Signal Transduction from the Endoplasmic Reticulum (ER) to the Cell Nucleus

Literature: Chapter 20, 24, Pollard and Earnshaw

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The ER serves several important functions, e.g.:
- entry for secretory and membrane proteins into the secretory pathway
- lipid synthesis
- folding and glycosylation of secretory and membrane proteins
- cholesterol synthesis

Various conditions can interfere with ER function (ER stress):
glucose starvation
cholesterol starvation
viral infection

In reponse to ER stress, **three distinct signal tranduction pathways** can be activated:

1. **Unfolded protein response (UPR)**
2. **ER-overload response (EOR)**
3. **ER-nucleus signalling by SREBPs**

Cells respond to stress situations by a decrease in general protein production and synthesis of proteins that may help to alleviate the stress. This up-regulation occurs mainly at the level of transcription.

1. Unfolded protein response (UPR)

How is the UPR induced?

UPR is induced by a variety of cellular insults that result in the accumulation of proteins in the ER:

- Inhibition of disulphide bond formation by reducing agents
- Inhibition of glycosylation by drugs such as tunicamycin
- Expression of aberrant proteins that do not fold properly
- Blocking ER to Golgi transport (e.g. Brefeldin A)
- Inhibition of protein degradation by the proteasome
- Ca^{2+} depletion of the ER

Which genes are regulated by the UPR?

UPR controls the transcription of genes encoding proteins catalyzing folding, assembly and modification of proteins in the ER lumen. These proteins were first identified as polypeptides that were highly induced upon glucose starvation in yeast (which impairs protein glycosylation and hence causes misfolding in the ER) and called glucose-regulated proteins (GRPs). GPR78, for example, encodes BiP, an ER-lumenal, HSP70 family member.

Table 1 ER chaperones regulated by the UPR

Mammalian	Yeast	Function
BiP (GRP78)	KAR2	hsp70 homologue
PDI (Erp59)	PDI	protein disulfide isomerase
GRP170		hsp70-like
GRP94		hsp90-like
ERP72		PDI-like
GRP58		PDI-like
	FKBP12	prolyl-*cis*-*trans*-isomerase
	LHS1	BiP-like
	EUG1	PDI-like
	ERO1	oxidoreductase

Using a functional genomic approach it was shown in yeast that more than 380 genes are upregulated by UPR. These did not only include ER chaperones:

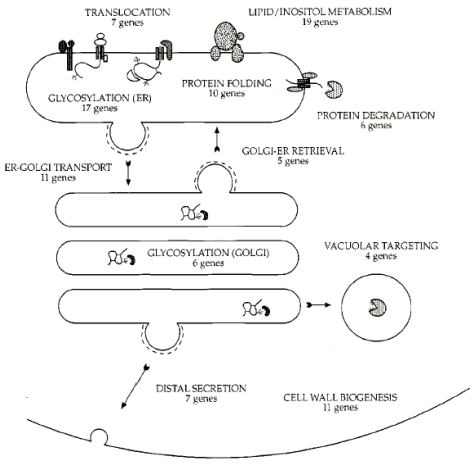


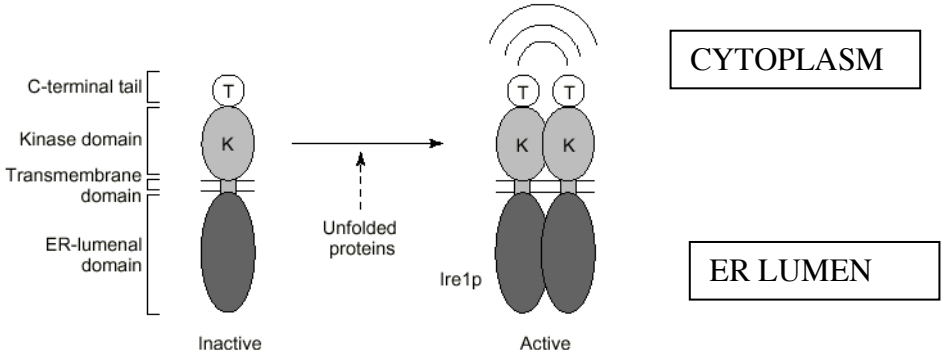
Figure 2. Many Aspects of Secretory Pathway Function Are Regulated by the UPR

A schematic diagram of the secretory pathway, with a classification of the genes identified as targets of the UPR. See Table 1 for details.

How does the presence of misfolded proteins in the ER activate transcription in the cell nucleus?

A. Sensing the accumulation of unfolded proteins in the ER lumen

The transmembrane kinase Ire1p is the key molecule in transmitting the signal from the ER lumen into a signal transduction cascade. In a similar fashion to growth factor receptors in the plasma membrane of higher eukaryotic cells, Ire1p is activated by phosphorylation and dimerisation.



Ire1p is a type I membrane protein of 1115 aa. The C terminus is located in the cytoplasm (or nucleus) and consists of Ser/Thr kinase domain and C-terminal tail, which resembles ribonucleases. The N-terminal domain is in the ER lumen and suggested to serve as a sensor to detect the accumulation of unfolded proteins.

Two models have been suggested:

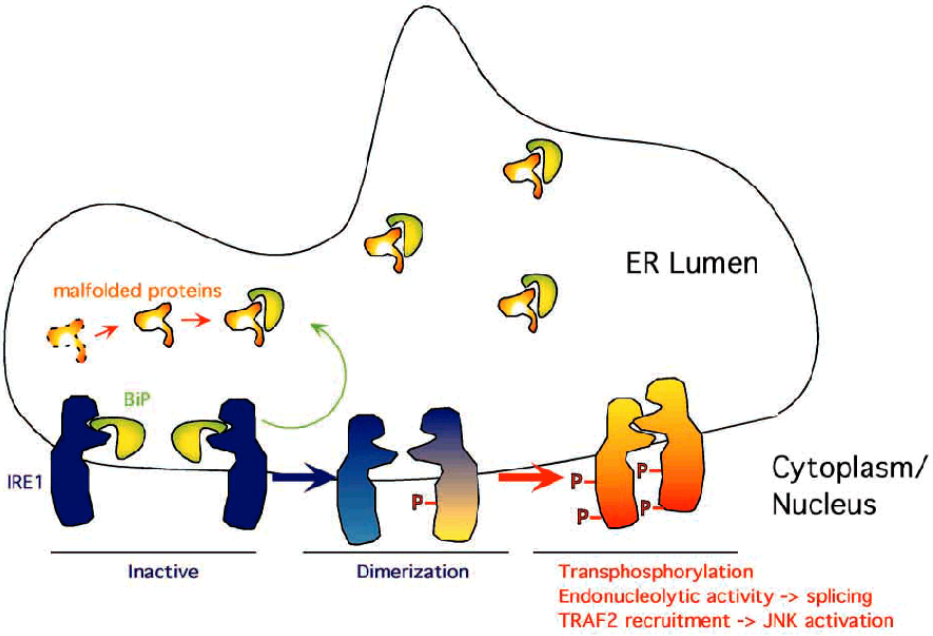


Fig. 2. Model for events important in IRE1 activation. BiP binding to the stress-sensing lumenal domain of IRE1 holds the protein in an inactive, monomeric, configuration. Accumulation of malfolded proteins in the ER lumen is associated with release of BiP from the lumenal domain and IRE1 dimerization, transautophosphorylation and activation of effector functions. These include, endonucleolytic activity that

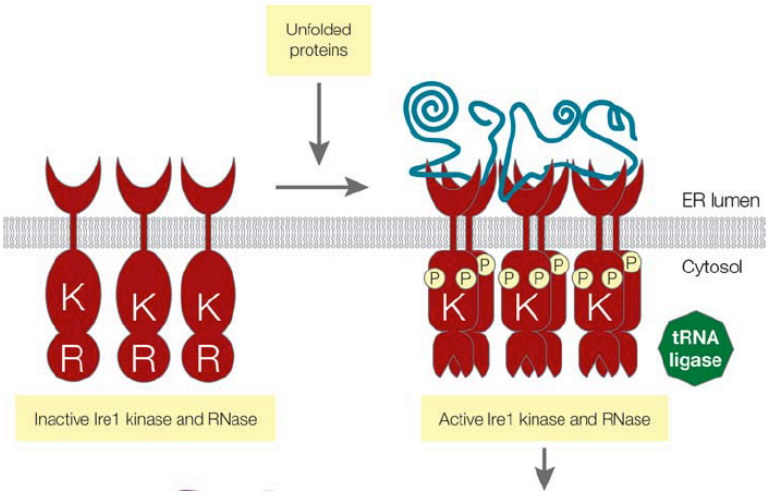


Figure 2 Mechanism of Ire1-mediated mRNA splicing in yeast. Unfolded proteins are recognized by the ER-lumenal domain of Ire1, leading to clustering of this stress sensor in the ER membranes. The Ire1 cytosolic domains become juxtaposed, in turn promoting transautophosphorylation by the kinase domain (K) and concomitant activation of the endoribonuclease domain (R). (Bernales et al., 2006)

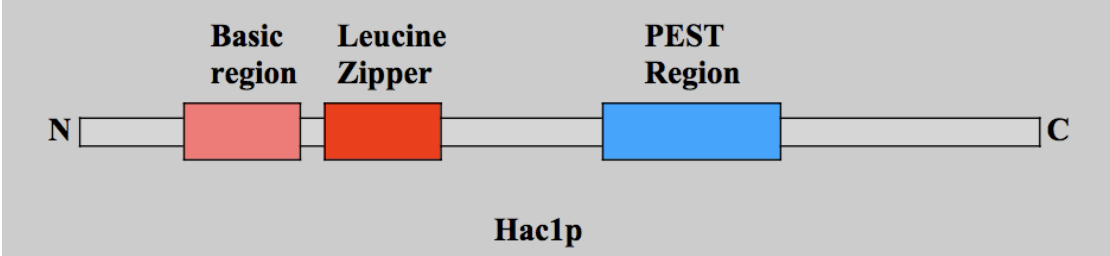
Recent evidence indicates that this model appears to be correct. For discussion see Gardner et al. 2013).

B. Transcriptional regulation of UPR-induced genes (UPRE and Hac1p)

The coordinate transcriptional induction of GRPs in mammalian cells suggested that this might be regulated by a common transcription factor. Deletion analysis of the yeast KAR2 (BiP) promoter identified a 22 bp element, the **UPR element (UPRE),** which is conserved in the promoters of all UPR target genes and required for their induction by ER stress. The UPRE can confer responsiveness to UPR to a heterologous promoter. The UPRE is characterised by short **E-box** like palindromic sequences (CANCNTG). (Basic helix-loop-helix (bHLH) regulatory proteins are known to bind to a single DNA consensus sequence referred to as an E-box. The E-box is present in the regulatory elements of many developmentally controlled genes.)

Regulation of transcription from UPRE-containing promoters in yeast is mediated by the **transcription factor Hac1p.** Hac1p belongs to the family of bZip transcription factors. bZIP transcription factors bind to DNA as homo- or heterodimers. Hac1p contains a DNA-binding domain and leucine zipper in its N-terminal region.

The C terminus contains a PEST-region rich in the amino acids Pro, Glu, Ser, Thr and Asp. PEST-regions destabilize proteins by targeting them into the ubiquitin-dependent proteolysis pathway. The half-life of Hac1p is 2 min. The short half-life of Hac1p ensures that UPRE- dependent transcription is rapidly down-regulated when UPR is no longer induced.



C. Regulation of Hac1p expression

UPRE-dependent transcription is regulated by the abundance of Hac1p. In the absence of unfolded proteins, Hac1p cannot be detected in yeast cells. However, transcription of Hac1p is independent of UPR activity and remains constant throughout the cell cycle. This suggested post-transcriptional regulation as a means for Hac1p protein expression.

Splicing of HAC1 mRNA

In the absence of unfolded proteins:
- $HAC1^{u}$ mRNA (u for uninduced) is present
- this mRNA cannot be translated !!!

In the presence of unfolded proteins:
- splicing of $HAC1^{u}$ mRNA to $HAC1^{i}$ mRNA (i for induced)
- translation of this mRNA would lead to the production of $Hac1p^{i,}$ however, little to none of this protein can be detected

Splicing of $HAC1^{u}$ mRNA is tightly regulated; processing occurs only when misfolded proteins accumulate in the ER lumen. It is so far the only known example of a regulated mRNA splicing event controlling the activity of a signal transduction pathway.

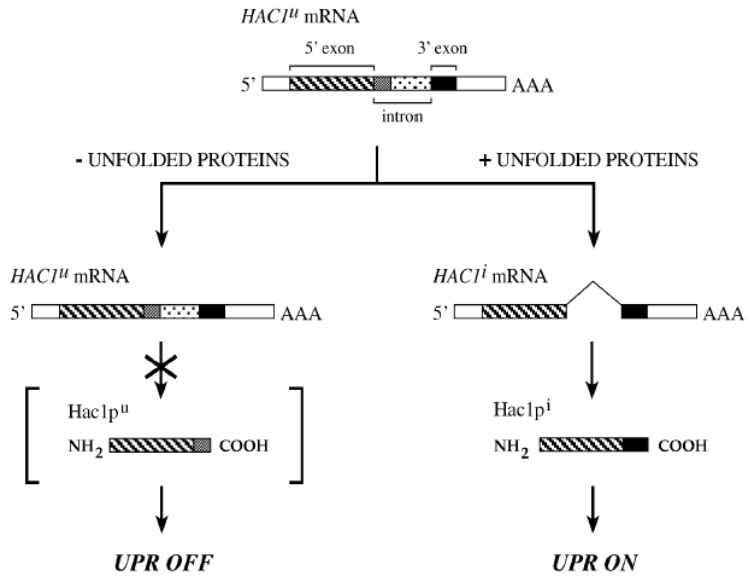


Figure 2 Splicing of *HAC1*^{u} mRNA. Removal of the intron alters the open reading frame of Hac1p. Thus the unspliced *HAC1*^{u} mRNA encodes Hac1p^{u} that contains a 10-amino acid tail encoded by intron sequences (*gray boxes*), which upon splicing is replaced by a different 18-amino acid tail encoded by 3′ exon sequences (*black boxes*). No detectable amounts of Hac1p^{u} are produced in cells because *HAC1*^{u} mRNA is not translated.

Translational Attenuation of HAC1^{u} mRNA

Unlike other pre-mRNAs, HAC1^{u} mRNA is stable and exported from the nucleus, escaping controls that retain other pre-mRNAs in this compartment. Although no protein is produced from this message, it co-sediments with polyribosomes suggesting that translational elongation is blocked. The intron in the HAC1^{u} mRNA was shown to cause the translational block.

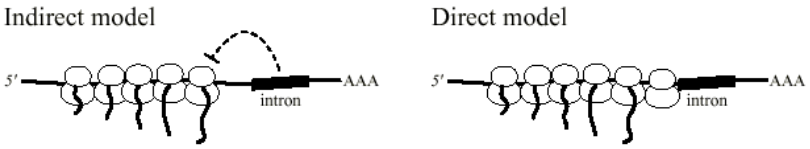
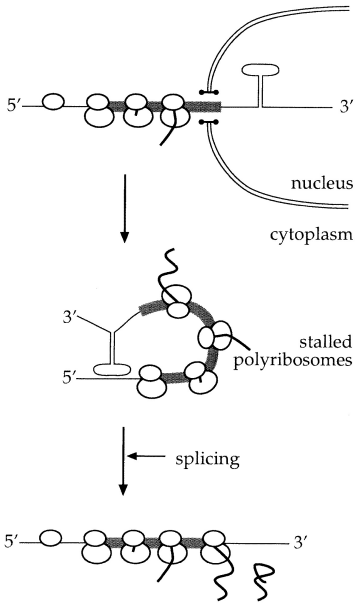


Figure 3 Model for translation attenuation by the *HAC1*^{u} mRNA intron. The Indirect model predicts that ribosomes and their associated nascent chains stall upstream of the intron. Consequently, no full-length protein is produced. According to the Direct model, the first ribosome stalls upon directly encountering the intron, presumably at a region of tight secondary structure. Further ribosomes and associated nascent chains stack behind it. If the intron is localized in the 3′ untranslated region (UTR) of a mRNA, this mechanism could work only if ribosomes continue moving down the 3′ UTR after protein synthesis has terminated.



Recently, it has been shown that base-pairing interaction between the intron and the 5' untranslated region is required and sufficient to block mRNA translation. Unspliced HAC1 mRNA is stable, located in the cytosol, and is associated with polyribosomes, yet does not produce protein, indicating that the ribosomes engaged on the mRNA are stalled. The polysomal, cytoplasmic pool of HAC1 mRNA is a substrate for splicing, suggesting that the stalled ribosomes may resume translation after the intron is removed.

Non-conventional splicing of HAC1 mRNA

Splicing of HAC1 mRNA is not catalyzed by the spliceosome but uses a unique splicing machinery composed of two proteins:

1. the bifunctional **Ire1p** transmembrane kinase/endonuclease : Ire1p is an **endonuclease** which cleaves $HAC1^u$ mRNA
   - the C-terminal tail of Ire1p displays about 30% sequence identity to the C-terminal domain of RNase L
   - RNase L is soluble, unspecific nuclease which is activated by 2'-5' oligoadenylates usually produced by cells infected with viruses

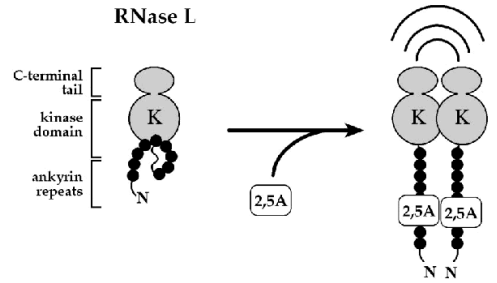


Figure 5 Similarities between Ire1p and RNase. Both RNase L, a soluble enzyme, and Ire1p, a transmembrane protein, contain a kinase domain followed by a C-terminal tail domain that is required for ribonuclease activity. The kinase (K) and the C-terminal tail domains show amino acid sequence similarity. The N-terminal regions of both molecules function to sense the upstream signal in their respective pathways, but show no homology. Binding of 2′-5′-linked oligoadenylates (2-5A) to two of the N-terminal ankyrin repeats of RNase L allows oligomerization and activation of the nuclease. Similarly, accumulation of unfolded proteins leads to oligomerization and activation of the kinase and nuclease activities of Ire1p.

   - the nuclease activity of Ire1p was directly demonstrated by assaying the activity of recombinantly expressed fusion protein consisting of the C-terminal half of Ire1p, which contains both the kinase and RNase domain and GST (glutathione-transferase, a dimer): GST-Ire1p (C) autophosphorylates in vitro and specifically cleaves $HAC1^u$ mRNA
   - it is assumed that Ire1p binds to the Hac mRNA as a dimer with each monomer recognizing one of the very similar stem-loop RNA structures present at the 5' and 3' splice sites of the RNA, respectively

Ire1p was originally identified as a regulatory protein in inositol response. Inositol is a precursor of the major structural phospholipids in yeast. The link of the two responses makes sense, as an increase in either the lumenal or the membranous components of the ER increase, the cell must compensate by coordinately increasing production of the remaining part of the organelle.

2. The step after cleavage of Hac-mRNA: the **tRNA ligase Rlg1p**

Rlg1p is tRNA ligase normally involved in the second step of pre-tRNA splicing, which is the ligation of the two tRNA halves generated by tRNA endonuclease. This tRNA ligase is also involved in joining the two halves of Hac1 mRNA.

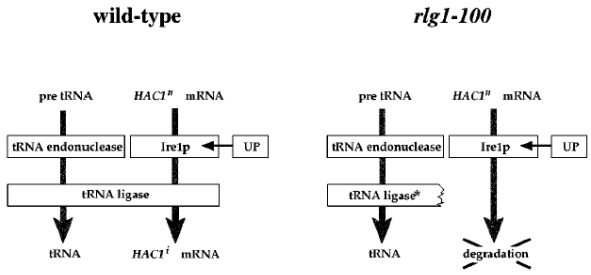


Figure 6 *rlg1-100* encodes a UPR pathway-specific loss-of-function mutant of tRNA ligase. tRNA ligase functions in two RNA processing pathways: the constitutive splicing of pre-tRNAs and the regulated splicing of *HAC1*^{u} mRNA. In cells bearing the *rlg1-100* allele, *HAC1*^{u} mRNA becomes selectively degraded when Ire1p is activated. Ire1p presumably initiates *HAC1*^{u} mRNA processing by cleaving at either or both splice junctions. The resulting fragments are unstable if their re-ligation is impaired. In contrast, pre-tRNA splicing is unaffected in the mutant strain, suggesting that the enzymatic functions of the mutant protein are intact. The *rlg1-100* mutation might therefore confer an inability to use *HAC1* mRNA fragments as substrates, or it could impair an interaction between Ire1p and tRNA ligase required for efficient processing. (UP, unfolded proteins).

Model

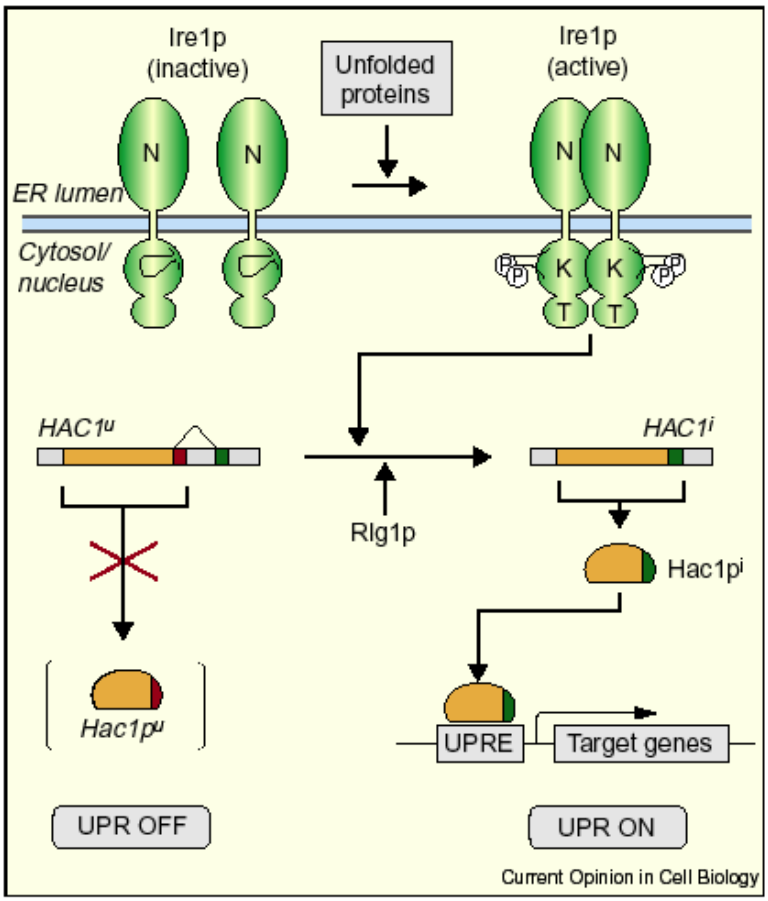


Fig. 1. A schematic of the unfolded protein response in yeast. Ire1p is a transmembrane serine-threonine kinase, oriented with the amino terminus (N) in the ER lumen and the carboxyl terminus in the cytosol. When unfolded proteins accumulate in the ER, Ire1p oligomerizes, trans-autophosphorylates via the cytosolic kinase domain (K) and activates the endonuclease in the tail domain (T). The endonuclease Ire1p cuts HAC1 mRNA at two sites, removing a nonclassical intron; the two exons are rejoined by Rlg1p (tRNA ligase). HAC1u ('uninduced') is not translated owing to the presence of the intron, and Hac1pu is not produced (brackets). After Ire1-mediated splicing, HAC1i mRNA is efficiently translated into Hac1pi, a transcriptional activator that upregulates expression of UPR target genes after binding to the unfolded protein response element (UPRE) in the promoters of genes encoding ER-resident chaperones and other proteins.(Patil and Walter 2001)

Tripartite management of unfolded proteins in the ER

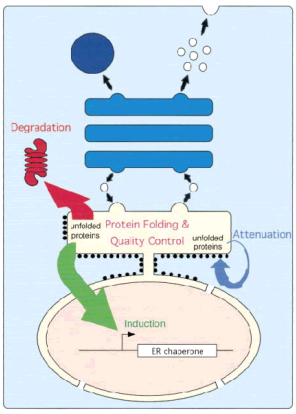


Figure 1. Three Cellular Responses to the Accumulation of Unfolded Proteins in the ER
The ER monitors the folding status of newly synthesized secretory and transmembrane proteins and controls their quality. Three cellular responses are activated to cope with the accumulation of unfolded proteins in the ER: transcriptional induction, translational attenuation, and degradation.

1. transcriptional induction of genes
2. ER associated degradation (ERAD)
3. translational attenuation

- continuous delivery of newly synthesized proteins is a burden to the ER when proper folding is prevented under ER stress conditions
- cells might be able to restore normal function more efficiently if they could suppress protein synthesis, and indeed translation is attenuated in response to the presence of unfolded proteins in the ER
- such a mechanism exists in metazoans (not in yeast)

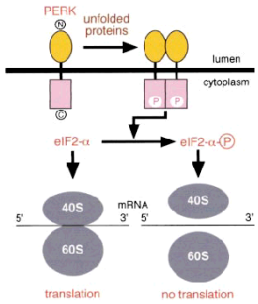


Figure 3. Mechanism for ER Stress–Induced Translational Attenuation
ER stress–induced oligomerization and autophosphorylation of PERK, a type I transmembrane protein kinase in the ER, result in phosphorylation of eIF2-α at serine 51, leading to inhibition of translation initiation.

Transmembrane kinase **PERK** (PEK) (stands for PKR like ER kinase)
- phosphorylates **eIF2α** upon induction of ER stress
- luminal domain resembles that of Ire1 and indeed the luminal domains of Ire1p and PERK are interchangeable (suggesting involvement of BiP in PERK activation)

2. ER-overload response

EOR is **activated by accumulation of membrane proteins** in the ER. This may be significant upon viral infection when the organelle is overcharged by the production of viral membrane proteins or, in several genetic diseases, such as cystic fibrosis, when altered proteins accumulate in the ER.
ER-overload activates the transcription factor NF-κB. Many activators of UPR also activate NF-κB in mammalian cells. However, there are UPR inducers, which do not activate NF-κB (e.g. DTT) and EOR inducers (e.g. over-expression of influenza virus hemeagglutinin).

TABLE 2. *ER stress conditions that activate nuclear factor κB*

ER overload by protein overexpression
Hepatitis B virus truncated middle HB surface antigen
Influenza virus hemagglutinin
Immunoglobulin μ-heavy chain
MHC class I
Adenovirus E3/19K
EPO receptor
Drugs perturbing ER function
Tunicamycin
2-Deoxyglucose
Monensin
Brefeldin A
Thapsigargin
Cyclopiazonic acid

MHC, major histocompatibility.

ER overload must release a signal from the organelle that reaches NF-kB, which resides in the cytoplasm. Pharmacological evidence suggested that the efflux of Calcium from the ER is required for ER overload-mediated NF-kB activation. How a change in the membrane protein to lipid ratio increases calcium permeability is unknown. Accumulation of membrane proteins may impair Ca^{2+}-ATPase function. Reactive oxygene intermediates also play a role. Pretreatment of cells with antioxidants prevents NF-κB activation by ER stress.

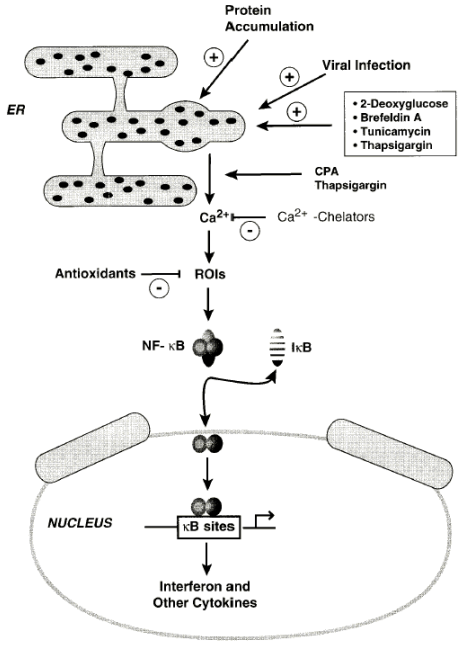


FIG. 2. ER-overload response. Accumulation of wild-type or misfolded proteins in ER leads to a release of Ca^{2+} from organelle. This causes production of reactive oxygen intermediates (ROI), which activate transcription factor nuclear factor κB (NFκB). Activation can be blocked by antioxidants and by Ca^{2+} chelators, or it can be activated by drugs that cause an active release of Ca^{2+} from ER. NFκB induces transcription of genes involved in inflammatory and immune responses. CPA, cyclopiazonic acid.

3. ER-nucleus signalling by SREBPs

Cells can obtain cholesterol, an essential cell membrane component, in two ways. One is the uptake of cholesterol-rich low-density lipoproteins (LDLs) by LDL receptors, which bind and internalize LDL from the plasma. Alternatively, almost all cells can synthesize cholesterol *de novo*.
Both cholesterol up-take and synthesis are subject to feed-back repression. Transcription of many genes the products of which are involved in cholesterol biosynthesis or uptake are inhibited by high intracellular sterol concentrations and induced upon sterol depletion.

Transcriptional induction requires a 10 bp DNA element, the **sterol regulatory element 1 (SRE-1),** in the promoters of sterol-regulated genes. A basic helix-loop-helix transcription factor **(SREBP-1)** was shown to activate transcription via the SRE-1.
SREBP-1 and the highly homologous SREBP-2 are trans-membrane proteins held in ER membrane by two trans-membrane domains, such that N- and C-terminal domains both face the cytoplasm. The N-terminal domain is the transcriptional activation domain whereas the C-terminal domain forms a complex with the protein **SCAP** (SREBP cleavage activating protein).

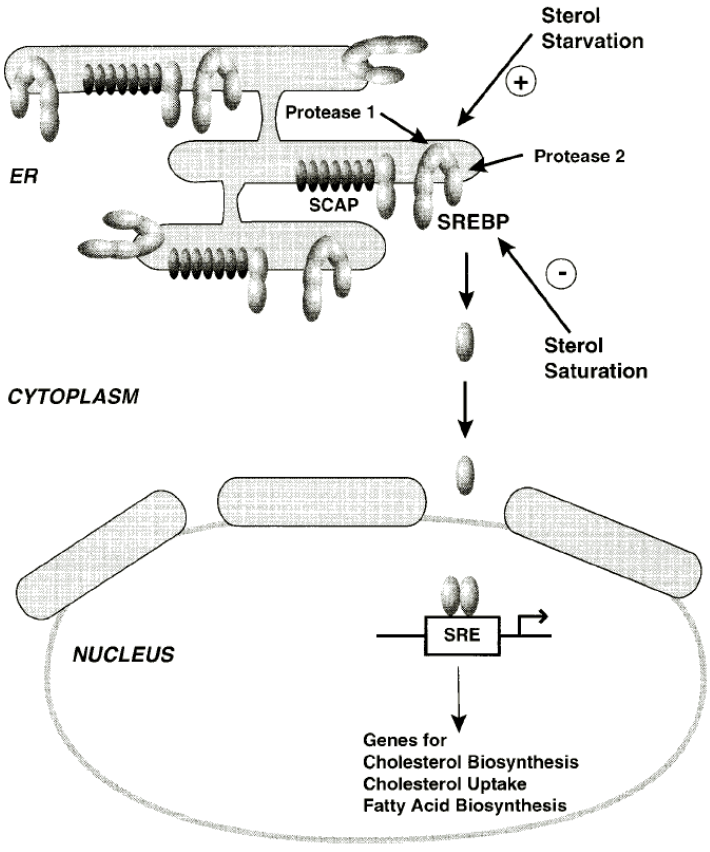
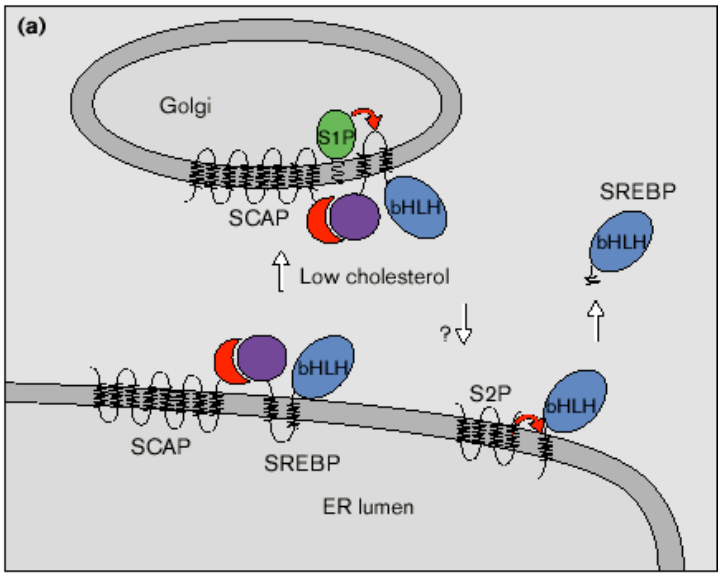


FIG. 3. Sterol response pathway. Synthesis and uptake of cholesterol is subject to feedback repression within cells. How cellular cholesterol level is sensed is not yet known. Depletion of cholesterol leads to proteolysis of membrane-bound transcription factor sterol regulatory element binding protein (SREBP). Proteases that cleave SREBP at 2 well-characterized sites within "luminal loop" in ER lumen and within first transmembrane domain have not been described. SREBP cleavage requires activity of SREBP cleavage-activating protein (SCAP), which has been shown to physically interact with COOH terminus of SREBP. SCAP possesses no enzymatic activity; however, its NH_2-terminal transmembrane domains may serve as cellular sterol sensor. Cleaved, active SREBP transcription factor homodimerizes and activates transcription of genes involved in cholesterol and fatty acid synthesis and uptake. SRE, sterol regulatory element.

Upon sterol depletion, SREBP is cleaved in two sequential proteolytic steps. The first cleavage occurs in the middle of the lumenal domain. The second cleavage is in the middle of the first trans-membrane region. The first cleavage is regulated by the presence of sterols (abolished in the presence of

sterols). The SCAP protein is instrumental in this regulation and may serve as the sterol sensor. In the absence of sterols, SCAP shuttles with SREBP from the ER to the Golgi. In the Golgi, SREBP encounters a dedicated membrane-bound protease called Site 1 protease (S1P). The N-terminal fraction remains membrane-associated until it is cleaved by Site 2 protease (S2P), which cleaves within the trans-membrane segment. Even though the activity of S2P is not under sterol control, S2P cannot act until the first cleavage by S1P has occurred.



(a) The SREBPs are ER-resident proteins that are shuttled to the Golgi in response to the need for more sterols. The amino-terminal transcriptionally active domain (bHLH) is liberated from its membrane-anchor by two consecutive proteolysis steps. The first cleavage reaction, performed by the Site 1 protease (S1P), takes place in the Golgi. The second cleavage reaction, by Site 2 protease (S2P), ultimately releases the transcription factor from the membrane. The

The released fragment of SREBP contains the basic DNA-binding domain as well as the leucine zipper dimerization motif. A dimer of two such fragments is then translocated into the nucleus by importin β.