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Review

Cell fate following ER stress: just a matter of "quo ante" recovery or death?

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Summary. The endoplasmic reticulum (ER) is a complex and multifunctional organelle. It is the intracellular compartment of protein folding, a complex task, both facilitated and monitored by ER folding enzymes and molecular chaperones. The ER is also a stress-sensing organelle. It senses stress caused by disequilibrium between ER load and folding capacity and responds by activating signal transduction pathways, known as unfolded protein response (UPR). Three major classes of transducer are known, inositol-requiring protein-1 (IRE1), activating transcription factor-6 (ATF6), and protein kinase RNA (PKR)-like endoplasmic reticulum kinase (PERK), which sense with their endoluminal domain the state of protein folding, although the exact mechanism(s) involved is not entirely clear. Depending on whether the homeostatic response of the UPR is successful in restoring an equilibrium between ER load and protein folding or not, the two possible outcomes of the UPR so far considered have been life or death. Indeed, recent efforts have been devoted to understand the life/death switch mechanisms. However, recent data suggest that what appears to be a pure binary decision may in fact be more complex, and survival may be achieved at the expenses of luxury cell functions, such as expression of differentiation genes.

Key words: Unfolded protein response, IRE, ATF6, PERK

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Introduction

The lumen of the ER is the first intracellular compartment reached by most secretory and membrane proteins (cargo proteins). The lumen of the ER provides an ideal environment where cargo proteins acquire the native conformation (folding) and a specific set of intrachain and, eventually, interchain disulphide bridges, a process known as oxidative folding. These are complex tasks, both facilitated and monitored by ER folding enzymes and molecular chaperones. Properly folded proteins will be exported out of the ER along the secretory pathway, whereas misfolded proteins will eventually be disposed of by an ER-associated protein degradation pathway (ERAD).

The load that cargo proteins impose on ER varies depending on physiological and pathological state. An imbalance between ER load and folding capacity is referred to as ER stress. So, ER stress can arise by an increase in protein load such as occurs in highly secretory cells such as B lymphocyte and pancreatic beta cells, when they differentiate to plasma cells or respond to glucose by increasing proinsulin synthesis. ER stress can be secondary to conditions that disrupt protein folding in the ER, such as hypoxia, inhibition of protein glycosylation, perturbation of the redox potential of the ER, Ca^{2+} depletion from the ER lumen and a variety of mutations that affect the ability of a protein to fold correctly.

When ER stress ensues, cells attempt to adjust the protein folding capacity to meet the new protein load or to counteract protein misfolding events through activation of transduction pathways that constitute the unfolded protein response (UPR). This response is activated by transducers, ER resident transmembrane

proteins that sense ER stress with their luminal domain and signal with their cytoplasmic domain. In mammalian cells, the UPR has three major functional components. The first component is the early and transient attenuation of protein synthesis that elicits a reduction of the protein load. The second component is the transcriptional activation of genes encoding, among others, components of the ER protein folding and degradation machinery. The third component is the induction of programmed cell death. Three classes of ER stress transducers have been identified: inositol-requiring protein-1 (IRE1), activating transcription factor-6 (ATF6), and protein kinase RNA (PKR)-like endoplasmic reticulum kinase (PERK) (Ron and Walter, 2007).

UPR signaling branches

The IRE branch

The UPR was first characterized in yeast, where a single pathway, emanating from IRE1p, constitutes the UPR. IRE1p oligomerizes in response to unfolded proteins allowing *trans*-autophosphorylation of the kinase domains, which constitute the only known substrate of the IRE kinase. *Trans*-autophosphorylation of the kinase domain activates the endonuclease activity of IRE1, which catalyzes the unconventional splicing of the mRNA of the transcription factor homologous to ATF/CREB1 (HAC1).

In metazoan cells, the UPR transducer IRE1 is well conserved (Wang et al., 1998; Shen et al., 2001; Hollien and Weissman, 2006) and, in mammalian cells, two IREs are present, IRE1α/ß [IRE1α is expressed ubiquitously (Tirasophon et al., 2000), whereas IRE1ß is expressed only in the gut (Bertolotti et al., 2001)]. In metazoans, X-box binding protein-1 (XBP1) substitutes HAC1 (Calfon et al., 2002) and the splicing event determines a frame shift with a resultant protein with the original N-terminal DNA-binding domain and an additional transactivation domain at the C-terminus (Yoshida et al., 2001; Calfon et al., 2002). In yeast, the *HAC1* mRNA intron represses translation (Ruegsegger et al., 2001) while in metazoans both the precursor and spliced form of XBP1 are translated (Calfon et al., 2002; Yoshida et al., 2006). However, the encoded proteins have different functional properties that derive from their above outlined different structure. XBP1 encoded in the spliced mRNA is more stable (Calfon et al., 2002) and works as a potent activator of UPR target genes (Yoshida et al., 2001), whereas the protein encoded by the precursor mRNA is labile and represses UPR target genes. Recently, novel insights into how HAC1/XBP1 mRNA is recognized by IRE1p/IRE1 have been reported. In unstressed yeast, HAC1 mRNA translation is blocked by a base pairing interaction between the *HAC1* intron and the *HAC1* 5' untranslated region (Ruegsegger et al., 2001). Upon ER stress, Ire1p molecules cluster into higher-order oligomers, to which unspliced *HAC1* mRNA is recruited by means of a conserved bipartite targeting stem loop contained in the 3' untranslated region (Aragon et al., 2009). In contrast, in mammals, the normally translated unspliced XBP1 protein associates with the ER membrane and recruits to the membrane XBP1 mRNA through a conserved region at its C-terminus. This provides close proximity between IRE1 and XBP1 mRNA facilitating, upon IRE1 activation, its splicing (Yanagitani et al., 2009).

In yeast, IRE1p and HAC1 function in a linear pathway, i.e., there is a substantial overlap of gene expression defect in cells that lacked either gene (Niwa et al., 2005). In metazoans, IRE1α and XBP1 knockout mice exhibit a similar embryonic lethality, suggesting that XBP1 mediates crucial functions of IRE1α (Reimold et al., 2000; Urano et al., 2000). However, expression profiling has shown that *C. elegans* with mutations in IRE1 and XBP1 display non-overlapping defects, suggesting that the IRE1-XBP1 pathway is not linear. In fact, mammalian IRE binds TNFR-associated factor 2 (TRAF2) and activates apoptosis signal-regulating kinase 1 (ASK1) and cJun-N-terminal kinase (JNK) pathway (Urano et al., 2000; Nishitoh et al., 2002). IRE1α also activates p38, ERK (Nguyen et al., 2004), and NF-κB pathways (Leonardi et al., 2002; Hu et al., 2006). The IRE1-ASK1/JNK pathway has been linked to the activation of apoptosis under irreversible ER stress (Nishitoh et al., 2002; Kim et al., 2009). Moreover, it has been shown that IRE1 in *D. melanogaster* cells, independently from XBP1 splicing, mediates the cleavage and degradation of mRNAs encoding proteins that traverse the secretory pathway (Hollien and Weissman, 2006), and that this pathway, called RIDD (Regulated IRE1-Dependent Decay), is conserved in mammalian cells (Hollien et al., 2009).

Spliced XBP1 controls the induction of a wide spectrum of UPR-related genes involved in protein folding, ERAD, cotraslational translocation and quality control (Lee et al., 2003; Shaffer et al., 2004). Using genome-wide approaches, the regulatory circuitry governed by XBP1 has recently been clarified. In addition to genes involved in constitutive maintenance of ER function in all cell types, unexpected targets were identified that link XBP1 to neurodegenerative and myodegenerative diseases, as well as to DNA damage and repair pathways, redox homeostasis and oxidative stress response (Acosta-Alvear et al., 2007).

In addition, XBP1 regulates the expansion of the secretory pathway by controlling phospholipids biosynthesis and ER/Golgi biogenesis (Shaffer et al., 2004; Sriburi et al., 2004).

The ATF6 branch

This pathway is initiated by ATF6α and ATF6ß, type II ER transmembrane proteins whose cytosolic domain encodes a bZIP transcriptional factor. Upon ER stress, ATF6s are translocated to the Golgi where they are cleaved by the same proteases that process the related sterol-regulated transcription factors, sterol regulatory

element binding proteins (SREBP-1/2). These proteases liberate the NH2-terminus of ATF6s, which migrates to the nucleus, where it activates transcription of target genes related to folding and ERAD among others, including the proapoptotic transcription factor CHOP, although, in the induction of CHOP, ATF4 predominates (Yoshida et al., 1998, 2000; Haze et al., 1999; Wang et al., 2000; Yamamoto et al., 2007). Wu et al. (2007) generated ATF6α -/- mice, while Yamamoto et al. (2007) have also generated ATF6ß -/- mice. Both find that ATF6α is nonessential for embryonic and postnatal development. Yamamoto et al. (2007) further demonstrated that ATF6ß-deficient animals develop normally, while the ATF6α/ß double knockout is an embryonic lethal. Interestingly, ATF6α heterodimerizes with XBP1 for the induction of unfolded protein response element (UPRE)-containing genes, such as Edem and HRD1, ERAD components (Wu et al., 2007; Yamamoto et al., 2007), and ATF6α may also modulate lipid biosynthesis and ER expansion under stress conditions (Bommiasamy et al., 2009).

Recently a number of ATF6α-related proteins have been identified. To date, five proteins-Luman (CREB3), Oasis (CREB3L1), BBF2H7 (CREB3L2), CREBH (CREB3L3), and Tisp40 (CREB4, CREB3L4)-share a region of high sequence similarity with ATF6α: a

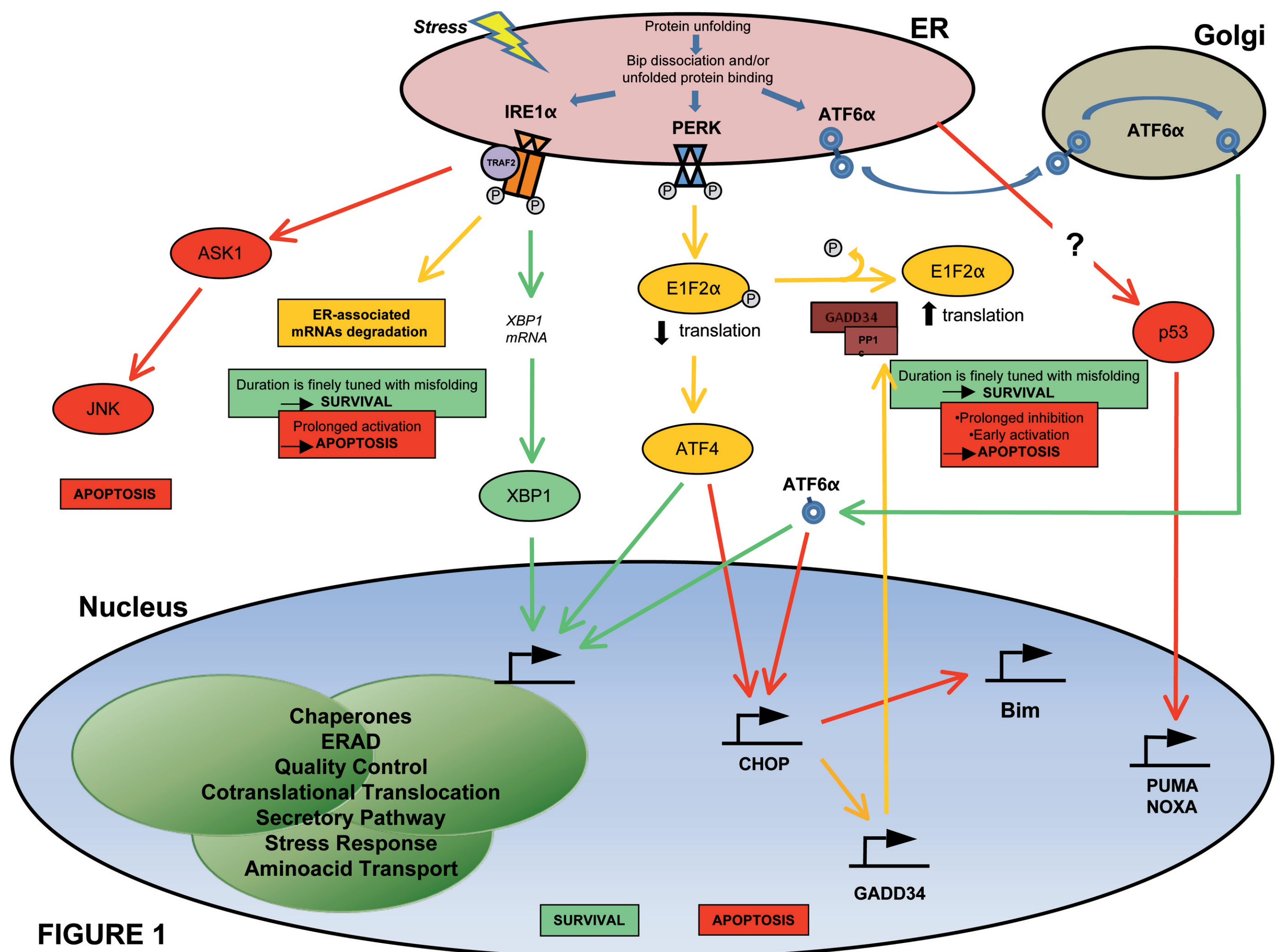

Fig. 1. The ER stress signaling pathways and the paradoxical coexistence of survival and death responses. Misfolded proteins accumulate in the ER and activate the major three stress sensors, either by titrating out BiP from their luminal domain or by direct binding of unfolded proteins to these domains. PERK, IRE1 and ATF6α activate both survival (green arrows) and death (red arrows) pathways. ATF6α activates transcription of both survival (molecular chaperones and ERAD) and proapoptotic genes (CHOP). The transcriptional arm of PERK is both protective (molecular chaperones and ERAD genes) and proapoptotic (CHOP). The transcriptional arm of IRE1 is protective, while activation of ASK1/JNK is proapoptotic. For RIDD (Regulated IRE1-Dependent Decay), and eIF2α dephosphorylation, the survival or apoptotic response is not a monomorph outcome, but rather varies in different contexts (yellow arrows). To be protective, the phosphorylation of eIF2α or activation of RIDD should be finely tuned with the duration of protein misfolding. It should not be longer (denying synthesis of necessary proteins) but should not be shorter, resuming ER load when protein folding has not been recovered.

transmembrane domain adjacent to a conserved bZIP region (Bailey and O'Hare, 2007). Their functions are still not completely understood.

The PERK branch

PERK is also a transmembrane resident ER protein (Harding et al., 1999). The luminal domain of PERK is sensing stress and it is phylogenetically related and functionally interchangeable with the luminal domain of IRE1. In response to ER stress, PERK oligomerizes, and the kinase domain is activated (Bertolotti et al., 2000). This leads to trans-autophosphorylation and phosphorylation of eIF2α, the eukaryotic initiation factor 2 complex, which recruits charged initiator methionyl tRNA to the 40S ribosomal subunit. Phosphorylated eIF2α is much less active, so protein synthesis is inhibited (Asano et al., 2002). This decreases the protein load of the ER.

PERK-dependent phosphorylation of eIF2α also contributes to transcriptional induction in the UPR. PERK plays a broad role in ER stress-induced gene expression, upregulating genes involved in the secretory pathway, stress response, resistance to oxidative stress, and amino acid transport (Harding et al., 2003). Part of this program is elicited by ATF4, a transcription factor downstream PERK (Harding et al., 2000b). In fact, under conditions of attenuated translation, the mRNA of ATF4 is efficiently translated because of its small upstream open reading frames (uORF) within the 5' untranslated region, which are skipped by ribosomes under these conditions (Harding et al., 2000b). Targets include CHOP, and ATF3.

The transcriptional program downstream PERK is entirely dependent upon eIF2α phosphorylation, since cells with a Ser51Ala mutation in eIF2α (which prevent the regulatory phosphorylation at Ser51) and PERK knock-out cells show a similar defect in gene expression (Scheuner et al., 2001; Harding et al., 2003). Moreover, the transcriptional program of a chemically activated PERK-kinase was entirely blocked by the Ser51Ala mutation in eIF2α (Lu et al., 2004). By contrast, as noted above, half of the PERK-dependent UPR target genes are ATF4-independent (Harding et al., 2003). PERK and eIF2α phosphorylations are subjected to rapid negative regulation. Best known is the de-phosphorylation of eIF2α. Two genes have been identified, *GADD34* (growth arrest and DNA-damage-inducible protein-34) and *CReP* (constitutive repressor of eIF2a phosphorylation). They encode the regulatory subunits of two phosphatase complexes that dephosphorylate eIF2α (Connor et al., 2001; Jousse et al., 2003). CReP constitutively operates eIF2α dephosphorylation (Jousse et al., 2003), whereas GADD34 is induced as part of the gene expression program activated by eIF2α phosphorylation, and therefore is part of a negative feedback loop on eIF2α phosphorylation (Ma and Hendershot, 2003).

Cross-talk exists between the UPR branches. XBP1 mRNA is induced by ATF6 and PERK signaling (Yoshida et al., 2000, 2001; Calfon et al., 2002; Lee et al., 2002) and by XBP1 itself (Yoshida et al., 2000, 2001), eliciting positive feedback between the ATF6 and IRE1 pathways.

ER stress and the induction of apoptosis

Cells irremediably damaged by ER stress activate the apoptotic program. There are several pathways that go between ER stress and the cell death machinery. Members of the BH3-only family are implicated in mediating apoptosis triggered by ER stress (Li et al., 2006; Puthalakath et al., 2007; Upton et al., 2008). BH3-only proteins are transcriptionally or post-translationally activated by ER stress and, in turn, activate proapoptotic Bax and Bak to initiate mitochondrial permeabilization. For instance, ER stress activates Bim through two novel pathways, involving protein phosphatase 2A-mediated dephosphorylation, which prevents its ubiquitination and proteasomal degradation and CHOP-mediated direct transcriptional induction (Puthalakath et al., 2007). Another signaling pathway linked to apoptosis under irreversible ER stress is the IRE1-ASK1/JNK pathway (Nishitoh et al., 2002; Kim et al., 2009). JNK can phosphorylate and inhibit the anti-apoptotic proteins Bcl-2, Bcl-XL and Mcl-1. Furthermore, JNK can also phosphorylate and activate several BH-3-only proteins, including Bid and Bim, to promote apoptosis (Weston and Davis, 2007).

The CHOP pathway is crucial in controlling cell death following ER stress, since deregulated CHOP activity compromises cell viability (Friedman, 1996) and cells lacking CHOP are significantly protected from apoptosis (Zinszner et al., 1998). The proapoptotic action of CHOP also has other explanations besides the link to direct effectors of cell death (McCullough et al., 2001; Puthalakath et al., 2007). CHOP positively regulates GADD34 and ERO1α, an ER oxidase (Marciniak et al., 2004). Therefore, CHOP, through GADD34, restores protein synthesis and protein load, favoring cell death, and, through ERO1α, promotes a strong oxidizing ER environment, increasing reactive oxygen species production.

Additional pathways that might contribute to ER-stress-induced apoptosis include interactions between ER-resident Bcl-2 family members and IRE1 (Hetz and Glimcher, 2009) and ER Ca^{2+} release, regulated by Bcl-2 family members, which converge to regulate inositol 1,4,5-triphosphate receptor (IP3R) activity (Kim et al., 2008). Released ER Ca^{2+} can influence apoptosis in the cytoplasm but also by direct transfer to mitochondria via highly specialized tethers that physically attach ER cisternae to mitochondria (Shneider 2005; Rizzuto et al., 2009; Cardenas et al., 2010; Csordas et al., 2010).

Sensing ER stress and transducing the signal.

The lumen of the ER is topologically equivalent to

the extracellular space. The ER stress transducers IRE1 and PERK, with their sensing and transducing properties, are equivalent to membrane receptors that transduce inside the cells the information codified by the occupancy of their extracellular domains by ligands. The question is how the luminal domains of IRE and PERK sense the folding state of cargo proteins.

It was originally proposed that IRE1α and the yeast homolog IRE1p are maintained, in normal conditions, in a deactivated monomeric state by the binding of BiP to their luminal domains (Bertolotti et al., 2000; Kimata et al., 2003). Accumulated unfolded proteins competitively titrate out BiP from the luminal domains of IRE, and this allows IRE to multimerize and autophosphorylate its cytosolic domain. This model was proposed on the basis that, in immunoprecipitation experiments, Ire1 interacts with BiP in unstressed cells and dissociates from BiP under ER stress conditions (Bertolotti et al., 2000; Kimata et al., 2004). However, mutants of IRE1p that lack the part of the luminal domain that is responsible for the binding of BiP retain regulation by ER stress, i.e., they are active under ER stress and inactive in its absence (Kimata et al., 2004; Credle et al., 2005; Oikawa et al., 2007). These data argue against an exclusive role of BiP in the regulation of IRE1p activity.

However, mutants of mammalian IRE1α that have low affinity for BiP, are significantly activated under unstressed conditions (Oikawa et al., 2009) and, in addition, in vitro, luminal fragments of IRE1α do not interact with unfolded proteins (Oikawa et al., 2009), at

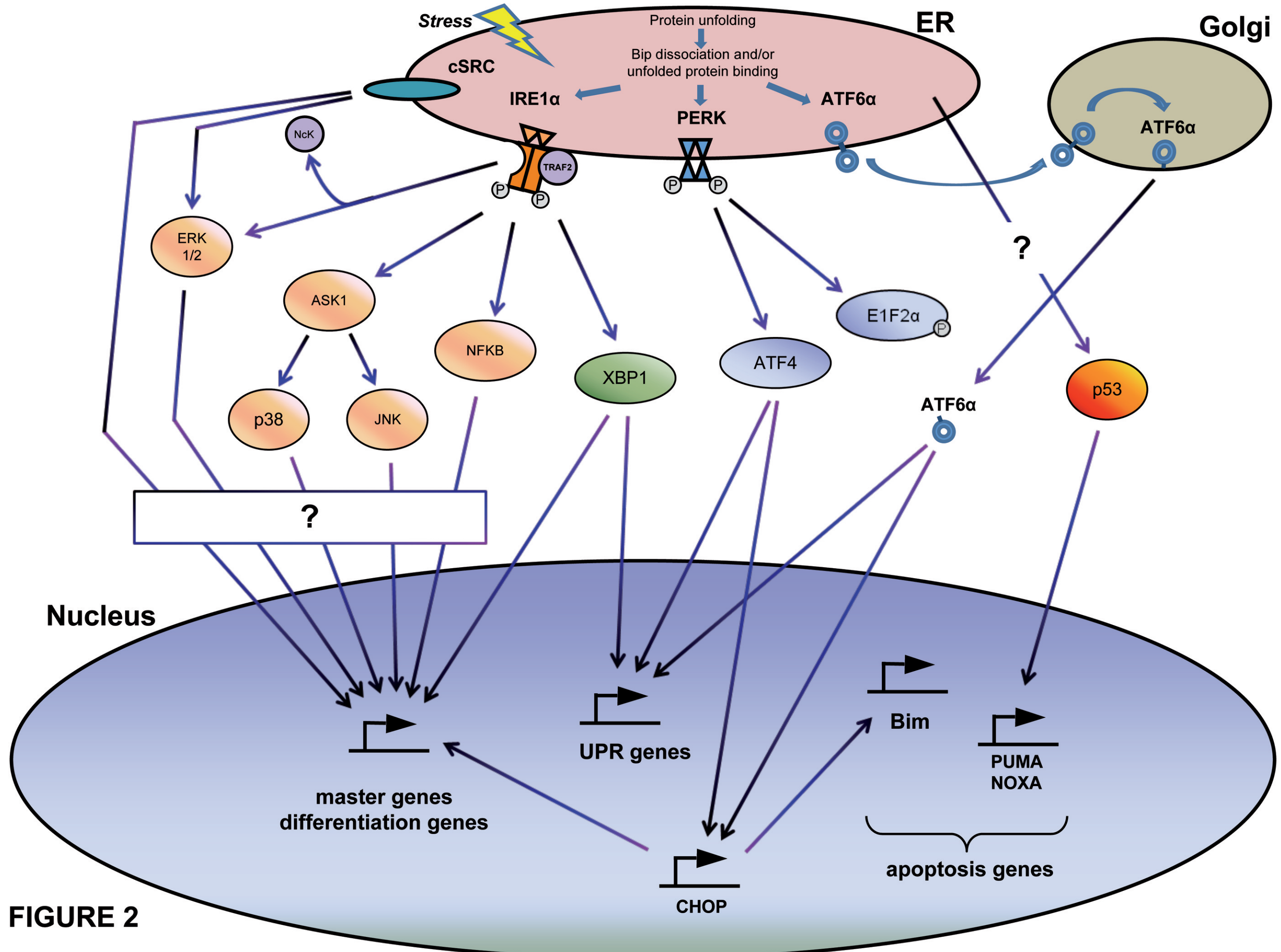

Fig. 2. The third cellular alternative following ER stress: cellular dedifferentiation. A medium intensity ER stress elicits cellular dedifferentiation. The signaling pathways involved in this response are beginning to emerge. Some are part of the "classical" UPR involving XBP1 (downregulation of Mist1 and repression of myogenic differentiation) and CHOP (downregulation of C/ebpα and repression of energy and lipid homeostasis in the liver). Another example is represented by proteins localized, albeit not exclusively, in the ER, but that do not participate in the UPR, such as src. Through src activation, ER stress causes thyroid dedifferentiation by downregulating TTF1 and Pax8. Other possible signal pathways may use JNK, NF-κB, or p38, all activated by IRE1.

variance with yeast IRE1p (Kimata et al., 2007). These results strongly suggest that regulation of mammalian IRE1α strongly depends on the dissociation of BiP.

An alternative model postulates that unfolded proteins bind the luminal domain of IRE1 and cause oligomerization and activation. The crystal structure of the luminal domain of IRE1p revealed that dimers form a central groove, reminiscent of the peptide binding domains of major histocompatibility complexes (MHCs). Mutational analyses suggest that further interaction between luminal domain dimers is required to form higher-order oligomers (Credle et al., 2005). Again at variance with IRE1p, the MHC-like groove of the otherwise similar luminal domain of human IRE1α may not be able to accommodate an unfolded protein peptide (Zhou et al., 2006). Recent data, however, recognized a role for BiP, in IRE1p activation. It has been proposed that the binding of BiP to IRE1p is responsible for the sequestration of inactive IRE1 molecules, thereby providing a barrier to oligomerization and activation, and favoring de-oligomerization and deactivation (Pincus et al., 2010). Thus, BiP binding is not regulating the switch of the UPR, but rather its sensitivity and dynamics in response to protein unfolding. This model seems in agreement with the previous observation indicating that IRE mutant lacking the BiP binding site displays higher sensitivity to various stresses (Kimata et al., 2004).

Other in vitro studies suggest a two step model in which BiP first dissociates from IRE1p leading to its oligomerization, and then directs the interaction of unfolded proteins with the luminal domain to orient IRE1 in an active cluster with full ribonuclease activity (Kimata et al., 2007).

Recently, the crystal structure of the cytosolic domain of IRE1p has been solved (Lee et al., 2008; Korennykh et al., 2009). The cytosolic domain forms high-order assemblies that are critical for IRE1p trans-autophosphorylation and RNAse activation (Korennykh et al., 2009), although previous studies of human IRE1α suggested that formation of dimers was sufficient to fully activate the enzyme (Bertolotti et al., 2000; Liu et al., 2000; Zhou et al., 2006). Oligomerization has also been demonstrated for mammalian IRE1α (Li et al., 2010), unifying, in this respect, the mode of IRE1 activation between yeast and metazoans.

The sensing mechanisms of PERK and ATF6 have been poorly investigated. The primary sequence of the luminal domains of IRE1 and PERK are similar and these domains are experimentally interchangeable (Bertolotti et al., 2000; Liu et al., 2000). Moreover, BiP binding to the luminal domains of PERK and ATF6 is ER-stress sensitive (Bertolotti et al., 2000; Chen et al., 2002; Shen et al., 2002). Finally, a MHC-I-like groove is also predicted to be present in PERK (Credle et al., 2005).

Targets of the ER stress branches

The transcriptional activation of genes encoding components of the ER protein folding machinery is mediated by the cis-acting ER stress response element (ERSE), which presents the consensus sequence CCAAT-N9-CCACG (Yoshida et al., 1998; Roy and Lee, 1999). ATF6 and XBP-1 both bind to this element, interacting with the CCACG part of ERSE when the general transcription factor NF-Y binds the CCAAT part (Yoshida et al., 2000, 2001). However, transcription from ERSE is activated in response to ER stress in IRE1-/- mouse embryonic fibroblasts (MEFs) (Yoshida et al., 2003), although Lee et al. (2003) reported only a small activation in XBP1-/- MEFs. Thus, in the case of ERSE, ATF6 shows some compensatory activity in the absence of XBP-1. Another ER stress responsive cis-acting element is the unfolded protein response element (UPRE, consensus sequence TGACGTGG/A). Originally identified through artificial binding site selection experiments as a sequence to which ATF6 binds (Wang et al., 2000), it actually represents a binding site NF-Y-independent for XBP1 (Yoshida et al., 2001). Transcription from UPRE is abolished in the absence of the IRE1-XBP1 pathway, indicating that XBP1 is necessary for UPRE-mediated transcriptional activation (Lee et al., 2002, 2003). In MEFs silenced for ATF6α the induction of UPRE and ERSE reporters was absent or significantly diminished (Lee et al., 2003). The role of ATF6α in the activation of UPRE reporters is explained by the recent demonstration that ATF6α/XBP1 heterodimers display higher affinity for UPRE-like elements than XBP1 homodimers (Yamamoto et al., 2007). A third ER stress responsive element is ERSE-II, the consensus sequence of which is ATTGG-N-CCACG (Kokame et al., 2001). ERSE-II allows both NF-Y-dependent binding of ATF6 and NF-Y-independent binding of XBPI (although XBPI binds ERSE-II also in the presence of NF-Y) (Yamamoto et al., 2004). Moreover, transcription from ERSE-II is partially inhibited in the absence of the IRE1-XBPI pathway, indicating that, in the case of ERSE-II, both ATF6 and XBPI contribute to transcriptional induction following ER stress (Yamamoto et al., 2004).

Analyzing the regulation of endogenous genes, it was found that while transcriptional induction of the major chaperones BiP and GRP94 occurs normally in the absence of the IRE1-XBPI pathway (Lee et al., 2002, 2003), that of ERAD genes, such as derlins, EDEM, Herp, and HRD1 (Lee et al., 2003; Yoshida et al., 2003; Yamamoto et al., 2004, 2008) and of the co-chaperones ERdj4 and p58^{IPK} (Lee et al., 2003) is inhibited to various degrees, suggesting a role for XBP1 on these promoters in a physiological setting. The IRE1-XBP1 insensitivity of molecular chaperone transcription is partly explained by the presence of ERSE in the promoters of BiP and GRP94 (Yoshida et al., 1998) and by the compensation by ATF6 of the XBP1 absence. In ATF6α-silenced MEFs, BiP transcriptional induction by ER stress was unaffected, and GRP94 induction only slightly reduced (Lee et al., 2003), suggesting that, in turn, XBP1, or other factors, can compensate for ATF6α absence. However, in XBP1-/- cells silenced for ATF6α,

BiP induction was only slightly affected, while GRP94 induction was obliterated (Lee et al., 2003). Thus, Bip induction did not require either XBP1 or ATF6α, while GRP94 induction requires either, but not both, ATF6α or XBP1. In fact, BiP mRNA induction was inhibited in MEF derived from PERK-/- and eIF2α homozygous mutant mice (Harding et al., 2000b; Scheuner at al., 2001). The PERK-eIF2α pathway was found to affect BiP transcription through ATF4 acting, together with ATF1 and CREB1, on a ATF/CRE-like sequence upstream of ERSEs (Luo et al., 2003).

Instead, the molecular basis of the IRE1-XBP-1 sensitivity of ERAD genes is not entirely clear, because the cis-acting elements responsible for XBP1 induction of mammalian ERAD components have not been clearly established. Thus, the responsiveness to the IRE1-XBP1 pathway of Herp is attributed an ERSE-II element in the promoter of Herp (Kokame et al., 2001; Yamamoto et al., 2004) and that of HRD1 to a novel cis-acting element called UPRE-II (Yamamoto et al., 2008).

The switch survival-death following ER stress

The UPR simultaneously transmits survival and apoptotic signals. While it is a common notion that strong ER stress leads to apoptosis, and mild ER stress results in adaptation and survival, the mechanisms by which cells decide whether to continue to attempt adaptation or to initiate apoptosis are poorly understood.

In an attempt to understand the mechanisms at the basis of the adaptive response, an experimental system in which UPR activation was dissociated from cell death was created (Rutkowski et al., 2006). It was shown that an adaptive response is not the result of a selective activation of proximal ER stress sensors, but rather is a consequence of preferential stabilities of mRNA and proteins that facilitate adaptation (molecular chaperones) respect those that facilitate cell death (CHOP, GADD34). Recently, data supporting a time factor have accumulated. Persistent, chronic ER stress activated only the PERK pathway, while it attenuated IRE signaling, although dephosphorylation of eIF2α, as an effect of GADD34 expression, was absent (Lin et al., 2007). In addition, chemical-genetic approaches were employed to activate selectively PERK or IRE signaling. Upon IRE activation, but in the absence of TRAF2-ASK1-JNK axis activation, cell viability increases (Lin et al., 2007), while following PERK activation, but in the absence of eIF2α dephosphorylation, cell viability decreases (Lin et al., 2009). These results lead to a model in which the switch in IRE1 signaling, coupled with unabated PERK activity, contributed to the transition from protective to proapoptotic UPR function. The role of eIF2α phosphorylation in cell survival and death is complex. Loss of PERK-mediated eIF2α phosphorylation markedly sensitizes cells to death from ER stress (Harding et al., 2000a, 2003; Scheuner et al., 2001). However, CHOP, which is transcriptionally induced by eIF2α phosphorylation (Harding et al., 2000b), promotes cell death (Zinszner et al., 1998; Oyadomari et al., 2002). CHOP induces GADD34, which participates in eIF2α dephosphorylation (Connor et al., 2001; Novoa et al., 2001, 2003; Brush et al., 2003; Ma and Hendershot, 2003). In some circumstances, dephosphorylation of eIF2α is harmful. In fact, GADD34 deletion protects ER stressed cells (Marciniak et al., 2004). Moreover, salubrinal, a compound that inhibits eIF2α de-phosphorylation (Boyce et al., 2005), and pre-emptive phosphorilation of eIF2α (Lu et al., 2004), both protect cell against ER stress. However, in other circumstances, eIF2α de-phosphorylation and protein synthesis recovery contributes to cell survival (Novoa et al., 2003).

These apparently contradictory results could be rationalized considering that 1) The translational block operated by phosphorylation of eIF2α is necessary for cells to survive the initial stress, 2) Successively, dephosphorylation of eIF2α is pro-apoptotic if it causes protein synthesis recovery in still stressed cells (i.e., the translational block is too short) and, conversely, 3) Extended eIF2α phosphorylation also has a pro-apoptotic effect denying translation of the majority of mRNA, among them those encoding important ER chaperones (Novoa et al., 2003) (i.e., the translational block is too long). Of course, it seems that the optimal duration of eIF2α phosphorylation is not a fixed time but must be finely tuned with the duration of protein misfolding, in turn likely dependent on the intensity and the rate of increment of ER stress.

Recently, IRE1α was implicated in the decay of ER-localized mRNAs in *D. melanogaster* (Hollien and Weissman, 2006) and in mammalian cells (Hollien et al., 2009), a process called RIDD (Regulated IRE1-Dependent Decay). The nuclease activity of IRE1α was required for both XBP1 splicing and RIDD, but this activity was not sufficient for RIDD. In fact, although XBP1 splicing can be induced by (4-amino-1-tert-butyl-3-[1'-naphthyl methyl]pyrazolo[3,4-d] pyrimidine) (1NM-PP1)-induced activation of the nuclease activity of the I642G mutant of hIRE1α (a kinase-dead mutant), RIDD activation requires hIRE1 (I642G) activation by ER stress. Therefore, the two outputs of IRE1's nuclease activity, XBP1 splicing and RIDD, can be differentially activated (Hollien et al., 2009). Also, these two outputs of IRE1's nuclease activity have been correlated with the adaptation/apoptosis switch (Han et al., 2009). These authors used doxycyline to induce expression and activation of WT IRE1α or expression of IRE1α (I642G), and also added 1NM-PP1, which only activates IRE1α (I642G). They called these two modes of IRE1 activation phosphotranfer and pseudokinase activation, respectively. Both modes trigger XBP1 splicing with similar kinetics, causing XBP1 protein to accumulate to levels mimicking those that occur during ER stress. However, only phosphotransfer activation triggers apoptosis (but kinase-active/nuclease-dead mutants do not) and in the same time activates RIDD. It was suggested therefore that the two outputs of IRE1 were

correlated with adaptation (XBP1 splicing) and apoptosis (RIDD). Thus, the activation of RIDD is reminiscent of eIF2α phosphorylation with respect to the adaptation/death switch. Acute activation of RIDD is protective, decreasing protein load, but sustained activation is proapoptotic, denying synthesis of indispensable proteins.

The third cellular alternative following ER stress: cellular dedifferentiation

Although much attention is presently given to switch adaptation/apoptosis, recent reports have contributed to the idea that adaptation does not necessarily mean full recovery of the pre-existing function. Systematic investigations of the variations of gene expression following ER stress have revealed an increased expression for about 14% (about 900) of the total genes investigated (6385) and a decreased expression for about 17% (about 1100 genes) (Kawai et al., 2004). About one-fourth/one-fifth of these genes were transcriptionally regulated. Therefore, ER stress also causes an extended transcriptional down-regulation. For example, transcriptional repression of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is mediated by binding of ATF6 to the CFTR minimal promoter region, in addition to DNA methylation and histone deacetylation (Bartoszewski et al., 2008).

In line with a transcriptional repression, it has been shown that ER stress dedifferentiates both primary and immortalized chondrocytes, downregulating collagen II and aggrecan (Yang et al., 2005). In vivo, in transgenic mice models expressing mutant collagen X, the UPR was activated in hypertrophic chondrocytes (Ho et al., 2007; Tsang et al., 2007; Rajpar et al., 2009). Chondrocytes survive ER stress, but terminal differentiation is interrupted, producing a chondrodysplasia phenotype resembling the phenotype observed in patients with metaphyseal chondrodysplasia type Schmid (MCDS), a disorder associated with heterozygous mutations in the collagen X affecting its folding. Interestingly, when a transgenic mouse was produced that used the collagen X promoter to drive expression of an ER stress-inducing protein (the cog mutant of thyroglobulin) in hypertrophic chondrocytes, the same phenotype was observed (Rajpar et al., 2009). This demonstrates that ER stress is a central pathogenic factor in the disease mechanism.

This effect of ER stress on cellular differentiation has been recently described in other cell types. Lens fiber cells, expressing both, ectopic collagen IV isoforms or mutant collagen IV, show an activated UPR and an altered differentiation, leading to cataract formation (Firtina et al., 2009). Cell death is a limited phenomenon in this system. Differentiated thyroid cells, experiencing mild drug-induced ER stress, show downregulation of thyroid markers, such as thyroglobulin and thyroperoxidase, and of their transcriptional activators, TTF1 and Pax8 (Ulianich et al., 2008). Interestingly, unfolding of the same protein, thyroglobulin, either caused by drugs (Ulianich et al., 2008) or by mutation (Rajpar et al., 2009), has analogous consequences on different cell types, that is, loss of differentiation markers. Since it has been demonstrated that alteration of thyroglobulin by drugs and by both cog (used by Rajpar et al., 2009) and rdw mutations, has similar consequences (alteration of oxidative folding with persistence of specific folding intermediates) (Di Jeso et al., 2003, 2005; Lee et al., 2008), the data of Ulianich et al. (2008) and of Rajpar et al. (2009), may suggest a mechanism that might contribute to determine cell fate. Thus, the unfolding of a specific protein, or of a subset of proteins, in the ER of a given cell type (insulin in pancreatic beta-cells, digestive enzymes in exocrine pancreatic cells) may play a role in the mode of UPR activation in these cells, which, in turn, may influence a specific outcome (recovery, dedifferentiation, death).

A specific mode of UPR activation in a given cell type may also help to explain the different roles of UPR components in organ physiology. XBP1-deficient embryos die in utero from severe liver hypoplasia, indicating that XBP1 is essential for the differentiation of hepatocytes (Reimold et al., 2000). In addition, XBP1 is required for the terminal differentiation of B lymphocytes to plasmacells (Reimold et al., 2001; Iwakoshi et al., 2003) while PERK-/- mice (Gass et al., 2007) or with a defect of eIF2α phosphorylation (Zhang et al., 2005) do not show any defect in B cell differentiation. Moreover, in differentiating B lymphocytes, PERK is quiescent (Gass et al., 2008; Ma et al., 2010). Together, the data on B lymphocytes differentiation indicate not only a requirement for XBP1 and dispensability of the PERK pathway, but also that the dispensable pathway is not, in fact, activated.

Mice lacking XBP1 in all organs except the liver (XBP1-/-;LivXBP1) die shortly after birth from a severe impairment in the production of pancreatic digestive enzymes, indicating a role of XBP1 in development of exocrine pancreatic cells (Lee et al., 2005). PERK-/- mice, and mice with homozygous or heterozygous mutations at the eIF2α phosphorylation site, show defects in beta-cells (Harding et al., 2001; Scheuner et al., 2001, 2005) and osteoblasts (Zhang et al., 2002), indicating a function of the PERK pathway in these cells. In contrast, XBP1-/-;LivXBP1 mice do not show any drastic change at birth in the endocrine pancreas.

As noted above, in a differentiating B lymphocyte, a distinct mode of UPR activation is present, so a specific cargo protein could be responsible. This protein is not immunoglobulin (Ig) since ER expansion via activation of the IRE1α and ATF6α pathways occurs before significant Ig synthesis occurs (van Anken et al., 2003). Moreover, B cells engineered to lack Ig production, nevertheless activate XBP1 and differentiate normally (Hu et al., 2009).

In the case of thyroid cells, ER stress negatively regulates master regulatory genes of thyroid differentiation, TTF1 and Pax8 (Ulianich et al., 2008).

Interestingly, it has been shown that XBP1 is able to inhibit several markers of myogenic differentiation and it does so by inhibiting Mist1, which is thought to negatively regulate MyoD (Acosta-Alvear et al., 2007). The effect of XBP1 is likely transcriptional (Acosta-Alvear et al., 2007). Moreover, unresolved ER stress suppresses expression of genes involved in maintaining energy and lipid homeostasis in the liver, by transcriptional inhibition of the master regulator *C/ebp· via* CHOP (Rutkowski et al., 2008). Therefore, inhibition of master regulatory differentiation gene seems to be a widely used strategy to transcriptionally reprogram gene expression that is linked to cell dedifferentiation.

Further studies are needed to understand how general this response is, the signal transmission pathways emanating from the ER that mediate this response, and their involvement, besides the examples that are beginning to emerge, outlined above, in human pathologies. Also, we have to understand if and how dedifferentiation and loss of function may be still, with the possibility of recovery, a better alternative than massive apoptosis, especially in organs with limited regenerative potential, or whether it represents an attempt to survive which paradoxically causes defects in organ physiology greater than the induction of apoptosis.

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