© 2014. Published by The Company of Biologists Ltd | Journal of Cell Science (2014) 127, 3649–3658 doi:10.1242/jcs.153643

COMMENTARY

Redox controls UPR to control redox

Davide Eletto[1,*], Eric Chevet[2], Yair Argon[1] and Christian Appenzeller-Herzog[3,*]

ABSTRACT

In many physiological contexts, intracellular reduction–oxidation (redox) conditions and the unfolded protein response (UPR) are important for the control of cell life and death decisions. UPR is triggered by the disruption of endoplasmic reticulum (ER) homeostasis, also known as ER stress. Depending on the duration and severity of the disruption, this leads to cell adaptation or demise. In this Commentary, we review reductive and oxidative activation mechanisms of the UPR, which include direct interactions of dedicated protein disulfide isomerases with ER stress sensors, protein S-nitrosylation and ER Ca^{2+} efflux that is promoted by reactive oxygen species. Furthermore, we discuss how cellular oxidant and antioxidant capacities are extensively remodeled downstream of UPR signals. Aside from activation of NADPH oxidases, mitogen-activated protein kinases and transcriptional antioxidant responses, such remodeling prominently relies on ER–mitochondrial crosstalk. Specific redox cues therefore operate both as triggers and effectors of ER stress, thus enabling amplification loops. We propose that redox-based amplification loops critically contribute to the switch from adaptive to fatal UPR.

KEY WORDS: Endoplasmic reticulum, Endoplasmic reticulum stress, Mitochondria, Protein disulfide isomerase, Reactive oxygen species, Unfolded protein response

Introduction

A fundamental property of Metazoa is their ability to send, receive and decode signals between neighboring tissues and the extracellular environment. As a prerequisite for such communication, eukaryotic cells developed a specialized endomembrane system known as the secretory pathway, in which peptide hormones, membrane receptors and other secretory cargo proteins are synthesized, folded and eventually released to the extracellular space. The endoplasmic reticulum (ER), where translation and folding of extracellular proteins occurs, is the first component of the secretory pathway (Gidalevitz et al., 2013). When ER homeostasis is compromised, a set of 'ER stress' signals is transmitted to the cytoplasm and nucleus. This ER-centric system of signal transduction is defined as the unfolded protein response (UPR), the primary function of which is to re-establish ER homeostasis (Hetz, 2012). If this fails, however, the very same signaling network is capable of sending death messages, leading to programmed cell death (Tabas and Ron, 2011).

The UPR in higher eukaryotes relies on three major signaling cascades originating from the ER membrane UPR sensors inositol-requiring protein-1 (IRE1α or IRE1β, herein referred to as IRE1; also known as ERN1), protein kinase PKR-like ER kinase (PERK; also known as EIF2AK3), and activating transcription factor-6α (ATF6). IRE1 is a ribonuclease that catalyzes non-canonical splicing of X-box binding protein 1 (XBP1) mRNA, a step that is required for the expression of this master transcription factor of UPR target genes (Calfon et al., 2002; Lee et al., 2002; Yoshida et al., 2001). The ribonuclease activity is also required for regulated IRE1-dependent mRNA decay (RIDD) (Hollien et al., 2009; Maurel et al., 2014). In addition, activated IRE1 can trigger the apoptosis signal-regulating kinase-1 (ASK1; also known as MAP3K5) to JUN N-terminal kinase (JNK) pathway (Urano et al., 2000). PERK is a kinase, the main target of which upon ER stress is the α subunit of the eukaryotic translation initiation factor-2 (eIF2α; also known as EIF2S1) (Harding et al., 2000b). Phosphorylated eIF2α, in turn, causes general attenuation of protein synthesis while promoting the expression of the UPR transcription factor ATF4 (Harding et al., 2000a). ATF6 is itself a transcriptional activator of UPR target genes. Normally confined to the ER membrane, it is activated upon ER stress and migrates to the Golgi where its cytosolic DNA-binding domain is freed through intra-membrane proteolysis (Haze et al., 1999).

A biochemical process that is crucial for ER protein homeostasis is the formation of disulfide bridges, which is often referred to as oxidative protein folding. Disulfide bonds are introduced into nascent proteins by ER-resident protein disulfide isomerases (PDIs) (Appenzeller-Herzog and Ellgaard, 2008), resulting in reduction of the latter. A number of pathways exist for the re-oxidation of PDIs, among these the best conserved – catalyzed by endoplasmic oxidoreductin 1 (Ero1α and Ero1β in human; encoded by *ERO1L* and *ERO1LB*, respectively) – exploit the oxidizing power of molecular oxygen (Box 1) (Bulleid and Ellgaard, 2011). At the same time, disulfide-reducing pathways are essential for the proof reading of incorrectly introduced disulfide bonds and for efficient degradation of ER proteins (Kojer and Riemer, 2014). As in probably every cell compartment (Schafer and Buettner, 2001), millimolar concentrations of the redox couple glutathione (GSH)–glutathione disulfide (Montero et al., 2013) are important for disulfide reduction and the maintenance of redox homeostasis in the ER (Appenzeller-Herzog, 2011). Thus, a healthy ER environment is characterized by a well-tuned curtailing of both oxidizing and reducing power.

In this Commentary, we summarize the consequences of redox disturbance for ER function. ER dysfunctions can cause cellular redox changes, which in turn feedback onto the ER to influence cell survival and death. These interrelations place redox disturbances at the interface of ER stress and disease.

[1]Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia and The University of Pennsylvania, Philadelphia, PA 19104, USA. [2]INSERM U1053, Université Bordeaux 33076 Segalen, Bordeaux, France. [3]Division of Molecular & Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, CH-4056 Basel, Switzerland.

*Authors for correspondence (davide.laptop@gmail.com; christian.appenzeller@unibas.ch)

Journal of Cell Science (2014) 127, 3649–3658 doi:10.1242/jcs.153643

Box 1. Sources of disulfide bonds in the ER

Oxidative folding – a process predominantly catalyzed in the ER – is defined as the acquisition of tertiary structure by a polypeptide chain through the formation of covalent disulfide crosslinks between specific cysteine residue side chains. Disulfide bond generation is an oxidative process, which necessitates the use of suitable electron acceptors. In the ER, molecular oxygen (O_2) and H_2O_2 are the principal electron acceptors for oxidative folding (besides a number of ill-characterized alternative pathways) (Bulleid and Ellgaard, 2011). O_2 assists disulfide bond generation, which is catalyzed by oxidases [e.g. of the Ero1 family (Ramming and Appenzeller-Herzog, 2012)], and H_2O_2 disulfide bond generation by the ER-resident peroxidases GPX7, GPX8 and peroxiredoxin IV (PrxIV; also known as PRDX4) (Ramming and Appenzeller-Herzog, 2013). Because a necessary by-product of Ero1 catalysis is H_2O_2, peroxidase-mediated disulfide bond formation through H_2O_2-reduction is also required to control Ero1-derived H_2O_2 and ER redox homeostasis. This control function has originally been ascribed to PrxIV (Tavender and Bulleid, 2010), but more recent findings suggest that (i) the GPX enzymes specifically reduce Ero1-derived H_2O_2 and that (ii) PrxIV targets an alternative source of H_2O_2 in the ER (Ramming et al., 2014). In most disulfide-generating pathways in the ER, members of the PDI family serve as the primary disulfide acceptor (Appenzeller-Herzog and Ellgaard, 2008; Gidalevitz et al., 2013). PDIs, in turn, can oxidize cysteine residues on substrates to mediate oxidative folding.

Box 2. Reactive oxygen species

In the cell, molecular oxygen (O_2) can react with free radicals (molecules with a single unpaired electron that are derived from internal metabolism or external exposure) or with enzymes catalyzing electron transfer reactions to form reactive oxygen species (ROS). These include, but are not limited to, superoxide anions, H_2O_2 and hydroxyl radicals. ROS rapidly interconvert in reactions that are catalyzed by enzymes (e.g. superoxide-dismutase-catalyzed conversion of superoxide anion into H_2O_2 and O_2) or metal ions (e.g. iron-catalyzed reduction of H_2O_2 to hydroxyl radicals mediated by Fenton chemistry). Physiological ROS concentrations fulfill important signaling roles as second messengers but, in excess, ROS can oxidize lipids (lipid peroxidation), cause oxidative protein cleavage (protein carbonylation) or damage DNA (Gough and Cotter, 2011). Such deleterious perturbations are referred to generically as 'oxidative stress'.

Reductive and oxidative insults trigger UPR

The early finding that glucose-regulated UPR target genes are induced by treatment with reducing agents, which antagonize the formation of disulfide bonds, was made before the actual discovery of the UPR (Kim and Lee, 1987). Nowadays, disulfide-reducing agents such as dithiothreitol (DTT) are widely being used as rapid inducers of ER stress (DuRose et al., 2006), and it has been shown that even an excess of the endogenous ER reductant GSH can activate the UPR by compromising oxidative protein folding (Kumar et al., 2011).

However, there is accumulating evidence that ER stress signaling is elicited also in response to oxidative triggers. Treatments that cause intracellular release of reactive oxygen species (ROS; Box 2) lead to pro-apoptotic ER stress (Dejeans et al., 2010; Inoue and Suzuki-Karasaki, 2013; Santos et al., 2009), a phenomenon that is being exploited in photodynamic cancer therapies (Garg and Agostinis, 2014). Like ROS generation, nitrosative stress is also linked to UPR-mediated cytotoxicity through the inactivation of active-site cysteine residues in PDIs through nitrosylation (Uehara et al., 2006).

In addition to these non-ER challenges, oxidative protein folding in the ER is assumed to be a source of ER over-oxidation and stress (Han et al., 2013; Marciniak et al., 2004; Tu and Weissman, 2004). Expression of a hyperactive Ero1α mutant causes detectable concentrations of its reaction by-product hydrogen peroxide (H_2O_2; Box 2) in the ER, along with mild UPR activation, which is exacerbated by knockdown of the ER-resident and UPR-inducible glutathione peroxidase 8 (GPX8) (Ramming et al., 2014). Accordingly, Ero1α-derived H_2O_2, rather than disulfide overproduction, appears to affect ER homeostasis. Similarly, overexpression of Ero1β in pancreatic β-cells causes ER stress (Awazawa et al., 2014). Whether H_2O_2 that is derived from endogenous Ero1 is sufficient to trigger ER stress is questionable (Appenzeller-Herzog, 2011; Ramming et al., 2014). Interestingly, peroxide sensing or quenching by GPX7 (a GPX8-related ER peroxidase, also known as NPGPx) (Ramming and Appenzeller-Herzog, 2013) promotes cell survival by stimulating the ER chaperone BiP (also known as GRP78 and HSPA5) (Wei et al., 2012).

Although the mechanism by which reductive challenge of the ER causes stress is mainly based on compromised disulfide bond formation and consequent protein misfolding, it is less clear how hyper-oxidizing conditions affect the ER. As discussed below, we propose that – besides sulfoxidation, nitrosylation or glutathionylation of key cysteine residues in ER lumenal proteins (e.g. in PDIs) – specific oxidation of ER membrane proteins has profound effects on Ca^{2+} homeostasis and UPR signaling.

Connections between the redox state and Ca^{2+}

Ca^{2+} influx into the ER is governed by ATP-dependent Ca^{2+} import pumps that belong to the sarcoendoplasmic reticulum Ca^{2+} transport ATPase (SERCA) family, whereas Ca^{2+} efflux from the ER is controlled by excitable Ca^{2+} release channels from the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) and, mainly in muscle and brain tissue, the ryanodine receptor (RyR) families, as well as a heterogeneous collection of Ca^{2+} leak pores (Berridge et al., 2003). Interestingly, different UPR-triggering stimuli, such as unsaturated fatty acids, DTT, cytokines or expression of misfolded ER proteins, result in loss of Ca^{2+} from the ER (Hara et al., 2014). This can occur owing to inhibition of SERCA, the opening of release pores, or both. Lower SERCA activity, e.g. following disturbed lipid metabolism, glucose starvation or inflammatory mediators, is a well-known phenomenon during ER-stress-dependent toxicity (Cardozo et al., 2005; Fu et al., 2011; Hara et al., 2014; Moore et al., 2011). Likewise, altered IP_3R activity and increases in passive Ca^{2+} leak from the ER are hallmarks of ER-stressed cells (Kiviluoto et al., 2013) (see also below).

ER Ca^{2+} pumps and IP_3R or RyR channels are subject to redox regulation, which we propose is an important component of ROS-driven ER stress. Sulfoxidation of the conserved SERCA cysteine residue 674 in response to ROS inhibits pump activity, whereas glutathionylation at Cys674 by nitric oxide activates SERCA (Raturi et al., 2014; Tong et al., 2010).

Journal of Cell Science

In the large IP_3R and RyR channel proteins, complex, yet incompletely defined redox mechanisms occur that involve several cysteine residues (Raturi et al., 2014). Nevertheless, ROS-mediated sensitization to submaximal doses of IP_3 agonist and cytosolic Ca^{2+}, which enhances ER Ca^{2+} release, is well documented (Bansaghi et al., 2014). Taken together, ROS can cause the depletion of Ca^{2+} from the ER, which disables Ca^{2+}-dependent ER chaperones, such as calnexin and calreticulin, and results in ER stress (Hetz, 2012).

Another connection between the redox state and Ca^{2+} that has potential implications for ER stress physiology has been demonstrated by the finding that short-term depletion of Ca^{2+} from the ER, by either inhibition of SERCA or activation of IP_3R, leads to an immediate drop in the reduction potential of the ER (i.e. rendering the ER lumen more reducing) (Avezov et al., 2013; Birk et al., 2013; Enyedi et al., 2010). Although the mechanistic basis for this drop remains to be explored, it is probable that lowering ER Ca^{2+} levels during chronic ER stress (Cardozo et al., 2005; Fu et al., 2011; Hara et al., 2014; Moore et al., 2011) mediates a hypo-oxidizing ER environment. With regards to the reductive activation of ATF6 (see below), ER hypo-oxidation, in turn, will stimulate stress signals.

Control of UPR activation is governed by specific PDI family members

The UPR sensors ATF6, IRE1 and PERK are activated in response to ER stress, which is mainly mediated through the lumenal domains of the sensors. BiP, which binds to all three sensors in their inactive state, is titrated off when the unfolded protein load rises in the ER, and UPR sensors can also directly sense unfolded proteins, leading to their activation (Walter and Ron, 2011). Two studies have recently described redox regulation of the lumenal domains of the ER sensors that is mediated by dedicated PDIs (Eletto et al., 2014; Higa et al., 2014). This regulation impacts the onset, amplitude and duration of signaling by each of the sensors, thus providing a mechanistically novel concept of sensor-specific UPR activation.

In the unstressed ER, ATF6 exists in the form of disulfide-bonded monomers, dimers or oligomers, which rely on cysteine residues 467 and 618 in the ER lumenal domain (Nadanaka et al., 2007) (Fig. 1A). When oxidized, the sensor remains in the ER, but in response to ER stress, the disulfide bonds in ATF6 are cleaved, and monomeric reduced ATF6 is released from the organelle (Fig. 1B). Not surprisingly, this occurs more rapidly in cells that are treated with DTT compared with cells that are treated with other pharmacological UPR triggers (DuRose et al., 2006; Higa et al., 2014; Nadanaka et al., 2007). In cells that have been depleted of PDIA5 (also known as PDIR), but not of other PDIs, oligomer dissociation, ER-to-Golgi migration and induction of ATF6 target genes are all inhibited, strongly suggesting that PDIA5 cleaves the disulfide bonds in ATF6 (Higa et al., 2014). ER-stress-induced dissociation of BiP from ATF6, however, occurs independently of PDIA5 (Higa et al., 2014) and is still a prerequisite for ER-to-Golgi transport of monomeric ATF6 in which cysteine residues 467 and 618 are mutated (Nadanaka et al., 2007). Thus, PDIA5-mediated reduction of ATF6 is necessary but not sufficient for Golgi targeting, which in addition requires the dissociation of BiP.

Unlike in the case of ATF6, the thiol–disulfide configuration of the three lumenal cysteine residues in IRE1 or the four lumenal cysteine residues in PERK (Fig. 1A) in their inactive states is not clear. However, similar to ATF6, the activities of IRE1 and PERK are controlled by thiol–disulfide exchange in their lumenal domains. Here, PDIA6 (also known as P5) is the specific catalyst, as indicated by the trapping of sensor–PDIA6 interchain disulfide bonds (Eletto et al., 2014). Depletion of PDIA6 increases the duration and amplitude of IRE1 or PERK signaling, causes profound hypersensitivity of cells to ER-stress-inducing agents and mediates a developmental block in *Caenorhabditis elegans* (Eletto et al., 2014). These observations indicate that PDIA6 terminates sensor activation and prevents exaggerated UPR signaling.

Although the lumenal cysteine residues in IRE1 are dispensable for signal initiation, they can engage in Cys148–Cys148 and Cys332–Cys332 interchain disulfide bonds that result in IRE1 dimers and higher order oligomers (Eletto et al., 2014; Liu et al., 2003). Formation of these interchain disulfide bonds is promoted by ER stress or by IRE1 overexpression (Eletto et al., 2014), which leads to spontaneous IRE1 clustering and activation (Han et al., 2013; Li et al., 2010b). ER-stress-induced IRE1 clustering could then promote the rearrangement of pre-existing intramolecular disulfide bonds (Fig. 1C). Alternatively, interchain disulfide bonds in IRE1 could be formed in response to an oxidative trigger such as ROS, which is consistent with the selective activation of IRE1 by the mitochondrial poison antimycin A (Mori et al., 2013). Irrespective of the precise mechanism, PDIA6 is required to revert this conversion and to inactivate IRE1 through nucleophilic attack, mainly at residue Cys148 (Eletto et al., 2014) (Fig. 1D). Accordingly, Cys148–Cys148 might act as a recognition motif for PDIA6, which in turn limits the signaling and resets the original inactive state of IRE1. To date, the roles of lumenal cysteine residues in PERK are poorly characterized.

Thus, the enzymatic activities of both PDIA5 and PDIA6 specifically act on the stress sensors in response to ER stress to reduce the disulfide bonds in the sensors. The molecular basis for the intriguing selectivity of the PDI–sensor pairs remains to be determined.

The discovery of the PDIA6–IRE1, PDIA6–PERK and PDIA5–ATF6 axes suggests that redox changes not only impact on ER homeostasis (as discussed above) but also on UPR signaling itself (Fig. 2). Accordingly, by promoting oxidation of UPR sensors or of PDIs, ROS signals could potentially inhibit ATF6 signaling while, at the same time, enhancing the duration of IRE1 and PERK signaling. In addition, it is conceivable that physiological triggers of mild ER hypo-oxidation selectively activate the ATF6 arm of the UPR. On a more general note, we propose that the different redox sensitivities of UPR sensors contribute to sensor-selective UPR settings and sensor-specific (in)activation kinetics.

The PDIA5–ATF6, PDIA6–IRE1 and PDIA6–PERK signaling axes promote cell survival

Stress sensor regulation mediated by PDIs probably influences cell physiology. Upregulation of both PDIA5 and PDIA6 is essential for chemoresistant cancer cell growth. Specifically, resistance of leukemic cells to the tyrosine kinase inhibitor Imatinib depends on PDIA5 and pro-survival ATF6 signaling (Higa et al., 2014), and non-small lung cancer cells require robust overexpression of PDIA6 (and PDIA4, also known as ERp72) to withstand treatment with cytotoxic Cisplatin (Tufo et al., 2014). It is attractive to speculate that these pro-survival roles of PDIA5 and PDIA6 in cancer are mainly due to redox regulation of UPR sensors, i.e. promoting pro-survival ATF6 signaling and inhibiting sustained, pro-apoptotic IRE1 or PERK signaling.

Journal of Cell Science (2014) 127, 3649–3658 doi:10.1242/jcs.153643

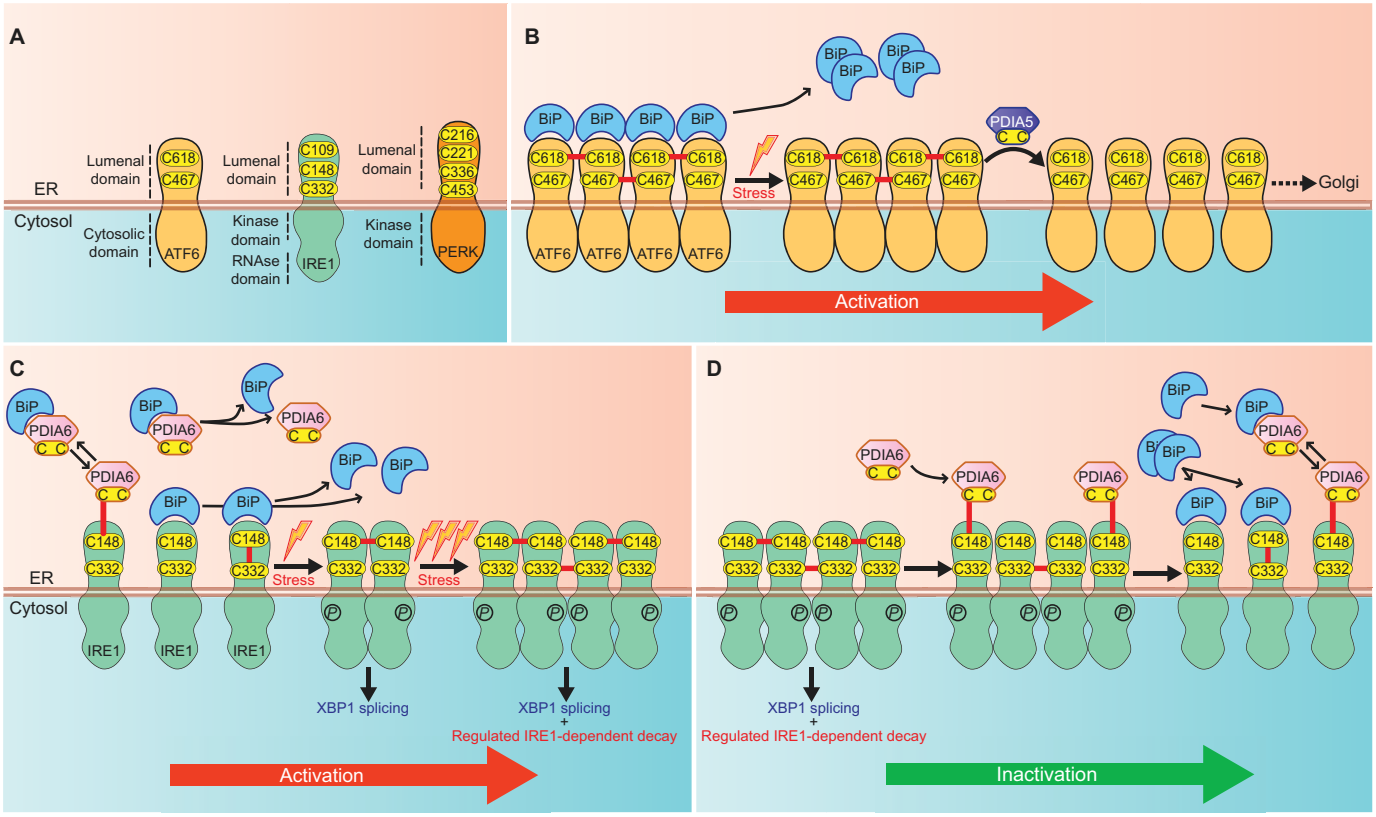

Fig. 1. Redox regulation of UPR sensors. (A) Schematic representation of the three major UPR sensors, ATF6, IRE1 and PERK. The conserved cysteine residues in the respective lumenal domains are indicated. (B) Activation of ATF6. In the unstressed ER, ATF6 forms disulfide-bonded (illustrated by the red line) oligomers (here depicted as a tetramer) that are bound by BiP. Upon ER stress, BiP is released and the ATF6 oligomer is reduced and disassembled in a PDIA5-dependent reaction. Reduced ATF6 can leave the ER and is proteolytically activated in the Golgi (not depicted). This process is irreversible because active ATF6 cannot be recycled to its inactive precursor. (C) Model for IRE1 activation. Under steady-state conditions (left), IRE1 monomers are bound to BiP. A subfraction of IRE1 might form intramolecular Cys148–Cys332 disulfide bonds or mixed disulfides with PDIA6 through Cys148. Under conditions of mild, tolerable stress, BiP dissociation allows the formation of IRE1 dimers that catalyze XBP1 splicing and that might be linked by Cys148–Cys148 (or Cys332–Cys332, not depicted). In parallel, ER stress probably causes the dissociation of BiP–PDIA6 complexes. When the stress is prolonged or exacerbated, IRE1 assembles into large clusters that induce XBP1 splicing and regulated IRE1-dependent decay (shown on the right). IRE1 cluster formation is accompanied by extensive disulfide crosslinking, which requires additional rounds of oxidation. (D) Hypothesized thiol–disulfide exchange reactions during inactivation of IRE1 signaling. Cys148–Cys148 bonds in IRE1 clusters are targeted by PDIA6, resulting in mixed-disulfide formation (middle) and reduction of IRE1 (right). Monomeric IRE1 is then again bound by BiP, thus restoring the steady-state situation. The validity of this model requires experimental verification. *P* represents phosphorylated residues.

Indeed, data for PDIA5- or PDIA6-mediated oxidative protein folding is scarce. It has been shown that, *in vitro*, PDIA5 catalyzes the folding of α_1-antitrypsin (Horibe et al., 2004) and associates with the chaperone calreticulin, which might hint to a role in glycoprotein folding (Vinaik et al., 2013). PDIA6 has been implicated in the folding of BiP substrates (Jessop et al., 2009), although its knockdown has no detectable consequences for oxidative protein folding in hepatocytes or pancreatic β-cells (Eletto et al., 2014; Rutkevich et al., 2010).

One remarkable difference between PDIA5 and PDIA6 is their copy number, with PDIA6 being at least tenfold more abundant, depending upon the cell type (Gidalevitz et al., 2013). PDIA6-mediated control of IRE1 and PERK signaling might be governed by low affinity interactions between the sensor and the enzyme, which could be one reason why PDIA6 is so abundant. Moreover, although PDIA5 also binds to BiP in detectable amounts (Jansen et al., 2012), the non-covalent complex between PDIA6 and BiP appears to be particularly prominent, as shown by several laboratories (Eletto et al., 2014; Jessop et al., 2009; Meunier et al., 2002). Thus, just like the UPR sensors themselves, PDIA6 might be sequestered (or recruited to the vicinity of BiP targets for the catalysis of oxidative folding) under basal conditions through interacting with BiP (Fig. 1C,D). However, a relatively lower amount of PDIA5 appears to suffice to guarantee efficient ATF6 activation. It is possible that PDIA5 is a better reductase than PDIA6, which displays more oxidizing power than other PDIs (Araki et al., 2013).

Collectively, we propose that the pro-survival effects of PDIA5 and PDIA6 can be attributed to their functions as reductants of disulfide bonds in UPR sensors. Genetic or oxidative interference with these functions can contribute to the execution of cell death.

ER stress can increase ROS generation

Although ROS trigger ER stress, they can also arise in response to ER stress in a cell-type-dependent manner (Appenzeller-Herzog, 2011) (Fig. 2). Yeast cells that are defective in clearing misfolded ER proteins, or PERK-knockout mouse fibroblasts that are subjected to ER stress, show prominent ROS levels and reduced viability (Harding et al., 2003; Haynes et al., 2004). In both model systems, ROS generation ceases upon inactivation of the

Journal of Cell Science (2014) 127, 3649–3658 doi:10.1242/jcs.153643

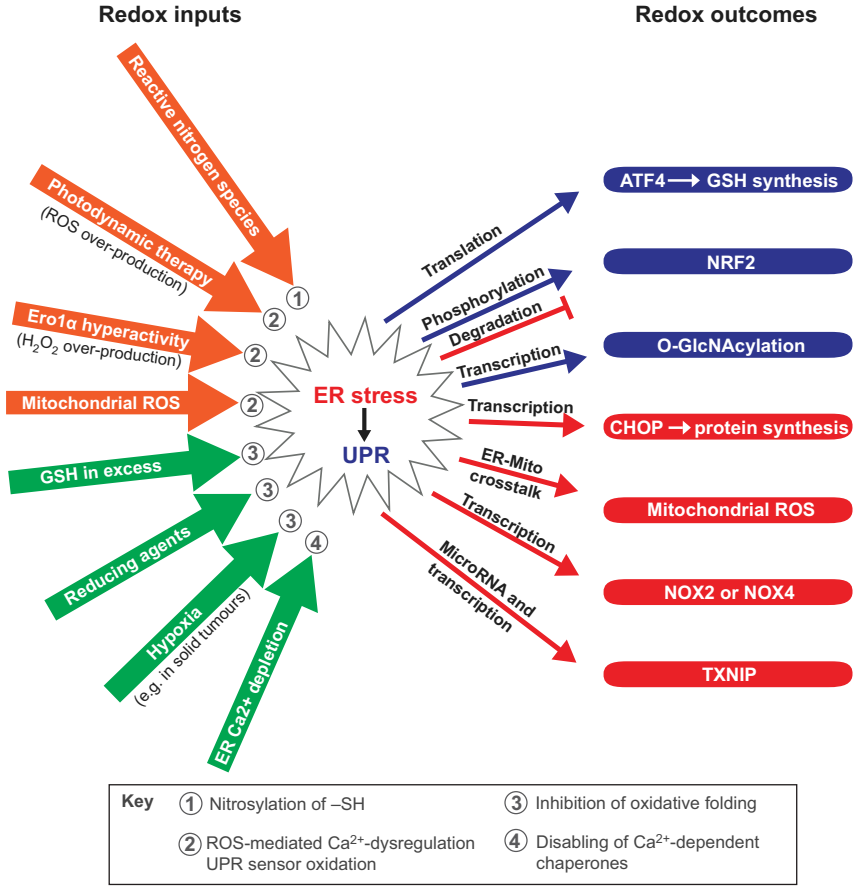

Fig. 2. Redox signals are upstream and downstream of ER stress and UPR. ER stress and UPR signaling ensue in response to a number of experimental or physiological redox inputs, which can be oxidizing or reducing (orange and green arrows, respectively). Putative mechanisms of ER stress induction are indicated in the indexed legend. UPR signaling, in turn, either stimulates or inhibits various redox-relevant effector processes with antioxidant (blue) or hyper-oxidizing (red) outcomes. UPR signaling triggers changes in translational or transcriptional regulation, phosphorylation, degradation, microRNA abundance or ER–mitochondrial (mito) crosstalk. GSH, reduced glutathione; ROS, reactive oxygen species; –SH, thiol groups.

mitochondrial electron transport chain, implying that ER–mitochondrial crosstalk plays a vital part in the redox stress response (see next section). Similarly, overexpression of misfolding-prone coagulation factor VIII induces severe ER stress and intracellular ROS, which can be rescued with the addition of a quencher of free radicals (Malhotra et al., 2008).

There is converging evidence that cellular hyper-oxidation during irremediable ER stress is, at least in part, orchestrated by the transcription factor C/EBP homologous protein (CHOP; also known as DDIT3) (Marciniak et al., 2004). At the transcriptional level, CHOP is targeted by ATF4, and ATF4 and CHOP co-regulate a panel of genes that are involved in protein synthesis upon prolonged stress (Han et al., 2013). Thus, phosphorylated-eIF2α-mediated translation attenuation that has been induced during the adaptive phase of UPR downstream of PERK is reverted, which ultimately increases ROS load and leads to cell death. *In vivo*, a diet containing antioxidants protects pancreatic β-cells from UPR- and protein synthesis-driven demise, suggesting a causal role of ER-stress-induced ROS in apoptosis progression (Back et al., 2009).

One CHOP target gene that contributes to ROS-dependent apoptosis in mammalian cells is *ERO1L* (Han et al., 2013; Li et al., 2009; Marciniak et al., 2004). Furthermore, nematode ERO-1 contributes to animal-wide ROS accumulation in response to ER stress and to decreased life span of ER-stressed animals (Harding et al., 2003; Marciniak et al., 2004). As Ero1-family enzymes are direct producers of ROS (Box 1) (Bulleid and Ellgaard, 2011), an apparently obvious explanation for these findings in mammalian cells and nematodes is that increased Ero1 activity following ER stress causes diffusion of Ero1-derived ROS to the cytoplasm. Testing this model in mammalian tissue culture, however, failed to support this notion and instead identified GPX8 as an ER-stress-regulated ER peroxidase that is crucial for the control of Ero1α-derived ROS and cell survival (Ramming et al., 2014). At second glance, it appears intuitive that ROS as a regular by-product of oxidative protein folding require tight control. Endogenous sources of ROS, which act in cellular signaling, such as NADPH oxidases (NOXs), are typically inducible (Gough and Cotter, 2011), but disulfide production by Ero1 is a constitutive housekeeping process.

In addition to its role in disulfide bond formation, Ero1α is a positive regulator of IP_3R. Accordingly, IP_3R-dependent Ca^{2+} flux to the cytosol and mitochondria is stimulated by induction of Ero1α (Anelli et al., 2012; Li et al., 2009). Increased cytosolic Ca^{2+} levels feed into the JNK pathway through Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and thereby lead to augmented ROS production through NOX2 (also known as CYBB) or NOX4, which in turn leads to oxidative stress and positive-feedback regulation of CHOP through double-stranded RNA-dependent protein kinase (PKR; also known as EIF2AK2) (Li et al., 2010a; Pedruzzi et al., 2004) (Figs 2 and 3). Mitochondrial Ca^{2+} elevation, however, increases the generation and release of mitochondrial ROS by various mechanisms, including opening of the mitochondrial permeability transition pore (MPTP) in the inner mitochondrial membrane (Figueira et al., 2013) (see below).

The ER-stress–mitochondrial signaling axis

Mitochondrial outer membrane permeabilization (MOMP) is the focal point of the intrinsic apoptosis pathway that is executed by

Journal of Cell Science (2014) 127, 3649–3658 doi:10.1242/jcs.153643

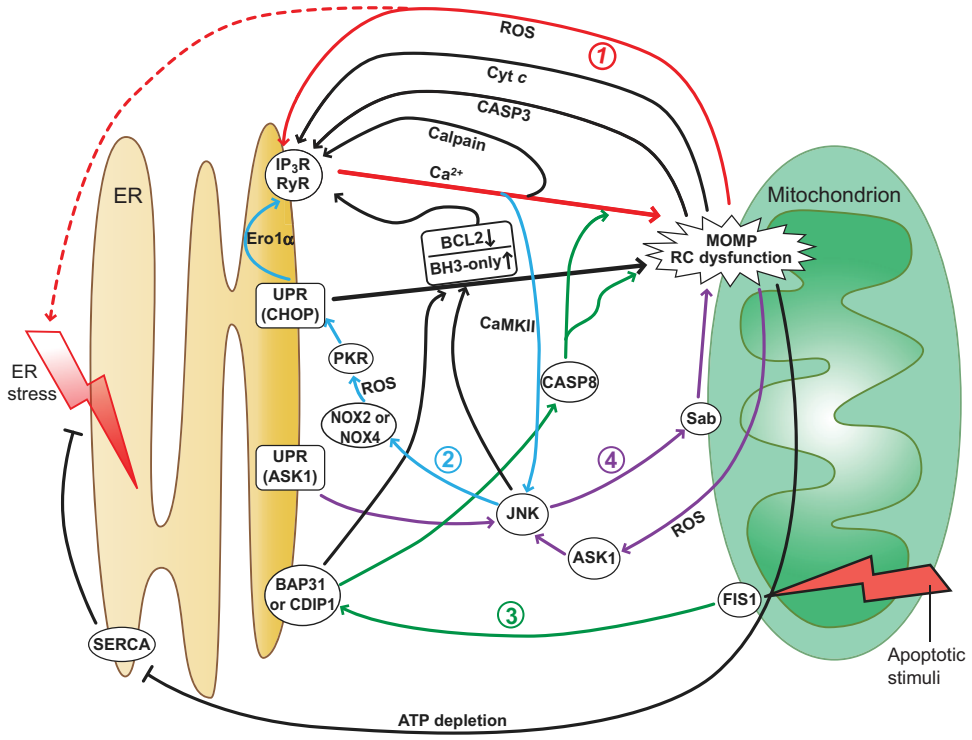

Fig. 3. Amplification loops in ER-centric pro-apoptotic signaling. Chronic ER stress or other apoptotic stimuli engage a variety of signaling loops, many of which localize to the ER–mitochondrion interface. Redox disturbances, as evidenced by an increase in ROS, are an essential component in many of these pathways. The following amplification loops are highlighted: (1, red) ER-stress-induced Ca^{2+} flux across the mitochondria-associated ER membrane (MAM) stimulates MOMP and respiratory chain (RC) dysfunction, leading to ROS increase, which positively feeds back on Ca^{2+} release channels or causes further ER stress (Raturi et al., 2014); (2, blue) an increase of cytosolic Ca^{2+} in response to ER stress triggers the CaMKII–JNK–NOX2/NOX4–PKR–CHOP pathway to amplify ER Ca^{2+} release through transcriptional induction of Ero1α (Li et al., 2010a; Pedruzzi et al., 2004); (3, green) activated FIS1 forms a complex with BAP31 (and possibly the ER-stress-inducible CDIP1), which activates caspase-8 (CASP8) to amplify pro-apoptotic Ca^{2+} and BCL2 input on mitochondria (Iwasawa et al., 2011; Namba et al., 2013); (4, violet) activation of ASK1, and thus JNK, downstream of ER stress is amplified by formation of a JNK–Sab complex (Sab is also known as SH3BP5), RC dysfunction and ROS-dependent activation of ASK1 (Win et al., 2014). For references and details on the pathways marked by black arrows refer to the main text. Cyt *c*, cytochrome *c*.

mitochondria-derived endonucleases, caspase-activating factors such as cytochrome *c*, and ROS (Figueira et al., 2013; Galluzzi et al., 2012). Two pathways mediate MOMP. First, the pro-apoptotic proteins BAX and BAK can assemble into oligomeric pore complexes in the outer mitochondrial membrane. Formation of these complexes is tightly controlled by protein–protein interactions with other factors, including the pro-MOMP BH3-only proteins and anti-MOMP BCL2 (Czabotar et al., 2014). Second, prolonged MPTP opening, for example in response to Ca^{2+} uptake, induces swelling of the mitochondrial matrix, ultimately leading to MOMP (Rizzuto et al., 2012). As discussed below, both of these pathways can be triggered through chronic irremediable ER stress (Fig. 3).

ER stress signals facilitate BAX and BAK oligomerization by inducing the expression of BH3-only proteins, including BIM, NOXA, PUMA, DR5 and CRK (Austgen et al., 2011; Cunha et al., 2012; Li et al., 2006; Puthalakath et al., 2007; Wali et al., 2014) and by repressing BCL2 (McCullough et al., 2001). Furthermore, the BH3-only protein BID is truncated or activated in response to ER stress in a pathway downstream of caspase-8 (Namba et al., 2013) or caspase-2 (Upton et al., 2008) – although the role of caspase-2 is debated (Sandow et al., 2014). The caspase-8 pathway is stimulated by the ER stress effector cell-death-inducing p53 target 1 (CDIP1), which forms a caspase-activating complex with the ER membrane protein BAP31 (also known as BCAP31) (Namba et al., 2013) and, presumably, the mitochondrial outer membrane protein FIS1 (Iwasawa et al., 2011). In addition, the CDIP1–BAP31 complex sequesters BCL2 (Namba et al., 2013). It is worth mentioning that BAX, BAK and some BH3-only proteins also reside on the ER membrane (in addition to mitochondria) where they are necessary for full and sustained activation of IRE1 through direct physical interactions (Hetz et al., 2006; Rodriguez et al., 2012), although the implication of these interactions at mitochondria is currently unclear.

ER–mitochondrial crosstalk is orchestrated at sites of close membrane apposition, termed mitochondria-associated ER membranes (MAMs) (van Vliet et al., 2014). So far, the only established signaling entity that is funneled from the ER to mitochondria at MAMs is Ca^{2+}. ER-to-mitochondria Ca^{2+} flux through a gateway comprising the IP_3R, voltage-dependent anion-selective channel protein 1 (VDAC1) and the mitochondrial Ca^{2+} uniporter (MCU) serves a dual role: at physiological amplitude, it stimulates mitochondrial ATP production, whereas at an excessive pathological amplitude, it initiates MOMP (Rizzuto et al., 2012). Several ER stress mechanisms influence mitochondrial function by boosting Ca^{2+} release from the ER. Firstly, a UPR-induced SERCA1 splice variant augments both resting and stimulated Ca^{2+} transmission to mitochondria (Chami et al., 2008). Secondly, CHOP-dependent

upregulation of Ero1α potentiates IP_3-induced Ca^{2+} release (Anelli et al., 2012; Kiviluoto et al., 2013; Li et al., 2009). Furthermore, the degradation of IP_3Rs and RyRs is inhibited during ER stress (Belal et al., 2012) and the ER membrane protein σ-1 receptor dissociates from BiP to chaperone and stabilize type 3 IP_3R at MAM (Hayashi and Su, 2007). Moreover, ER–mitochondrion contact zones are tightened in response to chronic ER stress, stimulating Ca^{2+}-dependent mitochondrial apoptosis (Csordás et al., 2006). It is possible that such tightening is facilitated by PERK, which is required for integrity and functionality of MAM (Verfaillie et al., 2012). Finally, although not formally proven, it is to be expected that UPR-dependent inhibition of anti-apoptotic Bcl-2 family proteins (by BH3-only proteins, see above) modulates IP_3-induced Ca^{2+} release (Parys, 2014). Taken together, these data demonstrate that the stimulated directed Ca^{2+} transport to mitochondria in response to ER stress can trigger MPTP opening and MOMP.

As exemplified by ROS-mediated amplification of ER Ca^{2+} release downstream of MOMP (Raturi et al., 2014), apoptotic ER–mitochondrial crosstalk entails various positive-feedback loops, including allosteric activation of IP_3R by cytochrome *c* (Boehning et al., 2003), caspase-3- or calpain-catalyzed activating cleavage of IP_3R (Alzayady et al., 2013) and inhibition of SERCA in response to cellular ATP depletion (Moore et al., 2011) (Fig. 3). In fact, it is probable that the propagation of MOMP through the mitochondrial network that precedes the 'point of no return' relies on these amplification loops, the generation of which is under the control of several signaling nodes.

Crosstalk to MAP kinases and antioxidant pathways

The UPR is interconnected with other redox-relevant pathways in addition to those highlighted above, i.e. NOX proteins and MOMP. One of these is initiated by ASK1 and leads to the activation of the mitogen-activated protein kinases (MAPKs) JNK or p38 (Matsuzawa and Ichijo, 2008). ASK1 responds to various apoptotic stimuli, including ROS. Mechanistically, inhibition of ASK1 by the major cytosolic antioxidant protein thioredoxin (TRX; also known as TXN) (Saitoh et al., 1998) or by glutathione S-transferase pi (GSTp) (Adler et al., 1999) is abolished upon ROS stimulus, or reactive cysteine residues in ASK1 itself are oxidized in response to ROS (Jarvis et al., 2012). In addition, ASK1 is activated by ER stress through IRE1 (Urano et al., 2000). Sustained activation of ASK1, and thus JNK, following ER stress crucially depends on JNK-dependent inhibition of mitochondrial respiration and resulting ROS production (Win et al., 2014) (Fig. 3). JNK also stimulates BIM and represses BCL2 through phosphorylation, which provides a further link to mitochondrial dysfunction (Lei and Davis, 2003; Putcha et al., 2003; Yamamoto et al., 1999).

Another redox-relevant pathway involves thioredoxin-interacting protein (TXNIP; also known as TBP-2 or VDUP1), a cytosolic ER stress effector that is upregulated both transcriptionally and by a de-repression mechanism that involves IRE1-catalyzed degradation of microRNA 17 (Lerner et al., 2012; Oslowski et al., 2012). TXNIP fulfills many functions, including negative regulation of mammalian target of rapamycin (mTOR) complex 1 (Jin et al., 2011), activation of the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome (Zhou et al., 2010) and inhibition of the antioxidant enzyme TRX (Nishiyama et al., 1999). The latter function is associated with higher susceptibility to oxidative stress (Liyanage et al., 2007), which again links UPR signaling with oxidative challenge.

However, in contrast to TXNIP-dependent TRX inhibition, UPR signals can also stimulate programs that have antioxidant effects to counterbalance oxidative injury (Fig. 2). These programs prominently include the antioxidant response that is orchestrated by nuclear factor erythroid 2-related factor 2 (NRF2; also known as NFE2L2) (Box 3). NRF2 can be directly phosphorylated and stabilized by PERK downstream of ER stress (Cullinan and Diehl, 2004). Conversely, in the context of ER stress during liver cirrhosis, NRF2 is degraded by an IRE1-regulated ubiquitin ligase (Wu et al., 2014). Extensive transcriptional crosstalk between UPR and the antioxidant response has been documented in *C. elegans* (Glover-Cutter et al., 2013), which establishes the basis for future investigations in mammalian models.

Together with CHOP, ATF4 is responsible for the upregulation of many genes that are involved in protein synthesis (Han et al., 2013; Harding et al., 2003), including genes encoding amino acid transporters that are essential for intracellular GSH synthesis. Consequently, *ATF4*-knockout cells display low GSH levels and are hypersensitive to oxidative challenge or amino acid withdrawal, highlighting an antioxidant function of the PERK–ATF4 pathway (Harding et al., 2003). Recently, stimulation of the hexosamine biosynthetic pathway, which provides the building blocks for stress-induced O-linked N-acetylglucosamine (O-GlcNAc) modifications that are involved in ROS defense, has been demonstrated downstream of IRE1 and XBP1 activation (Vincenz and Hartl, 2014). Taken together, both PERK and IRE1 effectors can contribute to ER-stress-regulated oxidant and antioxidant levels, whereas their relative specificities are likely to be context dependent.

Conclusions and perspectives

There is overwhelming evidence that in many cases ER-stress-related pathologies are accompanied or even caused by intracellular redox disturbances. Importantly, these disturbances can be upstream and downstream of ER stress or UPR and therefore have the potential to foster vicious circles that further amplify the initial disturbance (Figs 2 and 3). Depending on the context, the self-amplifying pathological redox imbalance can be oxidizing, as exemplified by ROS, or reducing, as outlined in the recent hypothesis that ER hypo-oxidation contributes to the etiology of diseases as diverse as type 2 diabetes, Alzheimer's

Box 3. The NRF2-dependent antioxidant response

The best-characterized antioxidant defense that counterbalances the harmful effects of reactive oxidants in mammalian cells is probably the NRF2-orchestrated transcriptional antioxidant response (Ma, 2013). In unstressed cells, NRF2 is constantly subjected to ubiquitylation and proteasomal degradation through interaction with the substrate adaptor protein kelch-like ECH-associated protein 1 (KEAP1). However, when reactive cysteine thiols in KEAP1 react with oxidants and electrophiles, the NRF2–KEAP1 complex dissociates, and the liberated NRF2 migrates to the nucleus where it transactivates genes harboring an antioxidant response element in their promoter region. NRF2 induces a panel of genes encoding antioxidant factors, but can also repress the transcription of genes encoding pro-oxidant machinery, such as TXNIP (Ma, 2013).

disease and cancer (Watson, 2014). However, it should be noted that connections between the redox state and UPR, so far, have been mostly derived from cell culture-based observations, and studies showing their *in vivo* relevance are scarce. Thus, examinations using suitable mouse models and genome-wide association and exome studies in humans are needed to fill this gap.

Does 'redox' provide a platform for therapeutic intervention? Although preclinical data suggest that treatment with free radical quenchers can improve ER-stress-dependent atrophy (Back et al., 2009; Malhotra et al., 2008), the translation of such therapies into the clinic is challenging (see, for example, Persson et al., 2014). One of the associated problems is illustrated by the dual role that NRF2 has in cancer; by increasing the endogenous antioxidant capacities, NRF2-inducing treatments lower the sensitivity to carcinogens but, at the same time, increase the risk of resistance against anti-cancer chemotherapy (Ma, 2013). More targeted interventions, such as BCL2 mimetics (Rong et al., 2009) or ASK1 inhibitors (Kim et al., 2009), might prove more useful to specifically interrupt pro-apoptotic redox amplification loops. On a different note, an emerging therapeutic concept is to kill solid tumors by redox attack, which is most prominently illustrated by the preclinical success of photodynamic therapies (Garg and Agostinis, 2014), as well as by the use of PDI inhibitors (Higa et al., 2014; Xu et al., 2014). Finally, defective insulin secretion and ER stress that is caused by folding-impaired genetic variants of proinsulin can be rescued by expression of hyperactive Ero1 (Wright et al., 2013), although increasing Ero1 activity in healthy β-cells predisposes them to developing ER stress and diabetes (Awazawa et al., 2014). Thus, targeted ER hyper-oxidation might be a viable therapeutic strategy in a subset of diabetic syndromes.

Apart from these translational challenges, the redox–UPR interplay will no doubt continue to elicit academic interest. Among the important questions to be addressed, we would like to highlight the nature and role of catalyzed thiol–disulfide exchange reactions within and between UPR sensor molecules (as tentatively proposed in Fig. 1). Moreover, it will be interesting to scrutinize the reactions of ROS with biological membranes during oxidative challenge and the potential function of lipid peroxides as second messengers, e.g. at the ER–mitochondrion interface (van Vliet et al., 2014). The final goal of a comprehensive description of the impact of redox on UPR and vice versa is still far ahead.

Competing interests

The authors declare no competing interests.

Funding

This work was supported by the University of Basel and the Swiss National Science Foundation (to C.A.-H.).

References

Adler, V., Yin, Z., Fuchs, S. Y., Benezra, M., Rosario, L., Tew, K. D., Pincus, M. R., Sardana, M., Henderson, C. J., Wolf, C. R. et al. (1999). Regulation of JNK signaling by GSTp. *EMBO J.* **18**, 1321-1334.

Alzayady, K. J., Chandrasekhar, R. and Yule, D. I. (2013). Fragmented inositol 1,4,5-trisphosphate receptors retain tetrameric architecture and form functional Ca2+ release channels. *J. Biol. Chem.* **288**, 11122-11134.

Anelli, T., Bergamelli, L., Margittai, E., Rimessi, A., Fagioli, C., Malgaroli, A., Pinton, P., Ripamonti, M., Rizzuto, R. and Sitia, R. (2012). Ero1α regulates Ca(2+) fluxes at the endoplasmic reticulum-mitochondria interface (MAM). *Antioxid. Redox Signal.* **16**, 1077-1087.

Appenzeller-Herzog, C. (2011). Glutathione- and non-glutathione-based oxidant control in the endoplasmic reticulum. *J. Cell Sci.* **124**, 847-855.

Appenzeller-Herzog, C. and Ellgaard, L. (2008). The human PDI family: versatility packed into a single fold. *Biochim. Biophys. Acta* **1783**, 535-548.

Araki, K., Iemura, S., Kamiya, Y., Ron, D., Kato, K., Natsume, T. and Nagata, K. (2013). Ero1-α and PDIs constitute a hierarchical electron transfer network of endoplasmic reticulum oxidoreductases. *J. Cell Biol.* **202**, 861-874.

Austgen, K., Johnson, E. T., Park, T. J., Curran, T. and Oakes, S. A. (2011). The adaptor protein CRK is a pro-apoptotic transducer of endoplasmic reticulum stress. *Nat. Cell Biol.* **14**, 87-92.

Avezov, E., Cross, B. C., Kaminski Schierle, G. S., Winters, M., Harding, H. P., Melo, E. P., Kaminski, C. F. and Ron, D. (2013). Lifetime imaging of a fluorescent protein sensor reveals surprising stability of ER thiol redox. *J. Cell Biol.* **201**, 337-349.

Awazawa, M., Futami, T., Sakada, M., Kaneko, K., Ohsugi, M., Nakaya, K., Terai, A., Suzuki, R., Koike, M., Uchiyama, Y. et al. (2014). Deregulation of pancreas-specific oxidoreductin ERO1β in the pathogenesis of diabetes mellitus. *Mol. Cell. Biol.* **34**, 1290-1299.

Back, S. H., Scheuner, D., Han, J., Song, B., Ribick, M., Wang, J., Gildersleeve, R. D., Pennathur, S. and Kaufman, R. J. (2009). Translation attenuation through eIF2alpha phosphorylation prevents oxidative stress and maintains the differentiated state in beta cells. *Cell Metab.* **10**, 13-26.

Bansaghi, S., Golenar, T., Madesh, M., Csordas, G., Ramachandrarao, S., Sharma, K., Yule, D. I., Joseph, S. K. and Hajnoczky, G. (2014). Isoform- and species-specific control of inositol 1,4,5-trisphosphate (IP3) receptors by reactive oxygen species. *J. Biol. Chem.* **289**, 8170-8181.

Belal, C., Ameli, N. J., El Kommos, A., Bezalel, S., Al'Khafaji, A. M., Mughal, M. R., Mattson, M. P., Kyriazis, G. A., Tyrberg, B. and Chan, S. L. (2012). The homocysteine-inducible endoplasmic reticulum (ER) stress protein Herp counteracts mutant α-synuclein-induced ER stress via the homeostatic regulation of ER-resident calcium release channel proteins. *Hum. Mol. Genet.* **21**, 963-977.

Berridge, M. J., Bootman, M. D. and Roderick, H. L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol.* **4**, 517-529.

Birk, J., Meyer, M., Aller, I., Hansen, H. G., Odermatt, A., Dick, T. P., Meyer, A. J. and Appenzeller-Herzog, C. (2013). Endoplasmic reticulum: reduced and oxidized glutathione revisited. *J. Cell Sci.* **126**, 1604-1617.

Boehning, D., Patterson, R. L., Sedaghat, L., Glebova, N. O., Kurosaki, T. and Snyder, S. H. (2003). Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. *Nat. Cell Biol.* **5**, 1051-1061.

Bulleid, N. J. and Ellgaard, L. (2011). Multiple ways to make disulfides. *Trends Biochem. Sci.* **36**, 485-492.

Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., Clark, S. G. and Ron, D. (2002). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* **415**, 92-96.

Cardozo, A. K., Ortis, F., Storling, J., Feng, Y. M., Rasschaert, J., Tonnesen, M., Van Eylen, F., Mandrup-Poulsen, T., Herchuelz, A. and Eizirik, D. L. (2005). Cytokines downregulate the sarcoendoplasmic reticulum pump Ca2+ ATPase 2b and deplete endoplasmic reticulum Ca2+, leading to induction of endoplasmic reticulum stress in pancreatic beta-cells. *Diabetes* **54**, 452-461.

Chami, M., Oulès, B., Szabadkai, G., Tacine, R., Rizzuto, R. and Paterlini-Bréchot, P. (2008). Role of SERCA1 truncated isoform in the proapoptotic calcium transfer from ER to mitochondria during ER stress. *Mol. Cell* **32**, 641-651.

Csordás, G., Renken, C., Várnai, P., Walter, L., Weaver, D., Buttle, K. F., Balla, T., Mannella, C. A. and Hajnóczky, G. (2006). Structural and functional features and significance of the physical linkage between ER and mitochondria. *J. Cell Biol.* **174**, 915-921.

Cullinan, S. B. and Diehl, J. A. (2004). PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *J. Biol. Chem.* **279**, 20108-20117.

Cunha, D. A., Igoillo-Esteve, M., Gurzov, E. N., Germano, C. M., Naamane, N., Marhfour, I., Fukaya, M., Vanderwinden, J. M., Gysemans, C., Mathieu, C. et al. (2012). Death protein 5 and p53-upregulated modulator of apoptosis mediate the endoplasmic reticulum stress-mitochondrial dialog triggering lipotoxic rodent and human β-cell apoptosis. *Diabetes* **61**, 2763-2775.

Czabotar, P. E., Lessene, G., Strasser, A. and Adams, J. M. (2014). Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat. Rev. Mol. Cell Biol.* **15**, 49-63.

Dejeans, N., Tajeddine, N., Beck, R., Verrax, J., Taper, H., Gailly, P. and Calderon, P. B. (2010). Endoplasmic reticulum calcium release potentiates the ER stress and cell death caused by an oxidative stress in MCF-7 cells. *Biochem. Pharmacol.* **79**, 1221-1230.

DuRose, J. B., Tam, A. B. and Niwa, M. (2006). Intrinsic capacities of molecular sensors of the unfolded protein response to sense alternate forms of endoplasmic reticulum stress. *Mol. Biol. Cell* **17**, 3095-3107.

Eletto, D., Eletto, D., Dersh, D., Gidalevitz, T. and Argon, Y. (2014). Protein disulfide isomerase A6 controls the decay of IRE1α signaling via disulfide-dependent association. *Mol. Cell* **53**, 562-576.

Enyedi, B., Várnai, P. and Geiszt, M. (2010). Redox state of the endoplasmic reticulum is controlled by Ero1L-alpha and intraluminal calcium. *Antioxid. Redox Signal.* **13**, 721-729.

Figueira, T. R., Barros, M. H., Camargo, A. A., Castilho, R. F., Ferreira, J. C., Kowaltowski, A. J., Sluse, F. E., Souza-Pinto, N. C. and Vercesi, A. E. (2013). Mitochondria as a source of reactive oxygen and nitrogen species: from molecular mechanisms to human health. *Antioxid. Redox Signal.* **18**, 2029-2074.

Fu, S., Yang, L., Li, P., Hofmann, O., Dicker, L., Hide, W., Lin, X., Watkins, S. M., Ivanov, A. R. and Hotamisligil, G. S. (2011). Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. *Nature* **473**, 528-531.

Galluzzi, L., Vitale, I., Abrams, J. M., Alnemri, E. S., Baehrecke, E. H., Blagosklonny, M. V., Dawson, T. M., Dawson, V. L., El-Deiry, W. S., Fulda, S. et al. (2012). Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ.* **19**, 107-120.

Garg, A. D. and Agostinis, P. (2014). ER stress, autophagy and immunogenic cell death in photodynamic therapy-induced anti-cancer immune responses. *Photochem. Photobiol. Sci.* **13**, 474-487.

Gidalevitz, T., Stevens, F. and Argon, Y. (2013). Orchestration of secretory protein folding by ER chaperones. *Biochim. Biophys. Acta* **1833**, 2410-2424.

Glover-Cutter, K. M., Lin, S. and Blackwell, T. K. (2013). Integration of the unfolded protein and oxidative stress responses through SKN-1/Nrf. *PLoS Genet.* **9**, e1003701.

Gough, D. R. and Cotter, T. G. (2011). Hydrogen peroxide: a Jekyll and Hyde signalling molecule. *Cell Death Dis.* **2**, e213.

Han, J., Back, S. H., Hur, J., Lin, Y. H., Gildersleeve, R., Shan, J., Yuan, C. L., Krokowski, D., Wang, S., Hatzoglou, M. et al. (2013). ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. *Nat. Cell Biol.* **15**, 481-490.

Hara, T., Mahadevan, J., Kanekura, K., Hara, M., Lu, S. and Urano, F. (2014). Calcium efflux from the endoplasmic reticulum leads to β-cell death. *Endocrinology* **155**, 758-768.

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

Harding, H. P., Zhang, Y., Bertolotti, A., Zeng, H. and Ron, D. (2000b). Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol. Cell* **5**, 897-904.

Harding, H. P., Zhang, Y., Zeng, H., Novoa, I., Lu, P. D., Calfon, M., Sadri, N., Yun, C., Popko, B., Paules, R. et al. (2003). An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol. Cell* **11**, 619-633.

Hayashi, T. and Su, T. P. (2007). Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. *Cell* **131**, 596-610.

Haynes, C. M., Titus, E. A. and Cooper, A. A. (2004). Degradation of misfolded proteins prevents ER-derived oxidative stress and cell death. *Mol. Cell* **15**, 767-776.

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

Hetz, C. (2012). The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat. Rev. Mol. Cell Biol.* **13**, 89-102.

Hetz, C., Bernasconi, P., Fisher, J., Lee, A. H., Bassik, M. C., Antonsson, B., Brandt, G. S., Iwakoshi, N. N., Schinzel, A., Glimcher, L. H. et al. (2006). Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1alpha. *Science* **312**, 572-576.

Higa, A., Taouji, S., Lhomond, S., Jensen, D., Fernandez-Zapico, M. E., Simpson, J. C., Pasquet, J. M., Schekman, R. and Chevet, E. (2014). Endoplasmic reticulum stress-activated transcription factor ATF6α requires the disulfide isomerase PDIA5 to modulate chemoresistance. *Mol. Cell. Biol.* **34**, 1839-1849.

Hollien, J., Lin, J. H., Li, H., Stevens, N., Walter, P. and Weissman, J. S. (2009). Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *J. Cell Biol.* **186**, 323-331.

Horibe, T., Gomi, M., Iguchi, D., Ito, H., Kitamura, Y., Masuoka, T., Tsujimoto, I., Kimura, T. and Kikuchi, M. (2004). Different contributions of the three CXXC motifs of human protein-disulfide isomerase-related protein to isomerase activity and oxidative refolding. *J. Biol. Chem.* **279**, 4604-4611.

Inoue, T. and Suzuki-Karasaki, Y. (2013). Mitochondrial superoxide mediates mitochondrial and endoplasmic reticulum dysfunctions in TRAIL-induced apoptosis in Jurkat cells. *Free Radic. Biol. Med.* **61**, 273-284.

Iwasawa, R., Mahul-Mellier, A. L., Datler, C., Pazarentzos, E. and Grimm, S. (2011). Fis1 and Bap31 bridge the mitochondria-ER interface to establish a platform for apoptosis induction. *EMBO J.* **30**, 556-568.

Jansen, G., Määttänen, P., Denisov, A. Y., Scarffe, L., Schade, B., Balghi, H., Dejgaard, K., Chen, L. Y., Muller, W. J., Gehring, K. et al. (2012). An interaction map of endoplasmic reticulum chaperones and foldases. *Mol. Cell Proteomics* **11**, 710-723.

Jarvis, R. M., Hughes, S. M. and Ledgerwood, E. C. (2012). Peroxiredoxin 1 functions as a signal peroxidase to receive, transduce, and transmit peroxide signals in mammalian cells. *Free Radic. Biol. Med.* **53**, 1522-1530.

Jessop, C. E., Watkins, R. H., Simmons, J. J., Tasab, M. and Bulleid, N. J. (2009). Protein disulphide isomerase family members show distinct substrate specificity: P5 is targeted to BiP client proteins. *J. Cell Sci.* **122**, 4287-4295.

Jin, H. O., Seo, S. K., Kim, Y. S., Woo, S. H., Lee, K. H., Yi, J. Y., Lee, S. J., Choe, T. B., Lee, J. H., An, S. et al. (2011). TXNIP potentiates Redd1-induced mTOR suppression through stabilization of Redd1. *Oncogene* **30**, 3792-3801.

Kim, Y. K. and Lee, A. S. (1987). Transcriptional activation of the glucose-regulated protein genes and their heterologous fusion genes by beta-mercaptoethanol. *Mol. Cell. Biol.* **7**, 2974-2976.

Kim, I., Shu, C. W., Xu, W., Shiau, C. W., Grant, D., Vasile, S., Cosford, N. D. and Reed, J. C. (2009). Chemical biology investigation of cell death pathways activated by endoplasmic reticulum stress reveals cytoprotective modulators of ASK1. *J. Biol. Chem.* **284**, 1593-1603.

Kiviluoto, S., Vervliet, T., Ivanova, H., Decuypere, J. P., De Smedt, H., Missiaen, L., Bultynck, G. and Parys, J. B. (2013). Regulation of inositol 1,4,5-trisphosphate receptors during endoplasmic reticulum stress. *Biochim. Biophys. Acta* **1833**, 1612-1624.

Kojer, K. and Riemer, J. (2014). Balancing oxidative protein folding: The influences of reducing pathways on disulfide bond formation. *Biochim. Biophys. Acta* **1844**, 1383-1390.

Kumar, C., Igbaria, A., D'Autreaux, B., Planson, A. G., Junot, C., Godat, E., Bachhawat, A. K., Delaunay-Moisan, A. and Toledano, M. B. (2011). Glutathione revisited: a vital function in iron metabolism and ancillary role in thiol-redox control. *EMBO J.* **30**, 2044-2056.

Lee, K., Tirasophon, W., Shen, X., Michalak, M., Prywes, R., Okada, T., Yoshida, H., Mori, K. and Kaufman, R. J. (2002). IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. *Genes Dev.* **16**, 452-466.

Lei, K. and Davis, R. J. (2003). JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. *Proc. Natl. Acad. Sci. USA* **100**, 2432-2437.

Lerner, A. G., Upton, J. P., Praveen, P. V., Ghosh, R., Nakagawa, Y., Igbaria, A., Shen, S., Nguyen, V., Backes, B. J., Heiman, M. et al. (2012). IRE1α induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. *Cell Metab.* **16**, 250-264.

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

Li, G., Mongillo, M., Chin, K. T., Harding, H., Ron, D., Marks, A. R. and Tabas, I. (2009). Role of ERO1-alpha-mediated stimulation of inositol 1,4,5-triphosphate receptor activity in endoplasmic reticulum stress-induced apoptosis. *J. Cell Biol.* **186**, 783-792.

Li, G., Scull, C., Ozcan, L. and Tabas, I. (2010a). NADPH oxidase links endoplasmic reticulum stress, oxidative stress, and PKR activation to induce apoptosis. *J. Cell Biol.* **191**, 1113-1125.

Li, H., Korennykh, A. V., Behrman, S. L. and Walter, P. (2010b). Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering. *Proc. Natl. Acad. Sci. USA* **107**, 16113-16118.

Liu, C. Y., Xu, Z. and Kaufman, R. J. (2003). Structure and intermolecular interactions of the luminal dimerization domain of human IRE1alpha. *J. Biol. Chem.* **278**, 17680-17687.

Liyanage, N. P., Fernando, M. R. and Lou, M. F. (2007). Regulation of the bioavailability of thioredoxin in the lens by a specific thioredoxin-binding protein (TBP-2). *Exp. Eye Res.* **85**, 270-279.

Ma, Q. (2013). Role of nrf2 in oxidative stress and toxicity. *Annu. Rev. Pharmacol. Toxicol.* **53**, 401-426.

Malhotra, J. D., Miao, H., Zhang, K., Wolfson, A., Pennathur, S., Pipe, S. W. and Kaufman, R. J. (2008). Antioxidants reduce endoplasmic reticulum stress and improve protein secretion. *Proc. Natl. Acad. Sci. USA* **105**, 18525-18530.

Marciniak, S. J., Yun, C. Y., Oyadomari, S., Novoa, I., Zhang, Y., Jungreis, R., Nagata, K., Harding, H. P. and Ron, D. (2004). CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev.* **18**, 3066-3077.

Matsuzawa, A. and Ichijo, H. (2008). Redox control of cell fate by MAP kinase: physiological roles of ASK1-MAP kinase pathway in stress signaling. *Biochim. Biophys. Acta* **1780**, 1325-1336.

Maurel, M., Chevet, E., Tavernier, J. and Gerlo, S. (2014). Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem. Sci.* **39**, 245-254.

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

Meunier, L., Usherwood, Y. K., Chung, K. T. and Hendershot, L. M. (2002). A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. *Mol. Biol. Cell* **13**, 4456-4469.

Montero, D., Tachibana, C., Rahr Winther, J. and Appenzeller-Herzog, C. (2013). Intracellular glutathione pools are heterogeneously concentrated. *Redox Biol.* **1**, 508-513.

Moore, C. E., Omikorede, O., Gomez, E., Willars, G. B. and Herbert, T. P. (2011). PERK activation at low glucose concentration is mediated by SERCA pump inhibition and confers preemptive cytoprotection to pancreatic β-cells. *Mol. Endocrinol.* **25**, 315-326.

Mori, T., Hayashi, T., Hayashi, E. and Su, T. P. (2013). Sigma-1 receptor chaperone at the ER-mitochondrion interface mediates the mitochondrion-ER-nucleus signaling for cellular survival. *PLoS ONE* **8**, e76941.

Nadanaka, S., Okada, T., Yoshida, H. and Mori, K. (2007). Role of disulfide bridges formed in the luminal domain of ATF6 in sensing endoplasmic reticulum stress. *Mol. Cell. Biol.* **27**, 1027-1043.

Namba, T., Tian, F., Chu, K., Hwang, S. Y., Yoon, K. W., Byun, S., Hiraki, M., Mandinova, A. and Lee, S. W. (2013). CDIP1-BAP31 complex transduces apoptotic signals from endoplasmic reticulum to mitochondria under endoplasmic reticulum stress. *Cell Rep.* **5**, 331-339.

Nishiyama, A., Matsui, M., Iwata, S., Hirota, K., Masutani, H., Nakamura, H., Takagi, Y., Sono, H., Gon, Y. and Yodoi, J. (1999). Identification of

thioredoxin-binding protein-2/vitamin D(3) up-regulated protein 1 as a negative regulator of thioredoxin function and expression. *J. Biol. Chem.* **274**, 21645-21650.
Oslowski, C. M., Hara, T., O'Sullivan-Murphy, B., Kanekura, K., Lu, S., Hara, M., Ishigaki, S., Zhu, L. J., Hayashi, E., Hui, S. T. et al. (2012). Thioredoxin-interacting protein mediates ER stress-induced β cell death through initiation of the inflammasome. *Cell Metab.* **16**, 265-273.
Parys, J. B. (2014). The IP3 receptor as a hub for Bcl-2 family proteins in cell death control and beyond. *Sci. Signal.* **7**, pe4.
Pedruzzi, E., Guichard, C., Ollivier, V., Driss, F., Fay, M., Prunet, C., Marie, J. C., Pouzet, C., Samadi, M., Elbim, C. et al. (2004). NAD(P)H oxidase Nox-4 mediates 7-ketocholesterol-induced endoplasmic reticulum stress and apoptosis in human aortic smooth muscle cells. *Mol. Cell. Biol.* **24**, 10703-10717.
Persson, T., Popescu, B. O. and Cedazo-Minguez, A. (2014). Oxidative stress in Alzheimer's disease: why did antioxidant therapy fail? *Oxid. Med. Cell. Longev.* **2014**, 427318.
Putcha, G. V., Le, S., Frank, S., Besirli, C. G., Clark, K., Chu, B., Alix, S., Youle, R. J., LaMarche, A., Maroney, A. C. et al. (2003). JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis. *Neuron* **38**, 899-914.
Puthalakath, H., O'Reilly, L. A., Gunn, P., Lee, L., Kelly, P. N., Huntington, N. D., Hughes, P. D., Michalak, E. M., McKimm-Breschkin, J., Motoyama, N. et al. (2007). ER stress triggers apoptosis by activating BH3-only protein Bim. *Cell* **129**, 1337-1349.
Ramming, T. and Appenzeller-Herzog, C. (2012). The physiological functions of mammalian endoplasmic oxidoreductin 1: on disulfides and more. *Antioxid. Redox Signal.* **16**, 1109-1118.
Ramming, T. and Appenzeller-Herzog, C. (2013). Destroy and exploit: catalyzed removal of hydroperoxides from the endoplasmic reticulum. *Int. J. Cell Biol.* **2013**, 180906.
Ramming, T., Hansen, H. G., Nagata, K., Ellgaard, L. and Appenzeller-Herzog, C. (2014). GPx8 peroxidase prevents leakage of H2O2 from the endoplasmic reticulum. *Free Radic. Biol. Med.* **70**, 106-116.
Raturi, A., Ortiz-Sandoval, C. and Simmen, T. (2014). Redox dependence of endoplasmic reticulum (ER) Ca^{2+} signaling. *Histol. Histopathol.* **29**, 543-552.
Rizzuto, R., De Stefani, D., Raffaello, A. and Mammucari, C. (2012). Mitochondria as sensors and regulators of calcium signalling. *Nat. Rev. Mol. Cell Biol.* **13**, 566-578.
Rodriguez, D. A., Zamorano, S., Lisbona, F., Rojas-Rivera, D., Urra, H., Cubillos-Ruiz, J. R., Armisen, R., Henriquez, D. R., Cheng, E. H., Letek, M. et al. (2012). BH3-only proteins are part of a regulatory network that control the sustained signalling of the unfolded protein response sensor IRE1α. *EMBO J.* **31**, 2322-2335.
Rong, Y. P., Barr, P., Yee, V. C. and Distelhorst, C. W. (2009). Targeting Bcl-2 based on the interaction of its BH4 domain with the inositol 1,4,5-trisphosphate receptor. *Biochim. Biophys. Acta* **1793**, 971-978.
Rutkevich, L. A., Cohen-Doyle, M. F., Brockmeier, U. and Williams, D. B. (2010). Functional relationship between protein disulfide isomerase family members during the oxidative folding of human secretory proteins. *Mol. Biol. Cell* **21**, 3093-3105.
Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K., Sawada, Y., Kawabata, M., Miyazono, K. and Ichijo, H. (1998). Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J.* **17**, 2596-2606.
Sandow, J. J., Dorstyn, L., O'Reilly, L. A., Tailler, M., Kumar, S., Strasser, A. and Ekert, P. G. (2014). ER stress does not cause upregulation and activation of caspase-2 to initiate apoptosis. *Cell Death Differ.* **21**, 475-480.
Santos, C. X., Tanaka, L. Y., Wosniak, J. and Laurindo, F. R. (2009). Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. *Antioxid. Redox Signal.* **11**, 2409-2427.
Schafer, F. Q. and Buettner, G. R. (2001). Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic. Biol. Med.* **30**, 1191-1212.
Tabas, I. and Ron, D. (2011). Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nat. Cell Biol.* **13**, 184-190.
Tavender, T. J. and Bulleid, N. J. (2010). Peroxiredoxin IV protects cells from oxidative stress by removing H2O2 produced during disulphide formation. *J. Cell Sci.* **123**, 2672-2679.
Tong, X., Evangelista, A. and Cohen, R. A. (2010). Targeting the redox regulation of SERCA in vascular physiology and disease. *Curr. Opin. Pharmacol.* **10**, 133-138.
Tu, B. P. and Weissman, J. S. (2004). Oxidative protein folding in eukaryotes: mechanisms and consequences. *J. Cell Biol.* **164**, 341-346.
Tufo, G., Jones, A. W., Wang, Z., Hamelin, J., Tajeddine, N., Esposti, D. D., Martel, C., Boursier, C., Gallerne, C., Migdal, C. et al. (2014). The protein disulfide isomerases PDIA4 and PDIA6 mediate resistance to cisplatin-induced cell death in lung adenocarcinoma. *Cell Death Differ.* **21**, 685-695.
Uehara, T., Nakamura, T., Yao, D., Shi, Z. Q., Gu, Z., Ma, Y., Masliah, E., Nomura, Y. and Lipton, S. A. (2006). S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature* **441**, 513-517.
Upton, J. P., Austgen, K., Nishino, M., Coakley, K. M., Hagen, A., Han, D., Papa, F. R. and Oakes, S. A. (2008). Caspase-2 cleavage of BID is a critical apoptotic signal downstream of endoplasmic reticulum stress. *Mol. Cell. Biol.* **28**, 3943-3951.
Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H. P. and Ron, D. (2000). Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* **287**, 664-666.
van Vliet, A. R., Verfaillie, T. and Agostinis, P. (2014). New functions of mitochondria associated membranes in cellular signaling. *Biochim. Biophys. Acta.* [Epub ahead of print] doi:10.1016/j.bbamcr.2014.03.009.
Verfaillie, T., Rubio, N., Garg, A. D., Bultynck, G., Rizzuto, R., Decuypere, J. P., Piette, J., Linehan, C., Gupta, S., Samali, A. et al. (2012). PERK is required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress. *Cell Death Differ.* **19**, 1880-1891.
Vinaik, R., Kozlov, G. and Gehring, K. (2013). Structure of the non-catalytic domain of the protein disulfide isomerase-related protein (PDIR) reveals function in protein binding. *PLoS ONE* **8**, e62021.
Vincenz, L. and Hartl, F. U. (2014). Sugarcoating ER Stress. *Cell* **156**, 1125-1127.
Wali, J. A., Rondas, D., McKenzie, M. D., Zhao, Y., Elkerbout, L., Fynch, S., Gurzov, E. N., Akira, S., Mathieu, C., Kay, T. W. et al. (2014). The proapoptotic BH3-only proteins Bim and Puma are downstream of endoplasmic reticulum and mitochondrial oxidative stress in pancreatic islets in response to glucotoxicity. *Cell Death Dis.* **5**, e1124.
Walter, P. and Ron, D. (2011). The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **334**, 1081-1086.
Watson, J. D. (2014). Type 2 diabetes as a redox disease. *Lancet* **383**, 841-843.
Wei, P. C., Hsieh, Y. H., Su, M. I., Jiang, X., Hsu, P. H., Lo, W. T., Weng, J. Y., Jeng, Y. M., Wang, J. M., Chen, P. L. et al. (2012). Loss of the oxidative stress sensor NPGPx compromises GRP78 chaperone activity and induces systemic disease. *Mol. Cell* **48**, 747-759.
Win, S., Than, T. A., Fernandez-Checa, J. C. and Kaplowitz, N. (2014). JNK interaction with Sab mediates ER stress induced inhibition of mitochondrial respiration and cell death. *Cell Death Dis.* **5**, e989.
Wright, J., Birk, J., Haataja, L., Liu, M., Ramming, T., Weiss, M. A., Appenzeller-Herzog, C. and Arvan, P. (2013). Endoplasmic reticulum oxidoreductin-1α (Ero1α) improves folding and secretion of mutant proinsulin and limits mutant proinsulin-induced endoplasmic reticulum stress. *J. Biol. Chem.* **288**, 31010-31018.
Wu, T., Zhao, F., Gao, B., Tan, C., Yagishita, N., Nakajima, T., Wong, P. K., Chapman, E., Fang, D. and Zhang, D. D. (2014). Hrd1 suppresses Nrf2-mediated cellular protection during liver cirrhosis. *Genes Dev.* **28**, 708-722.
Xu, S., Sankar, S. and Neamati, N. (2014). Protein disulfide isomerase: a promising target for cancer therapy. *Drug Discov. Today* **19**, 222-240.
Yamamoto, K., Ichijo, H. and Korsmeyer, S. J. (1999). BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. *Mol. Cell. Biol.* **19**, 8469-8478.
Yoshida, H., Matsui, T., Yamamoto, A., Okada, T. and Mori, K. (2001). XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* **107**, 881-891.
Zhou, R., Tardivel, A., Thorens, B., Choi, I. and Tschopp, J. (2010). Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat. Immunol.* **11**, 136-140.