Bob van de Water, Hong Liu and James L. Stevens

ER Stress Response Proteins and Modulation of Cell Death

Abstract

Severe environmental stress induces cell death. This is often associated with the upregulation of stress response proteins including endoplasmic reticulum (ER) stress proteins like the glucose regulated proteins (GRPs) GRP78 and GRP94. Upregulation of these proteins provides a tolerance phenotype against a variety of subsequent lethal conditions. The exact mechanism of the upregulation of these ER stress proteins, their role in protection against cell death and the mechanism of their protection will be discussed.

Introduction

Cellular exposure to environmental stresses such as ischemia/reperfusion, drugs, heat shock, environmental pollutants or toxic chemicals generally causes the upregulation of stress proteins in various intracellular compartments including the cytosol, the nucleus and the endoplasmic reticulum (ER). For example, a variety of cellular stresses upregulates the expression of transcription factors such as c-Myc, c-Jun, and c-Fos, and also members of the heat shock family, including heat shock proteins HSP70, HSP 90 and HSP110 and the glucose regulated proteins GRP78 and GRP94. Different stress conditions can lead to an increased expression of different sets of stress proteins. It seems likely that the pattern of stress proteins expression and the severity of the insult determines the outcome after a toxic insult: i.e. cell recovery or death. Therefore, it is important to understand the induction and the role of different stress proteins following cellular stress.

A variety environmental stress conditions lead to induction of ER stress proteins, including GRP78 and GRP94. Here we will describe the function and role of ER stress proteins in cellular protection in relation to both apoptotic and necrotic cell death. Particular attention will be given to the discrete signaling pathways through which the ER can regulate different modes of cell death caused by different toxicants.

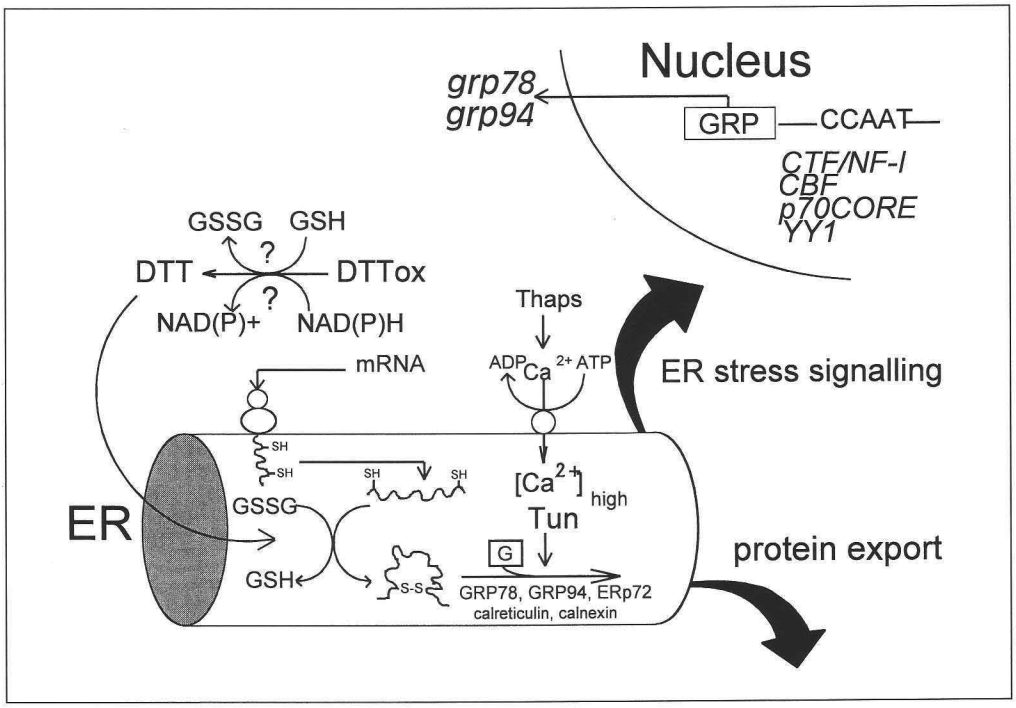

Fig. 1. Mechanisms of the induction of ER stress response proteins by different stress conditions. Perturbations of ER protein processing by DTTox, DTT, thapsigargin or tunicamycin, cause an accumulation of malfolded proteins in the ER. This results in the activation of different transcription factors that can activate specific elements in the promoter region of the genes ER stress protein. This leads to increased transcription and translation of ER stress proteins, including GRP78, GRP94, ERp72, calreticulin and PDI For further discussions see text.

ER Stress Response Proteins

ER Stress Proteins are Molecular Chaperones and Calcium Binding Proteins.

The ER is important in the processing of newly synthesised proteins made in the rough ER; proteins are folded and glycosylated prior to transport to the Golgi. The ER is also a major cellular free calcium store. $[Ca^{2+}]$ in the ER can be as high >1 mM; this is important for proper hormone responsive Ca^{2+} release as well as for normal ER protein processing.

Several proteins are involved in the processing of newly synthesised proteins in the ER. These include resident ER proteins such as GRP78/BiP and GRP94/endoplasmin, calreticulin, calnexin, protein disulphide isomerase (PDI), and Erp72 (Gething, 1994; Helenius, 1997). These ER proteins are retained in the ER due to their KDEL/HDEL retention motif (Fig. 1). They function as molecular chaperones and bind to partially folded newly synthesised, transmembrane and secretory proteins including integrins, viral proteins MHC class I and II (Gething, 1994; Helenius, 1997; Melnick,

1994). The different ER chaperones bind the unfolded proteins at discrete steps of the protein processing. For example, GRP78/BiP is involved in translocation of newly synthesised polypeptides across the ER membrane and stabilisation of partially folded or partially assembled proteins; PDI and ERp72 are involved in formation and rearrangement of disulphide bonds; calnexin and calreticulin bind predominantly (mono)glycosylated proteins, thereby preventing aggregation and promoting protein folding (Gething, 1994; Baksh, 1995; Herbert, 1995; Helenius, 1997).

Besides the chaperoning function most, if not all, of the ER chaperones are also Ca^{2+}-binding proteins (Michalak, 1992; Nigam, 1994). It is not entirely clear whether the calcium-binding capacity is necessary for the chaperoning function of ER stress proteins. However, perturbation of ER calcium by calcium ionophores or inhibition of the ER Ca^{2+}-ATPase with thapsigargin, interferes with the ER protein processing and causes ER stress protein induction (Gething, 1994). This indicates that there is a critical role for ER calcium homeostasis in ER protein processing.

Mechanism of ER Stress Protein Response

A variety of conditions induce an ER stress reponse, resulting in the upregulation of ER proteins including the GRPs, calreticulin, ERp72 and PDI (Dorner, 1991; Gething, 1994; Wenfeng, 1993). Expression of GRP78/BiP — the most abundant ER stress protein — is increased after glucose deprivation, perturbation of the ER calcium pool by calcium ionophores or thapsigargin, disturbance of the ER thiol-redox status with dithiothreitol (DTT) or β-mercaptoethanol, inhibition of protein glycosylation with tunicamycin and inhibition of vesicular transport with brefeldin A (Gething, 1994; Halleck, 1997; Kyu Kim, 1987; Wenfeng, 1993). All ER stress conditions have in common that they cause an accumulation of malfolded or unassembled proteins in the ER. Binding of GRP78 to these proteins, thereby lowering the amount of unbound GRP78, is believed to be the trigger for the ER stress response (Gething, 1994; Kozutsumi, 1988).

Although most of the studies on ER stress protein induction have been done *in vitro*, recent evidence indicates that also stressful conditions *in vivo* lead to an increase of GRP78. For example, ischemia/reperfusion injury of the brain in rats results in increased upregulation of *hsp70* and also *grp78* mRNA (Lowenstein, 1994). Moreover, we found that exposure of rats to nephrotoxic cysteine conjugates causes an upregulation of GRP78 protein in the rat renal cortex (Asmellash and Stevens, unpublished observation).

The signal transduction pathway involved in transmitting ER stress to the nucleus and activation of *grp78* transcription most likely involves several steps. First of all, the decrease in unbound GRP78, and possibly other chaperones, must be noticed by a sensor. Second, a mechanism should be present to transduce the signal from the sensor across the ER membrane to certain signal molecules in the cytoplasm; and, third, these signalling molecules should be able to either function as transcription factors themselves or activate other transcription factors that, in turn, will modulate transcription of ER stress proteins genes. In yeast an ER transmembrane protein kinase named IRE1, is required for the induction of the expression of ER stress proteins

(Cox, 1993). As yet, the mammalian homologue of IRE1 has not been identified. However, ER stress-induced upregulation of GRP78 does require both Tyr- and Ser/Thr-kinase activity and phorbol ester and cAMP do stimulate grp78 gene transcription (Cao, 1995; Hou, 1993; Prostko, 1991a).

Regulation of the promoter of GRP78 involves several transcription factors. CTF/NF-I is the best characterised and binds CCAAT or CCAAT-like motifs in the promoter of which the most proximal is important for transcriptional regulation of more upstream regulatory elements (Wooden, 1991) and is activated after exposure to tunicamycin, thapsigargin, A23187 and β-mercaptoethanol. Other transcription factors that bind the grp78 promoter have recently been identified and include CBF, p70CORE and YY1 (Li, 1994, 1997a; Roy, 1995). These interact with different stress regulatory elements of the GRP78/BiP core promoter region and binding results in transcriptional activation.

Suppression of stress-induced transcriptional activation of the grp78 promoter can also occur. The cold shock domain proteins dbpA and dbpB/YB-1 can prevent binding of the transcription factor YY1 to the stress inducible change region (SCIR) of the grp78 promoter, and thereby block the induction of the grp78 core element mediated by treatment of cells with A23187, thapsigargin or tunicamycin (Li, 1997b).

The promoter region of other ER stress proteins including GRP94, PDI, calreticulin, shares homology with the GRP78/BiP promoter and contain the Sp1 and CCAAT consensus sequences (Chang, 1989; McCauliffe, 1992). Since the promoter regions of different ER stress proteins share homology one might expect that other ER stress proteins are upregulated at the same rate as GRP78. This is however not the case. After ER stress GRP78 is the predominant ER stress protein upregulated. Therefore, it is likely that other factors also determine the pattern of upregulation of ER stress proteins.

ER Stress Response and Control of Cell Death

ER Stress Protein are Upregulated by Conditions that cause Cell Death

A variety of conditions that cause the induction of cell death also induce expression of ER stress proteins. For example, cytotoxicants, including, ionomycin, A23187 and thapsigargin, as well as deprivation of growth factors such as neuronal growth factor (NGF), or hypoxia/reoxygenation cause cell death in association with upregulation of GRP78 (Liu, 1997; McCormick, 1997; Aoki, 1997; Hori, 1996). These data suggest a relationship between cell death/survival and ER stress response. In some models induction of apoptosis by ER stress is associated with a lack of upregulation of ER stress proteins: thapsigargin-induced apoptosis in mouse lymphoma cells is associated with a lack of upregulation of GRP78 and GRP94; in contrast, cells that are resistant to thapsigargin have normal upregulation of GRPs (McCormick, 1997). Also competitive inhibition of a set of ER protein genes makes cells more sensitive to calcium ionophore treatment (Li, 1991). In the pig kidney renal epithelial cell line LLC-PK1, we also observed that thapsigargin causes apoptosis when the ER stress response is perturbed (see below). These data suggest that upregulation of GRPs

and/or other ER stress proteins is associated with cell survival, and that an insufficient upregulation will lead to cell death. Thus the upregulation of ER stress response proteins is critical for the eventual fate of the cell after a harmfull insult: survival or death.

Upregulation of ER Stress Protein provides Cellular Tolerance.

Various studies indicate that ER stress protein upregulation provides tolerance against a subsequent lethal insult. For example, upregulation of ER stress proteins by mild treatment with A23187, thapsigargin or tunicamycin provides a tolerant phenotype against subsequent lethal insults such as doxorubicin (Shen, 1987), etoposide (Chatterlee, 1994), oxidative stress (Gomer, 1991) and T-cell mediated cell killing (Sugawara, 1993). Although these harmful conditions may cause apoptosis, it has not been investigated whether preER stress protects by blocking necrosis, apoptosis or both. Moreover, the exact molecular mechanism by which ER stress proteins protect against otherwise lethal cell injury is largely unclear.

We have determined the mechanism underlying protection by ER stress against necrosis and apoptosis using different models. Ultimately, our goal is to understand how stress protein upregulation affects cell death after chemical damage *in vivo* and in the kidney in particular. In the first model, LLC-PK1 cells are treated with the alkylating agent iodoacetamide (IDAM). IDAM rapidly causes an upregulation of GRP78 in these cells (Liu, 1997). Also, IDAM depletes glutathione (GSH) in the cell and thereby increases the level of oxidative stress, followed by lipid peroxidation; the latter is involved in cell death by necrosis (Chen, 1991). Although prevention of lipid peroxidation with the lipophilic antioxidant N,N'-diphenyl-*p*-phenylenediamine (DPPD) inhibits necrosis it allows the onset of apoptosis (Chen, 1991; Liu, 1996; Van de Water, 1996). Because IDAM causes a rapid upregulation of GRP78 (Liu, 1997), indicating ER stress, we wanted to investigate the role of ER stress proteins in modulation of IDAM-induced cell death. Therefore, we pretreated cells with a variety of agents that induce an ER stress response. Upregulation of ER stress proteins by oxidized DTT (DTTox), thapsigargin or tunicamycin blocked both IDAM-induced necrosis and apoptosis in LLC-PK1 cells (Liu, 1997; Van de Water and Stevens, submitted).

In a second model we investigated the mechanism of thapsigargin-induced cell death in LLC-PK1 cells. In these cells thapsigargin itself does not cause apoptosis; however, thapsigargin treatment causes the upregulation of several ER stress proteins these conditions both GRP78, GRP94 but also calreticulin. When protein synthesis is inhibited with cycloheximide, thereby preventing upregulation of ER stress proteins, apoptosis is initiated (Van de Water and Stevens, submitted for publication). Cycloheximide itself did not cause cell death. Upregulation of ER stress proteins by tunicamycin, DTTox, A23187 or thapsigargin itself prior to thapsigargin/cycloheximide treatment, blocked apoptosis. These observations indicate that thapsigargin-induced ER stress activates different diverging signals: those that leading to the induction of ER stress proteins and those that are linked to the activation of the apoptotic machinery. Increased expression of ER stress proteins can modulate the signals that activate the apoptosis pathway.

Role of GRP78 in ER Stress-mediated Cytoprotection.

What are the ER stress proteins that modulate the apoptosis? GRP78 is the most abundant ER stress protein. The role of GRP78 in ER stress-mediated tolerant phenotype has been addressed in several studies. For example, overexpression of a targeted ribozyme directed to *grp94*, suppresses the expression levels of both GRP78 and GRP94 (Little, 1995). As a consequence, these cells are more sensitive against A23187, thapsigargin and tunicamycin-induced cell death. Similar observations were made in CHO cells that expressed a *grp78* antisense transcript (Li, 1992); and antisense *grp78*- mediated suppression of ER stress-induced upregulation of GRP78 eliminates the resistance to cell mediated cytotoxicity in fibrosarcoma B/C10ME cells (Sugawara, 1993). Importantly, in the latter case, these cells show less pronounced tumour progression in mice (Jamora, 1996).

We have analysed the role of GRP78 in IDAM-induced necrosis and apoptosis. For this purpose we have created LLC-PK1 cells that stably overexpress a 0.5 kbp *grp78* antisense (AS) transcript. In these cells the ER stress mediated upregulation of GRP78 is attenuated; this results in an attenuation of ER stress-mediated protection against IDAM-induced necrosis (Liu, 1997). However, in sharp contrast, AS*grp78*-LLC-PK1 cells are resistant against IDAM/DPPD-induced apoptosis; NEO vector control LLC-PK1 cells were killed at the same rate as normal LLC-PK1 cells. Interestingly, we found that these cells have increased levels of GRP94. In the AS*grp78* LLC-PK1 cells DTTox- induced upregulation of GRP78 is blocked. However, DTTox pretreatment is able to provide significant cytoprotection against apoptosis induced by high doses of IDAM (Table 1). This suggests that upregulation of ER stress proteins different from GRP78, also have a role in the cytoprotection against cell death. Moreover, our observation that DTTox pretreatment does not mediate cytoprotection in IDAM-induced necrosis but provides cytoprotection in IDAM-induced apoptosis indicates the involvement of distinct molecular pathways in ER stress-mediated tolerance against necrosis and apoptosis.

Altogether, these data indicate an important role for GRP78 in the ER stress mediated tolerance phenotype. However, other ER stress proteins are also important and may play a role in separate ER stress signaling pathways.

Mechanism of ER Stress Mediated Protection

GRP78-mediated Cytoprotection requires a Functional ATPase Domain.

The ATPase activity of GRP78 is important for cytoprotection. Like other hsp70 family members, GRP78 has ATPase activity (Gething, 1994). This activity is required for the release of associated proteins, as determined in vitro with recombinant, ATPase deficient, GRP78 (Wei, 1995). In line with this, expression of ATPase mutant GRP78 in COS cells causes disruption of the ER, which is dependent on the protein binding capacity of GRP78 (Hendershot, 1995). Overexpression of GRP78 itself is sufficient to protect against calcium ionophore induced cell death and is associated with an inhibition of ER stress-induced upregulation of GRP78 (Morris, 1997).

Table 1. Effect of antisense grp78 on DTTox-mediated cytoprotection against IDAM-induced cell death.

Cell line	Treatment	% Cell Death			
		IDAM			
		0 μM	100 μM	150 μM	200 μM
pkNEO (clone 10)	**IDAM**	6.3 ± 3.3^{A}	19.1 ± 0.6^{B}	$33.7 \pm 3.1^{C,D}$	39.7 ± 4.8^{D}
pkNEO (clone 10)	**preDTTox/IDAM**	7.3 ± 1.4^{A}	10.6 ± 3.6^{A}	17.5 ± 0.5^{B}	26.4 ± 1.3^{C}
pkASgrp78 (clone 10)	**IDAM**	3.3 ± 0.3^{A}	10.0 ± 0.4^{B}	12.3 ± 1.2^{B}	39.7 ± 4.4^{D}
pkASgrp78 (clone 10)	**preDTTox/IDAM**	4.2 ± 0.9^{A}	$7.9 \pm 1.5^{A,B}$	12.0 ± 1.5^{B}	25.2 ± 3.7^{C}

LLC-PK1 cells overexpressing an antisense *grp78* construct (pkASgrp78) or NEO-resistant control (pkNEO) were treated with 200 μM IDAM plus 20 μM DPPD for 45 min in EBSS. Thereafter, cells were allowed to recover in complete medium containing 20 μM DPPD. After 24 hr the % cell death was determined as described in Methods. Data are mean ± S.D. (n=3). Data are representative for two independent experiments. Significant differences were determined by ANOVA as described in Methods. Means with a different letter designation are significantly different ($p<0.05$).

In contrast, overexpression of the ATPase mutant GRP78, does not provide cytoprotection and does not block ER stress-mediated induction of *grp78* mRNA, indicating that the ATPase activity of GRP78 is necessary for the induction of a tolerant phenotype (Morris, 1997).

ER Stress Proteins as Calcium-binding Proteins

The ER stress proteins GRP78, GRP94, calnexin and calreticulin possess calcium binding capacity (Michalak, 1992; Nigam, 1994; Villa, 1991; Macer, 1988). Yet, little is known about the contribution of the calcium binding function in cytoprotection. Because cellular calcium perturbation is a critical factor in mechanisms of cell death in a variety of models (Liu, 1997; Van de Water, 1994), it is possible that ER stress-mediated cytoprotection is a result of a modulation of cellular calcium homeostasis.

Calreticulin is the best studied ER calcium binding protein. It has one high-affinity/low capacity (K_d=1.6 μM and B_{max} = 1 mol/mol) and several low capacity/high capacity (K_d=0.3-2.0 mM and B_{max}=20-50 mol/mol protein) Ca^{2+}-binding sites (Michalak, 1992). Overexpression of calreticulin in cells increases the calcium buffering capacity of the ER and blocks several hormone-mediated cellular calcium responses (Bastianutto, 1995; Camacho, 1995). ER stress increases the expression of calreticulin (Llewellyn, 1996; Plakidou-Dymock, 1994; Van de Water and Stevens, submitted for publication). We have used overexpression of calreticulin to study the role of ER calcium perturbations in the induction of both IDAM-induced necrosis and apoptosis. Overexpression of calreticulin blocks the IDAM-induced necrosis but has no effect on IDAM/DPPD-induced apoptosis (Liu et al, in press; Van de Water and Stevens; submitted). In contrast, calreticulin blocks apoptosis caused by thapsigargin treatment in the presence of cycloheximide. In the latter model buffering of intracellular calcium with the EGTA acetoxymethylester or removing extracellular calcium has no effect on thapsigargin/CHX-induced apoptosis of LLC-PK1 cells

(Van de Water and Stevens, submitted). These observations strongly suggest that emptying of ER calcium pools, and an increase of cytosolic Ca^{2+}, is primarily responsible for the initiation of thapsigargin- induced apoptosis. Yet, increases in cytoplasmic free Ca^{2+} related to ER calcium perturbations, may be linked to cytotoxicant-induced necrotic cell death (Liu, 1997; Van de Water, 1994).

Protection of Bcl-2 against apoptosis has been linked to modulation of the ER calcium pool (Distelhorst, 1996a, 1996b). Forced overexpression of Bcl-2 blocks thapsigargin-induced apoptosis in lymphoma cells (Distelhorst, 1996a; Lam, 1994), and Bcl-2 delays the emptying of the ER calcium pool after both thapsigargin or hydrogen peroxide treatment (Baffy, 1993; Lam, 1994). However, more recent studies indicate that Bcl-2 protection is independent of modulation of intracellular calcium stores (Reynolds, 1996). In our hands, overexpression of Bcl-2 has no effect on thapsigargin-induced cell death in LLC-PK1 cells. Yet, the ER calcium binding protein calreticulin blocks thapsigargin-induced apoptosis in these cells, indicating a direct role for ER calcium perturbations in thapsigargin-induced cell death (Van de Water and Stevens, submitted). These data indicate that Bcl-2 function is not simply related to calcium-perturbations; it may well be that Bcl-2 has different functions in different cell systems.

In summary, these data indicate that fundamental differences exist in the physiological role of ER calcium perturbations in necrosis and apoptosis. In addition, perturbations of ER calcium plays different roles in different models of apoptosis in different cell types.

Translational tolerance

Protein synthesis is controlled by a variety of translation initiation factors. Calcium-induced stress by calcium ionophore ionomycin or A23187 causes inhibition of protein synthesis, which is associated with phosphorylation of the eukaryotic translation initiation factor eIF2α at serine-51 (De Haro, 1996). Also other ER stresses including DTT, thapsigargin and EGTA cause a phosphorylation of eIF2α that is associated with inhibition of eIF2B (Brostrom et al., 1995; Prostko, 1992). eIF2B is a guanine nucleotide exchange factor which activity is closely linked to recycling of eIF2 (De Haro, 1996). The latter mediates the binding of Met-tRNA to the ribosome in a GTP-dependent manner. Phosphorylated eIF2α binds eIF2B thereby blocking its activity. After ionophore treatment the predominant stress protein upregulated is GRP78. Prior upregulation of ER stress proteins protects against subsequent ER stress-induced phosphorylation of eIF2α, and, thereby, provides translational tolerance (Brostrom, 1995). Also forced overexpression of GRP78 blocks the calcium ionophore-induced translational inhibition in association with a cytoprotective phenotype (Morris, 1997). Thus, these data suggest a direct link between ER stress mediated cytoprotection and translational control.

We also found that ER stress mediated cytoprotection against IDAM/DPPD-induced apoptosis is associated with translational tolerance phenotype. IDAM/DPPD treatment causes a drastic decrease in stress protein synthesis and protein synthesis in general. Pretreatment of cells with DTTox, thereby upregulating ER stress proteins,

did inhibit the IDAM/DPPD-induced block of protein synthesis (Van de Water and Stevens, submitted).

Recent data also suggest a link between translation regulation and the modulation of cell death. eIF4E is a cap binding protein that is involved in the transfer of mRNA to the 40S ribosomal subunit, a rate limiting step in translation initiation. Overexpression of eIF4E blocks apoptosis in growth factor restricted c-Myc overexpressing fibroblasts (Polunovsky, 1996). This protection is not linked to an increased expression of anti-apoptotic proteins such as Bcl-2 or Bcl-X_L. Intriguingly, eIF4E overexpression is associated with a transformed phenotype including the ability to form colonies in soft agar and tumorigenicity in nude mice (Polunovsky, 1996). Also, DAP-5, a recently cloned novel homologue of eukaryotic translation initiation factor 4G (p220) that binds eIF4E and promotes cap-binding independent translation, protects against cell death induced by interferon-γ when overexpressed in cells (Levy-Strumpf, 1997). These data indicate that modulation of translation might be a central event in the induction of apoptosis by diverse stimuli.

Activation of other Signalling Pathways after ER Stress

Activation of RNA Dependent Kinases

Transcriptional activation of ER stress protein genes is one signalling pathway that is activated after ER stress. However, other pathways are also activated. After ER stress, eIF2α is phosphorylated (see above). Phosphorylation of eIF2α occurs via a specific kinase. Different eIF2α kinases have been cloned, including the rabbit heme regulated protein kinase HRI, the human double stranded RNA dependent protein kinase, PKR, and the protein kinase GCN2 cloned from yeast (De Haro, 1996). Overexpression of dominant negative PKR prevents ER stress-induced phosphorylation of eIF2α and translational inhibition; this indicates a direct link between ER stress and PKR activation (Brostrom, 1996). The fact that eIF2α is phosphorylated after ER stress in association with apoptosis (Alcazar et al., 1995), suggest a role for eIF2 alpha kinases in modulation of cell death after ER stress.

Recent data indicate that activation of PKR by different conditions is linked to apoptosis. Thus, viral infection, interferon gamma exposure and dsRNA activate PKR and induce apoptosis (Takizawa, 1996). Moreover, overexpression of PKR itself causes a Bcl-2 dependent form of apoptosis that is mediated by ICE-like proteases (Lee, 1994, 1997). Bcl-2 does not block the PKR mediated inhibition of translation. Recent data indicate a direct role for PKR in mediating some forms of apoptosis. For example, PKR deficient fibroblasts from PKR null mice are resistant to apoptosis induced by dsRNA, TNF and lipopolysaccharide (Der, 1997). Similar observations were made in other cell types using overexpression of mutant PKR or using PKR antisense transcript (Takizawa, 1996; Yeung, 1996).

As yet, the relationship between ER stress and PKR activation in relation to cell death is unclear. Moreover, it will be interesting to see if ER stress proteins can modulate ER stress induced activation of PKR and thereby block the apoptosis.

NFκB Activation

NFκB is an important transcription factor involved in a variety of stress responses. It is present in the cytosol where it is inactive due to complex formation with IκB. Phosphorylation of IκB as a result of stimuli such as UV-radiation, inflammatory cytokines, and oxidative stress causes dissociation of the NFκB/IκB complex. NFκB is then translocated to the nucleus where it can transcriptionally activate specific target genes (Pahl, 1997).

ER stress can also induce activation of NFκB (Pahl, 1995, 1997). Thus, various stimuli such as tunicamycin, deoxyglucose, thapsigargin and brefeldin A cause NFκB activation. However, ER stress does not universally lead to NFκB activation. Thus, although DTT and mercaptoethanol upregulate GRP78, they do not activate NFκB, but, in contrast, inhibit its activation caused by other stimuli. Given that a variety of antioxidants block NFκB activation, it may be that thiol-based reductants that perturb the ER, fail to activate NFκB since they inhibit ROS production. On the other hand TNF is a good activator of NFκB, however, TNF does not upregulate GRP78 (Pahl, 1997a, 1997b).

These data indicate another potentially important route for ER stress related signalling in mechanisms that lead to a tolerant phenotype. it will be interesting to determine the role of ER stress proteins in the modulation of NFκB, and whether ER stress mediated NKκB activation is involved in the induction of a ER stress mediated tolerant phenotype against a variety of harmful conditions that lead to apoptosis or necrosis.

Summary and future directions

The above data provide a convincing link between ER stress response proteins and modulation of both necrosis and apoptosis. Most of the information on ER-mediated cytoprotection has been investigated in simple cell lines such as CHO cells or fibroblasts. Future effort should focus on the role of ER stress in cytoprotection against a different stresses in other more physiological relevant cells and with clinically relevant agents. For example, it will be important to investigate the mechanism of ER mediated cytoprotection against etoposide- and doxorubicin-induced cell death in more detail. In general, solid tumours are hypoxic, which causes upregulation of ER stress proteins. This, on its turn, can provide protection against cell death induced by etoposide, doxorubicin or cell-mediated toxicity.

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