Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls

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All eukaryotic cells have an extensive membranous labyrinth network of branching tubules and flattened sacs called the endoplasmic reticulum (ER). Approximately one-third of all cellular proteins are translocated into the lumen of the ER where post-translational modification, folding, and oligomerization occurs. The ER provides a unique oxidizing compartment for the folding of membrane and secretory proteins that are destined to the cell surface, as well as for proteins destined to other intracellular organelles, such as lysosomes and the Golgi compartment. Numerous cellular proteins reside within the ER through a mechanism that requires their continuous vesicle-mediated retrieval from post-ER compartments within the early secretory pathway. These ER-resident proteins are chaperones and catalysts of protein folding that form a matrix on which newly synthesized proteins attain their final conformation. The ER is also the site of synthesis of cellular lipids and sterols. In addition, the ER is the major signal-transducing organelle within the cell that continuously responds to environmental cues to release calcium. The ER is exquisitely sensitive to alterations in homeostasis, where, upon a variety of different stimuli, signals are transduced from the ER to the cytoplasm and the nucleus to eventually result in adaptation for survival or induction of apoptosis. The immediate response occurs at the translational apparatus, whereas changes in gene expression promote long-term adaptation or apoptotic cell death. Recent evidence supports findings that these signaling pathways influence pathogenesis associated with viral infection and genetic disease. The purpose of this review is to summarize what is presently known about the diversity of molecular signaling mechanisms that coordinate the complex ER stress response at the translational and transcriptional level in yeast and in higher eukaryotic cells.

The ER is a protein-folding compartment

The ER provides a unique oxidizing environment in which reside numerous protein chaperones facilitate and promote the productive folding of proteins and protein complexes despite the presence of high concentrations of protein that would otherwise interfere with productive folding reactions. These chaperones provide two important functions in protein folding (Gething and Sambrook 1992). First, there are proteins such as protein disulfide isomerase (PDI) and cis–trans prolyl isomerase that catalyze protein-folding reactions and increase the rate, without changing the pathway, at which proteins attain their final folded conformation. Second, there are protein chaperones that do not actively catalyze protein folding, but rather maintain proteins in a folding-competent state. These proteins prevent protein-folding intermediates from aggregating and stabilize energetically unfavorable conformations of polypeptides to minimize irreversible dead-end protein misfolding (Dill and Chan 1997). One of the best-characterized examples of this latter class is the immunoglobulin binding protein BiP. BiP was identified as an ER-localized protein that bound to heavy-chain immunoglobulins and inhibited their secretion in the absence of light chains in pre-B lymphocytes (Haas and Wahl 1983). In this manner, BiP prevents the secretion of incompletely assembled immunoglobulins. Independently, BiP was identified as a member of a protein family that is expressed at a high level in virally transformed cells and upon conditions of glucose deprivation (Lee 1987). Because their induced expression was a consequence of glucose deprivation, this protein family was named the glucose-regulated protein family, or GRPs. In the mid-1980s, it was shown that BiP is identical to GRP78 (Munro and Pelham 1986), the 78-kD family member of GRPs that has homology to the cytosolic Hsp70 stress protein.

As a protein-folding compartment the ER is exquisitely sensitive to alterations in homeostasis. Different stimuli signal through several protein kinases to up-regulate the protein-folding capacity of the ER. The GRPs proteins are expressed constitutively in all cells and their transcription is induced in response to a number of different stimuli that disrupt ER function. These stresses include calcium depletion from the ER lumen,
inhibition of asparagine (N)-linked glycosylation, reduction of disulfide bonds, expression of mutant proteins or protein subunits, or overexpression of some wild-type proteins, etc. These family members are now considered an ER stress-specific inducible family of proteins. When protein misfolding occurs and unfolded proteins accumulate and aggregate in the ER, there is a signal that selectively activates transcription of all the genes encoding GRPs as well as other ER-localized proteins such as PDI [Kozutsumi et al. 1988; Dorner et al. 1989]. This signal response was appropriately termed the unfolded protein response [UPR]. Protein products that are induced by the UPR are summarized in Table 1. Environmental stimuli that activate the UPR are summarized in Table 2.

The GRPs perform diverse roles to maintain ER function and facilitate protein folding. The numerous ER stress-inducible enzymes that catalyze isomerization reactions in protein folding include PDI and PDI-like activities and cis–trans prolyl isomerase [FKBPs]. The most well-characterized peptide-binding proteins that chaperone protein folding in the ER are BiP, designated KAR2 in *Saccharomyces cerevisiae*, and GRP94. BiP displays a weak ATPase activity that is stimulated by peptides that contain hydrophobic amino acids, such as Leu and Phe [Flynn et al. 1991; Blond-Elguindi et al. 1993a]. BiP has an ATPase domain in its amino terminus and a peptide-binding domain in its carboxyl terminus that have been modeled on the crystal structures of the heat-shock protein family members Hsc70 and DnaK, respectively (Flaherty et al. 1990; Zhu et al. 1996). BiP interacts transiently with exposed hydrophobic patches on protein-folding intermediates and is thought to prevent their aggregation while maintaining the protein in a folding-competent state. BiP interaction ensures that only properly folded and assembled proteins exit the ER compartment, a process known as ‘quality control’ [Hurtley and Helenius 1989]. Although yeast BiP is also required for protein translocation into the yeast ER lumen [Panzner et al. 1995], compelling evidence for a similar role for mammalian BiP does not exist. However, mammalian BiP does associate with the translocation apparatus and appears to maintain a permeability barrier before and soon after the initiation of polypeptide translocation into the ER [Hamman et al. 1998]. There are three gene encoding members of the DnaJ protein chaperone family, *SEC63*, *SCJ1*, and *JEM1*, that function to increase the rate of ATP hydrolysis by BiP in yeast. *SCJ1* and *JEM1* are induced by the UPR, and defects in these genes activate the UPR [Silberstein et al. 1998]. Members of the DnaJ family that are localized to the ER lumen in higher eukaryotic cells have not been identified.

Another polypeptide-binding protein that displays diverse functions is GRP94 or Grp96/endoplasmin, the ER paralog of Hsp90. GRP94 is also found in association with a number of protein-folding intermediates within the ER lumen and is likely to also display chaperone activity [Muresan and Arvan 1997]. The amino-terminal region of GRP94 is highly conserved with Hsp90, for which a three-dimensional structure is available (Prodomou et al. 1997). This conserved region serves as the peptide-binding site and displays ‘open’ and ‘closed’ con-

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**Table 1. Genes induced through the UPR**

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Mammals</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAR2</td>
<td>BiP/GRP78</td>
<td>HDEL (yeast)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KDEL (human)</td>
</tr>
<tr>
<td>LH51</td>
<td></td>
<td>HDEL</td>
</tr>
<tr>
<td>EUG1</td>
<td></td>
<td>KDEL</td>
</tr>
<tr>
<td>SCJ1</td>
<td></td>
<td>KDEL</td>
</tr>
<tr>
<td>JEM1</td>
<td></td>
<td>NFGL</td>
</tr>
<tr>
<td>ERO1</td>
<td></td>
<td>ERLL</td>
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<td>OPI3</td>
<td></td>
<td>IPKP</td>
</tr>
<tr>
<td>CHO1</td>
<td></td>
<td>KEEL</td>
</tr>
<tr>
<td>PD11</td>
<td>PDH</td>
<td>KDEL (yeast)</td>
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<tr>
<td></td>
<td></td>
<td>KEEL (human)</td>
</tr>
<tr>
<td>FKB2</td>
<td>FKB13</td>
<td>KSAA (yeast)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RTEL (human)</td>
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<tr>
<td>GT</td>
<td></td>
<td>KKNM</td>
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<tr>
<td></td>
<td></td>
<td>REEL (human)</td>
</tr>
<tr>
<td>GRP94</td>
<td></td>
<td>KDEL</td>
</tr>
<tr>
<td>GRP170</td>
<td></td>
<td>NDEL</td>
</tr>
<tr>
<td>ERP72</td>
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<tr>
<td>ERP59</td>
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<td>KDEL</td>
</tr>
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<td>ERP29</td>
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<td>KEEL</td>
</tr>
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<td>HSP47</td>
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<td>RDEL</td>
</tr>
<tr>
<td>calreticulin</td>
<td>KDEL</td>
<td></td>
</tr>
<tr>
<td>CHOP/GADD153</td>
<td>LHOQA</td>
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</tr>
</tbody>
</table>

The genes that are induced upon activation of the UPR are indicated. The ER-retrieval signals are indicated for the human species, except for GRP170, which is Chinese hamster. GT is present in *S. pombe* but absent in *S. cerevisiae*. 

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Table 2. Stimuli that activate the UPR, the EOR, and the phosphorylation of eIF-2α

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>UPR</th>
<th>EOR</th>
<th>eIF-2α-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Deoxyglucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tunicamycin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heavy metal ions</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Heavy chain immunoglobulin</td>
<td>+</td>
<td>+</td>
<td>N.D.</td>
</tr>
<tr>
<td>expression</td>
<td>+</td>
<td>+</td>
<td>N.D.</td>
</tr>
<tr>
<td>Brefeldin A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dithiothreitol</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Ca²⁺ ionophores</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Castanospermine</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Okadaic acid</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>tert-Butyldihydroperoxide</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>NF-κB/RelA/p65 overexpression</td>
<td>−</td>
<td>+</td>
<td>N.D.</td>
</tr>
<tr>
<td>Expression of influenza</td>
<td>−</td>
<td>+</td>
<td>N.D.</td>
</tr>
<tr>
<td>hemagglutinin</td>
<td>−</td>
<td>+</td>
<td>N.D.</td>
</tr>
<tr>
<td>Expression of coagulation</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>factor VIII</td>
<td></td>
<td></td>
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</tbody>
</table>

(+) Where the stimulus activates the response; (−) where the stimulus has no effect. (N.D.) Not determined.

Diversity in signaling from the ER lumen

In addition to glycoprotein folding and assembly, the ER is also the site for the synthesis of lipids and sterols. In *S. cerevisiae*, the UPR is also activated in response to lipid deprivation. Therefore, the UPR also coordinates the synthesis of lipids and new membrane structures as the demand arises. On sterol deprivation in the ER membrane, a signal is sent to induce transcription of sterol biosynthetic genes that contain a sterol-regulatory element (SRE) in their promoters [for recent review, see Brown and Goldstein 1997]. In this case, an ER membrane-bound transcription factor SREBP (SRE-binding protein) is released by sterol-regulated proteolysis and activates genes encoding products that are required for fatty acid and cholesterol biosynthesis. In addition to the UPR, the ER also generates a signal in response to excessive membrane protein traffic through that organelle. This frequently occurs on viral infection, when viral glycoproteins are produced in massive amounts. Under these conditions, the ER sends a signal to activate the transcription factor NF-κB to induce expression of immune response and pro-inflammatory genes, such as interferons and cytokines. This signaling pathway was termed the ER overload response (EOR; Pahl and Bauerle 1995). Evidence is accumulating that many diseases result from protein misfoldings in the ER that are due to mutations that disrupt protein folding or alterations in post-translational modifications [Thomas et al. 1995]. Under the wide variety of conditions that result in protein misfolding in the ER, the cellular response may be an important component of the pathophysiological process.

The coordinated synthesis of ER membrane and constituent proteins responds to environmental and physiological stimuli, as well as the differentiation state of the cellular response to ER stress.
Protein kinases that signal in response to unfolded protein in the ER

Over the last couple of years, significant progress was made in elucidating the signal transduction pathways that emanate from the ER. In many respects these pathways are conserved from yeast to humans and are designed to protect from environmental stresses. Two central mediators of the stress response in yeast are the protein kinases Gen2p and Ire1p. Gen2p is a dedicated eIF-2α kinase that is specifically activated on amino acid deprivation to induce the translation of a factor that is required for the transcription of genes encoding enzymes required for amino acid biosynthesis. Ire1p is the proximal sensor of the UPR in yeast that also has an endoribonuclease activity [Fig. 1]. In contrast, our understanding of the mammalian UPR remains an enigma where many of the hypotheses to be tested rely on paradigms identified in yeast. Recently, two homologs of yeast

The UPR in S. cerevisiae

Accumulation of unfolded protein in the ER of S. cerevisiae activates a signal transduction pathway that culminates in the transcriptional induction of genes encoding ER-localized protein-folding catalysts and protein chaperones to correct the protein-folding defect by increasing the folding capacity of that compartment [Fig. 2]. In addition, this response also activates transcription of genes involved in membrane biosynthesis. Conditions that overload the ER with unfolded and aggregated proteins include the expression of mutant proteins or unassembled protein subunits, overexpression of some wild-type proteins, or treatment with pharmacological agents that disrupt protein folding in the ER, such as tunicamycin to inhibit N-linked glycosylation or β-mercaptoethanol to inhibit disulfide bond formation. Experimentally, in S. cerevisiae as well as in mammalian cells, it also is possible to use conditional lethal temperature-sensitive mutants that have alterations in the ER glycosylation
Cellular response to ER stress

Figure 2. The UPR in S. cerevisiae. On accumulation of unfolded proteins in the ER, Ire1p kinase is activated, dimerizes, and trans-phosphorylates itself to elicit endoribonuclease activity. Ire1p then cleaves an intron from HAC1 mRNA and Rlg1p subsequently ligates the 5′ and 3′ ends together. This new form of HAC1 mRNA is efficiently translated to produce a protein with 18 new amino acids at the carboxyl terminus [green]. Hac1p then binds the UPRE and activate genes under UPRE control. The domains of Ire1p are as in Fig. 1. The red arrow indicates processes that occur when the UPR is off; the green arrows, processes that occur when the UPR is on.

and/or protein-folding machinery [i.e., tsSec53, which is phosphomannomutase (Kepes and Schekman 1988)]. However, in contrast to mammalian cells, depletion of calcium from the ER does not appear to activate the UPR in S. cerevisiae. Therefore, the mammalian UPR is more sophisticated in responding to a wider variety of stress signals with a wider diversity of downstream effects.

The induction of the ER-resident protein chaperones in response to the accumulation of unfolded protein is regulated at the level of transcription initiation. Analysis of the KAR2 [the S. cerevisiae homolog of mammalian BiP] promoter demonstrated that it was induced in response to both unfolded proteins in the ER and heat shock. These response elements are separable within the KAR2 promoter. A heat-shock element (HSE) resides upstream of a cis-acting 22-bp element, termed the unfolded protein response element [UPRE]. The UPRE, and not the HSE, was found upstream from all the genes that respond to the UPR in yeast (Kohno et al. 1993). The UPRE is necessary and sufficient to mediate the transcriptional induction, and mutagenesis studies confirmed the importance of this motif (Mori et al. 1992, 1998). A short consensus sequence within the UPRE is necessary and sufficient to mediate the transcriptional induction (Shamu and Walter 1995). Indeed, oligomerization and trans-autophosphorylation are required for activation of Ire1p in vitro and in vivo [Shamu and Walter 1996, Welihinda and Kaufman 1996].

Hac1p, a basic leucine zipper transcription factor that activates the yeast UPR

Genetic strategies were used by three groups independently to isolate Hac1p as the trans-acting factor that binds the UPRE (Cox and Walter 1996; Mori et al. 1996; Nikawa et al. 1996). HAC1 was originally isolated as a multicopy suppressor of a cdc10 mutant of Schizosaccharomyces pombe and was proposed to be a basic leucine zipper [bZIP] transcription factor with homology to the activating transcription factor [ATF]/cAMP-response element [CRE] binding protein [Nojima et al. 1994]. It is now known that Hac1p is a bZIP protein that binds the UPRE E-box motif upon ER stress (Cox and Walter 1996; Mori et al. 1996). Hac1p has a DNA-binding domain and leucine zipper in its amino terminus. Because bZIP transcription factors are known to bind DNA as homo- or heterodimers, it is likely that Hac1p binds the symmetrical UPRE as a homodimer. Indeed, the yeast two-hybrid system was used to demonstrate that Hac1p can dimerize [Nikawa et al. 1996]. The carboxyl terminus of Hac1p contains a PEST content [Pro, Glu, Ser, Thr rich] that might target the protein for ubiquitin conjugation and degradation by the 26S proteosome. Yeast carrying a deletion in HAC1 have the same phenotype as those lacking IRE1, being viable but sensitive to accumulation of
unfolded protein in the ER. Thus, IRE1 and HAC1 are in the same genetic pathway.

Activation of the UPR pathway is dependent on the cellular levels of Hac1p. The T1/2 of Hac1p is short, ~1–2 min in S. cerevisiae, and does not change on induction of the UPR [Chapman and Walter 1997; Kawahara et al. 1997]. However, in the absence of ER stress, Hac1p is not detected, although HAC1 mRNA is produced and is present on polysomes. Kawahara et al. (1997) and Cox et al. (1996) demonstrated that a 252-nucleotide 3′ untranslated region (3′ UTR) within HAC1 mRNA attenuates its own translation. On activation of the UPR, the 252-base intron is excised from the 3′ end of the mRNA. Removal of the intron sequence relieves a translation elongation block so that Hac1p is synthesized efficiently and accumulates [Chapman and Walter 1997; Kawahara et al. 1997]. Interestingly, Chapman and Walter (1997) demonstrated that the HAC1 252-base intron sequence attenuates not only its own translation but also the translation of a heterologous mRNA encoding green fluorescent protein (GFP) when placed behind the GFP-coding region within the 3′ UTR of the mRNA. It is not known how the attenuator inhibits translational elongation. Analysis of the intron indicated that it contains extensive secondary structure, possibly analogous to a tRNA, and this might prevent ribosome translocation along the mRNA and force ribosomes to pile up on the mRNA. Alternatively, the attenuator might feed back to directly inhibit elongation by the ribosome or may bind a trans-acting factor that inhibits elongation [Chapman et al. 1998].

The splicing reaction of HAC1 mRNA removes the last 10 amino acids at the carboxyl terminus of Hac1p and adds 18 amino acids to yield a 238-amino-acid protein. The 238-amino-acid Hac1p displayed a 12-fold greater transcriptional activation than the 230-amino-acid form of the protein [Kawahara et al. 1997]. The additional 18 residues inserted contain acidic amino acids and two potential serine phosphorylation sites that may be responsible for the greater transcriptional activation. Therefore, both the quantity and quality of Hac1p control activation from the UPRE.

Ire1p processes HAC1 mRNA by an unprecedented mechanism

On accumulation of unfolded proteins in the ER, the 252-base translational attenuator in HAC1 mRNA is removed. Sequence analysis of the 252-base intron sequence demonstrated that it did not have characteristics of pre-mRNA substrates. It did not contain the consensus 5′ and 3′ splice site junctions that form base pairs with snRNAs in the spliceosome that are found in pre-cursor mRNAs from S. cerevisiae and higher eukaryotes. In addition, it did not require the conventional pre-cursor mRNA processing machinery. Mutations in two spliceosome components [prp21as and prp8as] did not disrupt splicing of HAC1 mRNA [Cox and Walter 1996]. tRNA ligase was required in the final step of the HAC1 splicing reaction where the 5′ and 3′ exons are joined [Sidrauski et al. 1996]. Finally, mutation of the 5′ splice site prevented its cleavage but did not inhibit cleavage at the 3′ splice site [Cox and Walter 1996]. The spliceosome-mediated splicing reaction requires cleavage at the 5′ splice site for cleavage at the 3′ splice site junction. Thus, the characteristics of the splicing reaction were unprecedented.

The carboxy-terminal residues of yeast Ire1p have homology to the mammalian ribonuclease RNaseL. RNaseL is a soluble protein of the cytosol that contains 9 ankrin repeats at its amino terminus followed by a Ser/Thr kinase domain, with the ribonuclease catalytic site at the carboxy-terminal tail. RNaseL is a component of the interferon antiviral response [Player and Torrence 1998]. On viral infection, dsRNA is generated as an intermediate in viral replication cycles. dsRNA activates 2′–5′ oligoadenylate synthetase to generate 2′–5′ oligoadenylate polymers. The 2′–5′ oligo(A) polymers bind to the seventh and eighth ankrin repeats of RNaseL and induce oligomerization with subsequent activation of its nonspecific ribonuclease activity. Although RNaseL does not exhibit kinase activity, it does require the lysine that coordinates ATP in kinase subdomain 2 for functional RNase activity. Deletion studies support that the carboxy-terminal region of RNaseL contains the catalytic domain and the amino terminus may negatively regulate RNaseL activity in the absence of 2′–5′ oligoA [Dong and Silverman 1997].

Given the similarity in sequence and possible mechanism of activation between RNaseL and Ire1p, Sidrauski and Walter [1997] tested whether yeast Ire1p has an endonuclease activity. They demonstrated that yeast Ire1p is the endoribonuclease that cleaves HAC1 mRNA at the 5′ and 3′ splice sites within the 3′ end of HAC1 mRNA in vitro. The cleaved HAC1 exons are subsequently ligated by the tRNA ligase, Rlg1p [Sidrauski et al. 1996; Sidrauski and Walter 1997]. Sidrauski and Walter [1997] successfully reconstituted the IRE1 mRNA splicing reaction in vitro with only two components, Ire1p and Rlg1p. Yeast carrying a mutation in RLG1 [rlg1-100, a His-to-Tyr mutation at residue 148] are able to cleave HAC1 mRNA but are defective in the ligation step. Interestingly, this missense mutation only affects HAC1 mRNA ligation, whereas its ligase activity for tRNA splicing is not affected, supporting that different protein–RNA contacts are required for the different reactions [Sidrauski and Walter 1997].

Structural predictions of the HAC1 mRNA intron and flanking sequence identified that the 5′ and 3′ cleavage site both have stem structures with seven-membered loops. Indeed, synthetic oligonucleotides comprising only the stem–loop structures act as substrates for Ire1p-mediated cleavage in vitro [T.N. Gonzalez, C. Sidrauski, S. Doerfler, and P. Walter, in prep.]. Mutagenesis studies demonstrated that the cleavage was sequence specific and either cleavage could occur independently of the other [Kawahara et al. 1998]. It was possible to swap the 5′ and 3′ splice site junctions and retain cleavage specificity, although the resultant exons were not ligated by Rlg1p [Kawahara et al. 1998]. It was proposed that the 5′
and 3’ splice sites are juxtaposed in the final folded pre-HAC1 mRNA and that each subunit of a Ire1p homodimer cleaves at one of the sites [Chapman et al. 1998]. Given the simplicity of the splicing reaction and the minimal RNA sequence requirements to mediate splicing, it was surprising that when the HAC1 intron was placed within the 3’ end of GFP mRNA, it was not excised upon accumulation of unfolded proteins in the ER [Chapman and Walter 1997]. Therefore, this substrate was in a conformation that could not be recognized by Ire1p or did not have access to activated Ire1p. This observation suggests that there is more complexity to the reaction and that other factor(s) may be required to position the RNA substrate or otherwise facilitate the Ire1p-mediated splicing reaction in vivo.

Additional modifiers of the UPR: SAGA and PTC2

The yeast two-hybrid system was used to identify downstream effectors of Ire1p [Welihinda et al. 1997]. In this screen, GCN5 was identified as a specific interactor with the cytosolic domain of Ire1p. In eukaryotes, activation of transcription requires functional interaction between basal factors that occupy the TATA box and the activators that bind the upstream activating sequences. It is thought that this functional interaction is mediated by the transcriptional coactivators. Gcn5p [Ada4p] is a component of a multimeric transcription coactivator complex SAGA that is composed of Ada1p, Ada2p, Ada3p, Ada5p/Spt20p, Spt3, and Spt7 [Georgakopoulos and Thireos 1992; Marcus et al. 1994; Horiiuchi et al. 1995; Marcus et al. 1996, Grant et al. 1997]. Gcn5p is an acetyltransferase that facilitates transcription by acetylating histone to relax chromatin structures [Brownell et al. 1996]. Ada5p and Ada2p are known to functionally and/or physically interact with transcriptional activators containing acidic amino acid-rich patches and with the TATA-binding protein (TBP). Yeast strains lacking Gcn5p, Ada2p, or Ada3p were partially defective in activating transcription from both a lacZ reporter construct under control of the UPR and the endogenous KAR2 gene [Welihinda et al. 1997]. In contrast, strains carrying deletions in Ada5p were completely defective for the UPR, indicating a strict requirement for Ada5p in this response. However, heat shock-mediated induction of KAR2, from the HSE upstream of the UPR, was intact in all of these deletion strains [Welihinda et al. 1997]. These results demonstrate that there was not a general defect in transcriptional induction in the ada5 strain. Therefore, the Gcn5p/Ada complex is specifically required for the UPR but not for the heat-shock response at the same promoter. The Gcn5p/Ada2p/Ada3p/Ada5p complex may form a bridge between TBP and Hac1p bound to the UPR, ~100 bp upstream from the promoter.

The ire1, hac1, and ada5 null, and the rlg1-100 mutant strains shared common phenotypes being defective in the UPR and requiring inositol for growth. Preliminary in vitro studies suggest that Ire1p directly interacts with Ada5p and that Gcn5p directly interacts with Hac1p (A.A. Welihinda and R.J. Kaufman, unpubl.). Therefore, the original interaction detected between Gcn5p and Ire1p in the yeast two-hybrid system was probably indirect and mediated by endogenous Ada5p. The interaction of Ada5p with Ire1p is particularly interesting because strains deleted in either one have the same phenotype, being sensitive to tunicamycin treatment and inositol auxotrophy. It was surprising that ada5 deletion cells were defective in HAC1 mRNA processing in response to unfolded proteins in the ER [A.A. Welihinda and R.J. Kaufman, unpubl.]. This suggests a novel role for this transcriptional coactivator in HAC1 mRNA processing.

The first negative regulator of the UPR, Ptc2p, was also identified using the cytosolic domain of Ire1p as a bait in the yeast two-hybrid system [Welihinda et al. 1998]. The Ser/Thr phosphatase Ptc2p is a type 2C phosphatase that specifically binds Ire1p. Because there are no known inhibitors for this class of monomeric Mn2+- or Mg2+-dependent phosphatases, very little is known about their function. They appear to downregulate different stress responses, such as the PBS2/HOG1-MAP kinase pathway that is activated in response to osmotic and heat shock [Tsuiki et al. 1988; Maeda et al. 1993, 1994]. Ptc2p directly interacts with and dephosphorylates the phosphorylated form of Ire1p. In contrast, Ptc2p does not interact with unphosphorylated Ire1p, providing a mechanism to dissociate the phosphatase from the kinase once dephosphorylation occurs. Deletion of PTC2 elicited a three- to fourfold increase in UPR activity and augmented splicing of HAC1 mRNA, whereas overexpression of Ptc2p downregulated the UPR and attenuated HAC1 mRNA splicing [Welihinda et al. 1998]. Therefore, PTC2 negatively regulates the UPR. The activities of the phosphoprotein serine/threonine phosphatase (PPP) family are controlled by a regulatory subunit that localizes the phosphatase to its intracellular site of action [Barford 1996]. Because the type 2C phosphatases are monomers, they are not regulated by this mechanism. Ptc2p has a potential myristic acid addition site at its amino termini and also contains an Ire1p interaction domain that may regulate its membrane binding and localization within the cell [Welihinda et al. 1998]. Ire1p is the first substrate identified for the type 2C Ser/Thr phosphatases and confirms the role of the PTC2 family of phosphatases in stress responses.

Hac1p regulates membrane biogenesis

In yeast, membrane synthesis is coordinated with the synthesis of ER-resident proteins. Inositol-containing phospholipids are a major constituent of membranes in yeast. Phospholipid synthesis is regulated by the intracellular level of free inositol, where a decrease leads to the transcriptional induction of genes encoding enzymes required for phospholipid biosynthesis. Cox et al. [1997] demonstrated that the UPR is responsible for the coordinated synthesis of membranes and ER stress-induced proteins. A decrease in intracellular free inositol activates Ire1p to induce transcription of inositol biosynthetic genes that include inositol-1-phosphate synthase.
(INO1), phospholipid methyltransferase (OPI13), and phosphatidylserine synthase [CHO1]. On inositol starvation, either ire1 or hac1, as well as tgl1-100, mutant cells induce much lower levels of INO1 mRNA compared to wild-type cells and, more significantly, do not maintain INO1 mRNA synthesis, providing the basis for the inositol requirement for ire1, hac1, and tgl1-100 mutant strains. This may also provide the basis for the inositol requirement for ada5 deletion cells. In addition, activation of the UPR also induces INO1 transcription in an IRE1- and HAC1-dependent manner. Therefore, the UPR permits the coordinated expansion in both ER volume and protein content during ER stress.

Inositol regulation of membrane synthesis is mediated through a conserved 10-bp element named the UASINO that is necessary and sufficient for inositol-mediated induction of enzymes required for phospholipid biosynthesis. Expression is regulated through a transcriptional repressor, Opi1p [White et al. 1991], another member of the bZIP transcription factor family. opi1 mutant cells constitutively express the enzymes in lipid biosynthesis. Two transcriptional inducers for inositol biosynthetic genes are the bZIP factors, Ino2p and Ino4p, which bind the UASINO as a heterodimer [Hirsch and Henry 1986]. Cells that are double mutant in the UPR and opi1 constitutively express UASINO-regulated genes, but not UPRE-controlled genes (Cox et al. 1997). These results indicate that IRE1 and HAC1 are upstream of OPI1 and that the UPR and INO responses diverge downstream of HAC1. Based on these observations, Cox et al. (1997) proposed that the induced form of Hac1p (238AA) heterodimerizes with Opi1p to titrate out its inhibitory activity. However, to date there are no direct data that show Hac1p and Opi1p interact.

The mammalian UPR

Although the signaling mechanisms for the UPR in yeast are well characterized, the mechanisms for signaling the UPR in mammalian cells remain elusive. Based on the similarity of the signaling responses, both being induced by inhibition of N-linked glycosylation or reduction of disulfide bonds in the ER lumen, and both culminating in the transcriptional induction of ER chaperones, it was speculated that a signal transduction mechanism based on an Ire1p-like molecule should exist in higher eukaryotes. Indeed, within the last year two IRE1 homologs, IRE1α and IRE1β, were identified in both human and murine genomes. The cDNAs encoding human hIre1α and murine mIre1β were characterized to show they have characteristics expected for a mammalian IRE1 homolog [Tirasophon et al. 1998; Wang et al. 1998a]. The murine and human genetic loci have been termed ern1/ern2 and ERN1/ERN2, respectively. In contrast, a mammalian homolog of yeast HAC1 has not been identified. Thus, at present there appear to be a number of features conserved between S. cerevisiae and mammalian species. However, signaling the UPR in mammalian cells is more diverse than in yeast because additional pathways are activated that produce both protective as well as apoptotic responses.

hIre1αp is a 110-kD protein that, like its yeast counterpart, is constitutively expressed, but at a very low level. Although the mRNA can be detected in many tissues, it was not possible to detect the endogenous protein [Tirasophon et al. 1998]. Interestingly, the mRNA for IRE1α appears to be autoregulated by a process that requires the kinase and endoribonuclease activities of hIre1αp. Missense mutations within the coding region that disrupt either kinase or endoribonuclease activity increase the level of IRE1α mRNA by 10-fold [Tirasophon et al. 1998], leading to the hypothesis that the endonuclease activity of Ire1αp degrades its own mRNA. In contrast, overexpression of functional hIre1αp did not appear to autoregulate [Wang et al. 1998a].

hIre1αp contains high-mannose core oligosaccharides, a hallmark of ER-resident glycoproteins. Immunofluorescence studies revealed that hIre1αp and mIre1βp colocalize with the ER-resident protein chaperones. However, immunofluorescent staining of overexpressed hIre1αp suggested a preferential accumulation of protein around the nucleus. In addition, hIre1αp was shown to colocalize with RanGAP1p, a component of the nuclear pore complex [Tirasophon et al. 1998], suggesting that hIre1p is an ER membrane protein preferentially localized to the nuclear envelope, and possibly the nuclear pore complex. tRNA ligase was previously localized to the yeast nuclear pore complex [Clark and Abelson 1987]. The localization of these two mediators of the HAC1 mRNA splicing reaction near the nuclear pore complex suggests that splicing occurs on export of HAC1 mRNA from the nucleus. Consistent with this hypothesis, Walter and colleagues demonstrated that cytosolic unspliced HAC1 mRNA is not spliced upon induction of the UPR [Chapman et al. 1998].

Overexpression of either wild-type hIre1αp or mIre1βp constitutively activated the UPR, whereas the overexpression of a kinase-defective hIre1αp mutant downregulated the UPR [Tirasophon et al. 1998; Wang et al. 1998a]. In addition, overexpression of wild-type mIre1β induced apoptosis in the transfected cells [Wang et al. 1998a]. Taken together, these data demonstrate that the hIre1p family is required to activate the UPR pathway in mammalian cells and that mIre1βp overexpression and/or activation is sufficient to induce an apoptotic response.

A mammalian RNA substrate for hIre1p is not presently known. However, it was possible to test the nuclease activity of hIre1αp in vitro using the yeast HAC1 mRNA as a substrate. Surprisingly, the human protein was able to cleave the 5′ splice site of HAC1 mRNA at the identical site as the yeast Ire1p [Tirasophon et al. 1998]. The kinase activity of hIre1p was required for the in vitro HAC1 mRNA cleavage, supporting the theory that oligomerization and trans-autophosphorylation precede and are required for endoribonuclease activation. However, previous studies on the yeast Ire1p demonstrated that optimal endoribonuclease activity required an adenine nucleotide, and either AMP-PNP or ADP
stimulated the reaction as well [Sidrauski and Walter 1997]. It is possible that the yeast Ire1p protein produced in *Escherichia coli* was already autophosphorylated and activated upon expression. Therefore, it remains unknown whether adenine nucleotide binding and kinase activity are required to elicit the endoribonuclease activity. It is also possible that the yeast and mammalian Ire1 proteins differ in their mechanism of activation.

Although hIre1p cleaved the 5′ splice site of *HAH*1 mRNA, it did not cleave the 3′ splice site [Tirasophon et al. 1998]. This indicates an amazing conservation of substrate sequence specificity with also substrate sequence divergence between yeast and human Ire1p. Although endoribonuclease activity was not characterized for Ire1p, it was suggested that Ire1p may cleave the 3′ splice site of yeast *HAC1* mRNA. It was proposed that hIre1p may interact with Ire1p to create a heterodimer capable of cleaving both the 5′ and 3′ splice sites within a putative mammalian *HAC1* mRNA substrate [Tirasophon et al. 1998]. Although speculative, it is interesting to note that the archaean tRNA endonuclease is a tetramer in which the two homologous catalytic subunits are specific for either the 5′ or 3′ splice site of precursor tRNA molecules [Trotta et al. 1997].

The remarkable conservation of the protein kinase and the RNaseL domains of Ire1p between species suggests that both kinase and endoribonuclease activities of hIre1p are critical for transmitting the UPR signal from the ER to the nucleus. The functional requirement for endoribonuclease activity was tested by expression of a hIre1p mutant defective in endoribonuclease activity but with intact kinase activity. Overexpression of this hIre1p mutant did not activate the BiP promoter, but rather inhibited induction of the BiP promoter by tunicamycin treatment, when overexpressed in mammalian cells [W. Tirasophon, A.A. Welihinda, and R.J. Kaufman, unpubl.]. Therefore, the endoribonuclease activity of hIre1p appears to be required for signaling. These observations support the idea that a similar HAC1 mRNA-like processing reaction mediated by the Ire1p endonuclease may exist in higher eukaryotes as identified in *S. cerevisiae*. However, it is interesting that on expression of either unspliced or spliced yeast HAC1 mRNA at high level in COS-1 mammalian cells, these mRNAs were translated equally well [D.M. Foti, A.A. Welihinda, R.J. Kaufman, and A.S. Lee, in prep.], indicating that the translational attenuator in the yeast HAC1 intron cannot act in mammalian cells when expressed at a high level. Although differences in growth temperatures between yeast and mammals (30°C vs. 37°C) may affect the structure of HAC1 mRNA, these results suggest that the mechanism for HAC1 mRNA translational attenuation in mammalian cells may not be identical to that in yeast.

**The mammalian UPRE**

The *cis*-acting promoter element for the mammalian UPR appears significantly more complex than that of *S. cerevisiae*. Studies by Lee and coworkers demonstrated that multiple redundant elements are required. Two critical *cis*-acting elements identified, CORE and C1, were found to bind the zinc-finger transcription factors YY1 [Li et al. 1997a,b] and the histone-fold motif containing CCAAT-binding transcription factor CBF/NF-Y [Roy and Lee 1995; Roy et al. 1996], respectively. Overexpression of YY1 had little effect on basal expression from the BiP promoter but enhanced induction by ER stress [Li et al. 1997b]. However, the binding activity of neither CBF/NF-T nor YY1 changed upon ER stress, suggesting that DNA-binding activity of these factors is not regulated by ER stress. Because transcriptional induction is independent of intracellular calcium influx, it was proposed that UPR induction is mediated by calcium release from the ER [Roy and Lee 1995]. More recently, Yoshida et al. [1998] and Roy et al. [1999] noticed that these two elements share a common 19-nucleotide sequence motif [CCAAT(N)9CCAG] with a GC-rich 9-bp spacer. This finding supports the finding that BiP induction may be mediated by a common mechanism at each element [Yoshida et al. 1998; Roy and Lee 1999]. This element was termed the ER stress-response element (ERSE) and evidence supports that it is responsible for the transcriptional induction of BiP, GRP94, and calreticulin in response to ER stress [Yoshida et al. 1998]. A GCC triplet within the 9-bp spacer was also demonstrated to be required for stress inducibility [Roy and Lee 1999]. ERSE1 and ERSE2 in the ERP72 promoter are located within a 82-nucleotide ER protein traffic response element (ERTRE) identified by Green and coworkers, which is necessary and sufficient for ERP72 induction in response to ER stress [Srinivasan et al. 1993]. The ERSE can confer an orientation-independent stress inducibility to a heterologous promoter sequence. The existence of these *cis*-acting elements provides a mechanism for how the expression of only a limited set of genes is induced to promote protein folding in the ER and relieve ER stress.

Within the rat BiP promoter, ERSE3 [most distal to transcription start site, also called ERSE-163] and ERSE1 [most proximal to transcription start site, also called ERSE-98] are located within the CORE and C1 elements, respectively. In vivo footprinting within the rat BiP promoter detected ER stress-induced factor occupancy changes within ERSE3 [Li et al. 1994]. ERSE3 [GGCCAGCTTG], and neither ERSE1 nor ERSE2, is very similar to the yeast UPRE [GGACGCGTGG]. Indeed, overexpression of yeast Hac1p, either the 230-amino acid form or the 238-amino acid form, was able to transactivate the rat BiP promoter [D.M. Foti, A.A. Welihinda, R.J. Kaufman, and A.S. Lee, in prep.]. It was proposed that activation of the rat BiP promoter by YY1 required a mammalian homolog of yeast Hac1p to bind ERSE3. Therefore, the stress regulation would result from alterations in the cellular levels of Hac1p. A protein complex, termed ERSF, was recently described that binds ERSEs in a ER stress-inducible manner [Roy and Lee 1999]. The identification of the components of this complex should provide insight into the factors that regulate expression from the mammalian ERSE.

Using a yeast one hybrid screening approach, Yoshida et al. [1998] isolated a bZIP protein, ATF6. Although di-
rect binding of ATF6 to the ERSE was not demonstrated, overexpression of ATF6 constitutively induced the BiP promoter in an ERSE-dependent manner. In nonstressed cells, ATF6 was constitutively expressed as a 90-kD protein [Yoshida et al. 1998]. Interestingly, on ER stress the 90-kD protein was post-translationally processed to a 50-kD form that was proposed to be the transcriptionally active form of ATF6. However, there was no detectable change in the level or size of the ATF6 mRNA, suggesting that it is not a substrate for a mammalian Ire1p endoribonuclease-like activity. It was proposed that an mRNA encoding an ATF6-processing enzyme might be a substrate for mammalian Ire1p [Yoshida et al. 1998].

### Regulation of GRP induction

Transcriptional activation of ER-resident chaperones provides a protective effect in response to the accumulation of unfolded proteins in the ER. Preventing the induction of GRPs through transfection of BiP antisense RNA expression constructs, transfection of anti-BiP ribozymes, or transfection with multiple copies of the BiP promoter element to titrate out positively acting transcription factors increased sensitivity to ionophore, oxidative stress, hypoxia, and cell-mediated toxicity (Gomer et al. 1991; Li and Lee 1991; Li et al. 1992; Sugawara et al. 1993; Koong et al. 1994). In contrast, overexpression of BiP itself prevented transcriptional induction in response to ER stress and protected cells from death in response to calcium depletion from the ER [Dorner et al. 1992; Morris et al. 1997]. These studies suggest that BiP acts to alleviate the stress in the ER and/or to prevent generation of a death-inducing signal, presumably by preventing activation of Ire1p.

It is striking that all of the genes, except CHOP/GADD153 (GEBP homologous protein/growth arrest and DNA damage inducible), that are known to be induced by the UPR in mammalian cells encode ER-resident luminal proteins that are continuously retrieved from post-ER compartments by the Erd2p receptor (Lewis and Pelham 1990). Erd2p is a multiple-spanning transmembrane protein that is required for retrieval of proteins that escape the ER compartment. Presumably all proteins that are retrieved by the Erd2p receptor contain a tetrapeptide, termed KDEL, at their carboxyl terminus (Table 1). Surprisingly, in higher eukaryotes all of the UPR-induced genes encode proteins that contain a KDEL-like sequence. Therefore, there may be a functional association between the promoter elements for these genes and their carboxyl-terminal tetrapeptide retrieval signal. It is interesting to note that erd2 and ire1 are synthetic lethal in S. cerevisiae [Beh and Rose 1995].

The interpretation given to this result was that in the absence of ER retrieval, there is a requirement for induced ER chaperone gene expression. It may also be possible that the KDEL sequence is a negative regulatory ligand for Ire1p.

Transcription of the GRP genes is induced on ER stress, however, their transcription is also regulated by growth factor stimulation or withdrawal [Brewer et al. 1997]. During cytokine growth factor depletions, the expression of GRPs was reduced, however the UPR remained intact. The kinetics of activation of GRP gene transcription on growth factor addition was distinct from the UPR, suggesting that two independent signaling pathways exist for GRP induction [Brewer et al. 1997].

### The EOR

There are many similarities between yeast and mammalian cells in signaling ER chaperone induction in response to ER stress. However, signaling from the stressed ER in mammalian cells is considerably more diverse. ER stress signals generated in mammalian cells activates the EOR to induce proinflammatory and immune response gene expression. In addition, signaling the UPR in mammalian cells also activates a unique signal to induce cell death. Recent studies suggest that, on ER stress, there are two death-inducing signals that are generated. One signal is Ire1p-mediated transcriptional induction of CHOP/GADD153 and another signal is translational inhibition mediated through phosphorylation of the translation initiation factor eIF-2.

A number of ER stresses activate NF-κB to induce transcription of proinflammatory and immune response genes. However, signaling through NF-κB is distinct from signaling induction of GRPs. For example, overexpression of some wild-type proteins such influenza haemagglutinin induce NF-κB activation but have no effect on the UPR pathway [Liu et al. 1995] (Table 2). This signaling pathway was named EOR because it is activated by accumulation of membrane proteins in the ER. Studies support that overexpression of any transmembrane ER-localized protein will activate the EOR [Meyer et al. 1992; Pahl and Baueerle 1995, 1996; Pahl et al. 1996]. On overexpression of adenovirus E3/19K the protein accumulates in the ER and induces NF-κB. Mutation of the cytoplasmic dilysine ER-retrieval signal at the carboxyl terminus of adenovirus E3/19K permitted transport of the protein to the cell surface and the protein did not accumulate in the ER. This mutant E3/19K did not activate NF-κB, suggesting that ER retention was required [Pahl et al. 1996]. It was proposed that the induction of NF-κB in response to viral glycoprotein expression may represent a physiologically significant antiviral host response.

A number of stimuli that disrupt protein folding, such as 2-deoxyglucose, tunicamycin, calcium ionophores, etc., activate both the UPR and the EOR [Table 2]. However, because overexpression of some wild-type proteins or protein subunits also activate the UPR, the acronyms UPR and EOR are misnomers. These two pathways respond to different and overlapping types of ER stress and the common feature for their induction is not known. At least two signal-transducing molecules for the UPR are Ire1p and IreBP, whereas the signal transducing molecule for the EOR has yet to be identified. Identification of the putative ligand(s) within the ER that activate sig-

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**Table 1**: ER Stress-Induced Genes and Their Carboxy-Terminal Tetrapeptide Retrieval Signal

<table>
<thead>
<tr>
<th>Gene</th>
<th>Retrieval Signal</th>
<th>Stress Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>BiP</td>
<td>KDEL</td>
<td>ER Stress</td>
</tr>
<tr>
<td>CHOP/GADD153</td>
<td>KDEL</td>
<td>ER Stress</td>
</tr>
<tr>
<td>Erd2p</td>
<td>KDEL</td>
<td>ER Stress</td>
</tr>
<tr>
<td>Ire1p</td>
<td>KDEL</td>
<td>ER Stress</td>
</tr>
<tr>
<td>NF-κB</td>
<td>KDEL</td>
<td>ER Stress</td>
</tr>
</tbody>
</table>

**Table 2**: Comparison of UPR and EOR Signaling Pathways

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Induced Genes</th>
<th>Stress Conditions</th>
</tr>
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<tbody>
<tr>
<td>UPR</td>
<td>GRPs</td>
<td>ER Stress</td>
</tr>
<tr>
<td>EOR</td>
<td>NF-κB</td>
<td>ER Stress</td>
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</tbody>
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naling of the UPR and/or the EOR should aid in discriminating these signaling pathways.

It is possible to distinguish between the UPR and the EOR by pharmacological intervention. The phosphatase inhibitor okadaic acid potently induces NF-κB, whereas it has no significant effect on UPR induction. Okadaic acid does potentiate the UPR induction observed on depletion of calcium from the ER by thapsigargin treatment [Price et al. 1992]. Thapsigargin inhibits the ER-localized Ca$^{2+}$-dependent ATPase. The ER generates two second messengers in response to stress: calcium and reactive oxygen intermediates (ROIs). Most evidence supports that calcium release precedes ROI generation. Antioxidants inhibit NF-κB activation by all known inducers, including thapsigargin [Schreck et al. 1992a,b; Pahl and Baeuerle 1996]. Thapsigargin treatment induces a secondary rise in ROIs, which is inhibited by calcium chelators, suggesting that calcium release is required for ROI generation [Pahl and Baeuerle 1996]. Several mechanisms have been proposed to elicit calcium release and ROI generation in response to the EOR. Overexpression or alteration in membrane proteins may disrupt the ER membrane and permit calcium or glutathione (a weak oxidant) to leak out. Alternatively, membrane alterations may affect the activity of the Ca$^{2+}$–ATPase to block calcium uptake by the ER. Several oxidases localized to the ER membrane (cyclo-oxygenases, lipoxygenases, cytochrome P450, etc.) produce ROIs as a side product of their peroxidase activities. Peroxidase inhibition by treatment with tepoxalin [Tam et al. 1995] prevented NF-κB activation by thapsigargin but not by phorbol esters or TNFα, suggesting that these peroxidase activities are important for the calcium-dependent activation of NF-κB [Pahl and Baeuerle 1996].

NF-κB is a heterodimer present in the cytoplasm in an inactive complex with IκB, the inhibitor of NF-κB. There are at least five NF-κB DNA-binding subunits (RelA, RelB, c-Rel, p50, and p52), as well as five IκB proteins that control the activity of NF-κB dimers [IκB-α, IκB-β, IκB-γ, IκB-δ, and IκB-ε] [Baeuerle and Henkel 1994]. Exposure of cells to number of stress inducers, such as bacterial or viral infections, ultraviolet- or gamma-irradiation, or proinflammatory cytokines [such as tumor necrosis factor α (TNFα)], induces the phosphorylation at serine residues 32 and 36 at the amino terminus of IκB. Phosphorylated IκB is targeted for ubiquitination and degradation by the 26S proteosome, releasing the NF-κB heterodimer to translocate to the nucleus and activate target genes. Two kinases were identified, IKKα and IKKβ, that heterodimerize and together with NF-κB-inducing kinase [NIK] form a high molecular weight complex of 700–900 kD. These kinases form a cascade that is required for IκB phosphorylation and subsequent degradation [Mercurio et al. 1997; Regnier et al. 1997; Woronicz et al. 1997; Zandi et al. 1997]. However, it is not known how this kinase complex is activated in response to different stimuli. In addition, the importance of these IκB kinases in signaling the EOR has not been evaluated.

Deletion of the Rel family members in the murine germ line have demonstrated the physiological impor-

tance of NF-κB activation for the proinflammatory response [Baeuerle and Baltimore 1996]. For example, mice with c-Rel deletion do not mount effective responses to various pathogens, and immune cells are less responsive for cytokine production. A number of studies support this idea that NF-κB protects cells from apoptosis [Van Antwerpel et al. 1996; C.Y. Wang et al. 1996]. Deletion of the RelA (p65) subunit causes early embryonic lethality because of apoptosis in the liver. Mouse embryo fibroblasts derived from RelA knockout mice show enhanced apoptosis in response to TNFα [Beg and Baltimore 1996]. Overall, these studies support an antiapoptotic role for NF-κB activation in cellular responses.

ER stress-mediated induction of CHOP/GADD153 promotes apoptosis

As stated earlier, inhibition of N-linked glycosylation or disruption of ER calcium stores leads to programmed cell death, although the mechanism is poorly understood [Larsson et al. 1993; Perez-Sala and Mollinedo 1995]. In response to ER stress, the transcription factor CHOP/GADD153 is induced at the transcriptional level. CHOP was originally identified as a gene induced on DNA damage and growth arrest [Fornace et al. 1988]. However, subsequent studies have demonstrated a strong correlation between development of ER stress and induction of CHOP [Bartlett et al. 1992; Chen et al. 1992; Price and Calderwood 1992; Sunnerhagen et al. 1995; Carlberg et al. 1996; Brewer et al. 1997; Drici et al. 1997]. CHOP induction closely parallels the time course of BiP induction, where maximal induction occurs after several hours. The CHOP promoter appears to have less well-conserved ERSE sequences that may function in ER-stress transcriptional induction. CHOP is also regulated at the post-translational level by phosphorylation. On phosphorylation on serine residues 78 and 81 by p38 MAP kinase, the transcriptional activation by CHOP increases [Wang and Ron 1996].

Most evidence supports that CHOP negatively regulates cell growth and may induce apoptosis. Overexpression of CHOP can lead to cell cycle arrest and apoptosis [Barone et al. 1994; Zhan et al. 1994]. Disruption of the CHOP gene by a chromosomal translocation t(12:16)(q13;p11) is associated with human myxoid liposarcoma. This translocation results in a fusion between a glycine-rich novel protein and CHOP [Crozat et al. 1993; Rabbitts et al. 1993]. Deletion of the CHOP gene in the mouse leads to a modest reduction in cell death on activation of the UPR [Zinszner et al. 1998]. Overexpression of BiP prevents CHOP induction, suggesting that elevated BiP may attenuate cell death [X.Z. Wang et al. 1996]. However, it is not known whether overexpression of BiP relieves ER stress or interferes with UPR signaling [Morris et al. 1997]. Transfection studies with hIre1 demonstrated that these signaling molecules can activate CHOP transcription, although the physiological significance of this pathway in CHOP activation is not known. Mice with a homozygous deletion in the CHOP gene displayed reduced apoptosis in renal epithelium in
depends on mRNA abundance, the number of ribosomes, and the sequences within the 5′ upstream open reading frames (ORFs) or of internal ribosome entry sites (IRESs). The stress-inducible form of the gene is expressed from an internal promoter and encodes an intracellular form of what is normally a secreted protein. Studies in CHOP and C/EBPβ knockout mouse embryo fibroblasts demonstrate that stress-induced expression of CA-VI is both CHOP and C/EBPβ dependent. A CHOP-responsive element was mapped to the inducible CA-VI promoter, and in vitro footprinting demonstrated that CHOP-C/EBP heterodimers bind to that site. The identification of DOC genes in the ER stress response may identify novel components in the cell death pathway and link ER stress to apoptosis.

Translational control in response to ER stress

When cells are subjected to ER stress, in addition to transcriptional controls there are immediate changes in the mRNA translational capacity of the cell. Most translational control occurs at the level of initiation. The rate of initiation events on a specific mRNA is dependent on the following: (1) the sequence context of the initiation AUG codon (Kozak 1991a); (2) the presence of or absence or upstream open reading frames (ORFs) or of internal ribosomal entry sites (IRESs); (3) the presence of RNA secondary structure upstream of the initiation site; and (4) the sequences within the 5′ and 3′ untranslated regions of the mRNA that might bind translational repressors or activators (Kozak 1991b; Sonenberg 1994; Sachs et al. 1997). The general rate of translation initiation in a cell depends on mRNA abundance, the number of ribosomes, the availability of initiator tRNA Met, and the amount and activity of eukaryotic translation initiation factors (eIFs). Reversible covalent modification of these eIFs is a fundamental mechanism that determines the rate of initiation. The most frequently used mechanisms for translational control are the reversible phosphorylation of eIF-4E and eIF-2.

eIF-4E is a cap-binding protein that exists free in the cell and also in the eIF-4F complex composed of eIF-4A, eIF-4B, and the large p220 subunit eIF-4G. The activity of eIF-4E is controlled by a set of eIF-4E-binding proteins (a recent review, see Sonenberg and Gingras 1998). The cap binding protein complex eIF-4E promotes ATP-dependent unwinding at the 5′ end of the mRNA. ATP is used by the helicase eIF-4A to facilitate ribosome binding to the 5′ end of the mRNA. Phosphorylation of eIF-4E promotes its ability to bind the 5′-methylguanosine capped structure at the 5′ end of the mRNA and thereby stimulates translation initiation. Regulation of eIF-4E is physiologically important as eIF-4E is present in limiting amounts [0.01–0.2/ribosome] compared with other initiation factors [0.5–3.0/ribosome]. Cell stresses such as heat shock and poliovirus infection reduce eIF-4E phosphorylation. In contrast, mitogenic stimuli increase eIF-4E phosphorylation. Overexpression of eIF-4E transforms NIH-3T3 cells to form tumors in nude mice, supporting a transforming activity for eIF-4E (Lazaris-Karatzas et al. 1990; Lazaris-Karatzas and Sonenberg 1992). The growth-promoting property of eIF-4E is attributed to the higher eIF-4E requirement for mRNAs that have extensive secondary structure within their 5′ UTR. Frequently, mRNAs that have such secondary structure encode growth factors, growth factor receptors, proto-oncogenes, and other growth-promoting molecules. Some viral RNAs, such as picornaviruses, have evolved a cap-independent mechanism for translation initiation that permits their mRNA to outcompete cellular mRNAs for translation initiation (Gradi et al. 1998). These viruses have an IRES that promotes ribosome binding in the absence of a 5′-methylguanosine-capped structure. Some picornaviruses, such as poliovirus, cleave eIF-4G and inactivate the eIF-4F complex to rapidly prevent cap-dependent translation initiation. Interestingly, some cellular mRNAs also harbor IRES elements and are translated in a cap-independent manner (Rhoads and Lamphere 1995). BiP was identified as an mRNA in poliovirus-infected cells that, in contrast to most cellular mRNAs, continued to be efficiently translated in the presence of cleaved eIF-4G. It is now known that BiP mRNA has an IRES that may provide BiP mRNA the ability to compete for translation under ER stress conditions that limit initiation on other cellular mRNAs (Macejak and Sarnow 1991). However, to date studies have not demonstrated that ER stress alters eIF-4F function to elicit preferential BiP mRNA translation.

The second major translational control occurs through phosphorylation of eIF-2, a heterotrimeric protein that is required to bring the initiator tRNA Met to the 40S ribosome (for review, see Pain 1996). Polypeptide chain synthesis initiates when the ternary complex of eIF-2, GTP, and initiator tRNA Met binds to the 40S ribosomal subunit to generate a 43S species. Subsequently, mRNA binds and the 60S ribosomal subunit joins to form the 80S initiation complex with the concomitant hydrolysis of GTP to GDP. For eIF-2 to promote another round of initiation, GDP bound to eIF-2 must be exchanged for GTP in a reaction catalyzed by the guanine nucleotide exchange factor eIF-2B. Phosphorylation of the α subunit of eIF-2 on serine 51 stabilizes the eIF-2/GDP/eIF-2B complex and prevents the GDP–GTP exchange reaction. Because eIF-2B is required for the exchange of GDP for GTP and because the cellular levels of eIF-2B are approximately one-tenth the levels of eIF-2, the exchange process is inhibited when only a fraction [i.e., 20%–30%] of eIF-2α is phosphorylated and in an inactive complex with eIF-2B. Therefore, phosphorylation of eIF-2α immediately reduces the level of functional eIF-2 and limits response to tunicamycin injection (Zinszner et al. 1998). In addition, there was less subsequent tissue regeneration. These results suggest that CHOP may signal death in response to ER stress and that it may also play a role in cellular regeneration.

CHOP encodes a small nuclear protein that can heterodimerize with C/EBP family members and inhibit C/EBP function (Ron and Habener 1992). Additionally, the CHOP–C/EBP heterodimer can activate downstream target genes (DOCs, for downstream of CHOP). Several genes that respond to CHOP were identified although their significance remains unknown (Wang et al. 1998b; Sok et al. 1999). Murine DOC1 encodes a carbonic anhydrase VI (CA-VI). The stress-inducible form of the gene is expressed from an internal promoter and encodes an intracellular form of what is normally a secreted protein. Studies in CHOP and C/EBPβ knockout mouse embryo fibroblasts demonstrate that stress-induced expression of CA-VI is both CHOP and C/EBPβ dependent. A CHOP-responsive element was mapped to the inducible CA-VI promoter, and in vitro footprinting demonstrated that CHOP-C/EBP heterodimers bind to that site. The identification of DOC genes in the ER stress response may identify novel components in the cell death pathway and link ER stress to apoptosis.
initiation events on all cellular mRNAs within the cell. Control through reversible phosphorylation of eIF-2α provides the cell with an efficient and rapid means to respond to variety of different stimuli.

ER stress signaling through eIF-2α kinases

The status of eIF-2α phosphorylation is controlled by several Ser/Thr protein kinases that are primarily dedicated to recognize eIF-2α as a substrate. To date, four specific eIF-2α kinases have been identified in the mammalian genome that are [1] the hemin-regulated inhibitor (HRI) of protein synthesis that is expressed in reticulocytes and is proposed to coordinate globin polypeptide synthesis with hemin availability [Chen 1993], [2] the general control of amino acid biosynthesis kinase (GCN2) that is activated upon amino acid deprivation to paradoxically increase translation of GCN4 mRNA encoding a transcription factor for genes involved in amino acid biosynthesis [Hinnebusch 1996], [3] the PKR that protects cells on viral infection; and [4] the recently identified PERK/PEK [Fig. 3]. Original observations demonstrated that on treatment of cells with agents that disrupt ER function, protein synthesis is inhibited, and this correlates with increased eIF-2α phosphorylation [Prostko et al. 1992] (Table 2). Although all known eIF-2α kinases are activated by different cellular stress conditions, present evidence supports that PERK, and possibly PKR, respond to stress in the ER.

PERK/PEK was recently identified by two groups independently [Shi et al. 1998; Harding et al. 1999]. It is an eIF-2α kinase that is localized to the ER and has an intralumenal domain that shares limited homology with the luminal domains of mammalian Ire1α and Ire1β [Fig. 4]. This kinase was demonstrated to phosphorylate eIF-2α at serine 51 in vitro and in vivo [Harding et al. 1999]. On overexpression of PERK, the translation of both its own mRNA and that of a reporter gene was inhibited. This is consistent with PERK-induced phosphorylation of eIF-2α and inhibition of mRNA translation. Treatment of cells with tunicamycin or thapsigargin increased autophosphorylation of the overexpressed PERK [Harding et al. 1999]. In contrast, treatment with arsenite, heat shock, or UV irradiation did not increase autophosphorylation of PERK. These results support that PERK is an eIF-2α kinase that is selectively activated on ER stress to prevent further translation. It should be noted that overexpression of BiP also prevented the ionophore-induced inhibition in protein synthesis, suggesting that BiP overexpression can inhibit signaling through PERK [Morris et al. 1997].

PKR is the interferon-inducible, dsRNA-activated pro-

Figure 3. Signaling through eIF-2α kinases to inhibit protein translation. A variety of stresses activate specific eIF-2α kinases to elicit an immediate inhibition of translation initiation. Solid arrows indicate stress response pathways where data support that the kinase occurs. Broken arrows represent stresses that activate an eIF-2α kinase, although the specific kinase has not been studied. The kinase domains are as in Fig. 1.

Figure 4. Alignment of the luminal domains of Ire1p and PERK. Shown are the luminal domain DNA sequences of cloned PERK cDNAs or genes from human (H.s.) and mouse (M.m.), the putative Perk from C. elegans (C.e.) [Harding et al. 1999], human Ire1α, murine Ire1β, Ire1 from S. cerevisiae (S.c.), and the putative Ire1 sequences from C. elegans and S. pombe. [Black] Identical residues; [gray] homologous residues. Dashes represent gaps. Numbers on the left and right represent the position of the first and last amino acid in each lane. The alignment is based on a BLAST search of GenBank with the luminal domain of human Ire1α.
tein kinase that is ubiquitously expressed in all mammalian cells [Samuel et al. 1997]. PKR is the most studied of the eIF-2α kinases. It was first identified as a mediator of the antiproliferative and antiviral actions of type 1 interferons. Transcription of PKR is induced upon treatment with interferon α or β and the protein is synthesized in a latent form. PKR contains two conserved dsRNA binding motifs in its amino terminus with the eIF-2α kinase activity in its carboxyl terminus [St. Johnston et al. 1992]. A variety of dsRNAs or highly structured RNA molecules generated during viral infection activate PKR from its latent form. On activation, PKR displays two well-known activities: autophosphorylation and phosphorylation of its physiological substrate, the α subunit of eIF-2. As a consequence of PKR activation, viral replication and cell growth are inhibited and cells may undergo differentiation. Increasing evidence supports an additional role for PKR in transcriptional regulation. PKR is implicated in the transcriptional regulation of dsRNA-activated genes such as interferon β [Zinn et al. 1988] through activation of the interferon regulatory factor 1 [IRF-1] and NF-κB [Kumar et al. 1994, 1997].

PKR is associated with rough ER membrane, likely through binding to the 60S ribosomal subunit in mammalian cells [Jimenez-Garcia et al. 1993; Jeffrey et al. 1995; Wu et al. 1998]. This localization suggested that PKR might signal in response to ER stress. However, with the recent identification of PERK, there is a need to re-evaluate the data that implicate PKR in the ER stress response. Studies have detected activated PKR in cells that were treated with calcium ionophores or inhibitors of the calcium-dependent ATPase [Prostko et al. 1995]. Expression of a dominant-negative catalytically inactive mutant PKR partially inhibited eIF-2α phosphorylation and partially protected cells from translational inhibition in response to different stimuli including [1] calcium depletion from the ER mediated by ionomycin, [2] reduction of disulfide bonds mediated by dithiothreitol, and [3] induction of the heat-shock response mediated by sodium arsenite [Prostko et al. 1995; Srivastava et al. 1995]. However, it must be considered that interpretations of experiments using dominant-negative mutants are limited as it is not known whether the mutant kinase is affecting the activity of endogenous PKR or another eIF-2α-related kinase. The best data that support a role for PKR in the ER stress response were all obtained from ER stress induced by depletion of ER calcium stores, which may activate several stress signaling pathways. In contrast, there was no effect on ER stress induced translational inhibition in mouse embryo fibroblasts derived from PKR-deficient mice that were treated with either tunicamycin or thapsigargin [Harding et al. 1999; S. Srivastava and R.J. Kaufman, unpubl.]. Although the majority of the data support a direct role for PERK in ER stress, it is possible that multiple eIF-2α kinases may signal in response to separate as well as overlapping ER stresses. Alternatively, the involvement of PKR in ER stress-induced eIF-2α phosphorylation may be secondary to PERK activation.

**Stress-induced eIF-2α phosphorylation activates apoptosis**

Most studies concerning the role of eIF-2α phosphorylation in the cellular stress response have been conducted with PKR. Interest in PKR was aroused when the groups of Sonenberg and Hovanessian demonstrated that expression of a catalytically inactive mutant PKR can transform NIH-3T3 cells to form tumors in nude mice [Koromilas et al. 1992; Meurs et al. 1993; Barber et al. 1995]. This observation was ascribed to a trans-dominant inhibitory effect of the mutant enzyme on the endogenous wild-type PKR and implicated PKR as a tumor suppressor gene. In addition, overexpression of a non-phosphorylatable Ser51Ala mutant eIF-2α [Donze et al. 1995], as well as the PKR inhibitors p58Ipak and HIV TAR RNA-binding protein (TRBP) also displayed transforming activity [Barber et al. 1994; Benkirane et al. 1997]. It was also demonstrated that overexpression of wild-type PKR can inhibit protein synthesis and cell growth in yeast and in mammalian cells [Chong et al. 1992; Thomas and Samuel 1992]. A tumor suppressor activity for PKR is consistent with a rearrangement detected in the PKR gene in a lymphocytic leukemia [Abraham et al. 1998]. In addition, the 60S ribosomal protein L18 is a PKR inhibitor that is overexpressed in colorectal cancer tissue [Barnard et al. 1993; Kumar et al. 1999]. Subsequently, studies demonstrated that the tumor suppressor activity of PKR could be accounted for by its ability to induce apoptosis when overexpressed and/or activated in mammalian cells [Lee and Esteban 1994; Takizawa et al. 1996; Der et al. 1997]. Overexpression of Bcl-2 was able to protect from PKR-induced apoptosis, suggesting that Bcl-2 is downstream of PKR in the apoptotic cascade [Lee et al. 1997].

To elucidate the role of eIF-2α phosphorylation in PKR-induced apoptosis, studies were performed with a Ser-51–Ala mutant eIF-2α that is resistant to phosphorylation. Overexpression of the Ser-51–Ala mutant transformed NIH-3T3 cells to form tumors in nude mice, similar to observations with catalytically inactive trans-dominant-negative mutant PKR [Donze et al. 1995]. Expression of this Ser-51–Ala mutant eIF-2α also protected NIH-3T3 cells from serum-deprivation- and TNFα-induced apoptosis [Srivastava et al. 1998]. In addition, expression of a Ser-51–Asp mutant of eIF-2α was sufficient to inhibit protein synthesis and induce apoptosis in COS-1 cells [Srivastava et al. 1998]. Apoptosis in these cells correlated with activation of caspase 3. These studies demonstrate a role for eIF-2α phosphorylation in apoptosis under certain conditions. It will be important to determine if overexpression or activation of PERK is sufficient to induce apoptosis. In addition, future studies are required to elucidate how a reduction in protein synthesis initiation can induce an apoptotic cascade. Three possibilities include [1] the translational inhibition of an antiapoptotic gene product, such as an inhibitor of apoptosis [IAP, Deveraux and Reed 1999], [2] a paradoxical preferential translation of an mRNA encoding an effector of apoptosis, similar to translational control of...
GCN4, or (3) a direct feedback from the translational apparatus (i.e., accumulation of free 40S ribosomes) directly into the proapoptotic pathway.

Viruses have evolved many gene products that act through different mechanisms to inhibit apoptosis [Shen and Shenk 1995]. These mechanisms are vital for viruses to establish their latent states. These antideath mechanisms include modulation of Bcl-2, inactivation of the tumor suppressor p53, and caspase inhibition. PKR was originally identified as a cellular antagonist to viral infections. PKR-mediated inhibition of protein synthesis would directly prevent virus protein production. However, PKR activation also provides additional antiviral activity by limiting viral spread through promoting apoptosis and subsequent autodigestion of the cell. As a consequence, viruses evolved numerous mechanisms to prevent PKR activation leading to apoptosis [Katze 1995]. Because it is possible that PERK will be activated by excessive viral glycoprotein expression, it is likely that viruses have evolved mechanisms to prevent PERK activation. The pathogenesis for those viruses that do not have effective means for inactivating PKR and/or PERK may be attributed to viral-induced host-cell apoptosis. If this turns out to be true, then therapeutic intervention to prevent PERK and/or PKR activation may limit certain types of viral pathogenesis.

Potential ligands that activate the Ire1 and/or PERK

Original comparison of yeast and human Ire1 luminal domains did not detect any regions of significant homology. With the identification of PERK it is now possible to align the luminal domains from all the species where they have been characterized [Fig. 4]. This alignment significantly suggests that a couple of sequence motifs were very conserved throughout evolution. The homology displayed between the luminal domains of PERK, Ire1α, and Ire1β suggests they use a similar mechanism to transduce ER stress, and possibly share a similar ligand. Expression of either the isolated luminal domain of m Ire1β or of PERK alone inhibited transcriptional activation from the CHOP promoter on tunicamycin treatment [Harding et al. 1999]. This shows that either the Ire1β or the PERK luminal domain can interfere with ER signaling for CHOP induction and suggests that both luminal domains respond to a similar signal on inhibition of glycosylation. It will be interesting to know if PERK and Ire1α/β can trans-activate each other.

Whereas the signals emanating from Ire1p and the cellular responses generated by them are being illuminated, the molecular events occurring within the ER lumen that activate Ire1p remain largely unknown. It is possible that Ire1p and PERK bind to exposed hydrophobic patches on unfolded proteins that are in the process of aggregating, and therefore become oligomerized and activated. It was also proposed that BiP may negatively regulate Ire1p [Mori et al. 1993]. Ire1p may sense the level of free BiP in the ER so that in the absence of unfolded proteins, BiP would be available to bind Ire1p and prevent its oligomerization and subsequent activation.

On accumulation of unfolded proteins, BiP would bind unfolded polypeptides allowing Ire1p to oligomerize. BiP is present in the ER in monomeric and oligomeric states. BiP protein in oligomers is also modified by phosphorylation and ADP ribosylation [Freiden et al. 1992; Blond-Elguindi et al. 1993b]. On accumulation of unfolded protein in the ER, BiP oligomers dissociate and the modifications are removed. It is possible that the status of BiP oligomerization and/or modification may influence Ire1p and/or PERK activation. However, to date there has been no demonstration that BiP actually interacts with Ire1p. A number of experiments suggest that BiP negatively regulates the UPR. In both yeast [Kohno et al. 1993] and mammalian cells [Malmstrom 1982] overexpression of BiP down-regulates the UPR. Because the BiP ATPase activity was required to down-regulate the UPR [Morris et al. 1997], it can be assumed that cycles of BiP binding and release are required to prevent activation of the UPR. Moreover, reduction of BiP levels is sufficient to induce the UPR [Dorner et al. 1988; Beh and Rose 1995]. However, because the level of free BiP inversely correlates with the level of unfolded proteins, these experiments do not provide insight into whether an increase in unfolded protein or a decrease in free BiP elicits UPR activation.

It is intriguing that both Ire1α and PERK are most highly expressed in pancreatic tissue [Tirasophon et al. 1998; Shi et al. 1999]. The primary function of the pancreas is to respond to blood glucose levels in order to maintain glucose homeostasis through regulation of absorption, gluconeogenesis, and glucose utilization. Immunohistochemical analysis detected PERK expressed only in pancreatic islets of Langerhans, which represent only 1%–2% of the mass of the pancreas. PERK was most highly expressed in the 5 cells of the pancreatic islets, representing ~5% of the islet cells. The 5 cells secrete somatostatin that regulates release of glucagon and insulin from the α and β cells of the islets, respectively. The blood glucose level is the single most important factor that affects protein synthesis and secretion from islet cells. The high-level PERK expression in the 5 cells of the islet might suggest a unique role for PERK in protein synthesis, or perhaps metabolic processes. Alternatively, perhaps the high-level expression is important to rapidly signal changes in blood glucose. The glucose-stimulated synthesis of insulin occurs immediately at the level of translation [Goodison et al. 1992; Gilligan et al. 1996]. This indicates that if PERK is the primary eIF-2α kinase in this tissue, it should respond to blood glucose levels. The islet cells of the pancreas may also be expected to express high levels of a PERK inhibitor to prevent eIF-2α phosphorylation and subsequent apoptosis. It is intriguing that the genes that respond to activation of Ire1p are also regulated by glucose availability. These observations suggest the possibility that glucose, or a derivative of glucose, may directly activate or prevent activation of the Ire1 and PERK family of protein kinases. Future experiments should determine whether PERK and/or Ire1p is a glucose sensor for the pancreas.
Protein misfolding in human disease

It is now known that many genetic diseases are caused by mutations that result in protein misfolding and accumulation within the ER [Thomas et al. 1995] (Table 3). At present it is not known whether any pathology associated with defective protein folding may result from activation of the ER stress responses. However, as the signaling mechanisms that occur on ER stress are elucidated, it will be possible to utilize pharmacological intervention to determine the role of the ER stress response in the pathology of these diseases.

The most common form of cystic fibrosis is due to a mutation (Δ508) in the cystic fibrosis transmembrane conductance regulator (CFTR) protein that results in retention of the protein in the ER and eventual degradation by the cytosolic 26S proteasomal machinery [Lukacs et al. 1994, Ward et al. 1995]. Cystic fibrosis patients develop progressive, cytokine-mediated inflammatory lung disease that is the major cause of morbidity and mortality [McElvaney et al. 1992]. The production of inflammatory cytokines may be a consequence of activation of the EOR.

Homozygous PiZZ α1-antitrypsin [α1-AT] disease affects 1 in 1800 live births and is a well-characterized cause of emphysema/destructive lung disease. A subgroup of affected individuals also develop chronic liver disease and hepatocellular carcinoma. This is the most common genetic cause of liver disease in children. The Z allele of α1-AT produces an abnormally folded molecule because of a Glu–342–Lys mutation that is retained in the ER and eventually degraded by the cytosolic 26S proteosome [Qu et al. 1996]. To study the etiology for the susceptibility to liver disease, a complementation approach was used in which skin fibroblast cell lines from PiZZ individuals with liver disease (susceptible hosts) and from PiZZ individuals without liver disease (protected hosts) were transfected with the PiZZ mutant α1-AT gene. The results demonstrated a delay in the ER degradation of the mutant α1-AT only in the fibroblasts from susceptible hosts. It was proposed that the subset of PiZZ α1-AT-deficient individuals is more susceptible to liver disease because of a second inherited trait that affects degradation of the protein accumulated in the ER [Wu et al. 1994]. To study the course of the disease in humans, transgenic mice were obtained that expressed the mutant allele of α1-AT. These animals displayed neonatal hepatitis and accumulation of the mutant α1-AT protein in the ER of hepatocytes [Carlson et al. 1989]. These studies suggest that the pathology associated with liver disease results from activation of stress responses that occur as a consequence of mutant protein accumulation in the ER.

Congenital hypothyroid goiter with deficient thyroglobulin is due to mutations in the thyroglobulin gene that result in accumulation of thyroglobulin in the ER of thyroid cells [Medeiros-Neto et al. 1996]. Microscopic analysis of the thyroid cells identified microscopically visible condensation of the ER, very similar to that observed in mammalian cells that occurs on accumulation of protein in the ER with concomitant activation of the UPR [Dorner et al. 1989]. Associated with mutant thyroglobulin retention within the ER was the induction of ER molecular chaperones, consistent with activation of the UPR.

Alzheimer’s disease is a slow-onset neurodegenerative disease that is characterized by the deposition of ordered protein aggregates in the brain. The β-amyloid precursor protein (APP) is processed in the ER to yield two products Aβ40 and Aβ42. The former accumulates in the trans-Golgi, whereas the latter accumulates in the ER. However, it is not known whether these amyloid deposits are the cause or the effect of the neurodegeneration. The expression of several proinflammatory genes is known to be upregulated in late-stage Alzheimer’s disease, suggestive of activation of the EOR. Early onset Alzheimer’s disease is associated with mutations in one of the two presenillin genes. Cells carrying a mutation in the presenillin 1 gene were hypersensitive to induction of apoptosis by several stimuli [Guo et al. 1997]. These observations suggest that mutations in these genes may contribute to amyloid deposition and activation of the UPR.

Concluding remarks

The diversity of signaling responses from the lumen of the ER in mammalian cells are modeled in Figure 5. Under a variety of different stress conditions that alter protein folding and/or trafficking through the ER, as-yet-unknown signals are generated to activate two families of protein kinases. Activation of the eIF-2α kinase PERK, and possibly PKR, immediately reduces the rate of translation initiation to prevent further accumulation of unfolded protein in the ER. At the same time, an adaptive response is activated through Ire1α to induce transcription of genes encoding protective functions that include ER protein chaperones and enzymes required for mem-

Table 3. Diseases resulting from protein misfolding

<table>
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<tr>
<th>Disease</th>
<th>Protein</th>
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<tr>
<td>α1-AT deficiency</td>
<td>α1-antitrypsin</td>
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<tr>
<td>Cystic fibrosis</td>
<td>CFTR</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>presenilins 1 and 2</td>
</tr>
<tr>
<td>Congenital hyperthyroid goiter class 2</td>
<td>thyroglobulin</td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td>LDL receptor</td>
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<tr>
<td>Carbohydrate-deficient glycoprotein syndrome</td>
<td>multiple proteins</td>
</tr>
<tr>
<td>von Willebrand’s disease</td>
<td>von Willebrand factor</td>
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<tr>
<td>Hemophilia A</td>
<td>coagulation factor VIII</td>
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<tr>
<td>Hemophilia B</td>
<td>coagulation factor X</td>
</tr>
<tr>
<td>Osteogenesis imperfecta</td>
<td>type I procollagen</td>
</tr>
<tr>
<td>Scurvy</td>
<td>collagen</td>
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<tr>
<td>Marfan syndrome</td>
<td>fibrillin</td>
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<tr>
<td>Tay-Sachs disease</td>
<td>β-hexosaminidase</td>
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<tr>
<td>Retinitis pigmentosa</td>
<td>rhodopsin</td>
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<tr>
<td>Leprechaunism</td>
<td>insulin receptor</td>
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<tr>
<td>Charcot-Marie-tooth type 1A</td>
<td>peripheral myelin</td>
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<td></td>
<td>protein PMP22</td>
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brane biogenesis. Presumably under conditions of severe ER stress the cell would not be able to adapt and therefore signals are generated to induce apoptosis. Phosphorylation of eIF-2α initiates an apoptotic signal. In addition, Ire1-mediated induction of CHOP elicits transcriptional activation of downstream genes that may inhibit cells from cycling and also induce apoptosis. In addition, under certain conditions, a signal of unknown nature is generated that activates the transcription factor NF-κB and induces its degradation. Subsequently, the heterodimeric NF-κB translocates to the nucleus and activates transcription of proinflammatory, immune response, and ant apoptotic genes. The kinase domains are as in Fig. 1.

The homology between PERK and IRE1 links ER stress-mediated translational attenuation to the ER stress-mediated transcriptional induction in higher eukaryotes. The luminal domain acts as a common sensing transducer that is attached to different downstream effectors, that is, eIF-2α kinase or Ire1p kinase/endoribonuclease. The homologies suggest that evolution has selected for duplication and divergence of these two functions. Thereafter, a coordinated balance of responses is required to maintain cell survival under conditions of ER stress.

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Cellular response to ER stress

Finally, it is noteworthy to consider that regulation mediated by both GCN2 and IRE1 occurs at the translational level on GCN4 mRNA and HAC1 mRNA to modulate the level of translation of two different bZIP transcription factors that ultimately stimulate protein and membrane biosynthesis in yeast. Gen2p has a domain that binds uncharged histidyl tRNA and phosphorylates eIF-2α to increase translation of GCN4 mRNA by permitting read-through of upstream ORFs. In contrast, HAC1 mRNA has a translational attenuator within its 3′ UTR that has a structure similar to a tRNA and is removed by Ire1p endoribonuclease in a reaction that is not too dissimilar from tRNA splicing. For these responses, control at the level of translation initiation provides a rapid response to ER stress that is not dependent on transcriptional induction.

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