Endoplasmic reticulum: Stress, signalling and apoptosis

Syed G. Hussain and Kolluru V. A. Ramaiah*

Department of Biochemistry, University of Hyderabad, Hyderabad 500 046, India

The synthesis, folding and processing of the secretory/membrane proteins by the endoplasmic reticulum (ER) requires the functioning of ER chaperones, maintenance of ER calcium pools, and an oxidative environment. Disruption of the ER functioning elicits an adaptive signalling cascade called the unfolded protein response (UPR). UPR is triggered by the activation of ER transmembrane proteins and modulated by ER chaperones. It comprises of general inhibition in protein synthesis, induction of ER chaperones, and components of ER-associated protein degradation. UPR is implicated in development, disease and virus infection. Incessant ER stress, through unknown mechanism(s) bolsters the proapoptotic potential of the UPR with a subsequent decline in the adaptation capabilities and initiates cell death. This review highlights the mammalian UPR signalling pathways in general, and the major players implicated in ER stress-induced apoptosis.

Keywords: Apoptosis, endoplasmic reticulum, signalling, stress.

THE endoplasmic reticulum (ER) is a subcellular organelle involved in the synthesis of secretory/membrane proteins and lipids. ER harbours a plethora of chaperones, lectins, foldases and carbohydrate-processing enzymes[1] (Box 1) to aid disulphide bond formation and glycosylation required to ensure correct protein folding and translocation of the secretory/membrane proteins. Chaperones maintain the proteins in a folding-competent state and prevent aggregation of the exposed hydrophobic amino acid side chains, while foldases catalyse protein folding. ER quality-control mechanisms ensure the recognition of authentic folding intermediates from terminally malfolded proteins by ER chaperones[1]. Retro-translocation of misfolded proteins through ER membranes for degradation by the 26S proteasome constitutes the ER-associated degradation (ERAD)[1]. Aggregation and escape of misfolded proteins from the 'ER quality-control' is associated with many conformational diseases[1,2].

Unlike cytosol, ER maintains a rich oxidative environment and high levels of Ca^{2+}. Most of the secretory proteins processed by ER undergo disulphide bond for-

mation that involves conversion of the –SH groups to S–S. This task is accomplished mainly through the activity of ER oxidase1 (ERO1), which oxidizes protein disulphide isomerase (PDI) that in turn transfers the oxidative equivalents to the newly made secretory proteins[1]. This process generates reactive oxygen species[3]. Hence professional secretory cells such as the pancreas that produce insulin, experience and resist tremendous oxidative stress. $ER-Ca^{2+}$ stores are maintained by Ca^{2+}-binding ER chaperones such as BiP/GRP78 (immunoglobulin-binding protein/glucose-regulated protein), GRP94, calnexin, calreticulin, and Ca^{2+}-binding proteins such as calsequestrin, calreticulocalbin, and calstorin. Ca^{2+} is required for the functioning of many ER chaperones and its depletion leads to accumulation of unfolded proteins[4,5].

ER stress sensing, transduction and unfolded protein response

To maintain homeostasis against any ER dysfunction, the ER responds through a complex and coordinated adaptive signalling mechanism called the unfolded protein response (UPR)[6,7]. The UPR relays ER stress to the cytosol and nucleus to counter the imbalance in protein synthesis, folding, modification, translocation and degradation. Several physiological and pathological conditions such as nutrient or glucose deprivation, elevated protein synthesis, virus infection, disturbances in Ca^{2+} fluxes, and redox regulation have been shown to promote ER dysfunction and elicit UPR.

The UPR was discovered in early experiments that led to the induction of BiP/GRP78 in response to the accumulation of mutant unfolded viral haemaglutinin proteins or depletion of glucose[8]. Mutant unfolded proteins that cannot interact with BiP fail to induce BiP[9], thereby suggesting that the interaction between unfolded proteins and BiP plays a crucial role in evoking the ER stress response. The UPR also upregulates the expression of other chaperones and foldases such as PDI, GRP 170, and ERp 72 (ref. 10) (Box 1).

The UPR, an evolutionarily conserved mechanism to restore cellular homeostasis, is less complex in lower eukaryotes and primarily involves adaptive responses. The mammalian UPR is more complex, diverse and flexible, and signals through three ER-transmembrane proteins representing the three arms of the UPR. One of them is IRE-1

Box 1. Mammalian ER resident chaperones[1,4,5,7,33]

HSP-70 class	
BiP/GRP78	Assists protein folding, regulates ER stress transducers
GRP 170/ORP-150	Assists protein folding
HSP-90 class	
GRP 94/endoplasmin	Assists protein folding
HSP-40/DNA-J like	
Erdj1, 3, 4 and 5	Cochaperones that assist BiP
Sec63P	Cochaperone and ER translocon component
p58^{IPK}	Cochaperone and inhibitor of eIF2α kinases
Bacterial GrpE-like	
BAP	Assists in nucleotide exchange
Sil 1p	Assists in nucleotide exchange
Lectin chaperones	
Calnexin	Assists in glycoprotein folding
Calreticulin	Assists in glycoprotein folding
EDEM	Assists in glycoprotein degradation
Carbohydrate processing enzymes	Play a role in calnexin/calreticulin cycle
UGGT	Reglucosylation of unfolded proteins
α-Glucosidase I and II	Removal of glucose residues from N-glycan tags
α-Mannosidase I and II	Removal of mannose residues from N-glycan tags
Foldases	
Disulphide isomerases/oxidoreductases	
PDIs	Assists in oxidation/reduction of cysteine
ERp72, 61, 57 and 44	Disulphide bridges and isomerization
Ero1α and Ero1β	Transfers reducing equivalents to PDIs
FAD-dependent oxidases	
Fmo1p	Generates oxidizing equivalents in ER
Peptidyl-prolyl isomerases	
FKBP-13 and 65	Isomerize cis–trans peptidyl–prolyl bonds
S-Cyclophilin	
Cyclophilin B	
CCYLP	

ORP, Oxygen regulated protein; EDEM, ER degradation enhancing α-mannosidase-like protein; UGGT, UDP-Glc : Glycosyl transferase; PDI, Protein disulphide isomerases; FKBP, FK506-binding proteins; CCYLP, Cylophylin like protein.

resembling yeast IRE-1/ERN[11] (inositol-requiring and ER to nucleus signalling). The other two stress transducers are ATF6 (activated transcription factor 6)[12] and PERK (ER-resident PKR-like eIF2α kinase)[13,14] that are present in mammalian systems, but not in yeast. These ER stress transducers are inactive when BiP is bound to their luminal regulatory domains. Release of BiP is a prerequisite for their activation[15]. Over-expression of BiP suppresses the induction of ER stress responsive genes by the UPR[16]. Hence BiP is considered to be an ER stress sensor and a critical regulator of ER stress response[5,15]. ER stress-induced adaptive signalling cascade includes two arms: translational attenuation followed by transcriptional induction that includes an antioxidant gene expression programme, and/or ER chaperones mediated by the ER stress transducers, viz. PERK, IRE-I and ATF6.

Analysis of the UPR signalling pathways has also become possible using various pharmacological agents such as tunicamycin, an inhibitor of protein glycosylation; cycloheximide, an inhibitor of translational elongation; thapsigargin that affects ER Ca^{2+} levels; redox agents such as DTT, GSH or GSSG that influence disuphide bond formation; brefeldin A, an inhibitor of ER to Golgi protein transport, and inhibitors of protein degradation such as bortezomib[6,7,17].

IRE-1

Mammalian IRE-1 is a type-1 ER transmembrane bifunctional glycoprotein having serine/threonine kinase and endoribonuclease activities in its cytosolic domain[11]. Two

isoforms, α and β, have been identified in mammals[11,18]. IRE-1α is ubiquitously expressed[18] and β-expression is limited to gut epithelial cells[19]. BiP bound to the luminal domains of IRE-1 is released in response to ER stress, promoting[15,18] dimerization and subsequent activation of IRE-1. The recently reported crystal structure of the luminal domain of yeast IRE-1 reveals that dimerization of monomers forms a central groove that resembles the peptide-binding domain of major histocompatibility complex (MHC). Based on mutational analyses of the amino acids that face into the groove, it is suggested that oligomerization of yeast IRE-1 requires binding of the unfolded proteins to the dimers[20]. In contrast, human IRE-1α activation is proposed to be independent of unfolded protein binding and dependent on dimerization-induced autophosphorylation and subsequent activation of RNAse[21]. Active endoribonuclease splices XBP-1 (X-Box binding protein 1) mRNA in mammals[22] and HAC1 (homologous to ATF/CREB1) mRNA in yeast[7]. The unspliced human XBP-1 mRNA contains an ORF1 coding for 261 amino acids and ORF2 encoding 222 amino acids that partially overlaps with ORF1. Splicing of the intron results in a translational frame shift at amino acid 165 in ORF1. Splicing also removes the C-terminal 97 amino acids from ORF1 and adds on 212 amino acids of ORF2 to the N-terminal of ORF1. The splicing events result in the formation of a 376 amino acid XBP-1 protein, with a novel C-terminus that acts as a potent basic leucine zipper transcription factor, in contrast to a 261-amino acid XBP-1 protein encoded by unspliced XBP-1 mRNA[22]. While the mechanism of activation of IRE-1 is somewhat similar both in yeast and mammals, the ligase catalysing the joining reaction of the exons in XBP-1 is not known. In yeast, a tRNA ligase catalyses the joining of the two exons of HAC1 mRNA[23]. Further, HAC1 mRNA splicing is a cytoplasmic event, whereas XBP-1 mRNA splicing by mammalian IRE-1 may be a nuclear event because of the extension of IRE-1 cytoplasmic domain into the nucleoplasm[24]. Spliced XBP-1 mRNA encodes active bZIP transcription factor that upregulates genes of ER chaperones[25], including p58[IPK], a negative regulator of PERK[25], components of ERAD[26], and of lipid biosynthesis[27]. The IRE-1/XBP-1 arm plays an important role in plasma B-cell differentiation and liver development[10].

PERK

Its structure and activation share several similar features with IRE-1; however, it lacks the endoribonuclease activity[14]. Activation of PERK attenuates general translation through the phosphorylation of ser[51] residue in the alpha-subunit of heterotrimeric translational initiation factor 2 (eIF2)[14]. Hence PERK activation reduces ER synthetic capacity and inflow of the folding clients into an over-burdened ER. Phosphorylated eIF2α forms a complex with eIF2B, a rate-limiting, heteropentameric GDP/GTP ex-

change factor, inhibits its ability to recycle eIF2-GDP to eIF2-GTP and thereby impairs translation[28]. Phosphorylation of eIF2α, however, upregulates the translation of ATF4 (activated transcription factor 4)[29] which in turn induces genes involved in amino acid transport, glutathione biosynthesis, redox regulation[29] and CHOP (CCAAT/enhancer binding protein (C/EBP)[30] homologous protein) or growth arrest and DNA damage-inducible gene-153 (GADD-153). CHOP, however, signals apoptosis[30]. PERK also phosphorylates Nrf2 (Nuclear Factor-E2 related factor 2), a transcription factor that induces genes involved in antioxidation, detoxification of enzymes, immune signalling, protein trafficking and degradation, chaperones, cell growth and survival[31]. Although the antioxidant responses elicited by eIF2α-ATF4/Nrf2 converge at several points, they diverge at CHOP, primarily a proapoptotic transcription factor. While ATF4 promotes CHOP expression, Nrf2 inhibits it[31].

ATF6

It is a type-2 transmembrane protein with its N-terminal domain in the cytoplasm and the C-terminus in the ER lumen[32]. Unlike PERK and IRE-1, the ER luminal domain of ATF6 carries Golgi localization signals that are masked by BiP in unstressed conditions. In response to ER stress, BiP release facilitates ATF6 translocation to Golgi[32]. Only the under-glycosylated 90 kDa ATF6 monomer with reduced disulphide linkage is transported to Golgi, where it is proteolysed by S1P and S2P proteases to yield an active N-terminal 50 kDa domain (N-ATF6/p50ATF6) that translocates to the nucleus[33,34]. The first cleavage by S1P occurs in the luminal domain of Golgi on the serine protease 1 site of ATF6 and the second cleavage called regulated intramembrane proteolysis, by S2P, occurs in the transmembrane region[35]. A similar mechanism of activation is also observed in the activation of bacterial transcription factor σE[35]. Processed ATF6β inhibits processed ATF6α through heterodimerization[36]. In addition, many ATF6-like ER transmembrane proteins are activated by regulated intramembrane proteolysis. These are OASIS (old astrocyte specifically induced substance), CREBH, a liver-specific factor, Tisp40 (transcript induced in spermiogenesis 40), Luman/LZIP/CREB3, and CREB4 (ref. 36). ATF6-induced genes include ER chaperones, CHOP, p58[IPK], Herp (hyperhomocysteinemia-induced ER stress responsive protein) and PDI[32,35].

Among the adaptive responses, translational attenuation mediated by the PERK pathway appears to be the immediate event, followed by transcriptional induction of ER stress-responsive genes. Other evidences, however, suggest it is not necessary that all adaptive pathways are invoked in tandem and activation of a subset of pathways can regulate the ER stress response. For example, IRE-1, but not the PERK pathway, is activated in the maturation of B-cells[10].

The following *cis*-acting promoter elements are required for the transactivation ability of the ER stress-induced b-ZIP transcription factors[32]. XBP-1 binds to CRE-like (GATGACGTG(T/G)N3(A/T)T), ERSE-1 (CCAATN9CCACG) and UPRE elements (TGACGTGG/A). ATF4 binds to ATF/CRE element (TGACGT(C/A)(G/A) and ARE element (G/C) TGAC/TN3GC (A/G). ATF6 binds to ATF/CRE, ERSE-1 and ERSE-II elements (AATTGGNCCACG).

UPR in development and disease

The UPR plays an important role in embryonic development, maturation of secretory cell types such as antibody-producing plasma cells, osteoblasts that secrete collagen, and insulin-secreting pancreatic β-cells. It is also implicated in normal biological processes like aging, liver development, sleep deprivation, and in nutrient sensing in yeast[6,10,37]. Glucose limitation that activates the UPR and induces ER chaperone synthesis in mammalian cells, also represses cholesterol synthesis. Hence the ability of the ER to sense the availability of glucose, lipids, oxygen and energy may serve as an important site of integration of nutrient signalling. Early embryonic development is associated with ER stress and requires chaperone activity for viability. BiP is induced at 3.5 days during embryonic development and BiP knockout is lethal to embryos. These embryos have implantation problems, fail to grow in culture, and undergo severe apoptosis[38]. Plasma cells carrying IRE-1 mutations that cannot process XBP-I fail to efficiently secrete antibodies, and the secretion of antibodies is restored upon spliced XBP-1 expression[39]. Activation of c-Jun N-terminal kinase (JNK) by IRE-1 and subsequent phosphorylation at ser[307] of insulin receptor substrate-1 (IRS-1) leads to insulin resistance[6,40]. Analyses of homozygous and heterozygous knockout mouse cells in culture, and humans suffering from Walcott Rallison syndrome, an infantile type-1 diabetes, reveal that IRE-1-XBP-1, IRE1-JNK, PERK-eIF2α phosphorylation, and p58[IPK] play an important role in the functioning of β-cells and in promoting type-1 and type-2 diabetes/obesity-linked insulin resistance[6,40]. ER stress is also implicated in hypoxic resistance and tumour progression[41], several conformational and neurodegenerative disorders[2], atherosclerosis, viral infection, ischaemia-induced cell injury, and probably in mental disorders[6,35].

In higher eukaryotes, the ER stress response is shown to evoke an anti-inflammatory and immune response by activation of NF-kB, a transcription factor that in turn induces inflammation and immune genes critical for host defence, cell growth, and pro/antiapoptotic processes[40].

ER stress and apoptosis

Incessant ER stress beyond the limits of adaptation can trigger the proapoptotic potential of the UPR. The suicide of unhealthy cells via apoptosis represents the last resort of multicellular organisms to clear the non-functional cells[42]. Cell death results in loss of cell/tissue function and may be the primary reason for the manifestation of disease in several ER stress-related disorders. Although the exact mechanism(s) is not known, the ER stress-induced apoptosis is mediated by the mitochondria (intrinsic pathway) and/or through the activation of proapoptotic downstream kinases that are triggered typically in the death-induced receptor mediated extrinsic apoptotic pathway.

Since mitochondria are juxtaposed to the ER, in many cases ER stress is communicated to the mitochondria, and ER stress-induced apoptosis is mediated through a dysfunction in the mitochondria. Recent work suggests that BCL2 protein family regulates the ER-Ca^{2+} release and communication of ER stress signal to the mitochondria. Persistent ER stress can also induce a switch in the UPR signalling from prosurvival to proapoptotic pathways, like the induction of CHOP, a proapoptotic transcriptional factor and GADD34 (a cofactor of eIF2α phosphatase), through the PERK-eIF2α pathway, and activation of proapoptotic kinases such as ASK1 (apoptosis signal-regulating kinase) and JNK (c-Jun-N-terminal kinase) through the IRE-1 pathway. The major players involved in ER stress-induced apoptosis and their roles are described below.

Role of BCL2 proteins

Some of the BCL2 family members (BCL2, BIK, BAX, and BAK), regulators of mitochondrial-induced apoptosis, also reside on the ER, and play a regulatory role in the apoptotic crosstalk between the ER and the mitochondria[43–45] (Box 2). The current understanding suggests that the proapoptotic proteins, BAX and BAK, act as gatekeepers of apoptosis regulating cytochrome *c* release from the mitochondria, and their activities are triggered by the activator proteins of the BH-3-only group (tBID, BIM, PUMA). The antiapoptotic members of BCL2 proteins (BCL2, BCLx$_L$, MCL1) indirectly keep the proapoptotic BAX or BAK in check, presumably by sequestering the activator proteins of BH-3-only proteins. Inactivator proteins of the BH-3-only group (BAD, NOXA, BMF, BIK/BLK and HRK) can also regulate BAX and BAK activity by displacing the activators from the antiapoptotic proteins[43].

Antiapoptotic BCL2 proteins: ER-targetted BCL2 sequesters activator BH-3-only proteins and protects against many forms of apoptosis, including the BAX-promoted apoptosis[44–46]. BCL2 inhibits the oligomerization of BAX, disruption of mitochondrial membrane potential, and release of mitochondrial cytochrome *c*[44–46]. BI-1 (BAX inhibitor 1), an antiapoptotic protein, sup-

Box 2. BCL2 protein family

Proteins with conserved BCL2 homology (BH) domains constitute the BCL2 protein family. The multi-domain members are grouped as proapoptotic/antiapoptotic. Proteins with BH-3 domain only, are all proapoptotic.
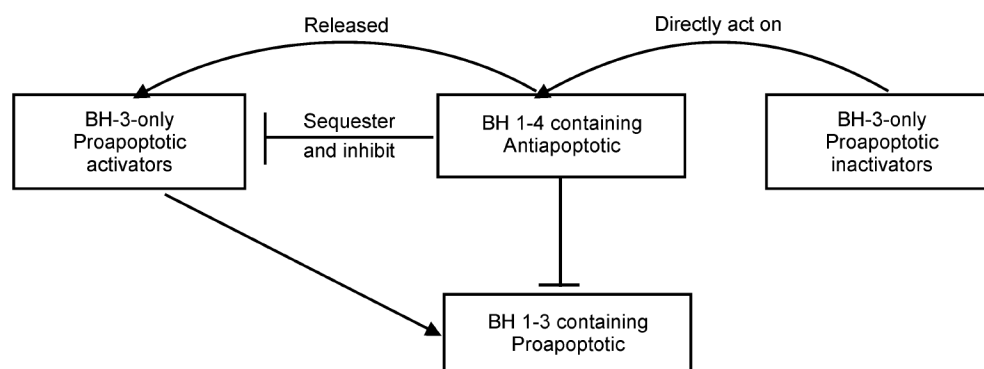
Antiapoptotic: BCL2, BCLx$_L$, MCL1, A1, BCL$_W$; all the four have conserved BH 1–4 homology domains[43,44].

Proapoptotic: BAX, BAK, BOK/MTD contain only BH 1–3 domains[43,44].

The proapoptotic BH-3-only domain containing proteins regulate the activity of these anti-/proapoptotic proteins and are grouped as follows:

Activators: BIM, t-BID, PUMA[43,44].

Inactivators: BAD, BIK, BMF, HRK/DPS, NOXA, BNIP3[43,44].

Released   Directly act on

| BH-3-only Proapoptotic activators | Sequester and inhibit | BH 1-4 containing Antiapoptotic | | BH-3-only Proapoptotic inactivators |

BH 1-3 containing Proapoptotic

BCL2, B cell leukaemia/lymphoma 2; BCLx$_L$, Bcl-x long; BCL$_W$, BCL-2 widely expressed; MCL-1, Myeloid cell leukaemia 1; BAK, BCL2 homologous antagonist/killer; BAX, BCL2 associated X-protein; BOK, BCL2 related ovarian killer; BIM, BCL2 interacting mediator of cell death; t-BID, Truncated active fragment of BID (BH-3 interacting domain death agonist); PUMA, p53 upregulated modulator of apoptosis; BAD, BCL2 associated death promoter; BIK, BCL2 interacting killer; BMF, BCL2 modifying factor; HRK, Product of *harakiri*; NOXA, Neutrophil NADPH oxidase factor; BNIP3, BCL2-binding protein Nip3.

presses cell death mediated by BAX over-expression. It does not associate with BAX, but interacts with antiapoptotic BCL2 and BCLx$_L$ proteins[44]. Further, homozygous mutant mice lacking BI-1 are found to be sensitive to ER stress and over-expression results in resistance[44]. BI-1 expression is linked to a reduction in ROS generation and reduced activation of ER stress sensors and regulator(s) involved in the UPR[47]. The mechanism involves the expression of heme oxygenase 1 through the activation of the PERK–Nrf2 pathway[47].

Proapoptotic BCL2 proteins: BAX/BAK deficiency protects cells from ER stress-induced apoptosis, whereas expression of ER-targetted BAK in BAX/BAK double knockout cells promotes apoptosis[46,48]. The induction of p53 transcription factor in response to DNA damage, or other ER stress activators, induces some of the BH-3-only proteins like BIK, PUMA, and NOXA[44]. Homozygous PUMA- and NOXA-deficient cells are resistant to ER stress-mediated apoptosis[49]. BIK, a BCL2-interacting killer, localized primarily on the ER, enhances the recruitment of BAX and BAK on the ER that signals ER-Ca^{2+} release, and mitochondrial-mediated apoptosis[44].

BIM, a BH3-only protein, and an activator of proapoptotic BAX and BAK, inhibits antiapoptotic BCL2 and MCL1 but has a higher affinity for the latter. BIM is induced in response to ER stress[44]. It is bound to dyenin motor complexes of the microtubule cytoskeleton in resting conditions. In response to ER stress, BIM is released from dyenin, and translocated to the ER. Apparently phosphorylation of BIM by JNK facilitates its release from dyenin and the subsequent activation of BAX and BAK[35,42]. In contrast, BCLx$_L$, an antiapoptotic protein binds BIM and inhibits its translocation to the ER[35]. BIM$^{-/-}$ cells resist ER stress-induced apoptosis, supporting the idea that it is a proapoptotic protein[50].

Role of ER-Ca^{2+} load and its release

Cellular Ca^{2+}, primarily stored in the ER, is maintained by SERCA (Sarcoplasmic Endoplasmic Reticulum Ca^{2+} ATPases) that actively pumps cytosolic Ca^{2+} into the ER, lectin chaperones that bind Ca^{2+}, and by inositol 1,4,5-triphosphate (IP3) or ryanodine receptor that passively releases Ca^{2+} from ER[51–53]. Ca^{2+} release generates high

calcium microdomains (~50–100 µM) at the ER–mitochondrial junctions that activate a low-affinity mitochondrial Ca^{2+} uniporter, resulting in mitochondrial Ca^{2+} uptake. This is possible because of physical and physiological coupling of ER and mitochondria[53,54]. ER-Ca^{2+} release channels are opened by Ca^{2+} and/or membrane-derived lipid inositol[44]. A disturbance in ER–mitochondrial Ca^{2+} levels that enhances mitochondrial Ca^{2+} uptake triggers disruption in membrane potential, activation of a mitochondrial permeability transition pore (PTP), cytochrome c release and apoptosis[44,53]. Reduction in ER-Ca^{2+} concentration offers protection against apoptosis[44]. For example, reduction of ER-Ca^{2+} load/stores by reducing extracellular Ca^{2+} levels and removal of calreticulin, a Ca^{2+} binding ER chaperone protects cells from apoptosis. In contrast, increasing ER-Ca^{2+} load/stores by over-expressing SERCA and calreticulin sensitizes the cells to apoptosis[55,56]. Further, ceramide or arachidonic acid produced by active phospholipase A in response to calcium influx into the cell, induces ROS production, ER-Ca^{2+} release, mitochondrial Ca^{2+} uptake, and apoptosis[57,58]. The magnitude of ER-Ca^{2+} release during stress depends on ER-Ca^{2+} load rather than on free Ca^{2+}. Further, the ability of the cells to the transfer ER-Ca^{2+} to mitochondria defines the severity of mitochondrial apoptosis. Agents that promote the uptake of released ER-Ca^{2+} by the mitochondria accentuate the process[46,53].

BCL2-family proteins regulate ER-Ca²⁺: Over-expression of BCL2 protects against thapsigargin (inhibitor of SERCA)-induced apoptosis. This is probably mediated by its inhibitory interaction with SERCA, inhibition of IP3-mediated ER-Ca^{2+} release, and down-regulation of calreticulin and SERCA expression[53,55]. Over-expression of proapoptotic BAX and BAK proteins that are localized both on mitochondrial and ER membranes, results in Ca^{2+} mobilization from the ER to the mitochondria and apoptosis[53,59]. BAX and BAK-deficient mouse embryonic fibroblasts (MEFs) have lowered ER-Ca^{2+} load, reduced mitochondrial Ca^{2+} uptake and are highly resistant to Ca^{2+}-dependent apoptotic stimuli. Correction of ER-Ca^{2+} load in these double knock-out cells by over-expressing SERCA restores cell death mediated by agents such as ceramide, arachidonic acid, and oxidative stress that causes the release of calcium from the ER[60]. The decline in ER-Ca^{2+} in the BAX/BAK double knockout cells may be due to the inhibition of SERCA, and passive leak of ER calcium through hyperphosphorylated IP3 receptors. Further, absence of BAX and BAK also enhances the interaction between antiapoptotic BCL2 and the IP3 receptor. Inhibition of BCL2 and IP3 receptor via RNA interference reduces ER-Ca^{2+} leak and restores ER-Ca^{2+} in these double knockout cells[44]. BAP31 (B-cell receptor-associated protein 31), an ER-transmembrane protein that exists in complex with cytosolic procaspase-8, is cleaved during ER stress. The cleaved proapoptotic p20-BAP31 can in-

duce ER-Ca^{2+} release and apoptosis. However, chelation of intracellular Ca^{2+} with BAPTA offers protection[44]. Anti-apoptotic BCL2 and $BCLx_L$ associate with the BAP31–procaspase-8 complex and inhibit their activation[44]. Cleavage of BAP31 and apoptosis is also inhibited in calnexin-deficient cells, suggesting that BAP31 activation may require calnexin[61]. Spike, a BH-3-only protein interacts with BAP31 and inhibits its interaction with anti-apoptotic proteins[44].

Targets of released ER-Ca²⁺: Release of ER-Ca^{2+} into the cytosol triggers calpain-mediated (Ca^{2+}-dependent protease) ER-resident caspase-12 cleavage and activation[44], and also enhances the proapoptotic potential of a BH-3-only protein, BAD, through its dephosphorylation mediated by a Ca^{2+}-dependent phosphatase, calcineurin[62]. Expression of ER-targetted BAX leads to depletion in ER-Ca^{2+} and activation[63] of caspase-12, whereas over-expression of BCL2 inhibits ER stress-induced caspase-12 activation and apoptosis[64] (Figure 1 b).

Activation of IRE-1-JNK

IRE-1-XBP-1 arm is the last prosurvival branch of the UPR[18,42]. However, persistent stress triggers the proapoptotic pathway of IRE-1 that recruits the kinase adaptor TRAF2 (tumour necrosis factor receptor-associated factor 2) and activates kinases such as ASK1 and JNK[65,66] (Figure 1 a). IRE-1–TRAF2 interaction also activates ATF3, a transcriptional repressor that triggers apoptosis[67]. Phosphorylation and oligomerization of IRE-1 promotes its interaction with, and clustering of, TRAF2. Mutation in the IRE-1 kinase domain disrupts this interaction. IRE-1 activation and TRAF2 recruitment[65,66] facilitate the formation of the heterotrimeric complex of IRE-1–TRAF2-ASK1 and activation of ASK1. ASK1 activates MKK3 and MKK6 that target p38 MAP kinases, and also MKK4 and MKK7 that target JNK[68]. It is not known whether the p38 kinase cascade has a prosurvival role, however, JNK activation mediates proapoptotic effects. IRE-1$^{-/-}$ fibroblasts are defective in apoptosis mediated by JNK activation and similarly, ASK$^{-/-}$ neurons resist ER stress-induced apoptosis. Over-expression of ASK1 sensitizes the cells to apoptosis[35,42,65].

Tumour necrosis factor receptor1 (TNFR1), a receptor similar to that involved in TNFα-induced apoptosis, also affects JNK activation through its interaction with IRE-1. TNFR1 null cells fail to activate JNK[35]. Surprisingly, TRAF2$^{-/-}$ cells are more sensitive to apoptosis suggesting that TRAF2–IRE-1 interaction has a dual role[35]. It is not known whether the IRE-1 interacting proteins, JIK (c-JUN-N-terminal inhibitory kinase) and JAB1 (Jun activation domain-binding protein 1), play a role in the pro- and antiapoptotic affects of IRE-1. JIK appears to facilitate IRE-1–TRAF2 interaction whereas JAB1 impedes the interaction[42]. However, in severe ER stress IRE-1–JAB1 in-
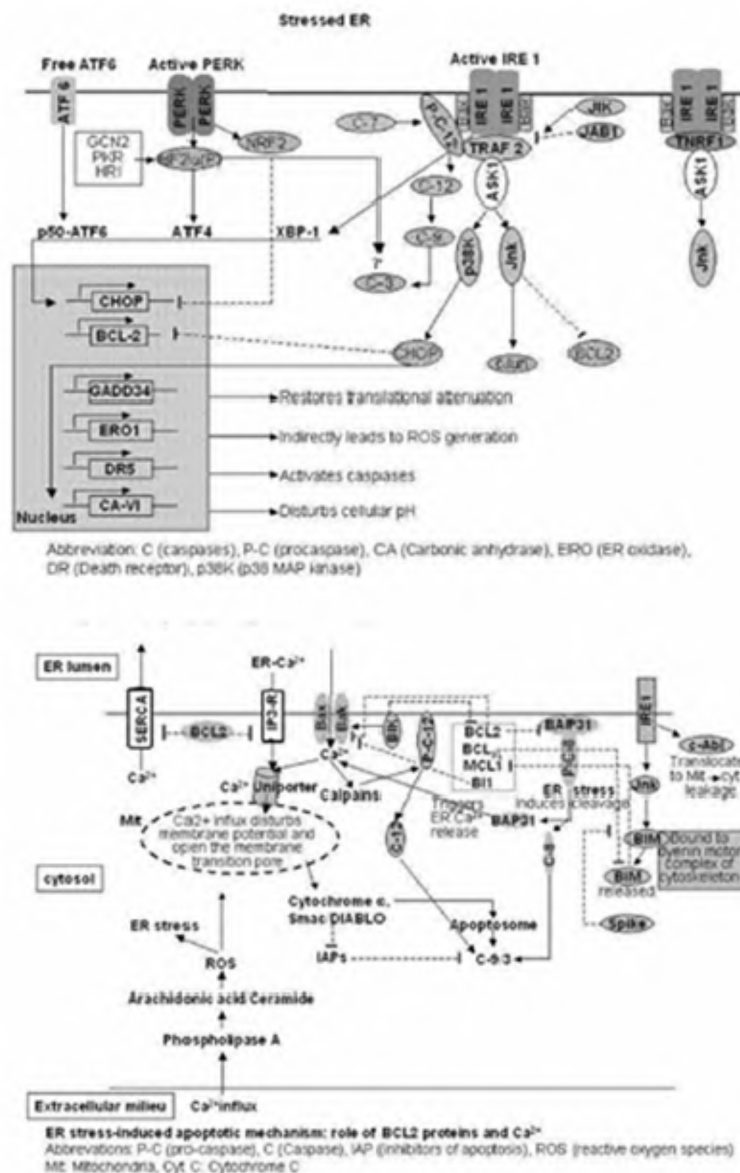
Figure 1. ER stress-induced apoptotic mechanisms. *a*, Role of ER stress transducers: Activation of ATF6, PERK and IRE1 leads to the generation of three *b*-ZIP transcription factors p50ATF6, ATF4, and XBP-1 respectively, that induce proapoptotic CHOP. ATF4 translational induction by active PERK or by cytosolic eIF2α kinases is mediated through the phosphorylation of ser[51] residue in eIF2α. PERK also phosphorylates transcription factor Nrf2 that stimulates the expression of genes involved in antioxidant response similar to ATF4. However, Nrf2, unlike ATF4 inhibits CHOP expression. CHOP-induced genes may promote apoptosis. PERK activation appears to be the most immediate adaptive event in ER stress that inhibits general translation and decreases the folding client influx into the ER. Proapoptotic BH 1–3 domain proteins BAX and BAK interact with the cytosolic domain of IRE-1 and modulate its activity. Active IRE-1 recruits TRAF2 that interacts with and activates ASK1. Active ASK1 phosphorylates JNK and p38 MAP kinase. Active JNK activates c-Jun, a proapoptotic transcription factor. p38 MAPK phosphorylates and activates CHOP. IRE-1 interacting proteins, JIK and JAB1, modulate IRE-1 and TRAF2 interaction. JIK promotes while JAB-1 impedes the interaction. IRE-1–TRAF2 complex also recruits and activates ER-associated procaspase-12. *b*, Role of BCL2 protein family and Ca²⁺: SERCA pumps calcium into the ER and inositol–3 phosphate receptors mediate the passive release of ER-Ca²⁺ release into the cytosol. BAX and BAK oligomerization on ER membranes may also facilitate ER-calcium release. Released ER-Ca²⁺ is taken up by the mitochondria and results in mitochondrial membrane depolarization, opening of the mitochondrial transition pore and release of small proapoptotic molecules like cytochrome *c* and Smac/DIABLO that leads to the assembly of apoptosome and activation of the caspase cascade. ER-Ca²⁺ release can also activate cytosolic calpain (Ca²⁺-dependent protease) and ER-associated caspase-12. Caspase-7 also translocates to the ER during stress and activates caspase-12. Extracellular Ca²⁺ influx into cytosol activates phospholipase A, which generates arachidonic acid or ceramide that induces ER stress or mitochondrial apoptosis through ROS generation. ER-localized BAP31 forms a complex with procaspase-8. ER stress-induced activation of BAP31 and caspase-8 initiates ER-Ca²⁺ release, and activation of downstream caspases respectively. BCL2 inhibits BAP31 interaction with procaspase-8. Active JNK phosphorylates BIM and facilitates its dissociation from the cytoskeleton and active BIM inhibits antiapoptotic MCL1 and BCL2 proteins. ER resident BIK inhibits BCL2 and facilitates BAX/BAK oligomerization on the ER. The interplay among the antiapoptotic BCL2 proteins, inactivator and activator BH3-only proteins and proapoptotic BAX and BAK modulates the ER-Ca²⁺ release and apoptotic process.

teraction is diminished[42], facilitating IRE-1 interaction with TRAF2–ASK1.

IRE-1α signalling is defective in BAX/BAK-deficient cells. Expression of BAK in these double-knockout cells restores ER stress response, XBP-1 splicing, and JNK activation[69]. In response to ER stress, active JNK1 phosphorylates BCL2 and inactivates it[42]. Phosphorylated BCL2 fails to inhibit the proapoptotic BH-3-only proteins and hence loses its control on ER-Ca^{2+} fluxes[70]. Down-regulation of BCL2 transcription and its inactivation by phosphorylation promotes the activity of proapoptotic BCL2 family members. Phosphorylated BCL2 is predominantly localized near the ER and is a target for proteasome. Dephosphorylation by PP2A protects BCL2 from proteasome degradation[71]. The interaction of IRE-1 and JNK activation is also linked to the activation of c-Abl, a tyrosine kinase, found on the ER apart from the nucleus and cytoplasm. c-Abl translocates to the mitochondria where it affects the release of cytochrome c in response to ER stress. c-Abl$^{-/-}$ cells resist ER stress[35].

CHOP

It is also known as C/EBPζ, GADD-153 or DDIT-3 (DNA-damage inducible transcript-3)[30]. CHOP is primarily proapoptotic and among the highest inducible genes during ER stress. For maximal induction of CHOP, however, all ER stress-response pathways are required because its promoter is regulated by four cis-acting elements[30] that can be transactivated by ATF4, ATF6 and XBP-1 and also by ATF2 and ATF3. Its induction is almost completely attenuated in cells lacking PERK or expressing the non-phosphorylatable form of eIF2α, suggesting that PERK–eIF2α phosphorylation plays a dominant role in CHOP induction during ER stress[72,73]. Since CHOP is downstream to ATF4-eIF2α phosphorylation, activation of other eIF2α kinases can also lead to CHOP induction. CHOP can heterodimerize with other bZIP transcription factors such as C/EBPs, ATF3 and ATF4, and can act as a transcriptional repressor or an activator[30]. CHOP expression leads to the induction of proapoptotic proteins such as GADD34 (cofactor of eIF2α phosphatase), ERO1 (endoplasmic reticulum oxidoreductin 1), DR5 (death receptor-5), carbonic anhydrase VI and TRB3 (Tribbles related protein-3)[35,40,42,74]. While ERO1 is an ER luminal protein, the others are cytosolic. In many ER stress models, induction or over-expression of CHOP sensitizes cells to ER stress-induced apoptosis, whereas CHOP deletion offers protection[30,40]. The proapoptotic effects of CHOP in ER stress may be mediated by the induction of GADD34, a cofactor of eIF2α phosphatase that dephosphorylates eIF2α[75,76]. Translational restoration in stressed and the overburdened ER aggravates ER stress by enhancing the folding client load. Alternatively the synthesis of proapoptotic proteins due to translational restoration

may trigger apoptosis[42]. GADD34 null mice resist ER stress-mediated nephrotoxicity induced by tunicamycin, like the CHOP null animals[75]. In contrast, GADD34 may also play a prosurvival role because mutations in GADD34 sensitize cells to thapsigargin treatment, substantiating the premise that GADD34-mediated translational restoration is required for adaptive cell-survival measures[40]. In mouse models of proteolipid protein mutation (Pelizaeus–Merzbacher leukodystrophy), interestingly over-expression of CHOP protects mice from apoptosis[40]. These findings raise the possibility that the CHOP–GADD34 signalling pathway has a dual role, protecting from and promoting cell death. Cells deficient of C/EBPβ, a major dimerization partner of CHOP, are resistant to ER stress-induced apoptosis[77], suggesting that CHOP's prosurvival and proapoptotic affects are modulated, mediated by protein–protein interactions[30].

It is likely that CHOP-induced cell death is mediated by other targets of CHOP, like ERO1α that helps PDI in disulphide bond formation. This oxidative protein-folding process involves the transfer of electrons to molecular oxygen and hence CHOP over-expression results in ROS generation, ER stress and cell death[4,40]. The ER stress-induced apoptosis mediated by the CHOP–GADD34–ERO1 pathway may be more pronounced in cells with elevated protein synthesis. Additionally, CHOP induced DR5 activates caspases and carbonic anhydrase VI affects changes in cellular hydrogen ion concentration, promoting apoptosis[35,40]. In contrast, the CHOP-induced TRB3 protein promotes apoptosis because of its ability to interact with and inhibit the prosurvival Akt kinase[42]. Akt activation may also signal TRB to inhibit the CHOP activity in a feedback loop[42]. The transcriptional ability and proapoptotic potential of CHOP is increased upon phosphorylation of its ser[78] and ser[81] residues by p38 MAP kinase[42,78]. In contrast to the ability of CHOP to upregulate certain genes, it down-regulates BCL2 expression by interacting with cAMP responsive element-binding protein, CREB, which induces BCL2 expression[30,42,79]. Over-expression of BCL2 diminishes CHOP induction[79]. CHOP plays a more significant role after birth than during embryogenesis, because CHOP$^{-/-}$ mice are born and develop normally without any embryonic and organ defects[30]. Interestingly, CHOP expression is not observed in pancreatic β-cell death of PERK$^{-/-}$ or homozygous eIF2α^{S51A} mutant mice, suggesting a redundancy in ER stress-mediated apoptotic pathways[40].

Paradoxical effects of eIF2α phosphorylation

Phosphorylation of ser[51] in eIF2α is a stress signal and is a negative regulator of general translation, however, it upregulates translation of certain gene-specific mRNAs[29] like ATF4. Initially phosphorylation of eIF2α was shown to promote apoptosis, but subsequent studies have shown

that it also protects from apoptosis. In fact, our own studies with *Sf9* insect cells projected an ambiguous nature of eIF2α phosphorylation[80]. For example, treatment of insect cells with agents like UV irradiation or cycloheximide promoted both eIF2α phosphorylation and apoptosis[80]. In contrast, treatment with tunicamycin, an inhibitor of protein glycosylation and an ER stress-inducing agent, displayed a good amount of eIF2α phosphorylation but not apoptosis, suggesting that eIF2α phosphorylation does not always lead to apoptosis[80]. It is likely that insect cells, unlike mammalian cells, may not possess ER-stress induced apoptotic mechanism(s) and hence do not undergo apoptosis. Interestingly, eIF2α phosphorylation can be a cause or a consequence of apoptosis because we and others have observed that caspases can also activate eIF2α kinases[80,81].

Involvement of diverse eIF2α kinases like PKR (double-stranded RNA-dependent kinase), HRI (heme-regulated kinase), GCN2 (general control non-derepressible) and PERK[82] may also be the reason for paradoxical effects of eIF2α phosphorylation. HRI, PKR and GCN2 are cytosolic whereas PERK is an ER-resident eIF2α kinase. Several lines of evidence now indicate that ER stress-induced eIF2α phosphorylation protects cells against apoptosis[73,83,84]. Loss of eIF2α phosphorylation due to PERK deficiency or abrogation of eIF2α phosphorylation, overloads the ER synthetic capacity of pancreatic β-cells and promotes apoptosis[40,84]. Complementing these observations, over-expression of GADD34, a cofactor of protein phosphatase1, is shown to promote apoptosis[75]. It has already been suggested that a type-1 phosphatase is involved in the dephosphorylation of eIF2α in physiological conditions and in herpes simplex virus (HSV) infection[85,86]. Further, a recent study has shown that salubrinal, a selective chemical inhibitor of eIF2α phosphatase, protects PC12 cells from ER stress-induced apoptosis promoted by tunicamycin or herpes simplex virus (HSV) infection[83]. Interestingly, salubrinal is also shown to potentiate the deleterious effect of free fatty acid-induced pancreatic β-cell death[87], suggesting that it is critical to determine as to what level/extent of eIF2α phosphorylation can offer protection or ensure cell death.

Further, ER stress does not always induce eIF2α phosphorylation as has been observed in plasma cells, probably due to expression of p58[IPK]; a cochaperone induced in ER stress and a negative regulator of eIF2α phosphorylation. It relieves the translational block imposed by eIF2α phosphorylation[88], similar to GADD34. However, the p58[IPK]-deficient mice show opposing effects compared with GADD34 mutant mice. Their pancreatic β-cells are sensitive to apoptosis and develop diabetes like Perk$^{-/-}$ mice[89].

Consistent with such paradoxical effects, a recent study describes activation of PKR, a cytosolic eIF2α kinase, and expression of a phosphomimetic S51D eIF2α mutant in Hela cells is found sufficient to stimulate caspase-3 ac-

tivity and induce apoptosis[90]. It is suggested that in ER stress, apparently the death-inducing property of eIF2α phosphorylation is not observed because it relieves the synthetic burden of the ER and facilitates production of other factors that dephosphorylate eIF2α. Caspase activation mediated by eIF2α phosphorylation is linked to a general decline in translation and degradation of the short-lived antiapoptotic factors, such as p53 or inhibitors of apoptosis (IAPs)[90]. These studies suggest that the effects of eIF2α phosphorylation on apoptosis are dependent on concomitant and parallel signalling events mediated by ER/cytosolic stress-induced eIF2α kinases and may vary based on the severity of the stress, and cell types involved.

The diversity and coincident signalling activities in cellular responses to eIF2α kinases may be different because eIF2α kinases like PERK and PKR can also phosphorylate other substrates such as Nrf2 and IKK respectively, and phosphorylation of these substrates affects the inter-protein interaction with their partners[31]. For example, ER stress-activated PERK phosphorylates transcription factor Nrf2 in mammalian cells that activates genes is involved in redox maintenance[31]. The PERK–Nrf2 or PERK–eIF2α–ATF4-induced antioxidant response can counter the ROS generated through multiple pathways by ER stress[31]. Interestingly, Nrf2 is shown to inhibit CHOP expression[31], the proapoptotic downstream target of PERK–eIF2α–ATF4 (as mentioned above). During UPR, the ER-associated translation is found to be active in spite of elevated levels of eIF2α phosphorylation[91]. The dephosphorylation of ER-juxtaposed eIF2α mediated by cofactors of PP1c (protein-phosphatase 1c) such as CReP (a constitutive activator of PP1)[92], GADD34 (a cofactor of PP1)[76], and NCK, an adaptor protein with a Src homology (SRH2/3 domains), may be the reason for the paradoxical effects of ER-stress associated eIF2α phosphorylation on translation and apoptosis[93]. In a recent study in rats, we observed enhanced expression of GADD34, concomitant with a decline in eIF2α phosphorylation with aging[94]. Also, in contrast to young rats, aged rats displayed higher levels of proapoptotic factors such as CHOP and phosphoJNK, and reduced amounts of ER stress adaptive protein BiP, suggesting that with aging, the adaptive response of UPR declines and the apoptotic potential is enhanced[94]. It appears that the expression of GADD34, a cofactor of eIF2α phosphatase during persistent ER stress, overrides the protective effects of eIF2α phosphorylation and may signal apoptosis. Alternatively, persistent eIF2α phosphorylation promoted by cytosolic eIF2α kinases in non ER-stress conditions, may stimulate a caspase activity as has been mentioned above[90].

In addition, a recent study implicates PKR expression and activation in ER stress. The level and activity of nuclear PKR is elevated substantially compared to cytoslic PKR in tunicamycin-treated SK-N-SH human neuro-

blastoma cells[95]. Active PKR is detected in the nuclei of autopsied brain, spinal cord and hippocampal tissues of Alzheimer's, amylotrophic lateral sclerosis, and Huntington's patients respectively, suggesting a role for PKR in ER stress and neurodegeneration[95,96]. Further, an inhibitor of PKR has been shown to protect neuroblastoma cells against tunicamycin-induced ER stress[96]. It is not known whether active PKR is generated as a consequence of ER stress-induced caspase activation[81] or in itself is a stimulus to apoptosis, as has been recently suggested[90]. It is also likely that ER stress-mediated activation of caspases may have caused the processing, activation and nuclear localization of PKR.

Phosphorylation of eIF2α also regulates the translation of mRNAs that code for cellular antiapoptotic proteins (IAPs). For example, ER stress induces human IAP-2 at the translational level[97], whereas the translation of certain IAPs decreases in response to PKR-induced eIF2α phosphorylation during TNF-α-induced apoptosis[90]. TDAG-51 (T-cell death associated gene 51), a pleckstrin related protein involved in atherosclerosis is induced in response to the UPR through the PERK pathway and over-expression stimulates ER stress-induced apoptosis[35].

Caspases

In rodents, the ER localized caspase-12 is possibly a key mediator in ER stress-mediated apoptosis. In ER stress[18,98], IRE-1–TRAF2 interaction is implicated in the activation of procaspase-12. Indirectly, calpains can also activate procaspase-12 upon ER Ca^{2+} release[44]. IRE-1 expression is shown to trigger caspase-12 activation and apoptosis[18]. In addition, during ER-stress[99], caspase-7 translocates to ER and promotes the activation of caspase-1. Caspase-12^{-/-} mouse embryonic fibroblasts are partially resistant to ER stress-induced apoptosis and caspase-12 activation is observed in many animal models of ER-stress associated disease[99]. However, the lack of functional caspase-12 questions the role of caspase-12 in ER stress-induced apoptosis in humans[99]. Human caspase-4 has been shown to associate with ER and may substitute for caspase-12 in ER stress-induced apoptosis[42]. ER transmembrane proteins, BAP31 and BAR (bifunctional apoptosis regulator) that occur in complex with procaspase-8, BCL2 and BCLx$_L$ also regulate procaspase-8 activation in ER stress[100]. In addition, ER stress also induces the activation of other caspases[35,42,99].

Future perspectives

UPR maintains ER homeostasis and has a critical role in professional secretory cells and also in growth, development and disease. It confers survival advantage for tumours in hypoxic conditions. UPR-induced transcriptional and translational programmes vary in different cell types. The mechanisms that activate specific arms of the UPR in certain cell types, and that which evoke UPR during various physiological and pathological stress conditions are still ambiguous. The intricacies like the dual nature of CHOP–GADD34 and eIF2α phosphorylation, and the ER to mitochondria signalling in apoptosis have yet to be understood. Understanding the prosurvival to proapoptotic signalling mechanisms of UPR and the stabilities of UPR-induced products can be of enormous potential in designing treatment modalities for various UPR-related metabolic and neurodegenerative disorders, tumours and viral infections. Drugs or small molecules that target the JNK pathway selectively would be attractive candidates for tackling type-2 diabetes. Modulation of the ER-stress signalling pathways, PERK–eIF2α/Nrf2–ATF4 or IRE1-XBP-1, and PDI, offer promise to resist tumour progression during hypoxia[41]. Alternatively, vectors encoding suicide genes under the ERSE promoter elements can be designed to specifically target tumours. Since persistent and overactive UPR triggers apoptosis, drugs that further enhance ER stress in tumours can flip the adaptive responses into apoptotic events rapidly. Keeping in view the above rationale, proteasome inhibitors like bortezomib (approved by FDA) have been found to inhibit the IRE1-XBP-1 pathway in multiple myeloma cells and sensitize them to apoptosis[17]. Transcriptional factor Nrf2, a PERK substrate can also be targetted for prevention of tumours. This view is substantiated by the inability of Nrf2 knockout mice to respond to the protective action of some tumour chemopreventive agents[31]. Viral infections trigger ER stress and viruses have developed strategies to counter host eIF2α phosphorylation for their propagation. Hence inhibitors that target viral strategies can be applied to control viral infection. In fact, salubrinal, an inhibitor of eIF2α phosphatase has been shown to inhibit HSV infection in rats. Small molecules such as pharmacological chaperones attract future approaches to tackle ER stress-related storage and conformational diseases.

1. Sitia, R. and Braakman, I., Quality control in the endoplasmic reticulum protein factory. Nature, 2003, 426, 891–894.
2. Selkoe, D. J., Folding proteins in fatal ways. Nature, 2003, 426, 900–904.
3. Tu, B. P. and Weissman, J. S., Oxidative protein folding in eukaryotes: Mechanisms and consequences. J. Cell Biol., 2004, 164, 341–346.
4. Meldolesi, J. and Pozzan, T., The endoplasmic reticulum Ca^{2+} store: A view from the lumen. Trends Biochem. Sci., 1998, 23, 10–14.
5. Hendershot, L. M., The ER function BiP is a master regulator of ER function. Mt. Sinai J. Med., 2004, 71, 289–297.
6. Zhao, L. and Ackerman, S. L., Endoplasmic reticulum stress in health and disease. Curr. Opin. Cell Biol., 2006, 18, 444–452.
7. Kaufman, R. J., Stress signalling from the lumen of the endoplasmic reticulum: Coordination of gene transcriptional and translational controls. Genes Dev., 1999, 13, 1211–1233.
8. Lee, A. S., Coordinated regulation of a set of genes by glucose and calcium ionophore in mammalian cells. Trends Biochem. Sci., 1987, 12, 20–24.

9. Ng, D. T., Watowich, S. S. and Lamb, R. A., Analysis *in vivo* of GRP78–BiP/substrate interactions and their role in induction of the *GRP78–BiP gene. Mol. Biol. Cell.*, 1992, **3**, 143–155.

10. Wu, J. and Kaufman, R. J., From acute ER stress to physiological roles of the unfolded protein response. *Cell Death Differ.*, 2006, **13**, 374–384.

11. Tirasophon, W., Welihinda, A. A. and Kaufman, R. J., A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes Dev.*, 1998, **12**, 1812–1824.

12. Haze, K., Yoshida, H., Yanagi, H., Yura, T. and Mori, K., Mammalian transcription factor ATF6 is synthesized as a trans-membrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol. Biol. Cell*, 1999, **10**, 3787–3799.

13. Shi, Y., Vattem, K. M., Sood, R., An, J., Liang, J., Stramm, L. and Wek, R. C., Identification and characterization of pancreatic eukaryotic initiation factor 2 α-subunit kinase, PEK, involved in translational control. *Mol. Cell. Biol.*, 1998, **18**, 7499–7509.

14. Harding, H. P., Zhang, Y. and Ron, D., Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature*, 1999, **397**, 271–274.

15. Bertolotti, A., Zhang, Y., Hendershot, L. M., Harding, H. P. and Ron, D., Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nature Cell. Biol.*, 2000, **2**, 326–332.

16. Dorner, A. J., Wasley, L. C. and Kaufman, R. J., Overexpression of GRP78 mitigates stress induction of glucose regulated proteins and blocks secretion of selective proteins in Chinese hamster ovary cells. *EMBO J.*, 1992, **11**, 1563–1571.

17. Lee, A. H., Iwakoshi, N, N., Anderson, K. C. and Glimcher, L. H., Proteasome inhibitors disrupt the unfolded protein response in myeloma cells. *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 90946–90951.

18. Wang, X. Z., Harding, H. P., Zhang, Y., Jolicoeur, E. M., Kuroda, M. and Ron, D., Cloning of mammalian Ire1 reveals diversity in the ER stress responses. *EMBO J.*, 1998, **17**, 5708–5717.

19. Bertolotti, A. *et al.*, Increased sensitivity to dextran sodium sulfate colitis in IRE1β-deficient mice. *J. Clin. Invest.*, 2001, **107**, 585–593.

20. Credle, J. J., Finer-Moore, J. S., Papa, F. R., Stroud, R. M. and Walter, P., On the mechanism of sensing unfolded protein in the endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA*, 2005, **102**, 18773–18784.

21. Zhou, J., Liu, C. Y., Back, S. H., Clark, R. L., Peisach, D., Xu, Z. and Kaufman, R. J., The crystal structure of human IRE1 luminal domain reveals a conserved dimerization interface required for activation of the unfolded protein response. *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 14343–14348.

22. Yoshida, H., Matsui, T., Yamamoto, A., Okada, T. and Mori, K., XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell*, 2001, **107**, 881–891.

23. Sidrauski, C., Cox, J. S. and Walter, P., tRNA ligase is required for regulated mRNA splicing in the unfolded protein response. *Cell*, 1996, **87**, 405–413.

24. Lee, K. *et al.*, IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signalling the unfolded protein response. *Genes Dev.*, 2002, **16**, 452–466.

25. Lee, A. H., Iwakoshi, N. N. and Glimcher, L. H., XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol. Cell. Biol.*, 2003, **23**, 7448–7459.

26. Yoshida, H., Matsui, T., Hosokawa, N., Kaufman, R. J., Nagata, K. and Mori, K., A time-dependent phase shift in the mammalian unfolded protein response. *Dev. Cell*, 2003, **4**, 265–271.

27. Sriburi, R., Jackowski, S., Mori, K. and Brewer, J. W., XBP1: A link between the unfolded protein response, lipid biosynthesis,

28. and biogenesis of the endoplasmic reticulum. *J. Cell Biol.*, 2004, **167**, 35–41.

28. Ramaiah, K. V., Davies, M. V., Chen, J. J. and Kaufman, R. J., Expression of mutant eukaryotic initiation factor 2 alpha subunit (eIF-2 alpha) reduces inhibition of guanine nucleotide exchange activity of eIF-2B mediated by eIF-2 alpha phosphorylation. *Mol. Cell. Biol.*, 1994, **14**, 4546–4553.

29. Harding, H. P. *et al.*, An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol. Cell*, 2003, **11**, 619–633.

30. Oyadomari, S. and Mori, M., Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell. Death Differ.*, 2004, **11**, 381–389.

31. Cullinan, S. B. and Diehl, J. A., Coordination of ER and oxidative stress signalling: The PERK/Nrf2 signaling pathway. *Int J. Biochem. Cell Biol.*, 2006, **38**, 317–332.

32. Schroder, M. and Kaufman, R. J., ER stress and the unfolded protein response. *Mutat. Res.*, 2005, **569**, 29–63.

33. Nadanaka, S., Yoshida, H. and Mori, K., Reduction of disulphide bridges in the lumenal domain of ATF6 in response to glucose starvation. *Cell Structure Funct.*, 2006, **31**, 127–134.

34. Hong, M., Luo, S., Baumeister, P., Huang, J. M., Gogia, R. K., Li, M. and Lee, A. S., Underglycosylation of ATF6 as a novel sensing mechanism for activation of the unfolded protein response. *J. Biol. Chem.*, 2004, **279**, 11354–11363.

35. Yoshida, H., ER stress and diseases. *FEBS J.*, 2007, **274**, 630–658.

36. Thuerauf, D. J., Marcinko, M., Belmont, P. J. and Glembotski, C. C., Effects of the isoform-specific characteristics of ATF6alpha and ATF6beta on ER stress response gene expression and cell viability. *J. Biol Chem.*, 2007 (Epub ahead of print).

37. Kaufman, R. J., Scheuner, D., Schröder, M., Shen, X., Lee, K., Liu, C. Y. and Arnold, S. M., The unfolded protein response in nutrient sensing and differentiation. *Nature Rev. Mol. Cell Biol.*, 2002, **3**, 411–421.

38. Luo, S., Mao, C., Lee, B. and Lee, A. S., GRP78/BiP is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development. *Mol. Cell. Biol.*, 2006, **26**, 5688–5697.

39. Iwakoshi, N. N., Lee, A. H., Vallabhajosyula, P., Otipoby, K. L., Rajewsky, K. and Glimcher, L. H., Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. *Nature Immunol.*, 2003, **4**, 321–329.

40. Marciniak, S. J. and Ron, D., Endoplasmic reticulum stress signalling in disease. *Physiol. Rev.*, 2006, **86**, 1133–1149.

41. Koumenis, C., ER stress, hypoxia tolerance and tumour progression. *Curr. Mol. Med.*, 2006, **6**, 55–69.

42. Szegezdi, E., Logue, S. E., Gorman, A. M. and Samali, A., Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep.*, 2006, **7**, 880–885.

43. Galonek, H. L. and Hardwick, J. M., Upgrading the BCL-2 network. *Nature Cell Biol.*, 2006, **8**, 1317–1319.

44. Oakes, S. A., Lin, S. S. and Bassik, M. C., The control of endoplasmic reticulum-initiated apoptosis by the BCL-2 family of proteins. *Curr. Mol. Med.*, 2006, **6**, 99–109.

45. Häcki, J., Egger, L., Monney, L., Conus, S., Rossé, T., Fellay, I. and Borner, C., Apoptotic crosstalk between the endoplasmic reticulum and mitochondria controlled by Bcl-2. *Oncogene*, 2000, **19**, 2286–2295.

46. Thomenius, M. J. and Distelhorst, C. W., Bcl-2 on the endoplasmic reticulum: Protecting the mitochondria from a distance. *J. Cell Sci.*, 2003, **116**, 4493–4499.

47. Lee, G. H. *et al.*, Bax inhibitor-1 regulates endoplasmic reticulum stress-associated reactive oxygen species and heme oxygenase-I expression. *J. Biol. Chem.*, 2007 (Epub ahead of print).

48. Wei, M. C. *et al.*, Pro-apoptotic BAX and BAK: A requisite gateway to mitochondrial dysfunction and death. *Science*, 2001, **292**, 727–730.

49. Li, J., Lee, B. and Lee, A. S., Endoplasmic reticulum stress-induced apoptosis: Multiple pathways and activation of p53-upregulated modulator of apoptosis (PUMA) and NOXA by p53. *J. Biol. Chem.*, 2006, **281**, 7260–7270.

50. Morishima, N., Nakanishi, K., Tsuchiya, K., Shibata, T. and Seiwa, E., Translocation of Bim to the endoplasmic reticulum (ER) mediates ER stress signalling for activation of caspase-12 during ER stress-induced apoptosis. *J. Biol. Chem.*, 2004, **279**, 50375–50381.

51. Berridge, M. J., Lipp, P. and Bootman, M. D., The versatility and universality of calcium signalling. *Nature Rev. Mol. Cell Biol.*, 2000, **1**, 11–21.

52. Camello, C., Lomax, R., Petersen, O. H. and Tepikin, A. V., Calcium leak from intracellular stores – The enigma of calcium signalling. *Cell Calcium*, 2002, **32**, 355–361.

53. Demaurex, N. and Distelhorst, C., Cell biology. Apoptosis – the calcium connection. *Science*, 2003, **300**, 65–67.

54. Rizzuto, R. *et al.*, Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science*, 1998, **280**, 1763–1766.

55. Pinton, P., Ferrari, D., Rapizzi, E., Di Virgilio, F., Pozzan, T. and Rizzuto, R., The Ca^{2+} concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: Significance for the molecular mechanism of Bcl-2 action. *EMBO J.*, 2001, **20**, 2670–2690.

56. Nakamura, K. *et al.*, Changes in endoplasmic reticulum luminal environment affect cell sensitivity to apoptosis. *J. Cell. Biol.*, 2000, **150**, 731–740.

57. Scorrano, L., Penzo, D., Petronilli, V., Pagano, F. and Bernardi, P., Arachidonic acid causes cell death through the mitochondrial permeability transition. Implications for tumour necrosis factor-alpha aopototic signalling. *J. Biol. Chem.*, 2001, **276**, 12035–12040.

58. Penzo, D. *et al.*, Arachidonic acid released by phospholipase A(2) activation triggers Ca(2+)-dependent apoptosis through the mitochondrial pathway. *J. Biol. Chem.*, 2004, **279**, 25219–25225.

59. Nutt, L. K., Pataer, A., Pahler, J., Fang, B., Roth, J., McConkey, D. J. and Swisher, S. G., Bax and Bak promote apoptosis by modulating endoplasmic reticular and mitochondrial Ca^{2+} stores. *J. Biol. Chem.*, 2002, **277**, 9219–9225.

60. Scorrano, L., Oakes, S. A., Opferman, J. T., Cheng, E. H., Sorcinelli, M. D., Pozzan, T. and Korsmeyer, S. J., BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: A control point for apoptosis. *Science*, 2003, **300**, 135–139.

61. Zuppini, A., Groenendyk, J., Cormack, L. A., Shore, G., Opas, M., Bleackley, R. C. and Michalak, M., Calnexin deficiency and endoplasmic reticulum stress-induced apoptosis. *Biochemistry*, 2002, **41**, 2850–2858.

62. Wang, H. G. *et al.*, Ca^{2+}-induced apoptosis through calcineurin dephosphorylation of BAD. *Science*, 1999, **284**, 339–343.

63. Zong, W. X., Li, C., Hatzivassiliou, G., Lindsten, T., Yu, Q. C., Yuan, J. and Thompson, C. B., Bax and Bak can localize to the endoplasmic reticulum to initiate apoptosis. *J. Cell. Biol.*, 2003, **162**, 59–69.

64. Contreras, J. L. *et al.*, Coupling endoplasmic reticulum stress to cell death program in isolated human pancreatic islets: effects of gene transfer of Bcl-2. *Transplant. Int.*, 2003, **16**, 537–542.

65. Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H. P. and Ron, D., Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science*, 2000, **287**, 664–666.

66. Nishitoh, H. *et al.*, ASK1 is essential for JNK/SAPK activation by TRAF2. *Mol. Cell*, 1998, **2**, 389–395.

67. Zhang, C., Kawauchi, J., Adachi, M. T., Hashimoto, Y., Oshiro, S., Aso, T. and Kitajima, S., Activation of JNK and transcriptional repressor ATF3/LRF1 through the IRE1/TRAF2 pathway is implicated in human vascular endothelial cell death by homocysteine. *Biochem. Biophys. Res. Commun.*, 2001, **289**, 718–724.

68. Roux, P. P. and Blenis, J., ERK and p38 MAPK-activated protein kinases: A family of protein kinases with diverse biological functions. *Microbiol. Mol. Biol. Rev.*, 2004, **68**, 320–344.

69. Hetz, C. *et al.*, Pro-apoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1alpha. *Science*, 2006, **312**, 572–576.

70. Bassik, M. C., Scorrano, L., Oakes, S. A., Pozzan, T. and Korsmeyer, S. J., Phosphorylation of BCL-2 regulates ER Ca^{2+} homeostasis and apoptosis. *EMBO J.*, 2004, **23**, 1207–1216.

71. Lin, S. S. *et al.*, PP2A regulates BCL-2 phosphorylation and proteasome-mediated degradation at the endoplasmic reticulum. *J. Biol. Chem.*, 2006, **281**, 23003–23012.

72. Harding, H. P., Novoa, I. I., Zhang, Y., Zeng, H., Wek, R., Schapira, M. and Ron, D., Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol. Cell*, 2000, **6**, 1099–1108.

73. Scheuner, D. *et al.*, Translational control is required for the unfolded protein response and *in vivo* glucose homeostasis. *Mol. Cell*, 2001, **7**, 1165–1176.

74. Ohoka, N., Yoshii, S., Hattori, T., Onozaki, K. and Hayashi, H., TRB3, a novel ER stress-inducible gene, is induced via ATF4–CHOP pathway and is involved in cell death. *EMBO J.*, 2005, **24**, 1243–1255.

75. Marciniak, S. J. *et al.*, CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev.*, 2004, **18**, 3066–3077.

76. Novoa, I., Zeng, H., Harding, H. P. and Ron, D., Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. *J. Cell. Biol.*, 2001, **153**, 1011–1022.

77. Zinszner, H. *et al.*, CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev.*, 1998, **12**, 982–995.

78. Maytin, E. V., Ubeda, M., Lin, J. C. and Habener, J. F., Stress-inducible transcription factor CHOP/gadd153 induces apoptosis in mammalian cells via p38 kinase-dependent and -independent mechanisms. *Exp. Cell Res.*, 2001, **267**, 193–204.

79. McCullough, K. D., Martindale, J. L., Klotz, L. O., Aw, Z. Y. and Holbrook, N. J., Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. *Mol. Cell. Biol.*, 2001, **21**, 1249–1259.

80. Aparna, G., Bhuyan, A. K., Sahdev, S., Hasnain, S. E., Kaufman, R. J. and Ramaiah, K. V., Stress-induced apoptosis in *Spodoptera frugiperda* (*Sf9*) cells: Baculovirus p35 mitigates eIF2 alpha phosphorylation. *Biochemistry*, 2003, **42**, 15352–15360.

81. Saelens, X., Kalai, M. and Vandenabeele, P., Translation inhibition in apoptosis: Caspase-dependent PKR activation and eIF2-alpha phosphorylation. *J. Biol. Chem.*, 2001, **276**, 41620–41628.

82. Wek, R. C., Jiang, H. Y. and Anthony, T. G., Coping with stress: eIF2 kinases and translational control. *Biochem. Soc. Trans.*, 2006, **34**, 7–11.

83. Boyce, M. *et al.*, A selective inhibitor of eIF2α dephosphorylation protects cells from ER stress. *Science*, 2005, **307**, 935–939.

84. Harding, H. P. *et al.*, Diabetes mellitus and exocrine pancreatic dysfunction in perk$^{-/-}$ mice reveals a role for translational control in secretory cell survival. *Mol. Cell*, 2001, **7**, 1153–1163.

85. Babu, S. V. and Ramaiah, K. V., Type 1 protein phosphatase inhibitors reduce the restoration of guanine nucleotide exchange activity of eukaryotic initiation factor 2B inhibited reticulocyte lysates rescued by hemin. *Arch. Biochem. Biophys.*, 1996, **327**, 201–208.

86. He, B., Gross, M. and Roizman, B., The gamma(1)34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1 alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA activated protein kinase. *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 843–848.

87. Cnop, M. *et al.*, Selective inhibition of eukaryotic translation initiation factor 2 alpha dephosphorylation potentiates fatty acid-induced endoplasmic reticulum stress and causes pancreatic beta-cell dysfunction and apoptosis. *J. Biol. Chem.*, 2007, **282**, 3989–3997.

88. Yan, W., Frank, C. L., Korth, M. J., Sopher, B. L., Novoa, I., Ron, D. and Katze, M. G., Control of PERK eIF2α kinase activity by the endoplasmic reticulum stress-induced molecular chaperone P58IPK. *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 15920–15925.

89. Ladiges, W. C. *et al.*, Pancreatic β-cell failure and diabetes in mice with a deletion mutation of the endoplasmic reticulum molecular chaperone gene P58IPK. *Diabetes*, 2005, **54**, 1074–1081.

90. Scheuner, D. *et al.*, Double-stranded RNA-dependent protein kinase phosphorylation of the alpha-subunit of eukaryotic translation initiation factor 2 mediates apoptosis. *J. Biol. Chem.*, 2006, **281**, 21458–21468.

91. Stephens, S. B., Dodd, R. D., Brewer, J. W., Lager, P. J., Keene, J. D. and Nicchitta, C. V., Stable ribosome binding to the endoplasmic reticulum enables compartment-specific regulation of mRNA translation. *Mol. Biol. Cell*, 2005, **16**, 5819–5831.

92. Jousse, C., Oyadomari, S., Novoa, I., Lu, P., Zhang, Y., Harding, H. P. and Ron, D., Inhibition of a constitutive translation initiation factor 2 alpha phosphatase, CReP, promotes survival of stressed cells. *J. Cell. Biol.*, 2003, **163**, 767–775.

93. Latreille, M. and Larose, L., Nck in a complex containing the catalytic subunit of protein phosphatase 1 regulates eukaryotic initiation factor 2 alpha signalling and cell survival to endoplasmic reticulum stress. *J. Biol. Chem.*, 2006, **281**, 26633–26644.

94. Hussain, S. G. and Ramaiah, K. V. A., Reduced eIF2alpha phosphorylation and increased pro-apoptotic proteins in aging. *Biochem. Biophys. Res. Commun.*, 2007, **355**, 365–370.

95. Onuki, R. *et al.*, An RNA-dependent protein kinase is involved in tunicamycin-induced apoptosis and Alzheimer's disease. *EMBO J.*, 2004, **23**, 959–968.

96. Shimazawa, M. and Hara, H., Inhibitor of double stranded RNA-dependent protein kinase protects against cell damage induced by ER stress. *Neurosci. Lett.*, 2006 **409**, 192–195.

97. Warnakulasuriyarachchi, D., Cerquozzi, S., Cheung, H. H. and Holcik, M., Translational induction of the inhibitor of apoptosis protein HIAP2 during endoplasmic reticulum stress attenuates cell death and is mediated via an inducible internal ribosome entry site element. *J. Biol. Chem.*, 2004, **279**, 17148–17157.

98. Yoneda, T., Imaizumi, K., Oono, K., Yui, D., Gomi, F., Katayama, T. and Tohyama, M., Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumour necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J. Biol. Chem.*, 2001, **276**, 13935–13940.

99. Szegezdi, E., Fitzgerald, U. and Samali, A., Caspase-12 and ER-stress-mediated apoptosis: The story so far. *Ann. NY Acad. Sci.*, 2003, **1010**, 186–194.

100. Xu, C., Bailly-Maitre, B. and Reed, J. C., Endoplasmic reticulum stress: Cell life and death decisions. *J. Clin. Invest.*, 2005, **115**, 2656–2664.