA			
		Fos		CRE	
		FosB			
		Fra-1		CRE	
		Fra-1		AP-1	
		HLF			
		HP8			
		c-Jun			
		Jun		CRE	
		JunD		ATF/CRE ↓	Cyclin A ↓
		cMaf			
		Nrf1			
		Nrf2		ARE ↑	*HO-1* ↑

(*Continued*)

TABLE 3 (*Continued*)

bZIP protein[b]	Alias	Interaction partner	Modifications	DNA-binding site[c]	Target
		p21SNFT			
		ZF			
		ZIP kinase			
		CBP, TBP, TFIIB, RAP30		ATF/CRE ↑	
		HTLV-1 Tax protein		Three 21 bp repeat ↑	Viral mRNAs ↑
		$SCF^{\beta TrCP}$	Degradation		
					ATF3 ↑
					ca-Ha-ras ↓
ATF6α		ATF6α		ERSE-I ↑	GRP78 ↑
		ATF6β		ERSE-I ↓	GRP78 ↓
		NF-Y/CBF		ERSE-I ↑	CHOP ↑, GRP78 ↑; Herp ↑; $P58^{IPK}$, XBP-1 ↑
		NF-Y		ERSE-II ↑	Herp ↑
		XBP-1		ATF/CRE-variant [TGACGTG(G/A)] ↑	
		ZF			
		SREBP2, HDAC1		Sterol response element ↓	HMG-CoA-synthetase ↓
					LDLR ↓
					Squalene synthetase ↓
		SRF	Phosphorylation of ATF6 by p38 enhances transcription	Serum response element ↑	ANF ↑, c-Fos ↑
ATF6β	CREB-RP	ATF6β		ERSE-I ↑	GRP78 ↑
	G13	ATF6α		ERSE-I ↓	GRP78 ↓
		NF-Y/CBF		ERSE-I ↑	GRP78 ↑
CHOP	CHOP-10 gadd153	CHOP			
		ATF2			
		ATF3 ↓			
		ATF4 ↓		ATF/CRE ↓	
		ATF7			

(*Continued*)

TABLE 3 (*Continued*)

bZIP protein[b]	Alias	Interaction partner	Modifications	DNA-binding site[c]	Target
		B-ATF			
		C/EBPα ↓, LAP ↓			
		C/EBPβ ↓		C/EBP-binding site ↓	CHOP ↓
		C/EBPβ		PuPuPuTGCAAT(A/C)C CC ↑	
		C/EBPβ			*DOC1* (carbonic anhydrase VI) ↑
		C/EBPγ			
		C/EBPδ			
		C/EBPε			
		CREBPA			
		DBP			
		c-Fos		TRE (TGACTCA) ↑	Collagenase ↑
		Fos			
		HLF			
		HP8			
		c-Jun		TRE (TGACTCA) ↑	Collagenase ↑
		JunD		ATF/CRE ↑	Somatostatin ↑ JunD ↑
		MafG			
		MafK			
		p21SNFT			
		TEF			
		Hepatitis B virus X protein			
			Phosphorylation by casein kinase II		Activation ↓
			Phosphorylation by p38 on Ser79 and Ser81		Activation ↑
					Bcl-2 ↓ β-casein ↑ *DOC4* (similar to *Drosophila melanogaster* Tenm/Odz) ↑, *DOC6* (villin, gelsolin homologue) ↑

(*Continued*)

TABLE 3 (*Continued*)

bZIP protein[b]	Alias	Interaction partner	Modifications	DNA-binding site[c]	Target
Nrf2	*NFE2L2*	ATF4		ARE ↑	*HO-1* ↑
		c-Jun		ARE ↑	Collagenase ↑
		v-Jun		ARE ↑	Collagenase ↑
		JunB		ARE ↑	Collagenase ↑
		JunD		ARE ↑	Collagenase ↑
		hMAF		NF-E2 ↓↑	γ-globin ↓↑
		MafG		ARE ↓	*NQO1* ↓
		MafK		ARE ↓	*NQO1* ↓
		NFE2-p45			
		Keap1 ↓	Phosphorylation by ERK ↑, p38 ↑, PKC ↑, and atypical PKCι ↑		
		PMF-1		PRE ↑	Spemidine/ spermine-N'-acetyl-transferase ↑
				ARE ↑	Nrf2 ↑
XBP-1	HTF	XBP-1			
	TREB5	ATF6α		ATF/CRE-variant [TGACGTG(G/A)] ↑	
		c-Fos			
		ZF			
		NF-Y		ERSE-I ↑	*GRP78* ↑
		HTLV-1 Tax protein		Three 21-bp repeats ↑	Viral mRNAs ↑
				TGACGCAA ↑	C/EBPβ
				ATF/CRE ↑	MHC class II Aα ↑
				TRE ↑	MHC class II DRα ↑, MHC class II DPβ ↑

[a]Compiled from References 113, 169–171.

[b]Abbreviations are ANF, atrial natriuretic factor; FBP, fructose-1,6-bisphosphatase; HMG-CoA synthetase, 3-hydroxy-3-methylglutaryl coenzyme A synthetase; *HO-1,* heme oxygenase-1; LDLR, low-density lipoprotein receptor; MHC, major histocompatibility complex; *NQO1*, NAD(P)H:quinone oxidoreductase; PEPCK, phosphoenolpyruvate carboxykinase; PMF1, polyamine-modulated factor 1; SRF, serum response factor; and TAT, tyrosine aminotransferase.

[c]Consensus DNA binding elements: ATF/CRE (cAMP response element), TGACGT(C/A)(G/A); AP-1, TGA(C/G)TCA; ARE, antioxidant response element, (G/C)TGA(C/T)N_3GC(A/G); Enk-2/CRE-2, TGCGTCA; ERSE-I, CCAAT-N_9-CCACG; ERSE-II, AATTGG-N-CCACG; MARE, Maf recognition element, TGCTGAC(G)TCAGCA; NF-E2, GCTGAGTCA; PRE, polyamine-responsive element, TATGACTAA; SP-1, GGGGCGGAGA; and TRE, TPA (12-*O*-tetradecanoylphorbol-13-acetate) response element, TGCGTCA.

[d]Activating and repressing activities are indicated with a "↑" or "↓", respectively.

been reported (13). In addition, UPR signaling is elevated in ERAD-defective cells (88, 139) and in cells treated with proteasome inhibitors (103). These observations show that ERAD is functional in the absence of UPR signaling, and it is therefore very likely that, even without direct upregulation of ERAD by the UPR, a portion of unfolded proteins is degraded by ERAD during the proposed "folding only" phase of the UPR.

Upregulation of the Size of the ER

Proliferation of the ER is observed in cell types with high secretory capacity, e.g., pancreatic exocrine cells, hepatocytes, or plasma cells, during ER stress (3) or when wild-type (WT) and mutant membrane proteins are overexpressed (141). Thus, an empirical correlation between expansion of the ER and ER stress exists. In yeast, UPR-deficient strains are inositol auxotrophs (26). ER stress-induced transcription of *INO1* encoding inositol-1-phosphate synthase, a key enzyme in phospholipid biosynthesis, occurs in an *IRE1*- and *HAC1*-dependent manner (13, 26). Induction of membrane proliferation by expression of membrane proteins is in some, but not all (142), cases dependent on a functional UPR pathway (26, 143). Activation of *INO1* transcription by inositol starvation was reported to be dependent on *IRE1* and *HAC1* (26). From these observations, it was concluded that the UPR, through regulation of *INO1* expression, controls proliferation of ER membrane synthesis. However, recent work has shown that the role of the UPR in membrane biosynthesis is more complex. Expression of Acr1p, an inner mitochondrial membrane protein whose expression did not induce ER membrane proliferation, was lethal in *IRE1*-deficient yeast (143). Further, lethalities—the one caused by overexpression of Acr1p and another one caused by overexpression of the ER membrane proliferation inducing peroxisomal membrane protein Pex15p—were rescued by growth of yeast on oleate instead of galactose (143). These observations suggest that ER membrane proliferation is not regulated by the UPR and that the lethality seen in *ire1*Δ cells may be due to a more general defect in phospholipid metabolism. This conclusion is supported by more direct evidence from a study by Henry and coworkers (144). Using a *sec14*^{ts} *cki1* strain, which has an overproduction of inositol (Opi$^-$) phenotype due to elevated phosphatidic acid levels (Figure 7), they showed that the UPR was not involved in activation of *INO1* through the promoter site UAS$_{INO}$ in response to inositol starvation. The defect in sustained *INO1* mRNA levels during prolonged inositol starvation in UPR-defective cells, first reported by Cox et al. (26), was reproduced. However, this defect was also suppressed in *opi*$^-$ cells. After prolonged inositol starvation, CDP-diacylglycerol levels in *ire1*Δ and *hac1*Δ strains were increased compared to WT, and phosphatidic acid and phosphatidylinositol levels decreased. Again, in *opi*$^-$ cells these changes were reversed (144). Thus, the UPR does not directly control expression of *INO1*. It seems more likely that subtle defects in the ER membrane, where key enzymatic reactions in phospholipid synthesis take place (Figure 7), are present in *ire1*Δ and *hac1*Δ cells when starved for inositol (144).

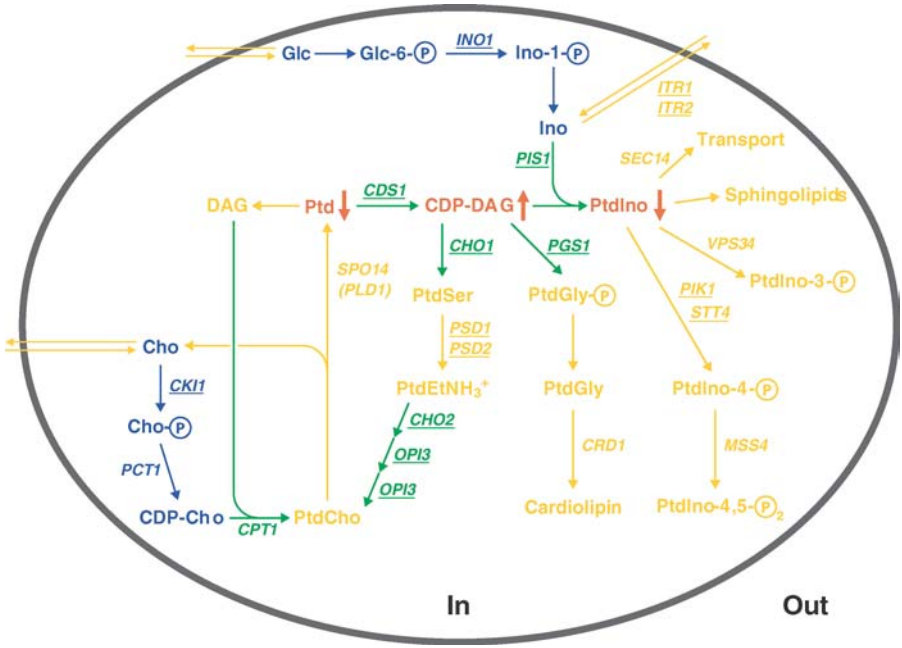


Figure 7 Phospholipid biosynthesis. Water soluble molecules, enzymes, and enzymatic reactions in the cytosol are in purple; membrane bound molecules, enzymes, and reactions at membrane compartments are in orange or red. Enzymes and reactions at the ER membrane are in green. Phospholipids whose levels are altered in *ire1*Δ and *hac1*Δ strains during inositol starvation are in red, and arrows (↓ or ↑) indicate if their levels are decreased or increased (144). Enzymes whose genes are repressed by inositol and choline in the growth medium are underlined. Abbreviations are Cho, choline; DAG, diacylglycerol; $EtNH_3^+$, ethanolamine; Glc, glucose; Gly, glycerol; Ino, inositol; Ptd, phosphatidic acid or phosphatidyl; and Ser, serine. Reprinted from *Mutation Research* (1c), copyright 2005, with permission from Elsevier.

These changes perturb phospholipid precursor pools, e.g., decrease the phosphatidic acid pool (Figure 7), which may be the cause for altered *INO1* transcription in *ire1*Δ and *hac1*Δ cells. This conclusion is supported by the recent finding that the soluble repressor of *INO1* transcription, Opi1p, is tethered to the ER membrane by binding to phosphatidic acid in the ER membrane and is held there in an inactive complex with Scs2p (145). An increase in the cellular inositol level converts phosphatidic acid into phosphatidylinositol (Figure 7), resulting in release of Opi1p from the ER membrane and in repression of *INO1*. Consistent with this model is that *scs2*Δ cells are inositol auxotrophs (145) and that overexpression of Scs2p suppresses the inositol auxotrophy of *ire1*Δ cells (145). Activation of the *INO1* locus is coupled with its relocalization from the nucleoplasm to the nuclear envelope (145a).

In mammals, fatty acid and cholesterol metabolism are regulated by sterol regulatory element (SRE)-binding proteins (SREBPs), a family of basic helix-loop-helix (bHLH) transcription factors consisting of SREBP1a, SREBP1b, and SREBP2. SREBPs localize to the ER membrane (146). There they form a complex with the tryptophan (W)-aspartic acid (D) repeat (WD repeat) domain of SREBP cleavage-activating protein (SCAP) and the protein INSIG-1 encoded by the insulin-induced gene 1. Changes in cellular lipid and cholesterol levels induce a conformational change in SCAP and dissociation of the SREBP-SCAP complex from INSIG-1. SCAP escorts SREBPs to the Golgi complex, where the cytosolic domains of SREBPs, harboring the bHLH domain, are proteolytically released by sequential action of S1P and S2P. The ER membrane resident bZIP transcription factor, ATF6, is also activated by proteolysis by S1P and S2P during ER stress. ATF6 can heterodimerize with SREBP2 and bind to the SRE in the low-density lipoprotein receptor (LDLR) promoter. ATF6 then recruits a transcriptional repressor, the histone deacetylase complex 1 (HDAC1), to the LDLR promoter (147). SREBP2 is primarily involved in stimulation of cholesterol synthesis (146). Suppression of cholesterol synthesis by ATF6 may adjust membrane fluidity during ER stress. However, it remains unclear whether a heterotrimeric ATF6-SREBP2-HDAC1 complex is formed on SRE or whether ATF6 alone recruits HDAC1 to SRE. It is also not known if changes in the composition of the ER membrane in UPR-deficient cells alter SREBP signaling. Finally, cholesterol toxicity in macrophages is signaled through the ER and appears to involve signaling through the UPR (148, 149).

The UPR in Unstressed Cells

Even cells considered healthy or unstressed experience ER stress due to unfolded proteins in the ER. For example, synthetic lethalities between the UPR and ERAD (13) or chaperone systems (53) have been reported in yeast, and increased UPR signaling in cells with defective ERAD was reported in yeast (88) and mammalian cells (136, 139). Exponentially growing yeast cultures splice between 3% and 30% of *HAC1* mRNA (22, 23), providing direct evidence for low-level activation of the UPR in healthy cells. This level of UPR activation may serve its well-established function to adjust the folding capacity of the ER to its folding load. Recent evidence has shown that UPR signaling in unstressed cells controls nutritional and differentiation programs (22) through control of the repression potential of the *RPD3-SIN3* HDAC (25). One class of genes specifically targeted by the *RPD3-SIN3* HDAC are genes defined by the promoter element URS1 (TCGGCGGCT), the DNA-binding site for the transcriptional regulator Ume6p (110). A global transcriptional profiling study showed that the majority of genes controlled by Ume6p are involved in carbon and nitrogen metabolism as well as meiosis in yeast (24). Indeed, spliced Hac1p repressed key metabolic genes, e.g., *ACS1* encoding acetyl coenzyme A synthethase, *CAR1* encoding arginase, and *INO1* (25), and early meiotic genes (22, 25) in an Ume6p- and HDAC-dependent manner. Furthermore, the level of UPR activation in unstressed cells is tightly linked to the

metabolic state of the cell. It is low on preferred, fermentable carbon sources (D-glucose and D-fructose), intermediate on disaccharides (D-maltose), and high on nonfermentable carbon sources, (acetate or ethanol) (22, 23). The level of *HAC1* splicing seen on various carbon sources is dependent on the presence of nitrogen sources. Induction of nitrogen starvation inhibited *HAC1* splicing within less than 5 min, and addition of ammonium salts to nitrogen-starved cells was sufficient to reactivate *HAC1* splicing with kinetics similar to those observed during induction of nitrogen starvation. Severe nitrogen starvation induces arginine catabolism, a process probably repressed by Hac1p through repression of *CAR1* (110). In diploid yeast, nitrogen starvation induces pseudohyphal growth. Consistent with the above observations, pseudohyphal growth was found to be derepressed in strains defective in the UPR (22). Inducers of the UPR, e.g., 2-deoxy-D-glucose or tunicamycin, inhibited pseudohyphal growth at sublethal concentrations in an *IRE1*- and *HAC1*-dependent manner, and overexpression of Hac1p repressed pseudohyphal growth, demonstrating that the UPR in response to nitrogen represses pseudohyphal growth (22). Furthermore, entry into meiosis, an alternative differentiation program of yeast to even more severe starvation conditions, was also repressed by the UPR in a similar way (22, 25). On the basis of these data, a role for the UPR in nutrient sensing and control of differentiation was proposed (Figure 8) (22).

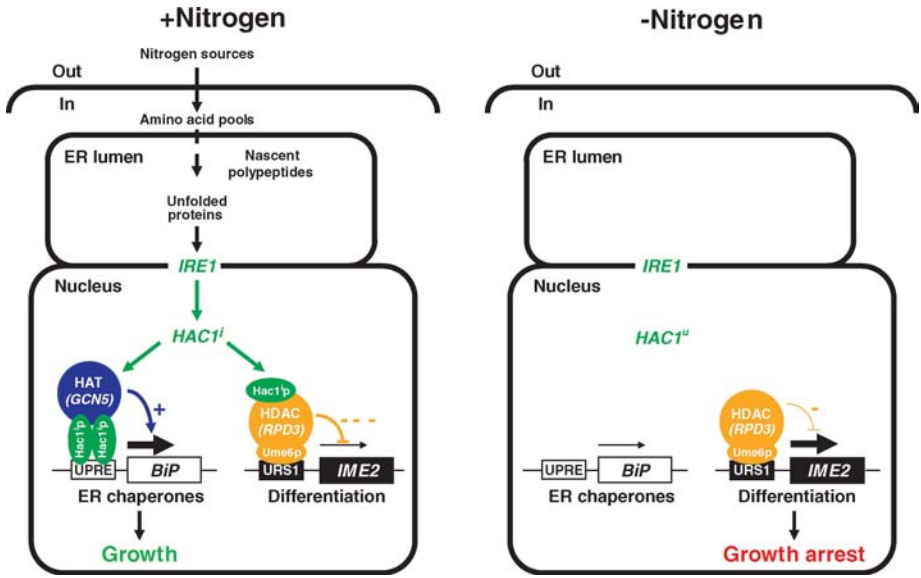


Figure 8 Control of starvation and differentiation responses by the UPR. Dashed lines indicate incompletely understood relationships. For simplicity only, the Hac1^{i}p species interacting with the HDAC is depicted as a monomer. For the same reason, only events related to the UPR are shown. Modified from Reference 25 and reprinted with permission from *EMBO Journal*.

Whereas a role for the UPR in control of nutritional and differentiation programs has been established for yeast, data to support a similar conclusion in mammalian systems are more sporadic. The major molecular chaperones of the ER, BiP/GRP78 and GRP94, are induced by glucose starvation (21) or anaerobiosis, suggesting that, as in yeast, the UPR is responsive to the nutritional state of a mammalian cell. Abrogation of PERK signaling through introduction of a *Ser51Ala* mutation into eIF2α in mice resulted in hypoglycemia, caused by decreased activity of enzymes catalyzing rate-limiting steps in gluconeogenesis, e.g., phosphoenolpyruvate carboxykinase, and a loss of the pancreatic β-cell population (18). These data are consistent with a model in which glucose sensing by the UPR controls proinsulin translation in pancreatic β-cells. In low glucose, possibly due to decreased ATP generation and altered synthesis of core N-linked oligosaccharides, protein folding in the ER is slow or partially inhibited, leading to activation of PERK, translational attenuation due to phosphorylation of eIF2α, and repression of proinsulin translation. When glucose levels rise, ATP generation and glycosylation become more efficient. This results in inactivation of the UPR and resumption of general and proinsulin mRNA translation. Thus, the UPR may contribute to glucose sensing in pancreatic β-cells (18). β-Cells may be predisposed to this sensing mechanism because of increased levels of IRE1 (69) and PERK (12), which allow for the detection of smaller fluctuations in the free BiP pool by IRE1 and PERK. However, the observations that are the basis of this model are also consistent with β-cell dysfunction, owing to loss of a great part of the β-cell population caused by ER stress-induced apoptosis (150). This conclusion is supported by the finding that proinsulin translation in response to glucose in islets isolated from WT PERK and *perk*$^{-/-}$ mice was indistinguishable in vitro (119). In addition, deletion of the proapoptotic UPR target gene CHOP delayed β-cell destruction and onset of diabetes associated with expression of a folding mutant of proinsulin in the Akita mouse (151). Further work will have to distinguish between these models.

A model system with involvement of the UPR in control of differentiation in a mammalian setting may be terminal differentiation of B cells into antibody-secreting plasma cells. This process is coupled to a 5- to 10-fold expansion of the ER (152) and considerable ER stress. Clearly, in this process, cellular differentiation, proliferation of the ER, and ER stress coincide. Indeed, XBP-1 has been shown to be essential for terminal B-cell differentiation (153). Splicing of *XBP-1* mRNA was observed during B-cell differentiation (8, 154). Recent studies demonstrated that spliced XBP-1 can induce phospholipid biosynthesis and membrane proliferation that accompany plasma cell differentiation (154a, 154b). These data indicate that activation of the UPR is required for terminal B-cell differentiation. Terminal differentiation of B cells is dependent on repression of c-*myc* (155) through recruitment of mammalian Rpd3p orthologs to the c-*myc* promoter by Blimp-1 (156). Further, the kinetics of activation of the UPR, splicing of *XBP-1* mRNA, repression of c-*myc*, and activation of Blimp-1, are similar (154). Together with the finding that the UPR controls HDACs in yeast, these observations raise the

possibility that UPR signaling drives differentiation or is important for maintaining the differentiated state in mammals.

ER Stress-Induced Apoptosis

Two major pathways contribute to control of apoptosis. The intrinsic pathway responds to intracellular insults, e.g., DNA damage. The extrinsic pathway responds to extracellular stimuli (Figure 9) and is triggered by self-association of cell surface receptors, recruitment of caspases, mainly caspase-8, and initiation of a caspase cascade. A balance between proapoptotic BH3-only proteins, e.g., Bad, Bak, and Bax, and antiapoptopic proteins, e.g., Bcl-2 proteins, controls the intrinsic pathway. When activated, the BH3-only proteins, Bak and Bax, oligomerize and insert themselves into the outer mitochondrial membrane to form a pore, resulting in release of cytochrome *c*. Cytochrome *c* then facilitates formation of a complex between Apaf-1 and procaspase-9, subsequent activation of a caspase cascade, and activation of the executioner caspase, caspase-3 (157). In analogy to the situation in the whole cell, we refer to the, presumably, receptor-independent pathway of ER stress apoptosis as the intrinsic pathway and to the receptor-dependent pathway as the extrinsic pathway.

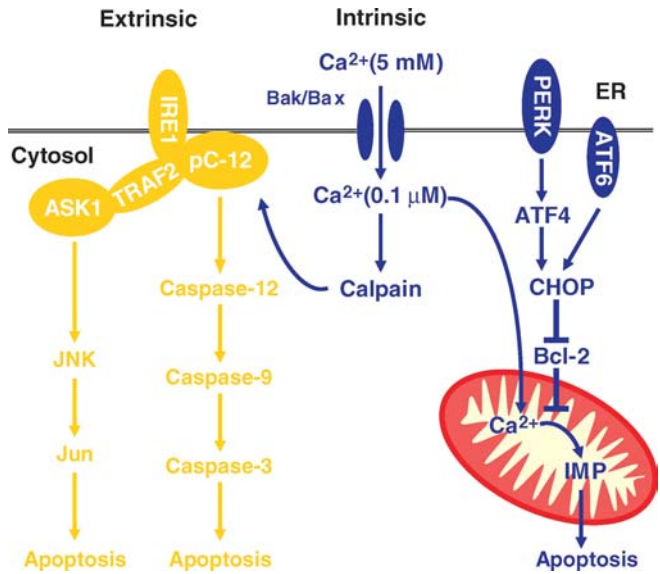


Figure 9 Apoptotic-signaling pathways activated by ER stress. IMP represents inner mitochondrial membrane potential. Modified and reprinted from *Mutation Research* (1c), copyright 2005, with permission from Elsevier.

THE INTRINSIC PATHWAY Insertion of oligomeric Bak and Bax into the ER membrane causes efflux of Ca^{2+} ions from the ER (77, 158) (Figure 9). The increase in the cytosolic Ca^{2+} concentration activates calpain (159), which cleaves and activates ER-localized procaspase-12 (159). Activated caspase-12 cleaves procaspase-9, and caspase-9 activates the executioner caspase, procaspase-3 (160). This pathway is independent of Apaf-1 and mitochondrial cytochrome *c* release (160). *caspase-12*$^{-/-}$ cells are partially resistant to apoptosis (16), which is consistent with a role for caspase-12 in ER stress-induced apoptosis.

Ca^{2+} released from the ER is rapidly taken up by mitochondria, which may lead to collapse of the inner membrane potential. Ultimately, Ca^{2+} influx into mitochondria opens the permeability transition pore (PTP), which is formed from a complex of the multiprotein voltage-dependent anion channel, the adenine nucleotide translocase, and cyclophilin-D (161). Cytochrome *c* is then released through the PTP into the cytoplasm, where the apoptosome is formed and procaspase-3 is activated. In addition, the PTP recruits Bax to the outer mitochondrial membrane (161). Overexpression of an antiapoptotic Bcl-2 family member, e.g., Bcl-X_L, blocked depolarization of the inner mitochondrial membrane in response to ER stress (162). ER stress activates ATF6 and synthesis of ATF4 after phosphorylation of eIF2α by PERK, which then induces expression of the proapoptotic bZIP transcription factor CHOP (163). CHOP represses transcription of antiapoptotic Bcl-2 and thus shifts the balance between pro- and antiapoptotic Bcl-2 family members toward the antiapoptotic family members (164); *chop*$^{-/-}$ cells were partially resistant to apoptosis (164), which is consistent with this model.

THE EXTRINSIC PATHWAY Overexpression of c-Jun N-terminal inhibitory kinase (JIK) increased phosphorylation of the tumor necrosis factor receptor-associated factor 2 (TRAF2) and interaction of TRAF2 with IRE1α (165). JIK also interacts with IRE1α (165). Formation of a trimeric complex between IRE1α, TRAF2, and apoptosis signal-regulating kinase 1 (ASK1) activates ASK1 (19) and c-Jun amino-terminal kinase and, subsequently, cell death (19). TRAF2 promotes clustering of procaspase-12 and is released from procaspase-12 upon ER stress, presumably by sequestering IRE1 (165), which was proposed to be a prerequisite for procaspase-12 activation (165). Thus, the extrinsic and intrinsic pathways interact. Whether IRE1β can substitute for IRE1α in transduction of apoptotic signals has not been investigated.

THE PATHOPHYSIOLOGY OF IMPAIRED ER AND UPR FUNCTION

Numerous diseases are caused by malfunction of the ER (Table 4). ER storage diseases were first classified on the basis of their biochemical cause by Kim & Arvan (6). Here we extend this classification to include diseases that are caused by malfunction of the ER transport machinery and defective UPR signaling.

TABLE 4 Conformational diseases related to the ER[a]

Disease	Affected protein[b]	Clinical manifestation
Class I[c]		
Cystic fibrosis	Cystic fibrosis transmembrane conductance regulator	Lung disease
Diabetes mellitus	Insulin receptor	Diabetes
Albinism/tyrosinase deficiency	Tyrosinase	Pigmentation defect
α_1-antichymotrypsin deficiency	α_1-antichymotrypsin	Lung disease, liver disease
α_1-antitrypsin deficiency without liver disease (Pi Z mutation)	α_1-antitrypsin	Lung disease
Autosomal dominant retinitis pigmentosa	Rhodopsin	Loss of peripheral and night vision
Congenital hypogonadotropic hypogonadism	Gonadotropin-releasing hormone receptor	Delayed sexual development
Congenital hypothyroidism and goiter	Thyroglobulin	Endocrine disease, developmental defect
Congenital hypothyroidism and goiter	Thyroid peroxidase	Endocrine disease
Congenital hypothyroidism and goiter	Thyroxine binding globulin	Endocrine defects
Congenital long QT syndrome	Voltage-gated potassium channel (HERG)	Heart disease
Congenital sucrase-isomaltase deficiency	Sucrase-isomaltase	Gastrointestinal disease
Crigler-Najjar disease	UDP-glucuronosyl-transferase	Liver disease
Diabetes insipidus, autosomal dominant neurohypophyseal	Adiuretin vasopressin (AVP)	Diabetes
Diabetes insipidus, X-linked nephrogenic	AVP receptor 2	Diabetes
Diabetes insipidus, autosomally inherited nephrogenic	Aquaporin-2	Diabetes
Diabetes mellitus	α-Subunit of insulin receptor	Diabetes
Fabrey disease	α-D-galactosidase	Neurological disease, endocrine defect
Familial hypercholesterolemia	LDL receptor	Vascular disease
Familial hyperchylomicronemia	Lipoprotein lipase	Vascular disease
Familial isolated hypoparathyroidism	Preproparathyroid hormone	Endocrine defects
Gaucher's disease	β-glucosidase	Hepatomegaly, splenomegaly, bone crisis

(*Continued*)

TABLE 4 (*Continued*)

Disease	Affected protein[b]	Clinical manifestation
Global polyendocrinopathy associated with obesity and infertility (*fat/fat* mouse)	Carboxypeptidase E	
Hemophilias A, B	Factors VIII and IX	Blood coagulation deficiency
Hereditary hemochromatosis	Hemochromatosis protein HFE	Liver disease
Hereditary hypofibrinogenemia	Fibrinogen	Liver disease
Hereditary myeloperoxidase deficiency	Myeloperoxidase	Cancer, immunodeficiency
Laron dwarfism	Growth hormone receptor	Developmental defects, endocrine defect
Lipoprotein a deficiency	Lipoprotein a	Cardiovascular disease
Obesity	Prohormone convertase 1	Obesity
Osteogenesis imperfecta	Type I procollagen	Skeletal deformity
Osteogenesis imperfecta	Decorin	Skeletal deformity
Parkinsonism, autosomal recessive juvenile	Pae I receptor	Neurodegenerative disease
Protein C deficiency	Protein C	Blood coagulation disease
Protein S deficiency	Protein S	Blood coagulation disease
Spondyloepiphyseal dysplasia due to hypochondrogenesis	Type II procollagen	Abnormal cartilage formation and growth
Spondyloperipheral dysplasia	Type II collagen	Skeletal dysplasia
Tay-Sachs disease	β-hexosaminidase	Neurological disease, endocrine defect
Type I hereditary angioedema	Complement C1 inhibitor	Immunodeficiency, skin disease
von Willebrand disease	von Willebrand factor	Blood coagulation deficiency
Class I[d]		
β-amyloid toxicity	β-amyloid	Neurodegenerative disease
α_1-antitrypsin deficiency with liver disease	α_1-antitrypsin	Lung disease, liver disease
Charcot-Marie-Tooth disease	Peripheral myelin protein PMP22	Neurological disease, degenerative muscle disease
Diabetes insipidus, autosomal dominant neurohypophyseal	Adiuretin vasopressin	Diabetes
Diabetes mellitus in the Akita mouse	Insulin 2	
Osteogenesis imperfecta	Type I procollagen	Skeletal deformity
Pelizaeus-Merzbacher leukodystrophy	Proteolipid protein	Neurological disease

(*Continued*)

TABLE 4 (*Continued*)

Disease	Affected protein[b]	Clinical manifestation
Presenile dementia/myoclonus	Neuroserpin	Dementia
Retroviral noninflammatory spongiform neurodegenerative disease (mouse)	Viral envelope protein	Neurodenegerative disease
Spondyloperipheral dysplasia	Type II collagen	Skeletal dysplasia
Class II[e]		
Abetalipoproteinemia	Apolipoprotein B/microsomal triglyceride transfer protein	Vascular disease
Combined coagulation factor V and VIII deficiency	Factor V, factor VIII/LMAN	Blood coagulation deficiency
Combined coagulation factor V and VIII deficiency	Factor V, factor VIII/MCFD2	Blood coagulation deficiency
Spondylo-epiphyseal dysplasia tarda	Sedlin/collagen	Skeletal defect
Class III[f]		
Bipolar disorder	XBP-1	Mood disorder
Colitis (mouse)	IRE1β	—
Diabetes mellitus (mouse)	PERK	—
Hypoglycemia (mouse)	eIF2α	—
Wollcott-Rallison syndrome	PERK	Diabetes; skeletal defects
Class IV[g]		
Polyglutamine diseases: dentatorubral-pallidoluysian atrophy, Huntington's disease, spinobulbar muscular atrophy, spinocerebellar ataxia	Proteasome	Neurodegenerative diseases

[a]Compiled from References 6, 7.

[b]When two proteins are listed, the WT protein whose loss of expression is the primary cause for the disease is listed first, followed by the mutated protein responsible for the loss of expression of the aforementioned proteins.

[c]Subclasses A and B: retention of proteins susceptible to ERAD in the ER.

[d]Subclass C: retention of degradation resistant proteins in the ER.

[e]A defective ER trafficking/processing machinery results in retention of WT cargo proteins in the ER.

[f]Defect in UPR signaling.

[g]Protective responses regulated by the UPR are inhibited or defective.

Class I diseases are caused by mutations in secretory client proteins that alter the folding properties of the affected protein. Three subgroups can be distinguished. In subgroup A, only transport of the affected protein through the secretory pathway is impaired, and the disease is caused by loss of function of the protein in its final destination. These proteins can either be functional or nonfunctional in their

predestined work place, but they do not reach their destination owing to prolonged interaction with molecular chaperones. The ΔF508 mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) is a prominent example of a largely functional protein that is retained in the ER and degraded by ERAD (20). However, for most diseases, the mutant proteins that are retained in the ER are biologically inactive (Table 4). In subgroup B, the mutant protein can still be incorporated into a multimeric complex composed of mutant and WT proteins. Owing to the presence of the mutant, unfolded protein the otherwise mature complex is biologically inactive or is retained in the ER. An example of this subclass is osteogenesis imperfecta caused by mutations in procollagen (166). A common characteristic of subclasses A and B is that they are susceptible to ERAD. Distinct from these subclasses of folding-incompetent proteins is a third subclass whose folding defects disrupt the function of the ER. In general, these mutant proteins are resistant to degradation by the proteasome, resulting in severe malfunction of the ER, cellular toxicity, and cell death. In such cases, a null allele may confer a mild phenotype caused by loss of protein function, whereas limited mutations, e.g., point mutations or frameshift mutations, can cause severe phenotypes due to disruption of ER function and activation of apoptotic ER stress-signaling mechanisms, as in the case of β-amyloid toxicity (16). Many of these diseases are therefore dominant. Expression of folding-incompetent, presumably degradation resistant, viral envelope proteins can be the cause for increased virulence of viral strains and result in robust activation of the UPR (9). In this subgroup, the UPR is part of the complex host-pathogen interaction. Recent work has shown that many diseases caused by proteins with folding defects can be cured with small membrane permeable chemical chaperones that stabilize the native conformation of the target protein (Table 5). An interesting alternative is the inhibition of the chaperone machinery through depletion of ER-luminal Ca^{2+}, which increased functional surface expression of ΔF508 CFTR (167).

Class II diseases are also caused by loss of function of specific proteins at their destination. However, in contrast to class I diseases, mutations in the trafficking machinery of the secretory pathway are responsible for retention of WT cargo molecules in the ER. A prominent example is the bleeding disorder, a combined factor V and VIII deficiency, in patients with mutations in the LMAN1-MCFD2 lectin complex (14) required for the transport of factors V and VIII from the ER to the Golgi complex.

Class III diseases are caused by a defective UPR-signaling machinery. In these diseases, one signaling pathway in the UPR is lost. For example, kinase-defective mutations in PERK are the cause for a severe early infancy insulin-dependent diabetes called Wollcott-Rallison syndrome (17). Another example is bipolar mood disorder, which is associated with mutations in the autostimulatory loop that regulates *XBP-1* transcription in response to ER stress (114).

In class IV diseases, UPR signaling itself is intact, but protective responses regulated by the UPR are disrupted, e.g., through poisoning of the proteasome by cytosolic polyglutamine repeats, which is associated with many neurodegenerative diseases (19). The concepts underlying classes III and IV can also be

TABLE 5 Chemical chaperones, receptor antagonists, and substrate analogs that stabilize the native conformation and increase productive folding of the protein in the ER

Protein	Drug or mode of action
α_1-antitrypsin	Osmolytes (4-phenylbutyric acid, glycerol)
Apolipoprotein a	6-aminohexanoic acid, proline
Aquaporin 2	Osmolyte (glycerol)
AVP receptor 2	Receptor antagonist
CFTR	Osmolytes (glycerol, trimethylamine *N*-oxide, D_2O)
δ-opioid receptor	Receptor antagonist
α-galactosidase	Galactose (competitive inhibitor)
β-glucosidase	Inhibitor
Gonadotropin-releasing hormone receptor	Receptor antagonist
HERG	Channel blocker
P-glycoprotein	Substrates, modulators
Rhodopsin	Retinal-based ligands
Tyrosinase	DOPA, substrates

advantageously used to induce apoptosis in cancers derived from cell types specialized in secretion. For example, small molecule inhibitors of the proteasome, e.g., bortezomib, are in clinical trials to treat myelomas (168). More specific results may be obtained with drugs that directly target ATF6, IRE1, or PERK.

FUTURE CHALLENGES

Mechanisms for signal transduction by ATF6, IRE1, and PERK have been established. However, a more detailed biochemical and biophysical characterization of the interaction of these proximal stress sensors with molecular chaperones is desirable. Important questions remain. Are all ER stress signals created equal? For example, is an ER stress response to a viral infection identical to ER stress experienced by a differentiating cell? Is regulation of ATF6, IRE1, and PERK by interaction with molecular chaperones really identical, or have the techniques used to study their activation simply been too crude to see differences in their activation? An unsolved and unaddressed mystery is also why, and if, one signaling pathway—seemingly at the same time—transduces survival and apoptotic signals. A comprehensive understanding of how a mammalian cell is reprogrammed during ER stress is also lacking, partly because not all players in adaptive responses (e.g., in ERAD) have been identified. To solve these remaining riddles, we will have to consider (*a*) the possibility that the ultimate response to ER stress is cell-type specific and subject to modulation according to the needs of each individual cell

type, (*b*) the prevailing set of bZIP transcription factors at the onset of ER stress, and (*c*) how much of a particular ER stress–inducible bZIP protein is spiked into this, cell-type specific, bZIP protein network during phases of acute ER stress. An empirical correlation between ER stress, proliferation of the ER, and cellular differentiation exists, e.g., in terminal B-cell differentiation. From the viewpoint of a cell, efficient coordination of these processes is highly desirable. Despite initial progress, especially in yeast, the question of how, and if, these diverse events are coordinated by the UPR remains to be answered.

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LITERATURE CITED

1. Selye H. 1985. *Basal Facts* 7:3–11
1a. Ron D, Oyadomari S. 2004. *Dev. Cell* 7:287–88
1b. Zhang K, Kaufman RJ. 2003. *Nat. Cell Biol.* 5:769–70
1c. Schröder M, Kaufman RJ. 2005. *Mutat. Res.* 569:29–63
2. Kaufman RJ, Wasley LC, Dorner AJ. 1988. *J. Biol. Chem.* 263:6352–62
3. Dorner AJ, Wasley LC, Kaufman RJ. 1989. *J. Biol. Chem.* 264:20602–7
4. Schröder M, Schäfer R, Friedl P. 2002. *Biotechnol. Bioeng.* 78:131–40
5. Kozutsumi Y, Segal M, Normington K, Gething M-J, Sambrook J. 1988. *Nature* 332:462–64
6. Kim PS, Arvan P. 1998. *Endocr. Rev.* 19:173–202
7. Aridor M, Hannan LA. 2000. *Traffic* 1:836–51
8. Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, et al. 2002. *Nature* 415:92–96
9. Dimcheff DE, Askovic S, Baker AH, Johnson-Fowler C, Portis JL. 2003. *J. Virol.* 77:12617–29
10. Walther-Larsen H, Brandt J, Collinge DB, Thordal-Christensen H. 1993. *Plant Mol. Biol.* 21:1097–108
11. Jelitto-Van Dooren EP, Vidal S, Denecke J. 1999. *Plant Cell* 11:1935–44
12. Harding HP, Zhang Y, Ron D. 1999. *Nature* 397:271–74
13. Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P. 2000. *Cell* 101:249–58
14. Nichols WC, Seligsohn U, Zivelin A, Terry VH, Hertel CE, et al. 1998. *Cell* 93:61–70
15. Davis RL, Shrimpton AE, Holohan PD, Bradshaw C, Feiglin D, et al. 1999. *Nature* 401:376–79
16. Nakagawa T, Zhu H, Morishima N, Li E, Xu J, et al. 2000. *Nature* 403:98–103
17. Delépine M, Nicolino M, Barrett T, Golamaully M, Lathrop GM, Julier C. 2000. *Nat. Genet.* 25:406–9
18. Scheuner D, Song B, McEwen E, Liu C, Laybutt R, et al. 2001. *Mol. Cell* 7:1165–76
19. Nishitoh H, Matsuzawa A, Tobiume K,

Saegusa K, Takeda K, et al. 2002. *Genes Dev.* 16:1345–55
20. Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, et al. 1990. *Cell* 63:827–34
21. Pouysségur J, Shiu RP, Pastan I. 1977. *Cell* 11:941–47
22. Schröder M, Chang JS, Kaufman RJ. 2000. *Genes Dev.* 14:2962–75
23. Kuhn KM, DeRisi JL, Brown PO, Sarnow P. 2001. *Mol. Cell. Biol.* 21:916–27
24. Williams RM, Primig M, Washburn BK, Winzeler EA, Bellis M, et al. 2002. *Proc. Natl. Acad. Sci. USA* 99:13431–36
25. Schröder M, Clark R, Liu CY, Kaufman RJ. 2004. *EMBO J.* 23:2281–92
26. Cox JS, Chapman RE, Walter P. 1997. *Mol. Biol. Cell* 8:1805–14
27. Dobson CM, Sali A, Karplus M. 1998. *Angew. Chem. Int. Ed. Engl.* 37:868–93
28. Anfinsen CB, Haber E, Sela M, White FH Jr. 1961. *Proc. Natl. Acad. Sci. USA* 47:1309–14
29. Stevens FJ, Argon Y. 1999. *Semin. Cell Dev. Biol.* 10:443–54
30. Palmiter RD. 1975. *Cell* 4:189–97
31. Rutkowski DT, Ott CM, Polansky JR, Lingappa VR. 2003. *J. Biol. Chem.* 278:30365–72
32. Wetmore DR, Hardman KD. 1996. *Biochemistry* 35:6549–58
33. Suzuki CK, Bonifacino JS, Lin AY, Davis MM, Klausner RD. 1991. *J. Cell Biol.* 114:189–205
34. Ou WJ, Bergeron JJ, Li Y, Kang CY, Thomas DY. 1995. *J. Biol. Chem.* 270: 18051–59
35. Corbett EF, Oikawa K, Francois P, Tessier DC, Kay C, et al. 1999. *J. Biol. Chem.* 274:6203–11
36. Fewell SW, Travers KJ, Weissman JS, Brodsky JL. 2001. *Annu. Rev. Genet.* 35: 149–91
37. Ellgaard L, Helenius A. 2003. *Nat. Rev. Mol. Cell Biol.* 4:181–91
38. Hosokawa N, Tremblay LO, You Z, Herscovics A, Wada I, Nagata K. 2003. *J. Biol. Chem.* 278:26287–94
39. Oda Y, Hosokawa N, Wada I, Nagata K. 2003. *Science* 299:1394–97
40. Sousa MC, Ferrero-Garcia MA, Parodi AJ. 1992. *Biochemistry* 31:97–105
41. Weng S, Spiro RG. 1993. *J. Biol. Chem.* 268:25656–63
42. Trombetta SE, Parodi AJ. 1992. *J. Biol. Chem.* 267:9236–40
43. Lin HY, Masso-Welch P, Di YP, Cai JW, Shen JW, Subjeck JR. 1993. *Mol. Biol. Cell* 4:1109–19
44. Feldheim D, Rothblatt J, Schekman R. 1992. *Mol. Cell. Biol.* 12:3288–96
45. Boisramé A, Kabani M, Beckerich JM, Hartmann E, Gaillardin C. 1998. *J. Biol. Chem.* 273:30903–8
46. Argon Y, Simen BB. 1999. *Semin. Cell Dev. Biol.* 10:495–505
47. Steel GJ, Fullerton DM, Tyson JR, Stirling CJ. 2004. *Science* 303:98–101
48. Fernández F, D'Alessio C, Fanchiotti S, Parodi AJ. 1998. *EMBO J.* 17:5877–86
49. Zapun A, Darby NJ, Tessier DC, Michalak M, Bergeron JJ, Thomas DY. 1998. *J. Biol. Chem.* 273:6009–12
50. Wang Q, Chang A. 2003. *EMBO J.* 22: 3792–802
51. Gillece P, Luz JM, Lennarz WJ, de La Cruz FJ, Römisch K. 1999. *J. Cell Biol.* 147:1443–56
52. Gething M-J. 1999. *Semin. Cell Dev. Biol.* 10:465–72
53. Tyson JR, Stirling CJ. 2000. *EMBO J.* 19:6440–52
54. Hightower LE, Sadis SE, Takenaka IM. 1994. In *The Biology of Heat Shock Proteins and Molecular Chaperones*, ed. RI Morimoto, A Tissieres, C Georgopoulos, pp. 197–207. Plainview, NY: Cold Spring Harbor Lab.
55. Hirschberg CB, Robbins PW, Abeijon C. 1998. *Annu. Rev. Biochem.* 67:49–69
56. Caramelo JJ, Castro OA, Alonso LG, De Prat-Gay G, Parodi AJ. 2003. *Proc. Natl. Acad. Sci. USA* 100:86–91
57. Taylor SC, Ferguson AD, Bergeron JJ,

Thomas DY. 2004. *Nat. Struct. Mol. Biol.* 11:128–34
58. Haze K, Yoshida H, Yanagi H, Yura T, Mori K. 1999. *Mol. Biol. Cell* 10:3787–99
59. Chen X, Shen J, Prywes R. 2002. *J. Biol. Chem.* 277:13045–52
60. Ye J, Rawson RB, Komuro R, Chen X, Dave UP, et al. 2000. *Mol. Cell* 6:1355–64
61. Wang Y, Shen J, Arenzana N, Tirasophon W, Kaufman RJ, Prywes R. 2000. *J. Biol. Chem.* 275:27013–20
62. Kokame K, Kato H, Miyata T. 2001. *J. Biol. Chem.* 276:9199–205
63. Yoshida H, Okada T, Haze K, Yanagi H, Yura T, et al. 2001. *Mol. Cell. Biol.* 21:1239–48
64. Shen J, Chen X, Hendershot L, Prywes R. 2002. *Dev. Cell* 3:99–111
65. Hong M, Luo S, Baumeister P, Huang J-M, Gogia RK, et al. 2004. *J. Biol. Chem.* 279:11354–63
66. Hong M, Li M, Mao C, Lee AS. 2004. *J. Cell. Biochem.* 92:723–32
67. Liu CY, Wong HN, Schauerte JA, Kaufman RJ. 2002. *J. Biol. Chem.* 277:18346–56
68. Liu CY, Schröder M, Kaufman RJ. 2000. *J. Biol. Chem.* 275:24881–85
69. Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. 2000. *Nat. Cell Biol.* 2:326–32
70. Liu CY, Xu Z, Kaufman RJ. 2003. *J. Biol. Chem.* 278:17680–87
71. Ma K, Vattem KM, Wek RC. 2002. *J. Biol. Chem.* 277:18728–35
72. Tirasophon W, Welihinda AA, Kaufman RJ. 1998. *Genes Dev.* 12:1812–24
73. Wang XZ, Harding HP, Zhang Y, Jolicoeur EM, Kuroda M, Ron D. 1998. *EMBO J.* 17:5708–17
74. Chen-Levy Z, Nourse J, Cleary ML. 1989. *Mol. Cell. Biol.* 9:701–10
75. Xu Q, Reed JC. 1998. *Mol. Cell* 1:337–46
76. Chae HJ, Kim HR, Xu C, Bailly-Maitre B, Krajewska M, et al. 2004. *Mol. Cell* 15:355–66
77. Zong WX, Li C, Hatzivassiliou G, Lindsten T, Yu Q-C, et al. 2003. *J. Cell Biol.* 162:59–69
78. Mathai JP, Germain M, Marcellus RC, Shore GC. 2002. *Oncogene* 21:2534–44
79. Mund T, Gewies A, Schoenfeld N, Bauer MK, Grimm S. 2003. *FASEB J.* 17:696–98
80. Adams JM, Cory S. 1998. *Science* 281: 1322–26
81. Eskes R, Desagher S, Antonsson B, Martinou JC. 2000. *Mol. Cell. Biol.* 20:929–35
82. Schlesinger PH, Gross A, Yin XM, Yamamoto K, Saito M, et al. 1997. *Proc. Natl. Acad. Sci. USA* 94:11357–62
83. Cox JS, Shamu CE, Walter P. 1993. *Cell* 73:1197–206
84. Mori K, Ma W, Gething M-J, Sambrook J. 1993. *Cell* 74:743–56
85. Sidrauski C, Walter P. 1997. *Cell* 90: 1031–39
86. Papa FR, Zhang C, Shokat K, Walter P. 2003. *Science* 302:1533–37
87. Tirasophon W, Lee K, Callaghan B, Welihinda A, Kaufman RJ. 2000. *Genes Dev.* 14:2725–36
88. Cox JS, Walter P. 1996. *Cell* 87:391–404
89. Lee K, Tirasophon W, Shen X, Michalak M, Prywes R, et al. 2002. *Genes Dev.* 16:452–66
90. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. 2001. *Cell* 107:881–91
91. Gonzalez TN, Sidrauski C, Dörfler S, Walter P. 1999. *EMBO J.* 18:3119–32
92. Sidrauski C, Cox JS, Walter P. 1996. *Cell* 87:405–13
93. McCraith SM, Phizicky EM. 1990. *Mol. Cell. Biol.* 10:1049–55
94. Culver GM, McCraith SM, Zillmann M, Kierzek R, Michaud N, et al. 1993. *Science* 261:206–8
95. Zillmann M, Gorovsky MA, Phizicky EM. 1991. *Mol. Cell. Biol.* 11:5410–16
96. Laski FA, Fire AZ, RajBhandary UL,

Sharp PA. 1983. *J. Biol. Chem.* 258: 11974–80
97. Spinelli SL, Malik HS, Consaul SA, Phizicky EM. 1998. *Proc. Natl. Acad. Sci. USA* 95:14136–41
98. Abelson J, Trotta CR, Li H. 1998. *J. Biol. Chem.* 273:12685–88
99. Rüegsegger U, Leber JH, Walter P. 2001. *Cell* 107:103–14
100. Winicov I, Button JD. 1982. *Mol. Cell. Biol.* 2:241–49
101. Mori K, Ogawa N, Kawahara T, Yanagi H, Yura T. 2000. *Proc. Natl. Acad. Sci. USA* 97:4660–65
102. Inada M, Guthrie C. 2004. *Proc. Natl. Acad. Sci. USA* 101:434–39
103. Lee AH, Iwakoshi NN, Anderson KC, Glimcher LH. 2003. *Proc. Natl. Acad. Sci. USA* 100:9946–51
104. Mori K, Sant A, Kohno K, Normington K, Gething MJ, Sambrook JF. 1992. *EMBO J.* 11:2583–93
105. Spode I, Maiwald D, Hollenberg CP, Suckow M. 2002. *J. Mol. Biol.* 319:407–20
106. Welihinda AA, Tirasophon W, Kaufman RJ. 2000. *J. Biol. Chem.* 275:3377–81
107. Welihinda AA, Tirasophon W, Green SR, Kaufman RJ. 1997. *Proc. Natl. Acad. Sci. USA* 94:4289–94
108. Kurdistani SK, Grunstein M. 2003. *Nat. Rev. Mol. Cell Biol.* 4:276–84
109. Ogawa N, Mori K. 2004. *Genes Cells* 9:95–104
110. Strich R, Surosky RT, Steber C, Dubois E, Messenguy F, Esposito RE. 1994. *Genes Dev.* 8:796–810
111. Goldmark JP, Fazzio TG, Estep PW, Church GM, Tsukiyama T. 2000. *Cell* 103:423–33
112. Kadosh D, Struhl K. 1997. *Cell* 89:365–71
113. Newman JR, Keating AE. 2003. *Science* 300:2097–101
114. Kakiuchi C, Iwamoto K, Ishiwata M, Bundo M, Kasahara T, et al. 2003. *Nat. Genet.* 35:171–75
115. Welihinda AA, Tirasophon W, Green SR, Kaufman RJ. 1998. *Mol. Cell. Biol.* 18:1967–77
116. Nguyên DT, Kebache S, Fazel A, Wong HN, Jenna S, et al. 2004. *Mol. Biol. Cell.* 15:4248–60
117. Oono K, Yoneda T, Manabe T, Yamagishi S, Matsuda S, et al. 2004. *Neurochem. Int.* 45:765–72
118. Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, Diehl JA. 2003. *Mol. Cell. Biol.* 23:7198–209
119. Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D. 2000. *Mol. Cell* 5:897–904
120. Brewer JW, Diehl JA. 2000. *Proc. Natl. Acad. Sci. USA* 97:12625–30
121. Kozak M. 2002. *Gene* 299:1–34
122. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, et al. 2000. *Mol. Cell* 6:1099–108
123. Yaman I, Fernandez J, Liu H, Caprara M, Komar AA, et al. 2003. *Cell* 113:519–31
123a. Vattem KM, Wek RC. 2004. *Proc. Natl. Acad. Sci. USA* 101:11269–74
123b. Lu PD, Harding HP, Ron D. 2004. *J. Cell Biol.* 167:27–33
123c. Jousse C, Bruhat A, Carraro V, Urano F, Ferrara M, et al. 2001. *Nucleic Acids Res.* 29:4341–51
124. Nguyen T, Sherratt PJ, Pickett CB. 2003. *Annu. Rev. Pharmacol. Toxicol.* 43:233–60
125. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, et al. 2003. *Mol. Cell* 11:619–33
126. Pahl HL, Baeuerle PA. 1995. *EMBO J.* 14:2580–88
127. Wu S, Tan M, Hu Y, Wang JL, Scheuner D, Kaufman RJ. 2004. *J. Biol. Chem.* 279:34898–902
128. Jousse C, Oyadomari S, Novoa I, Lu P, Zhang Y, et al. 2003. *J. Cell Biol.* 163: 767–75
129. Ma Y, Hendershot LM. 2003. *J. Biol. Chem.* 278:34864–73
130. Brush MH, Weiser DC, Shenolikar S. 2003. *Mol. Cell. Biol.* 23:1292–303
131. Yan W, Frank CL, Korth MJ, Sopher BL, Novoa I, et al. 2002. *Proc. Natl. Acad. Sci. USA* 99:15920–25

132. Kebache S, Cardin E, Nguyên DT, Chevet E, Larose L. 2004. *J. Biol. Chem.* 279:9662–71
133. Kebache S, Zuo D, Chevet E, Larose L. 2002. *Proc. Natl. Acad. Sci. USA* 99: 5406–11
134. Jiang HY, Wek SA, McGrath BC, Lu D, Hai T, et al. 2004. *Mol. Cell. Biol.* 24:1365–77
135. Okada T, Yoshida H, Akazawa R, Negishi M, Mori K. 2002. *Biochem. J.* 366:585–94
136. Lee AH, Iwakoshi NN, Glimcher LH. 2003. *Mol. Cell. Biol.* 23:7448–59
137. van Laar T, van der Eb AJ, Terleth C. 2001. *Curr. Protein Pept. Sci.* 2:169–90
138. Ma Y, Hendershot LM. 2004. *J. Biol. Chem.* 279:13792–99
139. Hori O, Ichinoda F, Yamaguchi A, Tamatani T, Taniguchi M, et al. 2004. *Genes Cells* 9:457–69
140. Yoshida H, Matsui T, Hosokawa N, Kaufman RJ, Nagata K, Mori K. 2003. *Dev. Cell* 4:265–71
141. Masuda A, Kuwano M, Shimada T. 1983. *Cell Struct. Funct.* 8:309–12
142. Hyde M, Block-Alper L, Felix J, Webster P, Meyer DI. 2002. *J. Cell Biol.* 156:993–1001
143. Stroobants AK, Hettema EH, van den Berg M, Tabak HF. 1999. *FEBS Lett.* 453:210–14
144. Chang HJ, Jones EW, Henry SA. 2002. *Genetics* 162:29–43
145. Loewen CJ, Gaspar ML, Jesch SA, Delon C, Ktistakis NT, et al. 2004. *Science* 304:1644–47
145a. Brickner JH, Walter P. 2004. *PLoS Biol.* 2:e342
146. Horton JD, Goldstein JL, Brown MS. 2002. *J. Clin. Investig.* 109:1125–31
147. Zeng L, Lu M, Mori K, Luo S, Lee AS, et al. 2004. *EMBO J.* 23:950–58
148. Feng B, Yao PM, Li Y, Devlin CM, Zhang D, et al. 2003. *Nat. Cell Biol.* 5:781–92
149. Li Y, Ge M, Ciani L, Kuriakose G, Westover E, et al. 2004. *J. Biol. Chem.* 279:37030–39
150. Zhang P, McGrath B, Li S, Frank A, Zambito F, et al. 2002. *Mol. Cell. Biol.* 22:3864–74
151. Oyadomari S, Koizumi A, Takeda K, Gotoh T, Akira S, et al. 2002. *J. Clin. Investig.* 109:525–32
152. Wiest DL, Burkhardt JK, Hester S, Hortsch M, Meyer DI, Argon Y. 1990. *J. Cell Biol.* 110:1501–11
153. Reimold AM, Iwakoshi NN, Manis J, Vallabhajosyula P, Szomolanyi-Tsuda E, et al. 2001. *Nature* 412:300–7
154. Iwakoshi NN, Lee AH, Vallabhajosyula P, Otipoby KL, Rajewsky K, Glimcher LH. 2003. *Nat. Immunol.* 4:321–29
154a. Sriburi R, Jackowski S, Mori K, Brewer JW. 2004. *J. Cell Biol.* 167:35–41
154b. Shaffer AL, Shapiro-Shelef M, Iwakoshi NN, Lee AH, Qian SB, et al. 2004. *Immunity* 21:81–93
155. Lin KI, Lin Y, Calame K. 2000. *Mol. Cell. Biol.* 20:8684–95
156. Yu J, Angelin-Duclos C, Greenwood J, Liao J, Calame K. 2000. *Mol. Cell. Biol.* 20:2592–603
157. Rutkowski DT, Kaufman RJ. 2004. *Trends Cell Biol.* 14:20–28
158. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, et al. 2003. *Science* 300:135–39
159. Nakagawa T, Yuan J. 2000. *J. Cell Biol.* 150:887–94
160. Rao RV, Castro-Obregon S, Frankowski H, Schuler M, Stoka V, et al. 2002. *J. Biol. Chem.* 277:21836–42
161. Crompton M. 1999. *Biochem. J.* 341: 233–49
162. Boya P, Cohen I, Zamzami N, Vieira HL, Kroemer G. 2002. *Cell Death Differ.* 9:465–67
163. Ma Y, Brewer JW, Diehl JA, Hendershot LM. 2002. *J. Mol. Biol.* 318:1351–65
164. McCullough KD, Martindale JL, Klotz LO, Aw TY, Holbrook NJ. 2001. *Mol. Cell. Biol.* 21:1249–59
165. Yoneda T, Imaizumi K, Oono K, Yui

D, Gomi F, et al. 2001. *J. Biol. Chem.* 276:13935–40
166. Lamande SR, Chessler SD, Golub SB, Byers PH, Chan D, et al. 1995. *J. Biol. Chem.* 270:8642–49
167. Egan ME, Glockner-Pagel J, Ambrose C, Cahill PA, Pappoe L, et al. 2002. *Nat. Med.* 8:485–92
168. Schenkein D. 2002. *Clin. Lymphoma* 3: 49–55
169. Hurst HC. 1995. *Protein Profile* 2:101–68
170. Hai T, Wolfgang CD, Marsee DK, Allen AE, Sivaprasad U. 1999. *Gene Expr.* 7: 321–35
171. Hai T, Hartman MG. 2001. *Gene* 273:1–11
172. Zhang K, Wong HN, Song B, Miller CN, Scheuner D, Kaufman RJ. 2005. *J. Clin. Investig*. 115(2):268–81

NOTE ADDED IN PROOF

It was recently reported (172) that IRE1α is required for immunoglobulin heavy and light chain rearrangements in early B lymphocyte differentiation. However, IRE1α did not require either protein kinase or endoribonuclease catalytic activities for this signaling. These results suggest there is a novel form of IRE1α signaling from the ER to the nucleus.

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