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Review

The role of MAPK signalling pathways in the response to endoplasmic reticulum stress ☆

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ABSTRACT

Perturbations in endoplasmic reticulum (ER) homeostasis, including depletion of Ca^{2+} or altered redox status, induce ER stress due to protein accumulation, misfolding and oxidation. This activates the unfolded protein response (UPR) to re-establish the balance between ER protein folding capacity and protein load, resulting in cell survival or, following chronic ER stress, promotes cell death. The mechanisms for the transition between adaptation to ER stress and ER stress-induced cell death are still being understood. However, the identification of numerous points of cross-talk between the UPR and mitogen-activated protein kinase (MAPK) signalling pathways may contribute to our understanding of the consequences of ER stress. Indeed, the MAPK signalling network is known to regulate cell cycle progression and cell survival or death responses following a variety of stresses. In this article, we review UPR signalling and the activation of MAPK signalling pathways in response to ER stress. In addition, we highlight components of the UPR that are modulated in response to MAPK signalling and the consequences of this cross-talk. We also describe several diseases, including cancer, type II diabetes and retinal degeneration, where activation of the UPR and MAPK signalling contribute to disease progression and highlight potential avenues for therapeutic intervention. This article is part of a Special Issue entitled: Calcium Signaling In Health and Disease. Guest Editors: Geert Bultynck, Jacques Haiech, Claus W. Heizmann, Joachim Krebs, and Marc Moreau.

1. Introduction

The endoplasmic reticulum (ER) is an extensive intracellular organelle that provides a site for protein modification and folding, as well as forming an intracellular Ca^{2+} store that plays a central role in signal transduction. Perturbations in the environment within the ER lumen, for example a reduction in luminal Ca^{2+} concentration or altered redox status, can affect protein folding and processing. A reduction in folding capacity and the accumulation of misfolded proteins within the ER activates a series of signalling pathways that are collectively known as the ER stress response or unfolded protein response (UPR). The UPR acts to modulate both the folding capacity and protein load in the ER to restore homeostasis. In doing so UPR signalling acts to promote survival and adaptation to ER stress but can also promote cell death in the event of excessive proteotoxic stimuli.

Recent research has shown that mitogen-activated protein kinase (MAPK) signalling pathways have a role in the response to ER stress (Fig. 1). Whilst some MAPK pathways are activated in response to ER stress and form part of the UPR, (such as p38 and c-Jun N-terminal kinase (JNK)), other members may promote adaptation, survival and resistance to ER stress (Fig. 1). In this review we introduce the core components of the UPR and MAPK signalling pathways and then review the interplay between these pathways, particularly as it pertains to cell survival or cell death.

2. The unfolded protein response (UPR)

UPR signalling promotes the expression of a wide range of target genes including ER-resident chaperones [1], components of the ER-

Abbreviations: Akt, Ak mouse thymoma oncogene, also known as protein kinase B, PKB; ANF, atrial natriuretic factor; AP, activator protein; ASK1, apoptosis signal-regulating kinase 1; ATF6, activating transcription factor 6; bZIP, basic leucine zipper; C/EBP, CCAAT/enhancer-binding protein; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CDK, cyclin dependent kinase; CHOP, C/EBP homologous protein; DR5, death receptor 5; eIF, eukaryotic initiation factor; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ERO1α, ER oxidase 1α; FOXO3, forkhead box O3; GADD34, growth arrest and DNA damage 34; GRP, glucose regulated protein; IL-6, interleukin-6; IP_3, inositol 1,4,5-triphosphate; IP_3R1, IP_3 receptor type 1; IRE1, inositol requiring protein 1; IRES, internal ribosome entry site; IRS1, insulin receptor substrate 1; JNK, c-Jun N-terminal kinase; Keap, Kelch-like ECH associating protein; MAPK, mitogen-activated protein kinase; MEFs, mouse embryonic fibroblast cells; MEK, MAPK or ERK kinase; MK-2, MAPK-activated protein kinase-2; MKK, MAPK kinase; MKKK, MAPK kinase kinases; NGF, nerve growth factor; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PERK, PKR-like ER kinase; Rb, retinoblastoma-associated protein; RIDD, regulated IRE1 dependent-decay; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase; Tg, thapsigargin; TNF, tumour necrosis factor; TRAF, TNF receptor-associated factor; UPR, unfolded protein response; XBP1, x-box binding protein 1

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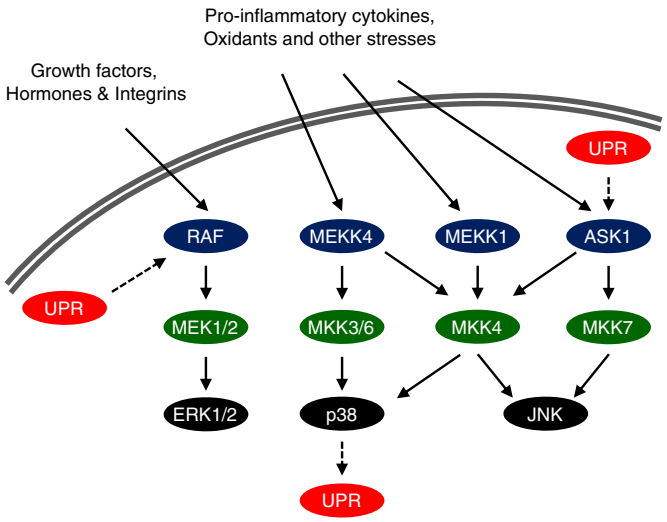

Fig. 1. Overview of MAPK signalling and points of cross-talk with the unfolded protein response. MAPKs are activated by a three-tier signalling cascade, involving MKKKs (blue), MKKs (green) and MAPKs (black). These MAPK signalling networks are activated in response to extracellular signals including growth factors, cytokines and a variety of cellular stresses including ER stress. Following ER stress, the UPR activates ASK1-MKK4/7-JNK signalling and also promotes ERK1/2 activation, although the mechanism for this is unknown. However, MEKK4-MKK3/4/6-p38 signalling also modulates the UPR via p38-dependent phosphorylation of CHOP and ATF6.

associated degradation system and the secretory pathway [2] which help cells to adapt to protein damage or misfolding or enact a cell death response if protein damage is too severe. Three major proteins are involved in sensing stress within the ER and activating UPR signalling within the cytosol and other organelles; these are the transmembrane proteins inositol requiring protein 1 (IRE1), PKR-like ER kinase (PERK) and activating transcription factor 6 (ATF6).

2.1. IRE1

The transmembrane ER stress sensor IRE1 is the most highly conserved branch of the UPR and is the only sensor present in all eukaryotes (Fig. 2); indeed in *Saccharomyces cerevisiae* IRE1 signalling is the sole arm of the UPR. In mammalian cells there are two isoforms [3]; IRE1α is ubiquitously expressed but IRE1β is only expressed abundantly in epithelial cells of the gastrointestinal tract [4,5]. The ER luminal domain of IRE1 is involved in sensing ER stress (see 2.6 below) whilst the cytosolic endoribonuclease and kinase domains are involved in transmitting ER stress signals to downstream effector pathways. Following ER stress, active IRE1 cleaves mRNAs encoding membrane and secretory proteins that would normally be trafficked through the ER, a process termed Regulated IRE1-Dependent Decay (RIDD), and thus contributes to a reduction in ER protein load [6,7]. This appears to involve non-specific binding to mRNA. In contrast, active IRE1 cleaves *x-box binding protein 1* (*XBP1*) mRNA in a site-specific manner to remove an intron, promoting its unconventional splicing [8] to generate an active basic leucine zipper (bZIP) transcription factor (Fig. 2b) [9–12]. XBP1 targets and promotes the transcription of genes encoding ER quality control proteins such as BiP [9,13] and those involved in the ER-associated degradation pathway [2].

The kinetics and amplitude of IRE1 signalling are regulated by a number of different proteins; for example IRE1 signalling is potentiated by protein tyrosine phosphatase-1B, as this is required for effective activation of IRE1-dependent signalling in response to ER stress in fibroblasts [14]. Interaction with cytosolic small heat shock proteins can also regulate IRE1 activity; for example HSP72 promotes activation of the endoribonuclease domain of IRE1 [15] whereas interaction with HSP90

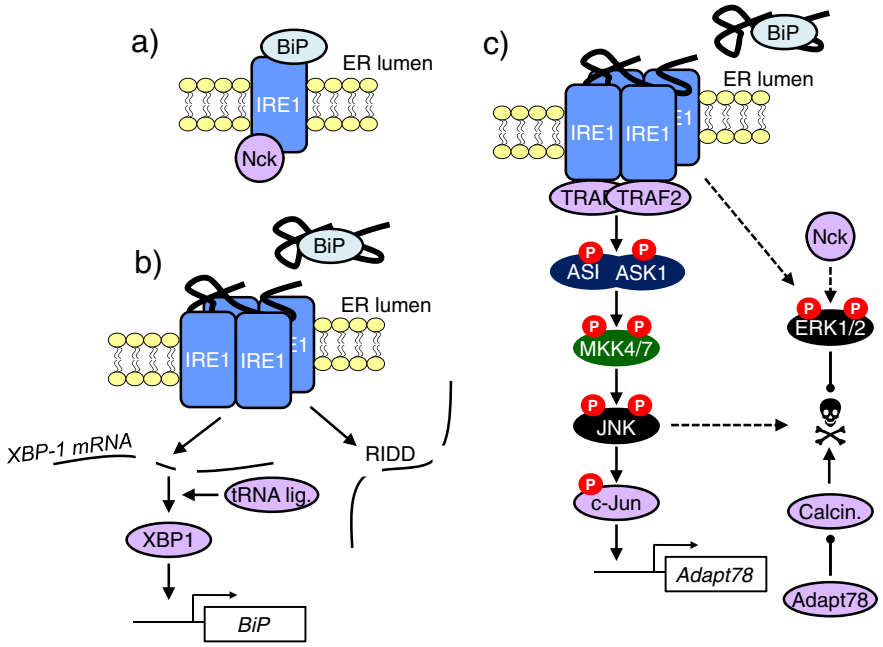

Fig. 2. Signalling to MAPK pathways downstream of IRE1. (a) In the absence of ER stress, IRE1 is ubiquitously expressed as an inactive monomer within the ER membrane. IRE1 is bound to the ER resident chaperone BiP, which inactivates IRE1 and reduces the sensitivity of IRE1 to ER stress inducers. Inactive IRE1 can also bind the adaptor protein Nck and this inhibits activation of ERK1/2 at the ER membrane. (b) Following ER stress, IRE1 is activated by direct interaction with unfolded proteins in the ER lumen and oligomerisation; this leads to activation of the cytosolic endoribonuclease domain of IRE1, site-specific cleavage of the *XBP1* mRNA and its non-canonical splicing by tRNA ligase (tRNA lig.) to express the active XBP1 transcription factor. Targets of XBP1 include the chaperone BiP, which increases protein folding capacity in the ER lumen. In addition, active IRE1 cleaves mRNAs encoding membrane and secretory proteins, reducing the ER protein load through Regulated IRE1-Dependent Decay (RIDD). (c) ER stress also promotes the IRE1-dependent activation of JNK and ERK1/2. IRE1-dependent oligomerisation of TRAF2 activates ASK1-MKK4/7-JNK signalling, which can promote survival through c-Jun-dependent expression of Adapt78 and inhibition of calcineurin (calcin.) or may promote cell death. IRE1 activation also promotes activation of pro-survival ERK1/2 signalling by promoting the dissociation of Nck from IRE1, though the mechanism for ERK1/2 activation has not been identified.

inhibits the proteasomal degradation of IRE1 [16]. Thus both HSP72 and HSP90 can increase IRE1 signalling through interaction with the cytosolic domains of IRE1. In addition, the cytosolic domains of IRE1 may also interact with the pro-apoptotic effector proteins Bax and Bak to promote IRE1 activation, possibly through stabilisation of active IRE1, and it has been proposed that this forms an interaction platform for pro-apoptotic signalling components [17]. On the other hand, interaction of IRE1 with Bax Inhibitor-1 reduces IRE1 activity, resulting in protection against ER stress-induced cell death [18]. Therefore, IRE1 activation is increased by interaction with pro-apoptotic family members (such as Bax and Bak), in contrast to the interaction with the pro-survival Bax Inhibitor-1, which reduces IRE1 activity. Finally, the pro-apoptotic BH3-only proteins BIM and PUMA have also been proposed to interact with, and promote the sustained activation of, IRE1 [19]. This suggests a direct mechanism for interaction between the UPR and the core apoptotic components. Furthermore, the cytosolic kinase and endoribonuclease domains of IRE1 can be differentially regulated by interacting proteins. For example, IRE1-dependent JNK activation is thought to be promoted by interaction with BAK and BIM or PUMA that are artificially targeted to the ER [20], although whether this applies to wild type versions of these BCL2 proteins is less clear. In contrast, interaction between IRE1 and HSP72 promotes XBP1 splicing independently of JNK activation [15]. In addition, inhibition of IRE1 with kinase inhibitors or IRE1-derived peptides can promote XBP1 splicing in the absence of RIDD [21,22].

2.2. PERK

PKR-like ER kinase (PERK) was originally identified as an ER stress-sensitive eukaryotic initiation factor (eIF) 2α kinase [23,24] and is required for attenuation of protein synthesis following ER stress (Fig. 3) [25]. PERK is a type-I transmembrane protein with a luminal N-terminus homologous to IRE1 (around 20% identity between luminal domains) and a cytosolic C-terminus homologous to the protein kinase PKR (around 40% identity) [23]. Similar to PKR, the kinase activity of PERK is stimulated by oligomerisation [26] and requires the autophosphorylation of Ser and Thr residues outside of the kinase domain following ER stress [27]. PERK phosphorylates and activates nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in response to ER stress. In unstressed cells, Nrf2 is bound to Kelch-like ECH associating protein 1 (Keap1) and is localised within the cytoplasm [28]; however, following ER stress PERK phosphorylates Nrf2 promoting its dissociation from Keap1 and allowing Nrf2 to translocate to the nucleus (Fig. 3a) [28,29]. Nuclear localisation of Nrf2 results in an increase in Nrf2-dependent transcription of antioxidant response element containing genes [29]. Indeed Nrf2 activity following ER stress promotes cell survival and redox homeostasis [30].

In addition to Nrf2, PERK phosphorylates eIF2α on Ser51 to inhibit the exchange of GDP for GTP within the eIF2 complex [23,24], thereby inhibiting recruitment of the ribosome to the mRNA bound pre-initiation complex. Whilst phosphorylation of eIF2α inhibits global protein translation, it actually promotes translation of mRNAs containing an internal ribosome entry site (IRES), which does not require all components of the pre-initiation complex for translation [31]. Many stress-responsive genes encode mRNAs with an IRES within their 5′ untranslated region and are therefore preferentially translated following conditions such as ER stress or amino acid starvation [31–33].

Another transcription factor that is activated in response to PERK activation is ATF4 [33,34]. *ATF4* translation proceeds efficiently despite eIF2α phosphorylation due to a series of short upstream open reading frames within the 5′ untranslated region [34]; thus ATF4 is activated

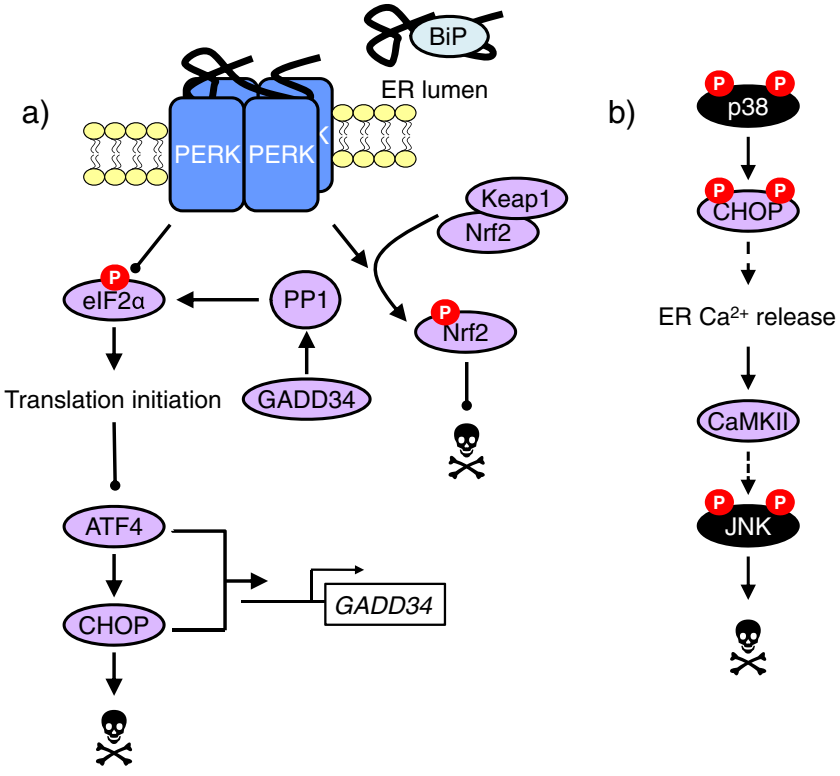

Fig. 3. Cross-talk between PERK signalling and MAPK pathways. (a) Following ER stress PERK is activated by direct interaction with unfolded proteins, dissociation of BiP and oligomerisation, which promotes activation of the cytosolic PERK kinase domain. PERK-dependent phosphorylation of Nrf2 promotes its dissociation from Keap1, allowing its translocation to the nucleus and increased transcriptional activity. Nrf2 increases the expression of several antioxidant enzymes which promote cell survival. In addition, eIF2α is phosphorylated at an inhibitory site by PERK, thus attenuating capdependent translation. Due to several short upstream open reading frames, ATF4 is efficiently expressed despite PERK-dependent eIF2α inhibition, and ATF4 promotes the expression of CHOP. CHOP expression is thought to sensitise cells to ER stress-induced death, and may have a pro-apoptotic role. Both ATF4 and CHOP promote the expression of GADD34, an activator of protein phosphatase 1 (PP1). PP1 dephosphorylates and reactivates eIF2α, promoting increased cap-dependent translation. (b) CHOP is also regulated by p38-dependent phosphorylation although it is unclear what role this phosphorylation has in response to ER stress. CHOP promotes ER Ca^{2+} release, subsequent activation of CaMKII and the ASK1-MKK4-JNK signalling cascade, leading to cell death.

by a number of stress signalling pathways that result in eIF2α phosphorylation, such as amino acid starvation and oxidative damage. ATF4 activates a common set of stress response genes and has a role in promoting adaptation to ER stress by modulating cellular metabolic activity [35]. One of the components that is expressed in response to ATF4 activation is the bZIP transcription factor C/EBP homologous protein (CHOP) (Fig. 3a) [34,36].

2.3. CHOP

CHOP expression is induced in response to a number of cellular stresses including amino acid starvation [37], genotoxic and mutagenic agents [38] and ER stress [39]. The expression of CHOP alters the composition of bZIP transcription factor complexes to inhibit transcriptional activation by CCAAT/enhancer-binding proteins (C/EBP) [40] and relieve transcriptional repression by ATF3 [41]. In addition to forming inhibitory heterodimers, CHOP also promotes transcription by targeting the CHOP-C/EBP complex to a specific DNA sequence [42].

In many cases CHOP induction is considered to be pro-apoptotic as CHOP promotes the expression of several apoptotic genes including the cell surface death receptor 5 (DR5) [43] and the BH3-only proteins BIM and PUMA [44,45], the latter components of the BCL2 protein family in the cell intrinsic death pathway. In addition, CHOP induces a decrease in the expression of the pro-survival protein BCL2 [46]. CHOP expression can also promote hyperoxidation of the ER lumen through increased expression of ER oxidase 1α (ERO1α) [46–48]. Hyperoxidation of the ER lumen following ER stress promotes inositol 1,4,5-triphosphate (IP_3)-induced Ca^{2+} release from the ER lumen due to activation of IP_3 receptor type 1 (IP_3R1) [49]. Oxidation of IP_3R1 is thought to release it from a repressive interaction with ERp44, thereby promoting increased IP_3R1 activation and release of Ca^{2+} from the ER lumen, which can promote cell death [50,51]. Thus CHOP can act indirectly to promote ER stress-induced release of Ca^{2+} from the ER lumen and cell death.

However, overexpression of CHOP in the absence of ER stress is not sufficient to promote cell death in many cell types including mouse embryonic fibroblasts (MEFs), suggesting that additional signalling (such as ATF4 activation) is required to activate ER stress-induced cell death [52]. Following ER stress, eIF2α phosphorylation attenuates global protein translation. However, both CHOP and ATF4 promote the expression of growth arrest and DNA damage 34 (GADD34), an activator of protein phosphatase 1, which reactivates global mRNA translation by promoting the dephosphorylation of eIF2α (Fig. 3a) [52–54]. This may then promote cell death due to the further increase in protein load, oxidative stress and ATP depletion [52]. Although it is unclear whether CHOP can directly promote cell death following ER stress, it is clear that expression of CHOP by the PERK signalling axis sensitises cells to additional cell death signals.

2.4. ATF6

Whilst PERK and IRE1 are functionally homologous ER stress sensors, ATF6 is a unique, constitutively expressed ER stress sensor and transcription factor present only in mammalian cells [3]. In mammalian cells, nearly all ER stress responsive genes contain a consensus sequence (CCAAT-N_9-CCACG) within the promoter region (where N is a GC-rich sequence), which acts as a binding site for several UPR-induced transcription factors [55,56]. ATF6 was identified alongside XBP1, as a bZIP transcription factor that can bind this consensus sequence [56], promoting the expression of genes that include BiP and CHOP [11,57]. Two isoforms of ATF6 have been identified; ATF6β is an inefficient transcriptional activator compared to ATF6α, but is a more stable protein (active ATF6α has a half-life of around 2 h compared to 5 h for active ATF6β) [58]. In addition, ATF6β can repress the ATF6α-dependent transcription of BiP following ER stress, perhaps due to competition at the promoter [58]. Therefore, the relative abundance of the two ATF6 isoforms appears to modulate ATF6-dependent ER stress signalling [59]. Deletion of ATF6β alone in mice had little impact on UPR-induced gene expression or cell viability, whereas deletion of ATF6α reduced the expression of BiP and other UPR-induced genes. However, double knockout of both isoforms of ATF6 was embryonic lethal, suggesting that ATF6β can compensate for the loss of ATF6α despite its lower transcriptional activity, and this is sufficient to promote resistance to ER stress during embryonic development in ATF6α knockout mice [60].

2.5. BiP

BiP, also known as glucose regulated protein 78 (GRP78) is a DnaK-type, ATP-dependent protein chaperone localised within the ER lumen where it promotes protein folding [61]. BiP protein has a long half-life (>48 h) [62] and also has a role in binding ER Ca^{2+} to buffer around 25% of the luminal Ca^{2+} load [63]. BiP activity is attenuated by the post-translational modification, ADP ribosylation. This is critical to ensure that the ER remains responsive to short-term fluctuations in unfolded protein load. Levels of unfolded protein within the ER are inversely correlated to the ADP ribosylation status of BiP, thus ensuring that BiP activity is attenuated when there is a reduction in unfolded protein load [64,65]. A reduction in ADP ribosylation of BiP promotes binding to unfolded proteins and association with an active multi-chaperone complex [65,66]. UPR activation also results in increased transcription of BiP through the activity of ATF6 and active XBP1 [9,11]. In this way ER stress promotes an increase in BiP activity through a combination of transcriptional and post-translational changes, resulting in increased ER protein folding capacity.

2.6. UPR activation

The demonstration that BiP binds unfolded proteins within the ER lumen and that this promotes its dissociation from IRE1, led to the proposal that the concentration of free BiP was an indicator of ER stress [1,67]. However in yeast, dissociation from BiP is not required for Ire1 activation following ER stress; rather, binding of BiP to the juxtamembrane region [68] promotes the inactivation of Ire1 following stimulation and also reduces the sensitivity of Ire1 to ER stress inducers [69]. Furthermore, the ER luminal domain of Ire1 was subsequently found to bind misfolded proteins directly, resulting in Ire1 oligomerisation [70–72]. Mutation of residues within a hydrophobic groove in the luminal domain of Ire1, that has a similar structure to the major histocompatibility complex, abolished binding of Ire1 to a model ER stress-inducing peptide and unfolded proteins in solution and reduced Ire1 activation [70–72]. In contrast, ER stress sensing is not as well characterised in metazoan cells. The crystal structure of the luminal domain of human IRE1 indicates a narrower hydrophobic groove compared to that in yeast Ire1 [73]. Therefore it is unclear whether direct interaction with unfolded proteins is required for metazoan IRE1 activation, although IRE1 forms higher order complexes following ER stress [26,74] which in yeast are promoted by interaction of Ire1 with unfolded protein [70–72,75].

Oligomerisation of the luminal domains of IRE1 allows binding of ATP to the cytosolic domain, inducing the activation of the cytosolic endoribonuclease/kinase domains of IRE1. Some studies suggest that IRE1 activation occurs via trans-autophosphorylation after IRE1 dimerisation [76]. An alternative theory is that assembly of IRE1 dimers into higher order oligomers following nucleotide binding is required for stabilisation of the endoribonuclease domain [77–79]. IRE1 mutants that are unable to trans-autophosphorylate retain endoribonuclease activity, yet mutants that are unable to bind ATP and ADP do not retain this activity [80,81]. However, phosphorylation may have a role in destabilising the higher order IRE1 oligomers [80,82] and may also promote association of adaptor proteins such as tumour necrosis factor (TNF) receptor-associated factor (TRAF) 2 with the cytosolic domains of IRE1 [83]. In summary, IRE1 is thought to be activated by oligomerisation, which in yeast is promoted in response to the direct interaction of Ire1 with unfolded proteins. In addition, binding of ATP to the cytosolic domain of IRE1, dissociation of BiP and trans-autophosphorylation also

contribute to IRE1 activation. The evidence for the different mechanisms of UPR activation and other contributing factors are further discussed by B. Gardner et al. [75].

PERK is anticipated to have a similar activation mechanism to metazoan IRE1 due to its sequence similarity within the core luminal domain that is crucial for IRE1 activation [70]. Similar to IRE1, active PERK forms higher order oligomers following ER stress [26], but there is currently no evidence that direct interaction with unfolded proteins is required for PERK activation [75]. In contrast to IRE1 and PERK, ATF6 undergoes reduction and dissociates into monomers following ER stress; dissociation of BiP from the luminal domain of ATF6 uncovers two golgi localisation sequences and this is required to promote the translocation of ATF6 to the golgi and its subsequent activation by proteolytic processing [84,85]. At the golgi membrane, ATF6 is cleaved by the golgi resident proteases S1P and S2P [86,87]. S1P first cleaves the luminal domain of ATF6, to leave a shortened luminal region adjacent to the golgi membrane. This allows the subsequent cleavage of the transmembrane domain of ATF6 by S2P, an intramembrane protease, and release of the active cytosolic domain of ATF6 (Fig. 4) [88], which translocates to the nucleus to drive gene transcription.

2.7. Pharmacological ER stress inducers

A variety of pharmacological agents are used to induce or mimic ER stress and have proved valuable in helping to define the UPR pathways; however, many of them also activate other signalling events within the cell so their use does come with some caveats. The sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) inhibitor, thapsigargin (Tg), is commonly used when studying ER stress. Tg treatment causes the release of Ca^{2+} from the ER, thereby undermining the protein folding capacity of the ER and activating the UPR. However, the increase in cytosolic free Ca^{2+} arising from Tg treatment has also been found to activate MAPK signalling pathways [89] and is likely to induce other signalling pathways. Other treatments that promote release of Ca^{2+} from the ER, including the Ca^{2+} ionophore A23187 have also been used to induce ER stress but with the same caveat as SERCA inhibitors. Inhibitors of N-linked glycosylation, such as tunicamycin, attenuate protein processing within the ER and thus promote the accumulation of misfolded protein within the ER lumen, resulting in ER stress. The disadvantage of this treatment is that inhibition of the production of a subset of proteins, in this case substrates of N-linked glycosylation, could deplete the components of several signalling pathways in the cell, thus eliciting changes in signalling pathways that are not directly involved in the UPR. The ER stressor brefeldin A inhibits protein transport from the ER to the golgi thus resulting in accumulation of proteins within the ER and activation the UPR. The main disadvantage of this treatment is the subsequent reduction in protein trafficking to the plasma membrane, which again may affect signal transduction and cell adhesion. Reducing agents such as dithiothreitol can also be used to induce ER stress through manipulation of the ER redox status and inhibition of disulphide bond formation. This promotes misfolding of proteins within the ER but also affects redox-dependent signalling throughout the cell, and particularly within mitochondria. In addition, redox changes within the ER lumen will be sensed by ER resident proteins including the IP_3R, thus manipulating Ca^{2+} release and subsequent signalling alongside UPR activation.

Amino acid analogues, such as the proline analogue azetidine, disrupt protein folding and are often used to induce ER stress. One disadvantage of this stressor is that it will also disrupt protein folding in the cytoplasm resulting in increased expression of cytosolic chaperone proteins and the inhibition of cell growth. ER stress can be more selectively induced by overexpression of proteins that are prone to misfolding, such as the human blood clotting factor VIII or amyloid-β derived peptides. Thus although expressing an exogenous sequence can increase the transcriptional and translational load, low levels of peptide expression is likely to be a more selective method for inducing ER stress. Through combined use of these ER stress inducers, it is possible to generate a robust model of the mammalian UPR.

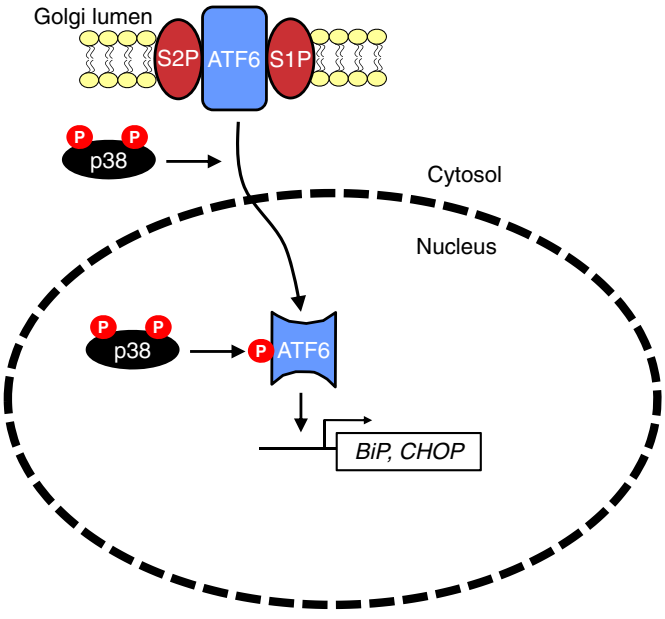

Fig. 4. Cross-talk between ATF6 signalling and MAPK pathways. Following ER stress, BiP dissociates from ATF6 uncovering two golgi localisation signals, which promote translocation of ATF6 monomers to the golgi membrane. At the golgi, ATF6 is cleaved by two golgi-resident proteases, S1P and S2P, releasing the active cytosolic domain. Active ATF6 protein promotes transcription of ER stress-responsive genes including *BiP* and *CHOP*, and its transcriptional activity is modulated by p38-dependent phosphorylation. It is presently unclear if p38 phosphorylates ATF6 before it enters the nucleus or, by analogy with other MAPK pathways, nuclear p38 phosphorylates ATF6 once it has entered the nucleus.

3. MAPK signalling pathways

The MAPKs are evolutionarily conserved, proline-directed Ser/Thr protein kinases, distantly related to the cyclin-dependent kinases. They include extracellular signal-regulated kinases 1 & 2 (ERK1/2), JNKs and the p38 family and are activated through three-tier kinase signalling cascades (Fig. 1) [90,91]. The MAPK subgroups are defined in part by the dual threonine/tyrosine motif in their activation loop, phosphorylation of which is required for their activation: ERK1/2 (Thr-Glu-Tyr), JNK (Thr-Pro-Tyr), and p38 (Thr-Gly-Tyr) [92–94]. These Thr-X-Tyr motifs are phosphorylated by select MAP kinase kinases (MKKs), which are in turn phosphorylated and activated by a number of MAP kinase kinase kinases (MKKKs). The biological outcomes of MAPK signalling are dependent on a variety of factors including the subcellular localisation of the MAPK (which is determined by scaffold proteins and determines the repertoire of substrates available), the magnitude and duration of MAPK activation and combinatorial signalling inputs provided by other pathways [90].

3.1. ERK1/2

ERK1/2 are activated upon phosphorylation by the dual specificity MKKs MAPK or ERK kinase (MEK) 1/2 (Fig. 1) [91]. ERK1/2 were originally identified as protein kinases activated in response to stimulation by extracellular signals such as insulin or nerve growth factor (NGF) [95,96]. They are now known to be activated following stimulation by virtually all growth factors including those acting via receptor tyrosine kinases, receptor serine kinases, cytokine receptors, integrins and G-protein coupled receptors. MEK1/2 are activated by the RAF protein kinases (Fig. 1) and this RAF-MEK1/2-ERK1/2 pathway is a major target for the RAS GTPases, products of the *RAS* proto-oncogenes.

The duration of ERK1/2 activation determines the outcome of signalling through this pathway in part by regulating the composition of the

transcription factor activator protein (AP)-1, and thus determining its DNA binding and transcriptional activity. Whilst transient ERK1/2 activation promotes the expression of c-Fos, sustained ERK1/2 activation is required for the expression of the AP-1 components Fra-1, Fra-2, c-Jun and JunB and promotes phosphorylation of the C-terminal, transactivation domain of c-Fos [97–99]. As a consequence, sustained activation of ERK1/2 in fibroblast cells promotes cyclin D1 expression and cell proliferation [99]. In contrast, treatment of PC-12 cells with NGF promotes a very strong and sustained ERK1/2 activation that leads to cell cycle arrest and terminal differentiation; in this case epidermal growth factor stimulates the transient activation of ERK1/2 and fails to promote differentiation [100].

In addition to control of cell proliferation or differentiation, ERK1/2 activation can clearly promote cell survival in the face of a number of stress stimuli most notably through the regulation of members of the BCL2 protein family. For example, ERK1/2 activation promotes the transcription of the pro-survival proteins MCL-1, BCL2 and BCL-X_L, most likely due to activation of the cAMP responsive element binding protein. In addition, ERK1/2 directly phosphorylates MCL-1 to promote its stabilisation. ERK1/2 signalling can also promote a decrease in the expression of several pro-apoptotic proteins including BIM, PUMA and BMF. For example, ERK1/2-dependent phosphorylation of BIM promotes its dissociation from the pro-survival proteins BCL-X_L and MCL-1 in addition to promoting its ubiquitination and turnover, whilst phosphorylation of forkhead box O3 (FOXO3), a transcriptional activator of both *BIM* and *PUMA*, promotes its ubiquitination and degradation, leading to the ERK1/2-dependent suppression of BIM and PUMA expression [101,102]. Finally, ERK1/2 signalling also promotes the phosphorylation and proteasome-dependent turnover of BAD. The various mechanisms by which ERK1/2 signalling can regulate BCL2 proteins to promote cell survival have been reviewed elsewhere [101,102].

3.2. JNK

The JNKs were initially identified as kinases that phosphorylated and activated the transactivation domain of the transcription factor c-Jun in response to UV treatment of cells [103]. There are three JNK genes which encode up to ten different JNK isoforms through alternative splicing; both JNK1 and JNK2 are ubiquitously expressed, whilst JNK3 is primarily expressed in the brain [93,104]. The JNK isoforms have different substrate specificity as shown by the preferential binding of c-Jun to JNK1 in comparison to other JNK isoforms [103]. The JNKs are activated in response to a variety of cellular stresses including UV, ionising radiation, methylating agents, translation inhibitors, heat shock and ER stress [104]. JNK activation requires dual phosphorylation of the Thr-Pro-Tyr motif by the upstream kinases MKK4 (which also activates p38) [105,106] and MKK7 (Fig. 1) [107]. However, the identification of alternative splice variants of MKK7, which are differentially regulated by upstream signals, adds to the complexity of JNK regulation [108].

JNK signalling can promote cell death through the de novo expression of death receptors and their ligands. However, JNK can also target components of the BCL2 family directly to effect cell death. For example, under basal (non-stressed) conditions the pro-apoptotic BH3-only protein, BIM is proposed to be sequestered by binding to the dynein motor complex; in response to stress, JNK phosphorylates BIM within the dynein light chain binding motif, promoting its release from the dynein motor complex to initiate apoptosis [109]. In addition JNK phosphorylates BCL2 at both Ser and Thr residues to promote cell cycle arrest at the G2/M-phase reducing the resistance of cells to cell death stimuli such as paclitaxel treatment [110]. Therefore, JNK activation is generally thought to be pro-apoptotic. However activation of JNK signalling alone is not sufficient to promote apoptosis [111] and JNK signalling can also have a pro-survival role [112]. Furthermore, JNK phosphorylation of BCL2 at Thr69, Ser70 and Ser87 can promote its dissociation from Beclin1, thus promoting autophagy [113] and potentially promoting cell survival.

3.3. p38

The p38 MAPKs were originally identified as proteins activated in response to cellular stresses such as hyperosmolarity, arsenite or taxol and pro-inflammatory cytokines [92,114]. They require dual phosphorylation of a Thr-Gly-Tyr motif for activation [105] and this is catalysed by MKK3, MKK4 and MKK6 but not MEK1/2 signalling (Fig. 1) [105,106,115]. There are four proteins in the p38 family; p38α and p38β are ubiquitously expressed, whereas p38γ and p38δ exhibit a more limited tissue distribution and have different substrate specificities compared to the more widely expressed family members [90].

Activation of p38 can either promote cell proliferation or induce cell cycle arrest, depending on the kinetics of activation and downstream signalling pathways activated. Following growth factor stimulation, p38 undergoes transient activation which induces cyclin D1 expression and phosphorylation of the transcriptional repressor, retinoblastoma-associated protein (Rb) [116]. Phosphorylation of Rb promotes its dissociation from E2F and increased transcription of S-phase entry genes therefore inducing cell proliferation [117]. In contrast, prolonged activation of p38, as is seen in response to cellular stresses such as anisomycin, arsenite or tunicamycin [116,118,119], leads to the activation of MAPK-activated protein kinase-2 (MK-2) and its substrate cAMP-responsive element-binding protein [116]. This promotes cell cycle arrest, perhaps through induction of the cyclin-dependent kinase (CDK) inhibitors p16, p21 or p27 [119–121]. In addition to cell cycle regulation, p38 activation can also promote cell death through phosphorylation of BIM at the same site as JNK [122] as well as promoting the expression of BIM through activation of the transcription factor FOXO3a [123]. Furthermore, p38-dependent phosphorylation of the transcriptional activator p53 promotes its stabilisation and transcriptional activity. The subsequent increase in apoptosis is likely to be due to the increased expression of p53-target genes, which includes expression of the pro-apoptotic proteins PUMA and NOXA [124].

4. ER stress and MAPKs

All three of the canonical MAPKs (ERK1/2, JNK and p38) are known to be activated in response to ER stress, each with different kinetics and with different roles [125,126]. Generally, ERK1/2 activation is considered to promote cell survival, but to what extent this protective role extends to ER stress is unclear. By analogy with its role in proliferation and differentiation, the role of ERK1/2 activation in ER stress signalling is likely to be determined by the kinetics of its activation, which will depend on the nature of the ER stress stimulus. In addition, ER stress induces an increase in both JNK and p38 activation [126], suggesting that these MAPKs may have a role in the response to ER stress [125]. Furthermore, ER stress-induced JNK activation is higher in hepatocytes derived from aged rat compared to their younger counterparts and results in greater cell death [126], suggesting a role for JNK signalling in ER stress-induced cell death. In some cell lines, inhibition of JNK reduces UPR activation, suggesting that JNK signalling can promote ER stress through an unidentified mechanism, leading to ER stress-induced cell death [127,128]. Therefore JNK signalling can increase ER stress and is also activated as a consequence of the UPR. Below we review the interplay between the UPR and MAPK signalling components and how this may contribute to cell survival or cell death decisions.

5. IRE1 and MAPK signalling

5.1. IRE1 activates JNK to promote cell death

In a variety of cell lines, ER stress has been found to induce JNK activation and this is thought to initiate a pro-apoptotic response. Whilst ER stress promotes the oligomerisation of IRE1 and its activation, IRE1 activation can be enforced through its overexpression, allowing activation of IRE1 in the absence of ER stress. This activation of IRE1 is sufficient

to activate JNK in cells and this requires the active IRE1 kinase domain but not the endoribonuclease domain. The adaptor protein TRAF2 was found to bind to IRE1 through a yeast two-hybrid screen. Following ER stress, IRE1 oligomerises, activating its kinase domain. This interacts with TRAF2 via its C-terminal TRAF domain promoting the clustering of the N-terminal effector domain of TRAF2 [83]. More recently, the IRE1-TRAF2 dependent activation of JNK following ER stress was found to require the MKKK apoptosis signal-regulating kinase 1 (ASK1). In support of this, MEFs and primary neurons from ASK1$^{-/-}$ mice showed a reduction in ER stress-induced JNK activation and were more resistant to ER stress-induced apoptosis [129]. Interaction with IRE1-TRAF2 promotes ASK1 oligomerisation [130] and induces a conformational change to promote intermolecular autophosphorylation of Thr845 within the ASK1 activation loop. Phosphorylation of Thr845 is required for the kinase activity of ASK1 and its subsequent activation of JNK signalling [131]. ASK1 has been shown to phosphorylate and activate MKK4/MKK7 and MKK3/MKK6, the upstream kinases for JNK and p38 respectively (Fig. 1) [132].

Aside from activation by TRAF2, ASK1 is regulated by interaction with a network of kinases and phosphatases in addition to binding proteins such as 14-3-3 [133]. Phosphorylation of ASK1 on Ser83 by the pro-survival protein kinases PIM1 or the Ak mouse thymoma oncogene (Akt, also known as protein kinase B) decreases ASK1 activity leading to a reduction in JNK activation and reduced sensitivity to stress-induced apoptosis [134,135]. ASK1 is inactivated by dephosphorylation of Thr845 and this may occur through protein phosphatase 5 activity, which binds to ASK1 in response to oxidative stress [136]. Furthermore, ASK1 dephosphorylation on Ser967 is required for dissociation from 14-3-3 and activation in response to stress [137]. It is currently unclear how this network of ASK1 regulators and binding proteins are co-ordinated in response to ER stress to promote ASK1 activation and subsequent JNK activation. However, oligomerisation of IRE1 forms a platform to promote signalling via TRAF2 and ASK1, leading to JNK activation following ER stress (Fig. 2c).

Intriguingly, whilst in most cases the IRE1-dependent activation of JNK is considered to be pro-apoptotic [129,134,135], inhibition of JNK2 activation in a human lymphoblast cell line (U937 cells) promoted cell death by apoptosis following ER stress. JNK2 activation was required for progression of autophagy and a reduction of unfolded protein load in the ER. Thus activation of JNK2 following ER stress promoted cell survival in these cells [138].

5.2. IRE1-dependent c-Jun activation inhibits ER stress-induced death

The transactivation domain of the transcription factor and proto-oncogene c-Jun is phosphorylated by JNK and this promotes c-Jun activation [103]. As a bZIP transcription factor, c-Jun can form both hetero- and homodimers as part of AP-1 transcription factor complexes [139]. Phosphorylation of both JNK and c-Jun increases following ER stress, and c-Jun is required for the transcription of Adapt78 in response to ER stress [140]. Adapt78 is an inhibitor of the Ca^{2+}-responsive protein phosphatase, calcineurin and so can act to decrease calcineurin activity following ER stress [140]. ER stress promotes the cleavage and activation of an ER-associated caspase, caspase 12 in rodents or caspase 4 in human cells (murine caspase 12 has 48% sequence identity to human caspase 4); this can contribute to ER stress-induced apoptosis [141,142]. Expression of c-Jun was shown to reduce caspase 12 cleavage and protect cells against Tg-induced cell death, although it is unclear whether the increased expression of Adapt78 and calcineurin inhibition has a role in this response [140]. In addition, calcineurin is activated by caspase-dependent cleavage and inhibition of calcineurin can attenuate Tg-induced apoptosis [143]; this suggests that calcineurin can promote ER stress-induced cell death, but may act downstream of caspase 12. Therefore, whilst JNK activation is implicated in promoting ER stress-induced apoptosis, it can also act to reduce ER stress-induced apoptosis through Adapt78 transcription and calcineurin inhibition (Fig. 2c).

5.3. IRE1-dependent ERK1/2 activation promotes survival

ERK1/2 activation is partially IRE1-dependent in MEFs treated with the proline analogue azetidine-2 carboxylic acid as an ER stress inducer and has also been re-capitulated in a cell-free ER stress assay in vitro. In this assay, depletion of the adaptor protein, Nck, from the system resulted in a reduction in ER stress-induced ERK1/2 activation. Nck was found to associate at the ER membrane with the cytosolic domain of IRE1 via its SH3 domains. At the ER membrane Nck inhibited ERK1/2 activation, whereas dissociation of Nck from the ER membrane following ER stress promoted the increased activation of ERK1/2 and correlated in vivo with increased cell proliferation and reduced apoptosis [144]. Thus whilst ER localised Nck interacts with inactive IRE1 and this inhibits ERK1/2 activation, dissociation of Nck from active IRE1 and the ER membrane promotes the cytosolic localisation of Nck and IRE1-dependent ERK1/2 activation (Fig. 2c). This suggests that activation of IRE1 promotes a conformational change within the cytosolic domains to promote both Nck dissociation from IRE1 and the subsequent activation of ERK1/2. However, it remains unclear how ERK1/2 is activated and if a direct interaction with either Nck or IRE1 is required as it is more likely that an upstream MKK is also necessary for ERK1/2 activation.

ER stress-induced ERK1/2 activation has also been shown to be partially IRE1-dependent in gastric cancer cells, as siRNA knockdown of IRE1 attenuated ERK1/2 activation following ER stress. In these cells, inhibition of ERK1/2 signalling promoted ER stress-induced apoptosis, perhaps through a reduction in BiP levels. ERK1/2 inhibition attenuated the ER stress-induced increase in BiP mRNA and protein levels and the reduction in BiP levels was sufficient to promote ER stress-induced apoptosis [145]. Thus signalling through the IRE1 axis of the UPR can promote activation of ERK1/2, which may have a pro-survival role following ER stress. However, in neither case is it clear how ERK1/2 signalling acts to promote cell survival during ER stress and how this relates to reports that ER stress initiates apoptosis by activating BIM or PUMA.

6. The PERK-CHOP signalling axis and MAPKs

6.1. p38-dependent phosphorylation of CHOP is required for CHOP activation

CHOP contains two serines within a favourable sequence context for MAPK phosphorylation. Mutation of Ser78 or Ser81 to alanine reduced the proportion of stress-induced CHOP phosphorylation, whilst the double mutation abolished phosphorylation. These sites were found to be phosphorylated by p38, but not by ERK2 or JNK3 both in an in vitro kinase assay and in a cell line overexpressing CHOP (Fig. 3b) [118]. Although phosphorylation of CHOP appeared to have no effect on its DNA binding activity or its dimerisation specificity, results from a transactivation domain reporter construct suggested that phosphorylation by p38 increases the activity of the CHOP transactivation domain [118]. This may have important implications for other C/EBP family proteins such as C/EBPβ, which are also phosphorylated by MAPKs [146]. MAPK signalling may alter the activity of a CHOP-C/EBP dimer through phosphorylation of either or both proteins in the complex and subsequent modification of transactivation domain activity. In vivo, p38-dependent phosphorylation of CHOP was required to inhibit differentiation of a pre-adipose cell line [118]. In addition, p38-dependent phosphorylation of CHOP was also required for maximal cell death in response to CHOP overexpression in HeLa cells [147]. Therefore, it seems likely that p38-dependent phosphorylation of CHOP alters both the activity of the transactivation domain and subsequently its affinity for binding partners, leading to an altered gene expression profile. However it is currently not known which genes

are dependent upon CHOP phosphorylation and thus are responsible for the altered cell phenotypes.

6.2. JNK is a downstream effector of the CHOP-CaMKII pathway

CHOP expression can promote the ER stress-induced release of Ca^{2+} due to hyperoxidation of the ER lumen and increased activation of IP_3R1 (Section 2.3). In macrophages, the ER stress-induced release of Ca^{2+} from the ER lumen results in the transient activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) [148]. This protein is then able to autophosphorylate on Thr287 and is further activated by ER stress-induced reactive oxygen species production, through oxidation of Met281/282 [148,149], which leads to sustained CaMKII activation, which can promote apoptosis through at least two pathways. Firstly, CaMKII is translocated to the surface of mitochondria, which leads to an accumulation of Ca^{2+} within mitochondria. Although the mechanism for this hasn't been identified, mitochondrial localised CaMKII may promote signalling to mitochondrial Ca^{2+} transporters or may promote increased ER-mitochondria contacts and thus greater ER-mitochondria Ca^{2+} transport. Increased mitochondrial Ca^{2+} uptake promotes loss of mitochondrial membrane potential allowing cytochrome c release and apoptosis. Secondly, CaMKII promotes JNK signalling, through activation of the MKKKs ASK1 [150] or TAK1 [151] and subsequent MKK4 activation (Fig. 3b). CaMKII-dependent JNK activation promoted a subsequent increase in Fas (a death receptor) expression and consequently an increase in the cell surface localisation of Fas [148]. In vivo, the knockout of CaMKII in mice reduced ER stress-induced apoptosis in macrophages, spleen and renal tubular epithelium. Renal function was maintained despite tunicamycin treatment in the CaMKII knockout mice [148], showing that ER stress-induced CaMKII activation is required for cell death. Therefore, in addition to activation by the IRE1-TRAF2-ASK1 pathway, JNK is also activated in response to CaMKII signalling. However, it is unclear whether the PERK-CHOP axis of the UPR has a role in the ER stress-induced Ca^{2+} release and CaMKII activation.

7. ATF6 and MAPK signalling

7.1. p38 phosphorylates ATF6 increasing its transcriptional activity

In atrial cardiomyocytes, growth factor stimulation promotes the p38-dependent expression of atrial natriuretic factor (ANF). Intriguingly, the promoter region for ANF does not contain a binding site for the previously recognised p38-inducible transcription factors, but can be activated by ATF6. Analysis of ATF6 showed that it can undergo phosphorylation and was a substrate for p38 in vitro. Furthermore, transfection of primary myocardial cells with ATF6 and p38α, promoted the transactivation activity of ATF6 in a luciferase reporter assay [152]. Sustained p38 activation resulted in increased ATF6 phosphorylation and promoted the activity of the transactivation domain of ATF6 at the *BiP* promoter (Fig. 4). Interestingly, following Tg treatment, induction of BiP expression occurred independently of p38 activation. On the other hand, p38 signalling was required for induction of BiP transcription in response to the ER stress inducer and proline analogue azetidine [153]. This suggests variation in the use of p38 depending on the ER stress inducer; whether this reflects the contributions made by other pathways activated by these various ER stressors is unclear.

8. ER stress and MAPK signalling in disease

ER stress and the resultant activation of signalling pathways arising from this has a key role in many diseases including neurodegeneration [154] and various cancer types [155]. In some cases, MAPK signalling has been identified as interacting with the ER stress response in specific disease models, as highlighted below.

8.1. Cell surface BiP and ERK1/2 activation

The protein chaperone BiP is ubiquitously expressed but its expression is increased following ER stress. Newly synthesised BiP has been found to translocate to the external surface of the plasma membrane perhaps due to association with DnaJ family members [156]. BiP on the cell surface of colon cancer, neuroblastoma and leukaemia cells [157] interacts with many proteins and may initiate RAF-MEK1/2-ERK1/2 signalling to promote tumour cell survival [158]. Indeed detection of over expressed or cell surface BiP has been proposed as a potential diagnostic marker in hepatocellular carcinoma and other cancers due to the detection of autoantibodies against BiP in patient samples [159].

8.2. ER stress and ERK1/2 signalling in cancer

Around 50% of melanomas contain an activating $BRAF^{V600E}$ mutation and exhibit hyperactivation of ERK1/2 and addiction to this pathway; therefore the generation of therapeutics that target the mutant kinase are of considerable interest. Vemurafenib is a $BRAF^{V600E}$ specific inhibitor that is effective in treating melanoma patients although acquired resistance to vemurafenib is a common problem. Interestingly, treatment with vemurafenib has been found to promote the release of Ca^{2+} from the ER in melanoma cells, thereby activating the UPR [160]. Knockdown of ATF4 reduced apoptosis arising from vemurafenib treatment, suggesting that vemurafenib promotes ER stress-induced apoptosis. However, melanoma cells with acquired resistance to vemurafenib were still sensitive to Tg-induced cell death implying only partial overlap between the mechanism of apoptosis induction with these treatments [160]. Further support for the combined use of ERK1/2 signalling pathway inhibitors and ER stressors comes from the finding that whilst some human melanoma cell lines are relatively insensitive to ER stress-induced or MEK1/2 inhibitor-induced cell death, treatment of these cells with a MEK1/2 inhibitor or with MEK1 targeted siRNA sensitised the cells to ER stress-induced apoptosis [161]. The mechanism for this varied between the melanoma cell lines. In some cases MEK1/2 inhibition resulted in a reduction in both BiP mRNA and protein levels. Caspase 4 is localised on the ER membrane and is cleaved and activated in response to treatment with ER stress inducers [142]. Inhibition of MEK1/2 promoted release of caspase 4 from BiP and so promoted its activation. The subsequent activation of caspase 4 required stimulation by an ER stressor, as MEK1/2 inhibition and addition of BiP siRNA was insufficient to promote cell death [161]. Therefore ERK1/2 activation protects melanoma cells against ER stress-induced cell death through increased BiP expression and inhibition of caspase 4 activation.

Another mechanism by which ERK1/2 activation may protect against ER stress-induced cell death is the ERK1/2-dependent up-regulation of MCL-1, a pro-survival BCL2 protein. The activation of IRE1 and ATF6 in melanoma cells following ER stress promoted the up-regulation of MCL-1 mRNA and protein, and this was partially blocked by inhibitors of ERK1/2 signalling. Following ER stress MCL-1 can bind to and neutralise the pro-apoptotic proteins PUMA and NOXA, preventing apoptosis, whereas loss of MCL-1 releases PUMA and NOXA (which are also up-regulated by ER stress), thereby sensitising cells to ER stress-induced apoptosis [162].

In support of a role for ERK1/2 activation in the resistance of melanoma cells to ER stress, a recent study investigated the effect of oncogenic $BRAF^{V600E}$ overexpression and increased ERK1/2 activation in melanocytes. Activation of ERK1/2 was found to promote the phosphorylation of eIF4E and thus induce an increase in protein synthesis in addition to increased IRE1 and ATF6 signalling. This suggests that acquisition of oncogenic $BRAF^{V600E}$ causes chronic ER stress. Survival and adaptation of cells to this chronic ER stress appear to be one of the changes required to promote melanoma pathogenesis [163].

It is intriguing that inhibition of $BRAF^{V600E}$ by vemurafenib treatment induced ER stress via release of Ca^{2+} from the ER and reduced protein folding capacity [160] but that overexpression of $BRAF^{V600E}$ also induced ER stress through increased protein load [163]. This indicates that contradictory changes to the cellular signalling network can perturb ER homeostasis resulting in ER stress. Indeed, manipulation of ER homeostasis has obvious therapeutic potential, particularly in combination with ERK1/2 inhibition in melanoma cells. However there is also the challenge that ER homeostasis can be unbalanced as an unanticipated consequence of cell treatment in both experimental and therapeutic settings.

In addition to melanoma cells, ERK1/2 activation also reduced ER stress-induced cell death in hepatocellular carcinoma cells where it acted alongside Akt signalling to promote cell cycle arrest following ER stress. Interestingly ER stress-induced Akt activation was inversely correlated to ERK1/2 activation suggesting cross-talk between these signalling pathways in the response to ER stress. This is likely due to the Akt-dependent inhibitory phosphorylation of the MKKK c-RAF on Ser259 and thus a reduction in ERK1/2 activation. However, after around 3 h of Tg or tunicamycin treatment there was a reduction in Akt activation and a subsequent increase in ERK1/2 activation in hepatocellular carcinoma cells. Although activation of either Akt or ERK1/2 signalling appeared to promote cell cycle arrest following ER stress, ERK1/2 activation was a more potent inhibitor of ER stress-induced cell death in hepatocellular carcinoma cells [164]. ER stress-induced loss of cyclin D1 was required for cell cycle arrest [164] but it is unclear how ERK1/2 signalling may contribute to this cell cycle arrest, especially as sustained ERK1/2 activation promotes cyclin D1 expression and cell cycle progression in fibroblast cells [99]. However, concurrent activation of the UPR will alter the context for ERK1/2 signalling and this may account for the contribution of sustained ERK1/2 signalling to ER stress-induced cell cycle arrest in hepatocellular carcinoma cells. Both the ERK1/2 and Akt signalling pathways also protected against ER stress induced cell death in human lung (H1299) and breast (Mcf-7) cancer cell lines [165]. However, Akt inhibition resulted in a greater increase in ER stress-induced cell death than ERK1/2 inhibition in these cell lines, due to the Akt-dependent increase in the pro-survival proteins c-IAP-2 and XIAP which inhibit ER stress-induced caspase activation [165]. Thus it is clear that ERK1/2 activation protects against ER stress-induced cell death through cell cycle arrest, up-regulation of pro-survival proteins including MCL-1 and/or increased BiP expression in a variety of human tumour cell lines.

On the other hand, other cancer cell lines show an ERK1/2-dependent increase in ER stress-induced cell death. In the human neuroblastoma cell line, SH-SY5Y, inhibition of ERK1/2 activation reduced Tg-induced cell death as judged by cell lysis and lactate dehydrogenase release. This effect was also seen in response to alternative ER stressors and suggested that ERK1/2 activation might promote ER stress-induced cell death in SH-SY5Y cells. Intriguingly, ERK1/2 inhibition had no effect on the proportion of cells with apoptotic markers, including DNA laddering, caspase 3 activation and poly ADP-ribose polymerase cleavage. This suggests that ER stress-induced ERK1/2 activation may promote either caspase-independent cell death or could act downstream of caspases to promote cell death [166]. The mechanism for cell death induced by ERK1/2 activation following ER stress is yet to be identified; however, ERK1/2 activation has also been shown to promote ER stress-induced cell death in the colorectal cancer cell line, HCT116. In these cells, Tg-induced cell death was caspase 8-dependent and activation of caspase 8 was in turn partially dependent on CHOP-induced DR5 expression [43,167]. Although ERK2 activation was found to promote Tg-induced cell death in HCT116 cells the mechanism for this cell death is unclear as PUMA, NOXA and BAD are reported to have a role in this cell death [167] but in most cases are repressed by ERK1/2 signalling [101,102].

The role of ERK1/2 activation in promoting ER stress-induced cell death in the colorectal cancer cell line HCT116 [167] and the neuroblastoma cell line SH-SY5Y [166] is in contrast to melanoma, breast cancer and a number of other cancer cell lines, where ERK1/2 activation has been shown to protect against ER stress-induced apoptosis [161,165].

8.3. Retinal degeneration in Drosophila

Activation of ER stress signalling has been identified in many degenerative diseases including human age-related retinal degenerative disease (retinitis pigmentosa), which is caused by the expression of mutant rhodopsin proteins. The expression of a similar mutant *rhodopsin-1* allele in *Drosophila* can be used to model this disease and was found to activate ER stress-induced apoptosis. The ER stress-induced cell death caused by expression of mutant Rhodopsin-1 was attenuated by the knockdown of CDK5 or MEKK1 [168]. CDK5 has been shown to be activated by a variety of stresses and increased activation of CDK5 promotes neurodegeneration in models for Alzheimer's [169] and Parkinson's [170] disease. When the UPR was interrogated, CDK5 activation did not affect the activation of ATF4 or splicing of *XBP1*, and may act downstream of UPR activation. Although the mechanism for ER stress-induced CDK5 activation is unclear, knockdown of CDK5 or MEKK1 was able to delay late onset retinal degeneration in a *Drosophila* model [168]. CDK5 is able to bind and phosphorylate MEKK1, resulting in its activation and subsequent JNK activation suggesting that JNK might be responsible for ER stress-induced CDK5-dependent cell death.

8.4. Atherosclerosis

The development of atherosclerotic lesions is associated with the recruitment of monocytes to the sub-endothelial space, which then differentiate into macrophages. Macrophages internalise cholesterol-rich lipoprotein and this is esterified and stored as cholesteryl-fatty acid esters [171]. Advanced lesions commonly show a build-up of unesterified cholesterol, which has been found to activate the UPR and can lead to apoptosis [172]. A common atherosclerosis model that involves the loading of macrophages with unesterified cholesterol induced a p38-dependent increase in CHOP, whereas other forms of ER stress induction, such as Tg or tunicamycin treatment result in p38-independent CHOP induction. In this model for atherosclerosis, activation of three pathways was required for induction of apoptosis; ER stress-induced JNK and p38 pathway activation, which subsequently led to CHOP induction and thirdly, activation of signalling downstream of the Scavenger Receptor Type A (also known as CD204) was required [173]. The signalling involved in CD204-induced cell death has not been clearly identified but may involve inhibition of PI3K-Akt or β-catenin signalling [174]. Further to the activation of JNK and p38 signalling, cholesterol trafficking to the ER in macrophages promoted the production of the inflammatory cytokines TNFα and interleukin-6 (IL-6). The increase in *TNFα* mRNA was CHOP-independent, but required the activation of p38 and JNK. In contrast, loss of CHOP reduced ERK1/2 activation and subsequent IL-6 production following free cholesterol stimulation [175]. This suggests a possible link between partially p38-dependent CHOP induction and ERK1/2 activation in macrophages.

8.5. Diabetes and JNK activation

In mouse models for obesity, liver and adipose tissue samples were found to have increased ER stress signalling and JNK activation. Furthermore, treatment of murine liver cells with an ER stressor decreased insulin-induced signalling, suggesting a possible link between ER stress, obesity and insulin sensitivity. Insulin is an activating ligand of both the insulin and insulin-like growth factor-1 receptors, which are both receptor tyrosine kinases. Activation of these receptors promotes binding and tyrosine phosphorylation of insulin receptor substrate 1 (IRS1) which initiates downstream signalling. Treatment of murine liver cells with an ER stressor promoted IRE1 activation and subsequent JNK

activation [176]. IRS1 undergoes Ser307 phosphorylation (equivalent to Ser312 on human IRS1) by JNK, which inhibits insulin-induced tyrosine phosphorylation of IRS1 and downstream signalling [177]. This clearly demonstrates a link between ER stress, JNK activation and insulin sensitivity. Indeed, mice that are heterozygous for the presence of XBP1 demonstrate higher levels of ER stress signalling, reduced sensitivity to insulin and were pre-disposed to develop type II diabetes [176]. Moreover, chemical chaperones such as 4-phenyl butyric acid that improve the folding capacity of the ER have been demonstrated to reduce UPR signalling in mouse obesity models and lower blood insulin and glucose levels closer to those in wild type mice. Indeed, markers for both obesity and type II diabetes are normalised in mouse models of obesity treated with chemical chaperones that reduce ER stress signalling [178]. Thus in mouse, high levels of UPR signalling and subsequent JNK activation promote reduced sensitivity to insulin and the development of type II diabetes.

8.6. ER stress pre-conditioning in ischemia

In models of ischemia within the kidney, activation of ERK1/2 signalling or inhibition of JNK protected against cell death due to oxidative stress [179]. Activation of the UPR has been identified during ischemia/reperfusion injury within the brain, heart and kidney [180]. In the post-ischemic kidney, cells were protected against subsequent ischemia-induced injury for up to 15 days and this was associated with loss of JNK and p38 signalling [181]. In a model for this transient ischemia-induced protection, renal epithelial cells were pre-conditioned by ER stress, which protected these cells against subsequent oxidative stress. The ER stress pre-conditioned cells showed greater activation of ERK1/2 signalling and a reduction in JNK activation following oxidative stress. This suggests that pre-conditioning cells by activation of the UPR modulates the balance of signalling through different MAPK pathways to promote cell survival following subsequent oxidative stress [179]. The mechanism for this 'cellular memory' effect is not currently known but likely reflects changes in the expression of genes controlling detoxification, damage repair or cell death.

9. Conclusions and future challenges

There are clearly strong links between the UPR and MAPK signalling networks. In some cases UPR signalling feeds into MAPK activation so that the MAPKs act as effector pathways; in addition, a number of interaction points allow cross-talk between these pathways. Activation of the UPR promotes IRE1-dependent ERK1/2 activation and JNK activation through IRE1-TRAF2-ASK1 signalling or due to ER stress-induced Ca^{2+} release. In addition, MAPK signalling can modulate the UPR through p38-dependent phosphorylation of CHOP and ATF6. In general, ERK1/2 activation is thought to promote cell survival whereas JNK and p38 signalling are considered pro-apoptotic. However, there are several exceptions to this; for example, IRE1-dependent JNK signalling promotes c-Jun activity and expression of the pro-survival protein Adapt78. In addition, although there are many cases where ERK1/2 signalling protects against ER stress-induced cell death (such as in melanoma or hepatocellular carcinoma), there are several cases where the opposite applies (including the colorectal cancer cell line, HCT116, or the neuroblastoma cell line SH-SY5Y). What determines whether MAPK-dependent signalling promotes cell proliferation, survival or cell death is unclear but by analogy with other studies the response to ER stress probably reflects differences in signal duration, signal magnitude, coincident signalling events and cell type-specific expression of MAPK effectors.

Although there are still several challenges in understanding the role of MAPK signalling and ER stress in some diseases, there are cases where there are indications that therapeutics to manipulate both UPR and MAPK signalling could be advantageous. For example, some melanoma lines are relatively insensitive to treatment with inhibitors of ERK1/2 activation or ER stressors individually but their combined use promoted ER stress-induced cell death [161]. Indeed, cells that were resistant to the $BRAF^{V600E}$ inhibitor vemurafenib were still sensitive to ER stress-induced cell death [160], again indicating that combining ERK1/2 pathway inhibitors and ER stressors should be investigated. On the other hand, JNK inhibition may promote protection against ER stress-induced cell death in retinal degeneration, ischemia and atherosclerosis. In addition, JNK inhibition and increased ER protein folding capacity due to chaperone expression could reduce inflammatory cytokine production in atherosclerosis. Furthermore, a reduction in ER stress signalling normalised obesity and type II diabetes markers in mice [178] suggesting potential for similar treatments in human cells. Whilst inhibitors of MAPK pathways are already well characterised and undergoing clinical evaluation, the UPR field has lagged behind in terms of drug discovery and potent and selective agents targeting the UPR components are relatively few. However, the recent description of a selective PERK inhibitor [182] and the emergence of a number of IRE1 inhibitors [183] should pave the way towards a greater understanding of the roles of PERK and IRE1 in disease.

However, the biggest challenge in developing treatments that manipulate UPR signalling or ER homeostasis in disease is avoiding off-target affects. Without targeting the ER stressor or UPR inhibitor to a subset of cells, off-target changes to ER homeostasis are likely to particularly affect secretory cells including insulin-producing pancreatic β cells and antibody-secreting B lymphocytes, due to the acute ER stress involved in these processes. In addition, understanding the mechanism of ER stress-induced cell death in different cells and how MAPK signalling interacts with this may provide further insight into the variation in response to ERK1/2 activation seen in different cancer cell types, in addition to highlighting further candidates for therapeutic intervention.

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