Journal Pre-proof

Crosstalk between UPR and mitochondria: The Triad of ER–Mitochondria Contacts, Ca^{2+}, and ROS

Ester Zito, György Hajnóczky

PII: S1043-6618(26)00343-9

DOI: https://doi.org/10.1016/j.phrs.2026.108428

Reference: YPHRS108428

To appear in: *Pharmacological Research*

Received date: 17 June 2026
Revised date: 28 August 2026
Accepted date: 29 August 2026

Please cite this article as: Ester Zito and György Hajnóczky, Crosstalk between UPR and mitochondria: The Triad of ER–Mitochondria Contacts, Ca^{2+}, and ROS, *Pharmacological Research,* (2026) doi:https://doi.org/10.1016/j.phrs.2026.108428

This is a PDF of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability. This version will undergo additional copyediting, typesetting and review before it is published in its final form. As such, this version is no longer the Accepted Manuscript, but it is not yet the definitive Version of Record; we are providing this early version to give early visibility of the article. Please note that Elsevier's sharing policy for the Published Journal Article applies to this version, see: https://www.elsevier.com/about/policies-and-standards/sharing#4-published-journal-article. Please also note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Journal Pre-proof

Crosstalk between UPR and mitochondria: The Triad of ER–Mitochondria Contacts, Ca^{2+}, and ROS

Ester Zito [1,2] & György Hajnóczky [3]

[1]Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy. Electronic address:ester.zito@marionegri.it

[2] Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy Electronic address: ester.zito@uniurb.it

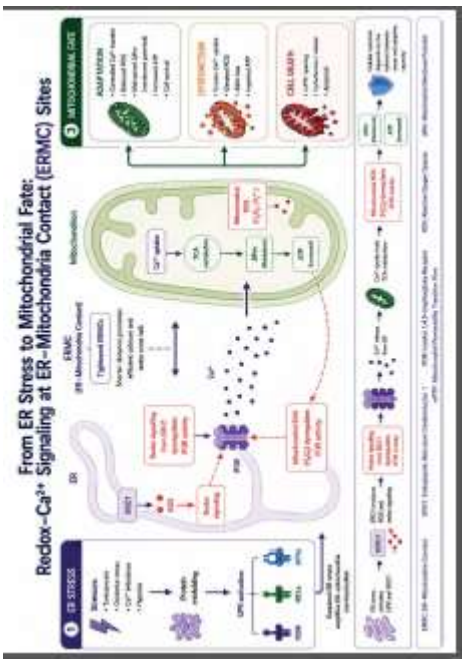
[3]Department of Pathology and Genomic Medicine, Thomas Jefferson University, Philadelphia, PA, USA. Electronic address: gyorgy.hajnoczky@jefferson.edu.

Journal Pre-proof

Abstract

Endoplasmic reticulum (ER) stress is triggered by several cellular perturbations causing protein misfolding, and activates the unfolded protein response (UPR), an initially adaptive signaling network that aims to restore ER and cellular homeostasis. Growing evidence indicates that UPR signaling extends beyond ER proteostasis, influencing mitochondrial function and bioenergetics through ER–mitochondria contact sites (ERMCs). The CHOP–ERO1A–IP_3R axis has a primary role in recruiting mitochondria to adaptive UPR. However, its sustained activation renders UPR signaling maladaptive, leading to mitochondrial dysfunction through both outer mitochondrial membrane permeabilization (OMMP) and mitochondrial permeability transition pore (mPTP) opening, ultimately contributing to irreversible cell injury and disease pathogenesis. Here, we examine the molecular mechanisms that govern adaptive and maladaptive UPR signaling and discuss how these ER-centered responses impinge on mitochondrial and cellular physiology. We analyze three major drivers of coupling mitochondrial function to UPR signaling: (i) enhanced ERMCs, (ii) IP_3R-mediated Ca^{2+} transfer from the ER to mitochondria, and (iii) bidirectional ROS/H_2O_2 exchange between the two organelles. We also discuss unresolved questions in the field and technological advances, including approaches to investigate ERO1-dependent redox nanodomains, ERO1 inhibitors and engineered ERMC linkers, that are advancing our understanding of ER–mitochondria crosstalk and revealing potential therapeutic opportunities. These insights may inform precision medicine strategies for diseases driven by chronic ER stress and mitochondrial dysfunction.

GRAPHICAL ABSTRACT



From ER Stress to Mitochondrial Fate: Redox–Ca^{2+} Signaling at ER–Mitochondria Contact Sites. The graphical abstract was prepared with assistance from ChatGPT (OpenAI) for design and visual organization purposes only. The scientific concepts, content, interpretation, and final validation of the figure were performed by the authors.

Key words: ER stress; ER–mitochondria contact sites (ERMCs); unfolded protein response (UPR); Ca^{2+} handling; mitochondrial permeability transition pore (mPTP); CHOP; ERO1; IP_3 receptor (IP_3R); pharmacological therapy

Journal Pre-proof

Introduction

The ER is central to protein folding, lipid synthesis, and intracellular Ca^{2+} storage. Impairment of ER proteostasis leads to accumulation of unfolded proteins and triggers ER stress, activating the UPR, an evolutionarily conserved signaling network [1]. In its adaptive phase, the UPR attenuates global protein synthesis, expands ER folding capacity, and enhances degradation of misfolded proteins. However, when stress is severe or unresolved, the UPR transitions into a maladaptive program that can lead to oxidative stress, inflammation and cell death.

Different forms of cellular stress are funneled through four specialized kinases that converge on the phosphorylation of eIF2α, thereby altering the transcriptional program (Fig. 1). Unfolded or misfolded proteins in the ER lumen are primarily sensed by the ER stress sensors PERK, ATF6, and IRE1 through BiP, thereby activating the UPR

(Fig. 2). PERK phosphorylates eIF2α, thereby attenuating global protein translation while selectively enhancing ATF4 translation. Mitochondrial stress is sensed through OMA1-mediated processing of DELE1, which activates HRI in the cytoplasm and leads to eIF2α phosphorylation, contributing to the integrated stress response (ISR). The ISR, by definition, is triggered by a broader range of insults, including disturbances in ER proteostasis and mitochondrial energetics. During the ISR, PERK and HRI are joined by GCN2, which responds to amino acid deprivation, and PKR, which responds to viral infection, to phosphorylate eIF2α (Fig. 1) [2].

Both adaptation and maladaptation are driven at least in part by the PERK–CHOP axis. CHOP induction promotes expression of ERO1A (hereafter ERO1) and GADD34, thereby enhancing oxidative protein folding within the ER while restoring protein synthesis despite ongoing stress [3]. However, excessive ERO1 activity can cause oxidative changes contributing to maladaptation.

Although UPR activation in response to ER stress has been studied for decades, many questions remain unanswered, including the role of mitochondria in shaping adaptive versus maladaptive ER stress responses.

In addition to converging with other stress-responsive pathways on eIF2α, the ER and mitochondria appear to coordinate their stress responses through local signaling at ER–mitochondria contact sites (ERMCs) (Figs. 1 and 3). These proteinaceous contact sites coordinate localized exchange of Ca^{2+} and redox signals, including hydrogen peroxide (H_2O_2), thereby coupling ER proteostasis to mitochondrial metabolism and cell fate decisions [4]. Moderate strengthening of ERMCs during transient stress can enhance local Ca^{2+} transfer to the mitochondria and ensuing stimulation of mitochondrial ATP production and support cell recovery [5 6]. It is conceivable that sustained PERK–CHOP–ERO1 signaling further enhances localized ERO1-dependent redox nanodomains at ERMCs, in which H_2O_2 modulates the redox state of Ca^{2+}-handling proteins. Such local redox remodeling could sensitize ER Ca^{2+} release channels, promote ERMC tightening, and thereby drive excessive Ca^{2+} transfer to mitochondria. The ER stress sensor IRE1 may also promote Ca^{2+} transfer from ER Ca^{2+} release channels to mitochondria [7]. The resulting mitochondrial Ca^{2+} overload and ROS amplification promote bioenergetic failure and commitment to cell death.

In this review, we propose that ERMCs function as ROS–Ca^{2+} amplification hubs positioned downstream of ERO1 and other ER stress responders that influence whether UPR signaling remains adaptive or becomes maladaptive. To develop this concept, we first provide an overview of organellar stress responses, outline how the UPR integrates transcriptional, translational and spatial signaling with mitochondria, and then examine how ERMC remodeling, together with Ca^{2+} handling and ROS signaling, drives mitochondrial threshold crossing and commitment to cell death.

From ER stress to adaptive and maladaptive UPR

ER stress arises when the protein-folding capacity of the ER is exceeded, resulting in the accumulation of misfolded or unfolded proteins within the ER lumen. This stress can be triggered by increased secretory protein demand, ER Ca^{2+} depletion, hypoxia, nutrient limitations, high levels of saturated fatty acids, defective protein degradation, or mutations that impair protein folding [8]. ER stress is sensed by three transmembrane

sensors on the ER membrane—IRE1, ATF6, and PERK—whose activation triggers the so-called UPR, a homeostatic signaling network initially aimed at restoring ER homeostasis (Fig. 2).

In its adaptive phase, the UPR coordinates corrective measures that expand protein folding and degradative capacity and reduce ER protein load to re-establish proteostasis. IRE1 catalyzes the unconventional splicing of XBP1 mRNA (involving the removal of an intron), generating a transcription factor that induces chaperones, ER-associated degradation (ERAD) components, and lipid biosynthetic enzymes (Fig. 2). IRE1 is also involved, through the IRE1-dependent decay (RIDD) of RNAs, in the degradation of several RNAs and thus directly contributes to decreasing the ER protein load (Fig2). However, although XBP1 splicing is generally considered adaptive, RIDD activity may become maladaptive under prolonged stress [9,10]. ATF6 translocates to the Golgi, where regulated intramembrane proteolysis releases its cytosolic transcription factor domain to promote ER expansion and proteostasis [11] [12] (Fig. 2). The PERK branch plays a central role in translational control [13], in regulation of ROS production but is also implicated in the anti-oxidant response through activation of NRF2 [14]. Upon activation, PERK phosphorylates eIF2α at serine 51, transiently attenuating global protein synthesis and thereby reducing ER client load [1]. This event also constitutes a core component of the ISR, a broader signaling network in which multiple kinases converge on eIF2α phosphorylation [2,15] (Fig. 1). Through this shared node, the ER stress response is mechanistically integrated with nutrient stress, viral stress, and mitochondrial dysfunction. Although eIF2α phosphorylation suppresses bulk translation, it selectively enhances translation of ATF4 (Fig. 2). ATF4 coordinates transcriptional programs governing amino acid metabolism and mitochondrial adaptation, including one-carbon metabolism, thereby further linking the ER stress response to mitochondrial function [16,17]. Mitochondrial stress caused by unfolded proteins and other cues, activates the mammalian UPRmt [18], which has been recently linked to DELE1 and HRI-mediated eIF2α phosphorylation and related ATF4 and CHOP activation (Fig. 1). Thus, mitochondrial proteotoxic stress results in CHOP upregulation, establishing CHOP as a key point of convergence between the UPRmt and the UPR into ER.
Importantly, ER–mitochondria integration is not solely transcriptional but also mediated by direct interorganellar communication. UPR signaling is locally organized at the ERMCs, where PERK and IRE1 are enriched and directly coordinate bioenergetic adaptation. At these junctions, PERK and IRE1 have been reported to interact with ERMC components and to stimulate mitochondrial bioenergetics [7,19,20] thereby coupling proteostasis to mitochondrial ATP production [5,6]. Collectively, the adaptive UPR preserves ER proteostasis while supporting mitochondrial bioenergetics, metabolic adaptation and cell survival during ER stress.

Under severe or prolonged stress— the quantitative determinants of which remain incompletely defined—the adaptive UPR can switch to a maladaptive program that promotes cellular dysfunction and death. Central to this switch is the PERK–ATF4–CHOP axis. Recently, CHOP was proposed as an adaptive mediator of ISR [21] but is also a pro-apoptotic transcription factor whose targets include GADD34 and ERO1 [3].

CHOP-induced GADD34 promotes dephosphorylation of eIF2α, restarting protein synthesis but increasing ER folding burden and, if stress remains unresolved, contributing to maladaptive signaling (Fig. 2).

ER oxidoreductin 1 (ERO1) is a flavoprotein central to oxidative protein folding by catalyzing disulfide bond formation in nascent ER proteins through protein disulfide isomerase (PDI) [22,23]. This reaction transfers electrons to O_2, generating stoichiometric amounts of H_2O_2—estimated to contribute up to ~25% of total cellular H_2O_2 [24]—thereby imposing a significant oxidative burden. ERO1-derived H_2O_2 is mitigated by ER antioxidant systems, including PRDX4, which not only utilizes H_2O_2 but also further sustains oxidative protein folding [25], thereby explaining the relative dispensability of ERO1 in healthy mammalian cells [26]. ERO1 activity is normally tightly regulated by four cysteines (Cys104, Cys131, Cys208, Cys241), which couple the formation of inhibitory intramolecular disulfide bonds to the ER oxidizing environment, thereby preventing excessive ERO1 activity [27]. However, sustained ERO1 activity, as observed with the hyperactive ERO1 (C104A/C131A) mutant, increases ER oxidative poise without triggering a broad antioxidant response, suggesting that when local antioxidant buffering becomes overwhelmed or impaired, ERO1 can shift local ER redox toward a hyperoxidizing state [28].

ERO1 is strategically enriched at ERMCs [29] and regulates ERMC function under conditions of ER stress [6] positioning this disulfide oxidase at the interface where UPR signaling converges on mitochondrial function. As a catalyst of disulfide bond formation in newly synthesized ER client proteins, ERO1 contributes to an adaptive response. However, when the local antioxidant buffering capacity becomes limiting, sustained ERO1 activity may drive local hyperoxidation and contribute to the transition toward maladaptive UPR. As a consequence, increased ERO1-derived H_2O_2 production at ERMCs might lead to localized oxidation of strategic target proteins at the ER–mitochondria interface. This might affect the oxidation-sensitive IP_3 receptors (IP_3R) [6] and ryanodine receptor 2 (RyR2), enhancing Ca^{2+} release from the ER [30]. In this redox context, Cys2496 and Cys2504 in the third luminal loop of IP_3R1 mediate their interaction with ERp44. Under ER stress conditions, the induced ERO1 may disrupt this interaction by promoting the dissociation of ERp44 from IP_3R1, thereby increasing Ca^{2+} release through the channel [31] [29]. Similarly, ERp44 binds to Cys4806 of RyR2, and ERO1-mediated dissociation of ERp44 from this complex triggers RyR2 hyperactivation [30]. Thus, by promoting activation of these Ca^{2+} channels, ERO1 may drive mitochondrial ROS/H_2O_2 accumulation, Ca^{2+} overload, respiratory dysfunction, and cell death signaling. Consistent with this model, during ER stress, CHOP promotes Ca^{2+}-dependent apoptosis through the ERO1–IP_3R pathway [32], whereas inhibition of ERO1 prevents this apoptotic response in mammals. Moreover, ERO1 knockdown in *C. elegans* also confers protection against ER stress–induced cell death by reducing ROS levels [16,33]. Thus, ERO1 may function at ERMCs as a molecular rheostat that shifts UPR signaling from adaptive to maladaptive; its upregulation or sustained activation may become detrimental, whereas its limitation preserves cellular viability.

In the following sections, we focus on three principal drivers of maladaptive UPR that impinge on mitochondrial function: ERMC tightening, IP3R-dependent mitochondrial Ca^{2+} overload, and H_2O_2/ROS signaling, which together compromise mitochondrial fitness and promote cell death. Several studies have linked ER stress to maladaptation and cell injury through mitochondrial permeability transition [34] [35] [36]. However, there is limited evidence specifically on ERO1 as the causative factor [37]. Other UPR mediators might also impinge on ERMC, Ca^{2+} and ROS signaling.

ERMCs as Dynamic Signaling Platforms

ERMCs, are specialized ER subdomains that establish close, non-fusogenic contacts with the outer mitochondrial membrane (OMM). Early biochemical fractionation studies identified ER-derived membranes co-purified with mitochondria and enriched in lipid biosynthetic enzymes, providing the first evidence of stable ER–mitochondria coupling. This fraction was named mitochondria-associated membranes (MAMs) [38,39]. Subsequent ultrastructural and proteolytic analyses demonstrated that ERMCs are discrete, protein-organized platforms [40]. Quantitative studies indicate that typically 10–20% of the mitochondrial surface is associated with the ER, with an intermembrane distance of <100 nm [4,41,42]. ERMCs are supported by tethering complexes of different lengths (10-30 nm) and shapes [40]. Tethering modules include complexes containing the IP_3R and the Voltage-Dependent Anion Channels (VDAC) connected by GRP75 [43] or other proteins (recently reviewed in [44]), which functionally couple ER Ca^{2+} release to mitochondrial uptake. Additional tethering complexes that do not directly involve IP_3Rs include Mfn2, PACS2, VAPB–PTPIP51, PDZD8–FKBP8, and several other proteins [43,45-50]. Beyond tethers, ERMCs are enriched in Ca^{2+} channels, lipid transfer proteins, and redox enzymes, thereby enabling spatially restricted exchange of Ca^{2+}, lipids, metabolites, and ROS between ER and mitochondria [51-55]. Importantly, ERMCs are increasingly recognized not only as platforms for Ca^{2+} and metabolic exchange but also as specialized signaling hubs that regulate lipid homeostasis and redox-dependent cell fate decisions. In this regard, recent evidence suggests that ERMCs may represent hotspots for lipid peroxidation, thereby contributing to the regulation of ferroptosis, a form of iron-dependent cell death driven by peroxidation to polyunsaturated fatty acids [56].

ERMCs are highly dynamic structures that remodel in response to metabolic demand and stress intensity [57,58]. During ER stress, ERMC architecture undergoes remodeling, often accompanied by increased contacts [40]. UPR promotes ER membrane expansion to increase folding capacity [59]. This expansion increases the probability of ER–mitochondria encounters and reduces local interorganellar spacing, thereby tightening ERMCs [5,40]. In adaptive settings, such remodeling enhances interorganellar signaling and metabolic coupling, thereby promoting ATP production required for proteostasis. However, under sustained or unresolved ER stress, ER expansion combined with persistent ERMC tightening might favor excessive Ca^{2+} and ROS flux toward mitochondria. Importantly, expansion of ERMCs may create conditions that facilitate positive feedback loops in which mitochondrial ROS production and Ca^{2+} dysregulation further amplify local exposure to Ca^{2+} and oxidative stress. Thus, structural plasticity that initially supports adaptation may ultimately become a driver of mitochondrial Ca^{2+} overload and dysfunction [60,61]. As already mentioned above, several components of the UPR are selectively enriched at ERMCs, highlighting these domains as potential signaling platforms for UPR [6,7,19,20] (Fig. 3). Along this line, PERK-ERO1-dependent tightening of ERMCs supports metabolic adaptation during transient stress but, when sustained, facilitates mitochondria-dependent apoptosis [6,19,32]. In addition to regulating ERMC architecture, PERK activation has also been linked to mitochondrial remodeling. Recent studies indicate that PERK-dependent signaling promotes mitochondrial hyperfusion, a protective adaptation that preserves mitochondrial function and bioenergetic capacity [62].

Among other ER stress sensors, IRE1 has been reported to control IP3R distribution at ERMC and form a complex with Sigma1R, thereby promoting cell survival during ER stress [7,63]. In summary, during adaptive UPR, structural and functional plasticity of ERMCs sustains metabolic coupling and ATP production in mitochondria required for proteostasis. However, under maladaptive UPR, persistent ERMC tightening and

amplified inter-organellar signaling shift the balance from metabolic support to mitochondrial stress. Excessive Ca^{2+} transfer and localized redox amplification at these junctions promote mitochondrial ROS accumulation, Ca^{2+} overload that might lead to cell death. Among the redox enzymes enriched at ERMC, ERO1 occupies a particularly strategic position, linking oxidative protein folding in the ER directly to mitochondrial Ca^{2+} and ROS dynamics [64,65]. Therefore, ERMC remodeling during ER stress creates a node through which sustained UPR signaling is translated into altered mitochondrial Ca^{2+} handling and redox dynamics.

IP3R-mediated Ca^{2+} transfer at ERMCs

The ER serves as the main intracellular Ca^{2+} store, maintaining a luminal [Ca^{2+}] of 100-500 μM, which is three to four orders of magnitude higher than the resting cytoplasmic [Ca^{2+}]. Upon release into the cytoplasm, ER-derived Ca^{2+} serves as dominant source of intracellular calcium signals. Howerer, high levels of luminal [Ca^{2+}] in the ER are required to maintain proper protein folding and sustained ER Ca^{2+} depletion induces the UPR. Although some molecular chaperons inside the ER, like calreticulin and calnexin are EF-hand Ca^{2+} binding proteins [66], and BIP has been connected to ER Ca^{2+} storage and Ca^{2+} depletion-induced stress [67,68], a dedicated luminal Ca^{2+}-binding protein that links protein folding with sensing of UPR induction remains to be found.

ER Ca^{2+} uptake, storage, and Ca^{2+} release are tightly controlled by sarco/endoplasmic reticulum Ca^{2+}-ATPases (SERCAs), which move Ca^{2+} from the cytoplasm into the ER lumen against a concentration gradient, and by Ca^{2+} channels (such as IP_3Rs and RyRs) that release ER Ca^{2+} into the cytoplasm. However, ER Ca^{2+} storage capacity is also influenced by the size of the ER and the abundance of luminal Ca^{2+}-binding proteins. The ER is present in almost all cells, and it contains the housekeeping SERCA2B Ca^{2+} pumps and IP_3R. In muscle tissues, the ER is complemented by the sarcoplasmic reticulum (SR) that is equipped with SERCA1 or SERCA2A Ca^{2+} pumps and RyRs [69]. The SR's Ca^{2+} transport function is well established, but its role in protein folding remains to be fully defined. Although the IP_3Rs can be activated selectively by IP_3, and certain RyRs can be activated through direct interactions with membrane voltage-sensing proteins; both IP_3R and RyR are biphasically controlled by cytoplasmic [Ca^{2+}] and are sensitive to post-translational modifications including phosphorylation and thiol redox modification. IP_3Rs dynamically distribute throughout the ER membrane [70], though they often concentrate at ERMCs [71], whereas RyRs are enriched at the Ca^{2+} release units of striated muscle are associated with plasma membrane proteins and can be scarce at the adjacent SR-mitochondrial contacts [72,73]. Because IP_3Rs are better characterized in the context of ER stress and ERMCs than RyRs, this chapter focuses on IP_3Rs, and only a few examples of RyR mechanisms are discussed.

Activation of mitochondrial Ca^{2+} uptake through the mitochondrial calcium uniporter complex (mtCU) during transient ER Ca^{2+} release depends on the mtCU being exposed to high Ca^{2+} concentrations that are attained only in the vicinity of an open IP_3R or RyR [74-76]. Exposure of mitochondria to 10–30 μM Ca^{2+} concentrations has been documented during IP_3R-mediated Ca^{2+} release, which is one to two orders of magnitude higher than the global cytoplasmic [Ca^{2+}] rise [41,77]. Therefore, positioning of IP_3R/RyR at ERMCs helps to couple mitochondrial Ca^{2+} uptake and metabolism to ER Ca^{2+} release [75,78]. Furthermore, IP_3R-mitochondrial Ca^{2+} transfer is supralinearly dependent on the ER Ca^{2+} loading state [79]. However, during ER stress, there is a sustained Ca^{2+} leak from the

ER and this, through saturation of the Ca^{2+} binding sites of the mtCU gatekeepers MICU1-2-3 lowers the [Ca^{2+}] threshold for mitochondrial Ca^{2+} uptake [80,81] leading to large mitochondrial Ca^{2+} loads. Importantly, mitochondrial Ca^{2+} uptake and extrusion can also locally control the Ca^{2+} dependent activation and inhibition of the IP_3R/RyR and in turn, modulate the calcium signal that is propagated to the mitochondria [44,82].

Three mammalian isoforms of the IP_3R (IP_3R1, IP_3R2, and IP_3R3) are encoded by distinct genes and exhibit tissue-specific expression and regulatory properties; however, all mediate Ca^{2+} efflux from the ER lumen into the cytosol [83]. IP_3Rs are large tetrameric channels embedded in the ER membrane consisting of a large cytosolic regulatory domain that binds IP_3 and a C-terminal channel-forming region [84]. Their activation requires Ca^{2+} at 100 nanomolar concentrations, whereas higher Ca^{2+} levels are inhibitory, resulting in a tight biphasic regulation by Ca^{2+} [85].

The IP_3R1 isoform contains 60 cysteine residues, including several redox-sensitive residues located in both cytosolic and luminal domains [86]. Some cysteines serve structural roles, whereas others exert regulatory functions [87-89]. PDI mediates tetramer formation of IP_3R1 through intermolecular disulfide bond formation between two luminal cysteines. Two additional luminal cysteines Cys2496 and Cys2504 in the third luminal loop of IP_3R1 are reportedly oxidized by the PDI/ERO1/ERp46 relay to promote receptor activation [87]. However, comprehensive redox proteomic analyses of IP_3R1 cysteines revealed that, among the two regulatory intraluminal cysteines previously proposed to form a stable disulfide bond, one was predominantly reduced while the other was partially oxidized. Although the intrinsic difficulty in detecting oxidized peptides cannot exclude the possibility that a cysteine residue was oxidized but not detected, these findings could suggest a dynamic or asymmetric redox regulatory mechanism between the two aforementioned cysteines [86].

On the contrary, ERdj5 reduces the regulatory disulfide bond between these two cysteines, thereby attenuating IP3R activity under both basal and ER-stressed, low-$[Ca^{2+}]_{ER}$ conditions [87]. A similar pattern is mediated by the oxidoreductase ERp44, which interacts with IP_3R1 under reducing and low $[Ca^{2+}]_{ER}$ conditions to inhibit ER Ca^{2+}release [31], opposing the ERO1-dependent IP_3R oxidation/activation and cell death [32]. Although thiol oxidation has been studied primarily in IP_3R1, IP_3R2 and IP_3R3 also contain cysteine residues, many of which occupy positions analogous to those in IP_3R1 and may also undergo redox regulation [90].

ER H_2O_2 is capable of targeting cysteines and modulating IP_3R oxidation and activation [91]. Locally generated H_2O_2, can also freely diffuse through the ER membrane, and modify redox-sensitive thiols in both luminal and cytosolic domains of IP_3Rs. Indeed, the presence of multiple oxidation-sensitive cysteines on the cytosolic face of the receptor may allow activation of IP_3Rs via extraluminal thiol modification. Thus, H_2O_2 produced outside the ER might also oxidize and activate IP3R. Indeed, H_2O_2 derived from the mitochondrial respiratory chain may directly oxidize cytosolic thiols of the IP_3R and modulate its IP_3-sensitivity [86,92]. Beyond direct redox regulation of IP_3Rs, NOX4, a ROS producing ezyme on the cytoplasmic surface of the ER engages also AKT to reduce IP_3R activity via phosphorylation-dependent mechanisms, thereby promoting cell survival [93]. RyRs are also activated by ROS and by ERO1-dependent redox signaling [30,94-97]. The sensitivity of IP_3R/RyR to both ER luminal oxidative poise

and mitochondrial bioenergetics at the ERMCs allows them to integrate inputs provided by both ER and mitochondria.

During adaptive UPR, ERMC tightening and controlled redox modulation of IP_3Rs enhance mitochondrial Ca^{2+} uptake in a calibrated manner, supporting ATP production required for proteostasis [5,6] . In contrast, during maladaptive UPR, CHOP-induced ERO1 upregulation may shift ER redox balance toward hyperoxidation, lowering the activation threshold of IP_3Rs and prolonging channel opening. Combined with increased ER–mitochondria physical coupling, this redox sensitization of IP_3R promotes mitochondrial Ca^{2+} overload and mPTP opening [98-101]. Thus, IP_3R functions as a redox-tunable inflection point at which adaptive ER–mitochondria communication may transit into mitochondrial dysfunction and cell death (Fig. 3).

Bidirectional ROS Signaling and Feed-Forward Amplification

ROS are generated in both the ER and mitochondria and function as diffusible redox signals coordinating inter-organellar communication and signaling [102] (Fig. 3). In the ER, ROS are produced through ERO1 in the lumen as a byproduct of oxidative protein folding or through NOX4 on the cytosolic side [64,103] and can also arise from lipid peroxidation of the ER membrane [104]. TMX2 and SEPN1 are oxidoreductases predominantly localized to the ER-facing side of ER–mitochondria contact sites (ERMCs), where they contribute to the redox regulation of interorganellar signaling and protein thiol homeostasis. Although they do not directly generate H_2O_2, they influence ER redox balance, at least in part through interactions with the ERO1-dependent oxidative folding machinery [105] [106]. In mitochondria, H_2O_2 primarily derives from superoxide anion generated at respiratory chain complexes I within the matrix and complex III on both the matrix and intermembrane space (IMS) sides of the inner mitochondrial membrane (IMM), and subsequently dismutated by superoxide dismutases [107]. Direct H_2O_2 generation can be mediated by p66Shc in the IMS during stress [108] and at the OMM by MAO-B [109].

Importantly, H_2O_2 is membrane-permeable, either by passive diffusion or via aquaporins, allowing it to diffuse across ER and mitochondrial membranes [110]. Evidence suggests that H_2O_2 generated in mitochondria can undergo retrograde diffusion toward the ER, influencing ER redox homeostasis and Ca^{2+} handling by affecting the activity of Ca^{2+} channels [92]. This retrograde redox signaling provides a mechanism by which even single mitochondria can modulate the activity of the neighboring ER Ca^{2+} release channels [92]. Mitochondrial superoxide anion and H_2O_2, and ERMC confined redox nanodomains might result from processes inherent to mitochondria but can also be stimulated by ER-originated [Ca^{2+}] spikes exerting a positive feedback on calcium oscillations [53].

H_2O_2 production is tightly balanced by scavenging mechanisms to maintain subtoxic levels. Robust H_2O_2 scavenging including glutathione peroxidase is present in the mitochondrial matrix, in the cytoplasm,in the intermembrane space of the ERMCs as well as in the ER lumen [111,112]. For example, the ER-resident enzymes GPx8 and PRDX4 prevent the diffusion of ERO1-derived H_2O_2 within and beyond the ER [113].

The most common targets of H_2O_2 are the cysteine thiol groups. Because IP_3Rs and RyRs contain redox-sensitive thiols on both luminal and cytosolic domains, H_2O_2 can

modulate channel activity on either side of the ER membrane [53]. The Ca^{2+} transporters SERCA pumps also have redox-sensitive cysteine thiols [114]. In addition, the redox sensitivity of their cysteine residues varies, and mild versus severe oxidative stress may produce different outcomes [115]. Other functional and structural components of the ERMC such as PERK, TOM70, SEPN1 and TMX2 are also redox regulated, and interact with Ca^{2+} transporters and channels; thus their redox modification might switch adaptation to maladaptation [105,116 117 106]. Therefore, the redox sensitivity of cysteines, combined with the membrane permeability of H_2O_2, enables effective—and often complex—redox regulation of Ca^{2+} channels and transporters across ERMCs.

In summary, ERMCs harbor a network of local ER- and mitochondria-derived H_2O_2 sources, antioxidant scavenging systems, and redox-sensitive targets that collectively regulate Ca^{2+} dynamics. The conditions are also given to set up amplification loops. For example, mitochondria-derived H_2O_2 can enhance IP_3R- or RyR-mediated Ca^{2+} efflux from the ER, increasing mitochondrial Ca^{2+} uptake [92,118]. Elevated mitochondrial Ca^{2+} stimulates respiratory chain activity but, when mitochondrial buffering capacity is exceeded, enhances electron leak, leading to further ROS generation. When this amplification surpasses cellular antioxidant defenses, excessive mitochondrial Ca^{2+} accumulation triggers mPTP opening, bioenergetic collapse, and apoptotic or necrotic cell death [100,119]. Thus, local H_2O_2 signaling between the ER and mitochondria constitutes a feed-forward amplification mechanism in which redox-dependent sensitization of Ca^{2+} release channels promotes mitochondrial Ca^{2+} accumulation. Under adaptive UPR conditions, this loop transiently supports metabolic compensation and proteostasis. In contrast, during maladaptive UPR, persistent H_2O_2/ROS trigger mitochondrial Ca^{2+} overload. In this context, the ER–mitochondria redox–Ca^{2+} circuit becomes a decisive checkpoint that shifts the cell from stress adaptation to irreversible apoptotic commitment (Fig.3).

UPR-initiated Mitochondrial Cell Death Pathways

Sustained activation of the pro-apoptotic transcription factor CHOP and its downstream effector ERO1 link maladaptive UPR signaling to mitochondrial-dependent cell death through two mechanistically distinct yet converging pathways: selective OMMP and opening of the mPTP [34,120-122]. Under conditions of ER stress, CHOP shifts the balance of BCL-2 family proteins toward apoptosis by repressing the transcription of anti-apoptotic members such as BCL-2, BCL-XL and MCL-1 while inducing pro-apoptotic BIM transcriptionally. These changes come together to support BAK/BAX pore formation, leading to cytochrome *c* release and subsequent activation of the caspase cascade [123-125].

In parallel, maladaptive UPR signaling promotes the opening of the mPTP, a high-conductance Ca^{2+}- and redox-sensitive channel located in the IMM [126]. Transient mPTP openings, often confined to single mitochondria, are manifested as transient mitochondrial depolarizations (flickers) even in unchallenged cells [127,128], and their frequency and duration increases very early during various cellular stress conditions [92,129]. Sustained pore opening causes dissipation of the mitochondrial membrane potential, matrix swelling, and bioenergetic failure. Downstream of CHOP, induction of ERO1 amplifies ER Ca^{2+} release and H_2O_2 at ER–mitochondria contact sites, resulting in excessive mitochondrial Ca^{2+} uptake and elevated ROS. Calcium overload might be the pivotal trigger of mPTP opening but Ca^{2+} often acts in synergism with

coincident perturbations [61,130]. ROS may oxidize critical thiol residues on mPTP-associated proteins, sensitizing the pore to Ca^{2+}-dependent permeability transition [98]. Vast literature suggests that both anti-and pro-apoptotic BCL-2 family proteins also control mPTP activation by multiple mechanism [131-134]. Persistent mPTP opening leads to matrix expansion, rupture of the IMM and OMM, release of apoptotic factors, collapse of membrane potential, and ATP depletion. Together, mitochondrial Ca^{2+} overload, oxidative stress and other factors synergistically lower the threshold for mPTP activation, culminating in irreversible mitochondrial dysfunction and cell death [121]. Thus, during maladaptive UPR signaling, CHOP–ERO1–mediated Ca^{2+} and redox dysregulation promotes mPTP opening and might cooperate with BCL-2 family–dependent OMMP, integrating IMM and OMM damage to amplify mitochondrial impairment and irreversibly commit the cell to death.

Limitations and conclusion

Although the precise mechanisms underlying the transition from adaptive to maladaptive UPR remain to be elucidated, several factors are likely to contribute, including the intensity of ER stress, mitochondrial Ca^{2+} overload, and ROS accumulation. Below we focus on ERO1's role in this framework. We propose that sustained ERO1 activity augments localized redox nanodomains at ERMCs, whose abundance may determine whether UPR signaling remains adaptive or progresses toward a maladaptive response (Fig. 3). This transition could be influenced by the balance between the local ERO1-derived H_2O_2 production and its scavenging by the ER-resident antioxidant enzymes GPX8 and PRDX4 [113]. When H_2O_2 production exceeds the antioxidant capacity of these enzymes, H_2O_2 accumulation may promote the transition from adaptive to maladaptive UPR signaling. Importantly, the threshold at which ERO1 signaling becomes maladaptive is likely to be cell type-specific. ERMC composition, IP_3R isoform expression, mitochondrial oxidative capacity, metabolic demands, and ER antioxidant buffering differ among tissues, potentially influencing the susceptibility of individual cell types to ERO1-dependent H_2O_2 and Ca^{2+} signaling. Highly oxidative tissues such as skeletal muscle, cardiac muscle, and neurons may therefore exhibit distinct thresholds for the transition from adaptive to maladaptive UPR compared with predominantly glycolytic cell types, emphasizing the need to define tissue-specific mechanisms of ERMC regulation.While modulation of IP_3R activity represents a plausible pathway linking ERO1 to altered ER–mitochondria Ca^{2+} transfer, ERO1 likely influences cell fate through multiple mechanisms. It remains still unclear whether the effects of ERO1 on IP_3R activity arise primarily from its enzymatic function as a disulfide oxidase, acting through PDI-mediated oxidation of regulatory cysteines, or from its generation of H_2O_2. Importantly, not all cysteine residues are equally susceptible to direct oxidation by H_2O_2. Cysteine reactivity depends strongly on the local amino acid environment, which influences thiol accessibility, pKa, and stabilization of reactive thiolate species. Consequently, PDI-mediated disulfide relay reactions and H_2O_2-mediated oxidation may target distinct subsets of cysteine residues within IP_3Rs. Furthermore, H_2O_2-dependent oxidation may generate different oxidative modifications, including sulfenic, sulfinic, or sulfonic derivatives, potentially leading to outcomes that differ from those produced by disulfide bond formation [112]. Thus, ERO1-dependent regulation of IP_3Rs may involve mechanistically distinct redox pathways that modify different cysteine populations and exert different effects on channel activity. The experimental elucidation of these two options, as well as the precise definition of the ERO1 threshold that renders the UPR maladaptive, remain

limited by technical constraints. These include the lack of reliable in vivo sensors for measuring ERO1-derived H_2O_2—and H_2O_2 in general—within the ER, although some attempts have been made to develop genetically encoded H_2O_2 sensors targeted to this compartment. However, their reliability and specificity towards H_2O_2 remain limited in the oxidizing ER environment hosting disulfide bond formation [135]. In addition, the inability to directly measure ERO1 activity in vivo further prevents accurate characterization of the ERO1-dependent transition between adaptive and maladaptive UPR. Future advances may include the development of genetically encoded H_2O_2 sensors specifically targeted to ERMCs, enabling real-time and spatially resolved monitoring of local ERO1-derived H_2O_2 dynamics at these subcellular domains.

Dysregulated ER–mitochondria communication is implicated in multiple pathological conditions (for a recent review see [44]), where UPR-driven remodeling of ERMCs may either support cellular adaptation or promote mitochondrial failure and cell death. Recent studies employing synthetic ERMC linkers demonstrate that ER–mitochondria proximity is dynamically tunable, with major consequences for Ca^{2+} transfer, redox signaling, and mitochondrial bioenergetics (for recent reviews see [4,41 136 137]). These findings suggest that ERMC functions depend on maintaining an optimal physical distance between the two organelles, for example the support of mitochondrial metabolism by local Ca^{2+} transfer. However, other ERMC functions such as phospholipid transfer or support of mitochondrial fission depend differently on ERMC gap width. This reinforces the concept that ERMCs act as adjustable signaling hubs, and that precision tuning of ER–mitochondria contacts could be harnessed as a disease-modifying therapeutic strategy [138]. In addition, excessive tightening of ERMCs may exacerbate Ca^{2+} overload and ROS transfer, promoting mitochondrial dysfunction and apoptosis. Therapeutic strategies should therefore aim not simply to increase ER–mitochondria coupling, but to fine-tune the architecture and nanoscale organization of ERMCs according to the disease context. Restoring physiological ERMC spacing may improve mitochondrial function in ER stress–associated myopathies, whereas deliberately enhancing short-range contacts could be exploited to promote apoptosis in cancer cells. Nonetheless, the ability to selectively manipulate and monitor ERMCs remains technically limited. Recent advances in synthetic biology, high-resolution imaging, genetically encoded linkers, and chemically inducible reversible tethering systems are beginning to overcome these limitations and may provide new opportunities for both mechanistic studies and therapeutic targeting of ERMC. However, current approaches primarily manipulate the physical proximity between the ER and mitochondria and may not fully recapitulate the molecular complexity of endogenous ERMCs, where distinct tethering complexes, lipid composition, and local signaling microdomains determine functional outcomes. In addition to providing different interorganellar distances, therapeutic synthetic ERMC linkers should ideally be inducible by clinically applicable compounds and fully reversible upon withdrawal of the inducing agent [139]. Furthermore, future studies should determine whether diseases associated with UPR drive pathology by irreversibly locking ERMCs into maladaptive configuration, or whether these alterations remain reversible and amenable to correction through engineered ERMC linkers/spacers.

Interorganellar Ca^{2+} transfer at ERMC could also be a potential therapeutic target. Potent inhibitors are available to inhibit mitochondrial Ca^{2+} uptake, but their specificity and plasma membrane permeability remain debated [140 141]. The FDA-approved compound benzethonium[142], and MCU-i11 [141,143] have been validated to inhibit

Journal Pre-proof

mitochondrial Ca^{2+} uptake in many intact cells. Although these compounds could be potentially useful for studying the role of mitochondrial Ca^{2+} uptake in ER stress paradigms, their inhibition would not be specific to ERMC-mediated Ca^{2+} transfer, and mitochondrial Ca^{2+} uptake regardless of its source would be affected.

Another important question is whether combining ERMC-targeting approaches with interventions that restore proteostasis—such as the chemical chaperones TUDCA and 4-PBA or ERO1 inhibitors—may provide synergistic therapeutic benefits. In this regard, ERO1 represents an attractive therapeutic target because of its dual role in regulating ER redox homeostasis and ERMC organization. Notably, genetic deletion of ERO1 in a preclinical mouse model of SEPN1-related myopathy restored sarcoplasmic reticulum–mitochondria contacts in diaphragm muscle, demonstrating a direct role for ERO1 in ERMC regulation [144]. Together with the potential benefits of chemical chaperones such as TUDCA and 4-PBA, these findings raise the possibility that combining proteostasis-restoring interventions with approaches that modulate ERMC architecture may provide synergistic therapeutic benefits across a range of ER stress-associated diseases, including cancer, neurodegenerative disorders, diabetes, and muscle diseases. However, the mechanisms by which ER–mitochondria communication contributes to disease are likely to be highly tissue-specific. In neurodegenerative disorders, excessive ER-to-mitochondria Ca^{2+} transfer and oxidative stress may promote neuronal death; in diabetes, altered ERMC signaling contributes to β-cell dysfunction and insulin resistance; in skeletal muscle disorders, impaired ERMC architecture compromises mitochondrial bioenergetics and contractile function; whereas in cancer, enhanced ERMC coupling may either sustain mitochondrial metabolism or sensitize cells to apoptosis, depending on the tumor context. A better understanding of these context-dependent mechanisms will be important for the development of tissue-specific biomarkers and therapeutic strategies that selectively target ERMC [65,144-151].

Competing interest

The authors declare that they have no conflict of interest.

Acknowledgments

This study was supported by AIRC IG 28733, Telethon TS001324 and Giving Strength to E.Z., and NIH grant RO1 DK125897 to G.H.. Thanks to Nicolas Demaurex and Amaldo Carreras-Sureda for expert advice, and Victor Hugo Sanchez-Vazquez for help with the graphic design.

REFERENCES:

1. Walter, P., and Ron, D. (2011). The unfolded protein response: from stress pathway to homeostatic regulation. Science *334*, 1081-1086. 334/6059/1081 [pii] 10.1126/science.1209038.

2. Pakos-Zebrucka, K., Koryga, I., Mnich, K., Ljujic, M., Samali, A., and Gorman, A.M. (2016). The integrated stress response. EMBO Rep *17*, 1374-1395. 10.15252/embr.201642195.

3. 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. 18/24/3066 [pii]
10.1101/gad.1250704.
4. Csordas, G., Weaver, D., and Hajnoczky, G. (2018). Endoplasmic Reticulum-Mitochondrial Contactology: Structure and Signaling Functions. Trends Cell Biol *28*, 523-540. 10.1016/j.tcb.2018.02.009.
5. Bravo, R., Vicencio, J.M., Parra, V., Troncoso, R., Munoz, J.P., Bui, M., Quiroga, C., Rodriguez, A.E., Verdejo, H.E., Ferreira, J., et al. (2011). Increased ER-mitochondrial coupling promotes mitochondrial respiration and bioenergetics during early phases of ER stress. J Cell Sci *124*, 2143-2152. jcs.080762 [pii]
10.1242/jcs.080762.
6. Bassot, A., Chen, J., Takahashi-Yamashiro, K., Yap, M.C., Gibhardt, C.S., Le, G.N.T., Hario, S., Nasu, Y., Moore, J., Gutierrez, T., et al. (2023). The endoplasmic reticulum kinase PERK interacts with the oxidoreductase ERO1 to metabolically adapt mitochondria. Cell Rep *42*, 111899. 10.1016/j.celrep.2022.111899.
7. Carreras-Sureda, A., Jana, F., Urra, H., Durand, S., Mortenson, D.E., Sagredo, A., Bustos, G., Hazari, Y., Ramos-Fernandez, E., Sassano, M.L., et al. (2019). Non-canonical function of IRE1alpha determines mitochondria-associated endoplasmic reticulum composition to control calcium transfer and bioenergetics. Nat Cell Biol *21*, 755-767. 10.1038/s41556-019-0329-y.
8. Schroder, M., and Kaufman, R.J. (2005). ER stress and the unfolded protein response. Mutat Res *569*, 29-63. 10.1016/j.mrfmmm.2004.06.056.
9. Han, D., Lerner, A.G., Vande Walle, L., Upton, J.P., Xu, W., Hagen, A., Backes, B.J., Oakes, S.A., and Papa, F.R. (2009). IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell *138*, 562-575. 10.1016/j.cell.2009.07.017.
10. 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. 10.1016/j.tibs.2014.02.008.
11. Ye, J., Rawson, R.B., Komuro, R., Chen, X., Dave, U.P., Prywes, R., Brown, M.S., and Goldstein, J.L. (2000). ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell *6*, 1355-1364. 10.1016/s1097-2765(00)00133-7.
12. 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. 10.1091/mbc.10.11.3787.
13. Harding, H.P., Zhang, Y., and Ron, D. (1999). Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature *397*, 271-274. 10.1038/16729.
14. Cullinan, S.B., Zhang, D., Hannink, M., Arvisais, E., Kaufman, R.J., and Diehl, J.A. (2003). Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. Mol Cell Biol *23*, 7198-7209. 10.1128/MCB.23.20.7198-7209.2003.
15. Taniuchi, S., Miyake, M., Tsugawa, K., Oyadomari, M., and Oyadomari, S. (2016). Integrated stress response of vertebrates is regulated by four eIF2alpha kinases. Sci Rep *6*, 32886. 10.1038/srep32886.
16. 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. S1097276503001059 [pii].
17. Celardo, I., Lehmann, S., Costa, A.C., Loh, S.H., and Miguel Martins, L. (2017). dATF4 regulation of mitochondrial folate-mediated one-carbon metabolism is neuroprotective. Cell Death Differ *24*, 638-648. 10.1038/cdd.2016.158.
18. Costa-Mattioli, M., and Walter, P. (2020). The integrated stress response: From mechanism to disease. Science *368*. 10.1126/science.aat5314.
19. Verfaillie, T., Rubio, N., Garg, A.D., Bultynck, G., Rizzuto, R., Decuypere, J.P., Piette, J., Linehan, C., Gupta, S., Samali, A., and Agostinis, P. (2012). PERK is

required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress. Cell Death Differ *19*, 1880-1891. 10.1038/cdd.2012.74.
20. Munoz, J.P., Ivanova, S., Sanchez-Wandelmer, J., Martinez-Cristobal, P., Noguera, E., Sancho, A., Diaz-Ramos, A., Hernandez-Alvarez, M.I., Sebastian, D., Mauvezin, C., et al. (2013). Mfn2 modulates the UPR and mitochondrial function via repression of PERK. EMBO J *32*, 2348-2361. 10.1038/emboj.2013.168.
21. Kaspar, S., Oertlin, C., Szczepanowska, K., Kukat, A., Senft, K., Lucas, C., Brodesser, S., Hatzoglou, M., Larsson, O., Topisirovic, I., and Trifunovic, A. (2021). Adaptation to mitochondrial stress requires CHOP-directed tuning of ISR. Sci Adv *7*. 10.1126/sciadv.abf0971.
22. Frand, A.R., and Kaiser, C.A. (1998). The ERO1 gene of yeast is required for oxidation of protein dithiols in the endoplasmic reticulum. Mol Cell *1*, 161-170. S1097-2765(00)80017-9 [pii].
23. Pollard, M.G., Travers, K.J., and Weissman, J.S. (1998). Ero1p: a novel and ubiquitous protein with an essential role in oxidative protein folding in the endoplasmic reticulum. Mol Cell *1*, 171-182. S1097-2765(00)80018-0 [pii].
24. Tu, B.P., and Weissman, J.S. (2002). The FAD- and O(2)-dependent reaction cycle of Ero1-mediated oxidative protein folding in the endoplasmic reticulum. Mol Cell *10*, 983-994. S1097276502006962 [pii].
25. Zito, E., Melo, E.P., Yang, Y., Wahlander, A., Neubert, T.A., and Ron, D. (2010). Oxidative protein folding by an endoplasmic reticulum-localized peroxiredoxin. Mol Cell *40*, 787-797. S1097-2765(10)00848-8 [pii] 10.1016/j.molcel.2010.11.010.
26. Zito, E., Hansen, H.G., Yeo, G.S., Fujii, J., and Ron, D. (2012). Endoplasmic Reticulum Thiol Oxidase Deficiency Leads to Ascorbic Acid Depletion and Noncanonical Scurvy in Mice. Mol Cell *48*, 39-51. S1097-2765(12)00697-1 [pii] 10.1016/j.molcel.2012.08.010.
27. Ramming, T., Kanemura, S., Okumura, M., Inaba, K., and Appenzeller-Herzog, C. (2016). Cysteines 208 and 241 in Ero1alpha are required for maximal catalytic turnover. Redox Biol *7*, 14-20. 10.1016/j.redox.2015.11.004.
28. Hansen, H.G., Schmidt, J.D., Soltoft, C.L., Ramming, T., Geertz-Hansen, H.M., Christensen, B., Sorensen, E.S., Juncker, A.S., Appenzeller-Herzog, C., and Ellgaard, L. (2012). Hyperactivity of the Ero1alpha oxidase elicits endoplasmic reticulum stress but no broad antioxidant response. J Biol Chem *287*, 39513-39523. 10.1074/jbc.M112.405050.
29. Anelli, T., Bergamelli, L., Margittai, E., Rimessi, A., Fagioli, C., Malgaroli, A., Pinton, P., Ripamonti, M., Rizzuto, R., and Sitia, R. (2012). Ero1alpha regulates Ca(2+) fluxes at the endoplasmic reticulum-mitochondria interface (MAM). Antioxid Redox Signal *16*, 1077-1087. 10.1089/ars.2011.4004.
30. Hamilton, S., Terentyeva, R., Bogdanov, V., Kim, T.Y., Perger, F., Yan, J., Ai, X., Carnes, C.A., Belevych, A.E., George, C.H., et al. (2022). Ero1alpha-Dependent ERp44 Dissociation From RyR2 Contributes to Cardiac Arrhythmia. Circ Res *130*, 711-724. 10.1161/CIRCRESAHA.121.320531.
31. Higo, T., Hattori, M., Nakamura, T., Natsume, T., Michikawa, T., and Mikoshiba, K. (2005). Subtype-specific and ER lumenal environment-dependent regulation of inositol 1,4,5-trisphosphate receptor type 1 by ERp44. Cell *120*, 85-98. S0092867404011535 [pii] 10.1016/j.cell.2004.11.048.
32. 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. jcb.200904060 [pii] 10.1083/jcb.200904060.
33. 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. 10.1016/j.molcel.2004.08.025 S1097276504005118 [pii].
34. Deniaud, A., Sharaf el dein, O., Maillier, E., Poncet, D., Kroemer, G., Lemaire, C., and Brenner, C. (2008). Endoplasmic reticulum stress induces calcium-

dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis. Oncogene *27*, 285-299. 10.1038/sj.onc.1210638.
35. Dou, G., Sreekumar, P.G., Spee, C., He, S., Ryan, S.J., Kannan, R., and Hinton, D.R. (2012). Deficiency of alphaB crystallin augments ER stress-induced apoptosis by enhancing mitochondrial dysfunction. Free Radic Biol Med *53*, 1111-1122. 10.1016/j.freeradbiomed.2012.06.042.
36. Zheng, Y., Qu, H., Xiong, X., Wang, Y., Liu, X., Zhang, L., Liao, X., Liao, Q., Sun, Z., Ouyang, Q., et al. (2019). Deficiency of Mitochondrial Glycerol 3-Phosphate Dehydrogenase Contributes to Hepatic Steatosis. Hepatology *70*, 84-97. 10.1002/hep.30507.
37. Lomovsky, A., Baburina, Y., Odinokova, I., Kobyakova, M., Evstratova, Y., Sotnikova, L., Krestinin, R., and Krestinina, O. (2020). Melatonin Can Modulate the Effect of Navitoclax (ABT-737) in HL-60 Cells. Antioxidants (Basel) *9*. 10.3390/antiox9111143.
38. Vance, J.E. (1990). Phospholipid synthesis in a membrane fraction associated with mitochondria. J Biol Chem *265*, 7248-7256.
39. Mannella, C.A., Buttle, K., Rath, B.K., and Marko, M. (1998). Electron microscopic tomography of rat-liver mitochondria and their interaction with the endoplasmic reticulum. Biofactors *8*, 225-228. 10.1002/biof.5520080309.
40. Csordas, G., Renken, C., Varnai, P., Walter, L., Weaver, D., Buttle, K.F., Balla, T., Mannella, C.A., and Hajnoczky, G. (2006). Structural and functional features and significance of the physical linkage between ER and mitochondria. J Cell Biol *174*, 915-921. jcb.200604016 [pii] 10.1083/jcb.200604016.
41. Csordas, G., Varnai, P., Golenar, T., Roy, S., Purkins, G., Schneider, T.G., Balla, T., and Hajnoczky, G. (2010). Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface. Mol Cell *39*, 121-132. 10.1016/j.molcel.2010.06.029.
42. Giacomello, M., and Pellegrini, L. (2016). The coming of age of the mitochondria-ER contact: a matter of thickness. Cell Death Differ *23*, 1417-1427. 10.1038/cdd.2016.52.
43. Szabadkai, G., Bianchi, K., Varnai, P., De Stefani, D., Wieckowski, M.R., Cavagna, D., Nagy, A.I., Balla, T., and Rizzuto, R. (2006). Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels. J Cell Biol *175*, 901-911. jcb.200608073 [pii] 10.1083/jcb.200608073.
44. Cartes-Saavedra, B., Ghosh, A., and Hajnoczky, G. (2025). The roles of mitochondria in global and local intracellular calcium signalling. Nat Rev Mol Cell Biol *26*, 456-475. 10.1038/s41580-024-00820-1.
45. de Brito, O.M., and Scorrano, L. (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature *456*, 605-610. 10.1038/nature07534.
46. Merkwirth, C., and Langer, T. (2008). Mitofusin 2 builds a bridge between ER and mitochondria. Cell *135*, 1165-1167. 10.1016/j.cell.2008.12.005.
47. De Vos, K.J., Morotz, G.M., Stoica, R., Tudor, E.L., Lau, K.F., Ackerley, S., Warley, A., Shaw, C.E., and Miller, C.C. (2012). VAPB interacts with the mitochondrial protein PTPIP51 to regulate calcium homeostasis. Hum Mol Genet *21*, 1299-1311. 10.1093/hmg/ddr559.
48. Nakamura, K., Aoyama-Ishiwatari, S., Nagao, T., Paaran, M., Obara, C.J., Sakurai-Saito, Y., Johnston, J., Du, Y., Suga, S., Tsuboi, M., et al. (2025). Mitochondrial complexity is regulated at ER-mitochondria contact sites via PDZD8-FKBP8 tethering. Nat Commun *16*, 3401. 10.1038/s41467-025-58538-3.
49. Simmen, T., Aslan, J.E., Blagoveshchenskaya, A.D., Thomas, L., Wan, L., Xiang, Y., Feliciangeli, S.F., Hung, C.H., Crump, C.M., and Thomas, G. (2005). PACS-2 controls endoplasmic reticulum-mitochondria communication and Bid-mediated apoptosis. EMBO J *24*, 717-729. 10.1038/sj.emboj.7600559.
50. Hirabayashi, Y., Kwon, S.K., Paek, H., Pernice, W.M., Paul, M.A., Lee, J., Erfani, P., Raczkowski, A., Petrey, D.S., Pon, L.A., and Polleux, F. (2017). ER-mitochondria tethering by PDZD8 regulates Ca(2+) dynamics in mammalian neurons. Science *358*, 623-630. 10.1126/science.aan6009.

51. Vance, J.E. (2014). MAM (mitochondria-associated membranes) in mammalian cells: lipids and beyond. Biochim Biophys Acta *1841*, 595-609. 10.1016/j.bbalip.2013.11.014.
52. Wiedemann, N., Meisinger, C., and Pfanner, N. (2009). Cell biology. Connecting organelles. Science *325*, 403-404. 10.1126/science.1178016.
53. Booth, D.M., Enyedi, B., Geiszt, M., Varnai, P., and Hajnoczky, G. (2016). Redox Nanodomains Are Induced by and Control Calcium Signaling at the ER-Mitochondrial Interface. Mol Cell *63*, 240-248. 10.1016/j.molcel.2016.05.040.
54. Csordas, G., and Hajnoczky, G. (2009). SR/ER-mitochondrial local communication: calcium and ROS. Biochim Biophys Acta *1787*, 1352-1362. S0005-2728(09)00205-9 [pii]
10.1016/j.bbabio.2009.06.004.
55. Voeltz, G.K., Sawyer, E.M., Hajnoczky, G., and Prinz, W.A. (2024). Making the connection: How membrane contact sites have changed our view of organelle biology. Cell *187*, 257-270. 10.1016/j.cell.2023.11.040.
56. Sassano, M.L., Tyurina, Y.Y., Diokmetzidou, A., Vervoort, E., Tyurin, V.A., More, S., La Rovere, R., Giordano, F., Bultynck, G., Pavie, B., et al. (2025). Endoplasmic reticulum-mitochondria contacts are prime hotspots of phospholipid peroxidation driving ferroptosis. Nat Cell Biol *27*, 902-917. 10.1038/s41556-025-01668-z.
57. van Vliet, A.R., and Agostinis, P. (2016). When under pressure, get closer: PERKing up membrane contact sites during ER stress. Biochem Soc Trans *44*, 499-504. 10.1042/BST20150272.
58. Feng, Q., and Kornmann, B. (2018). Mechanical forces on cellular organelles. J Cell Sci *131*. 10.1242/jcs.218479.
59. Schuck, S., Prinz, W.A., Thorn, K.S., Voss, C., and Walter, P. (2009). Membrane expansion alleviates endoplasmic reticulum stress independently of the unfolded protein response. J Cell Biol *187*, 525-536. 10.1083/jcb.200907074.
60. Carraro, M., and Bernardi, P. (2023). The mitochondrial permeability transition pore in Ca(2+) homeostasis. Cell Calcium *111*, 102719. 10.1016/j.ceca.2023.102719.
61. Baumgartner, H.K., Gerasimenko, J.V., Thorne, C., Ferdek, P., Pozzan, T., Tepikin, A.V., Petersen, O.H., Sutton, R., Watson, A.J., and Gerasimenko, O.V. (2009). Calcium elevation in mitochondria is the main Ca2+ requirement for mitochondrial permeability transition pore (mPTP) opening. J Biol Chem *284*, 20796-20803. 10.1074/jbc.M109.025353.
62. Sassano, M.L., van Vliet, A.R., Vervoort, E., Van Eygen, S., Van den Haute, C., Pavie, B., Roels, J., Swinnen, J.V., Spinazzi, M., Moens, L., et al. (2023). PERK recruits E-Syt1 at ER-mitochondria contacts for mitochondrial lipid transport and respiration. J Cell Biol *222*. 10.1083/jcb.202206008.
63. 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. 10.1371/journal.pone.0076941.
64. Zito, E. (2015). ERO1: A protein disulfide oxidase and H2O2 producer. Free Radic Biol Med *83*, 299-304. S0891-5849(15)00018-0 [pii]
10.1016/j.freeradbiomed.2015.01.011.
65. Bassot, A., Violy, L., Gorka, L., Cigalotto, L., Namasivayam, B., Mikaelian, I., St Johnston, E., Han, C., Gadet, R., Alpha-Bazin, B., et al. (2026). ERO1a fosters glioblastoma aggressiveness and metabolic flexibility by regulating mitochondria-associated membrane dynamics. Nat Cell Biol. 10.1038/s41556-026-01980-2.
66. Tjoelker, L.W., Seyfried, C.E., Eddy, R.L., Jr., Byers, M.G., Shows, T.B., Calderon, J., Schreiber, R.B., and Gray, P.W. (1994). Human, mouse, and rat calnexin cDNA cloning: identification of potential calcium binding motifs and gene localization to human chromosome 5. Biochemistry *33*, 3229-3236. 10.1021/bi00177a013.
67. Preissler, S., Rato, C., Yan, Y., Perera, L.A., Czako, A., and Ron, D. (2020). Calcium depletion challenges endoplasmic reticulum proteostasis by destabilising BiP-substrate complexes. Elife *9*. 10.7554/eLife.62601.

Journal Pre-proof
68. Lievremont, J.P., Rizzuto, R., Hendershot, L., and Meldolesi, J. (1997). BiP, a major chaperone protein of the endoplasmic reticulum lumen, plays a direct and important role in the storage of the rapidly exchanging pool of Ca2+. J Biol Chem *272*, 30873-30879. 10.1074/jbc.272.49.30873.
69. Vangheluwe, P., Raeymaekers, L., Dode, L., and Wuytack, F. (2005). Modulating sarco(endo)plasmic reticulum Ca2+ ATPase 2 (SERCA2) activity: cell biological implications. Cell Calcium *38*, 291-302. 10.1016/j.ceca.2005.06.033.
70. Lock, J.T., Smith, I.F., and Parker, I. (2019). Spatial-temporal patterning of Ca(2+) signals by the subcellular distribution of IP(3) and IP(3) receptors. Semin Cell Dev Biol *94*, 3-10. 10.1016/j.semcdb.2019.01.012.
71. Bartok, A., Weaver, D., Golenar, T., Nichtova, Z., Katona, M., Bansaghi, S., Alzayady, K.J., Thomas, V.K., Ando, H., Mikoshiba, K., et al. (2019). IP(3) receptor isoforms differently regulate ER-mitochondrial contacts and local calcium transfer. Nature communications *10*, 3726. 10.1038/s41467-019-11646-3.
72. Franzini-Armstrong, C., and Boncompagni, S. (2011). The evolution of the mitochondria-to-calcium release units relationship in vertebrate skeletal muscles. J Biomed Biotechnol *2011*, 830573. 10.1155/2011/830573.
73. Franzini-Armstrong, C. (2007). ER-mitochondria communication. How privileged? Physiology (Bethesda) *22*, 261-268. 10.1152/physiol.00017.2007.
74. De Stefani, D., Rizzuto, R., and Pozzan, T. (2016). Enjoy the Trip: Calcium in Mitochondria Back and Forth. Annu Rev Biochem *85*, 161-192. 10.1146/annurev-biochem-060614-034216.
75. Rizzuto, R., Pinton, P., Carrington, W., Fay, F.S., Fogarty, K.E., Lifshitz, L.M., Tuft, R.A., and Pozzan, T. (1998). Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses. Science *280*, 1763-1766.
76. Csordas, G., Thomas, A.P., and Hajnoczky, G. (1999). Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. EMBO J *18*, 96-108. 10.1093/emboj/18.1.96.
77. Giacomello, M., Drago, I., Bortolozzi, M., Scorzeto, M., Gianelle, A., Pizzo, P., and Pozzan, T. (2010). Ca2+ hot spots on the mitochondrial surface are generated by Ca2+ mobilization from stores, but not by activation of store-operated Ca2+ channels. Mol Cell *38*, 280-290. 10.1016/j.molcel.2010.04.003.
78. Katona, M., Bartok, A., Nichtova, Z., Csordas, G., Berezhnaya, E., Weaver, D., Ghosh, A., Varnai, P., Yule, D.I., and Hajnoczky, G. (2022). Capture at the ER-mitochondrial contacts licenses IP(3) receptors to stimulate local Ca(2+) transfer and oxidative metabolism. Nat Commun *13*, 6779. 10.1038/s41467-022-34365-8.
79. Csordas, G., Weaver, D., Varnai, P., and Hajnoczky, G. (2024). Supralinear Dependence of the IP(3) Receptor-to-Mitochondria Local Ca(2+) Transfer on the Endoplasmic Reticulum Ca(2+) Loading. Contact (Thousand Oaks) *7*, 25152564241229273. 10.1177/25152564241229273.
80. Csordas, G., and Hajnoczky, G. (2003). Plasticity of mitochondrial calcium signaling. J Biol Chem *278*, 42273-42282. 10.1074/jbc.M305248200.
81. Basso, E., Rigotto, G., Zucchetti, A.E., and Pozzan, T. (2018). Slow activation of fast mitochondrial Ca(2+) uptake by cytosolic Ca(2). J Biol Chem *293*, 17081-17094. 10.1074/jbc.RA118.002332.
82. 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. 10.1038/nrm3412.
83. Mikoshiba, K. (2007). IP3 receptor/Ca2+ channel: from discovery to new signaling concepts. J Neurochem *102*, 1426-1446. 10.1111/j.1471-4159.2007.04825.x.
84. Baker, M.R., Fan, G., Arige, V., Yule, D.I., and Serysheva, II (2023). Understanding IP(3)R channels: From structural underpinnings to ligand-dependent conformational landscape. Cell Calcium *114*, 102770. 10.1016/j.ceca.2023.102770.
85. Schmitz, E.A., Takahashi, H., and Karakas, E. (2022). Structural basis for activation and gating of IP(3) receptors. Nat Commun *13*, 1408. 10.1038/s41467-022-29073-2.

86. Joseph, S.K., Booth, D.M., Young, M.P., and Hajnoczky, G. (2019). Redox regulation of ER and mitochondrial Ca(2+) signaling in cell survival and death. Cell Calcium *79*, 89-97. 10.1016/j.ceca.2019.02.006.
87. Fujii, S., Ushioda, R., and Nagata, K. (2023). Redox states in the endoplasmic reticulum directly regulate the activity of calcium channel, inositol 1,4,5-trisphosphate receptors. Proc Natl Acad Sci U S A *120*, e2216857120. 10.1073/pnas.2216857120.
88. Hajnoczky, G., Robb-Gaspers, L.D., Seitz, M.B., and Thomas, A.P. (1995). Decoding of cytosolic calcium oscillations in the mitochondria. Cell *82*, 415-424. 10.1016/0092-8674(95)90430-1.
89. Berridge, M.J. (2016). The Inositol Trisphosphate/Calcium Signaling Pathway in Health and Disease. Physiol Rev *96*, 1261-1296. 10.1152/physrev.00006.2016.
90. 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. 10.1074/jbc.M113.504159.
91. Li, G., Scull, C., Ozcan, L., and Tabas, I. (2010). NADPH oxidase links endoplasmic reticulum stress, oxidative stress, and PKR activation to induce apoptosis. J Cell Biol *191*, 1113-1125. 10.1083/jcb.201006121.
92. Booth, D.M., Varnai, P., Joseph, S.K., and Hajnoczky, G. (2021). Oxidative bursts of single mitochondria mediate retrograde signaling toward the ER. Mol Cell *81*, 3866-3876 e3862. 10.1016/j.molcel.2021.07.014.
93. Beretta, M., Santos, C.X., Molenaar, C., Hafstad, A.D., Miller, C.C., Revazian, A., Betteridge, K., Schroder, K., Streckfuss-Bomeke, K., Doroshow, J.H., et al. (2020). Nox4 regulates InsP(3) receptor-dependent Ca(2+) release into mitochondria to promote cell survival. EMBO J *39*, e103530. 10.15252/embj.2019103530.
94. Aley, P.K., Porter, K.E., Boyle, J.P., Kemp, P.J., and Peers, C. (2005). Hypoxic modulation of Ca2+ signaling in human venous endothelial cells. Multiple roles for reactive oxygen species. J Biol Chem *280*, 13349-13354. 10.1074/jbc.M413674200.
95. Andersson, D.C., Betzenhauser, M.J., Reiken, S., Meli, A.C., Umanskaya, A., Xie, W., Shiomi, T., Zalk, R., Lacampagne, A., and Marks, A.R. (2011). Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging. Cell Metab *14*, 196-207. 10.1016/j.cmet.2011.05.014.
96. Suzuki, Y.J., Cleemann, L., Abernethy, D.R., and Morad, M. (1998). Glutathione is a cofactor for H2O2-mediated stimulation of Ca2+-induced Ca2+ release in cardiac myocytes. Free Radic Biol Med *24*, 318-325. 10.1016/s0891-5849(97)00227-x.
97. Hidalgo, C., Bull, R., Behrens, M.I., and Donoso, P. (2004). Redox regulation of RyR-mediated Ca2+ release in muscle and neurons. Biol Res *37*, 539-552. 10.4067/s0716-97602004000400007.
98. Hajnoczky, G., Csordas, G., Das, S., Garcia-Perez, C., Saotome, M., Sinha Roy, S., and Yi, M. (2006). Mitochondrial calcium signalling and cell death: approaches for assessing the role of mitochondrial Ca2+ uptake in apoptosis. Cell Calcium *40*, 553-560. 10.1016/j.ceca.2006.08.016.
99. Pinton, P., Giorgi, C., Siviero, R., Zecchini, E., and Rizzuto, R. (2008). Calcium and apoptosis: ER-mitochondria Ca2+ transfer in the control of apoptosis. Oncogene *27*, 6407-6418. onc2008308 [pii] 10.1038/onc.2008.308.
100. Hajnoczky, G., Csordas, G., Madesh, M., and Pacher, P. (2000). Control of apoptosis by IP(3) and ryanodine receptor driven calcium signals. Cell Calcium *28*, 349-363. 10.1054/ceca.2000.0169.
101. Breckenridge, D.G., Stojanovic, M., Marcellus, R.C., and Shore, G.C. (2003). Caspase cleavage product of BAP31 induces mitochondrial fission through endoplasmic reticulum calcium signals, enhancing cytochrome c release to the cytosol. J Cell Biol *160*, 1115-1127. 10.1083/jcb.200212059.
102. Rhee, S.G. (1999). Redox signaling: hydrogen peroxide as intracellular messenger. Exp Mol Med *31*, 53-59. 10.1038/emm.1999.9.

103. Wu, R.F., Ma, Z., Liu, Z., and Terada, L.S. (2010). Nox4-derived H2O2 mediates endoplasmic reticulum signaling through local Ras activation. Mol Cell Biol *30*, 3553-3568. 10.1128/MCB.01445-09.
104. Saimoto, Y., Kusakabe, D., Morimoto, K., Matsuoka, Y., Kozakura, E., Kato, N., Tsunematsu, K., Umeno, T., Kiyotani, T., Matsumoto, S., et al. (2025). Lysosomal lipid peroxidation contributes to ferroptosis induction via lysosomal membrane permeabilization. Nat Commun *16*, 3554. 10.1038/s41467-025-58909-w.
105. Chen, J., Yap, M.C., Bassot, A., Pascual, D.M., Makio, T., Zimmermann, J., Mast, H., Bhat, R., Fleury, S.G., Fan, Y., et al. (2025). The ER thioredoxin-related transmembrane protein TMX2 controls redox-mediated tethering of ER-mitochondria contacts. Cell Rep *44*, 116486. 10.1016/j.celrep.2025.116486.
106. Chernorudskiy, A., Varone, E., Colombo, S.F., Fumagalli, S., Cagnotto, A., Cattaneo, A., Briens, M., Baltzinger, M., Kuhn, L., Bachi, A., et al. (2020). Selenoprotein N is an endoplasmic reticulum calcium sensor that links luminal calcium levels to a redox activity. Proc Natl Acad Sci U S A *117*, 21288-21298. 10.1073/pnas.2003847117.
107. Turrens, J.F. (2003). Mitochondrial formation of reactive oxygen species. J Physiol *552*, 335-344. 10.1113/jphysiol.2003.049478.
108. Giorgio, M., Migliaccio, E., Orsini, F., Paolucci, D., Moroni, M., Contursi, C., Pelliccia, G., Luzi, L., Minucci, S., Marcaccio, M., et al. (2005). Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. Cell *122*, 221-233. 10.1016/j.cell.2005.05.011.
109. Antonucci, S., Di Sante, M., Tonolo, F., Pontarollo, L., Scalcon, V., Alanova, P., Menabo, R., Carpi, A., Bindoli, A., Rigobello, M.P., et al. (2021). The Determining Role of Mitochondrial Reactive Oxygen Species Generation and Monoamine Oxidase Activity in Doxorubicin-Induced Cardiotoxicity. Antioxid Redox Signal *34*, 531-550. 10.1089/ars.2019.7929.
110. Sorrentino, I., Galli, M., Medrano-Fernandez, I., and Sitia, R. (2022). Transfer of H(2)O(2) from Mitochondria to the endoplasmic reticulum via Aquaporin-11. Redox Biol *55*, 102410. 10.1016/j.redox.2022.102410.
111. Nguyen, V.D., Saaranen, M.J., Karala, A.R., Lappi, A.K., Wang, L., Raykhel, I.B., Alanen, H.I., Salo, K.E., Wang, C.C., and Ruddock, L.W. (2011). Two endoplasmic reticulum PDI peroxidases increase the efficiency of the use of peroxide during disulfide bond formation. J Mol Biol *406*, 503-515. S0022-2836(10)01382-3 [pii]
10.1016/j.jmb.2010.12.039.
112. Zito, E. (2013). PRDX4, an endoplasmic reticulum-localized peroxiredoxin at the crossroads between enzymatic oxidative protein folding and nonenzymatic protein oxidation. Antioxid Redox Signal *18*, 1666-1674. 10.1089/ars.2012.4966.
113. 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. S0891-5849(14)00031-8 [pii]
10.1016/j.freeradbiomed.2014.01.018.
114. Li, Y., and Camacho, P. (2004). Ca2+-dependent redox modulation of SERCA 2b by ERp57. J Cell Biol *164*, 35-46. 10.1083/jcb.200307010 jcb.200307010 [pii].
115. Yoboue, E.D., Sitia, R., and Simmen, T. (2018). Redox crosstalk at endoplasmic reticulum (ER) membrane contact sites (MCS) uses toxic waste to deliver messages. Cell Death Dis *9*, 331. 10.1038/s41419-017-0033-4.
116. Madesh, M., and Hajnoczky, G. (2001). VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release. J Cell Biol *155*, 1003-1015. 10.1083/jcb.200105057.
117. Casas-Martinez, J.C., Samali, A., and McDonagh, B. (2024). Redox regulation of UPR signalling and mitochondrial ER contact sites. Cell Mol Life Sci *81*, 250. 10.1007/s00018-024-05286-0.
118. Fauconnier, J., Thireau, J., Reiken, S., Cassan, C., Richard, S., Matecki, S., Marks, A.R., and Lacampagne, A. (2010). Leaky RyR2 trigger ventricular arrhythmias

in Duchenne muscular dystrophy. Proc Natl Acad Sci U S A *107*, 1559-1564. 0908540107 [pii]
10.1073/pnas.0908540107.
119. Eisner, V., Csordas, G., and Hajnoczky, G. (2013). Interactions between sarco-endoplasmic reticulum and mitochondria in cardiac and skeletal muscle - pivotal roles in Ca(2)(+) and reactive oxygen species signaling. J Cell Sci *126*, 2965-2978. jcs.093609 [pii]
10.1242/jcs.093609.
120. Tait, S.W., and Green, D.R. (2010). Mitochondria and cell death: outer membrane permeabilization and beyond. Nat Rev Mol Cell Biol *11*, 621-632. 10.1038/nrm2952.
121. Bernardi, P., Rasola, A., Forte, M., and Lippe, G. (2015). The Mitochondrial Permeability Transition Pore: Channel Formation by F-ATP Synthase, Integration in Signal Transduction, and Role in Pathophysiology. Physiol Rev *95*, 1111-1155. 10.1152/physrev.00001.2015.
122. Scorrano, L., Oakes, S.A., Opferman, J.T., Cheng, E.H., Sorcinelli, M.D., Pozzan, T., and Korsmeyer, S.J. (2003). BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. Science *300*, 135-139. 10.1126/science.1081208.
123. Zinszner, H., Kuroda, M., Wang, X., Batchvarova, N., Lightfoot, R.T., Remotti, H., Stevens, J.L., and Ron, D. (1998). CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev *12*, 982-995.
124. 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. 10.1016/j.cell.2007.04.027.
125. 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. 10.1128/MCB.21.4.1249-1259.2001.
126. Bernardi, P., and Forte, M. (2007). The mitochondrial permeability transition pore. Novartis Found Symp *287*, 157-164; discussion 164-159.
127. Duchen, M.R., Leyssens, A., and Crompton, M. (1998). Transient mitochondrial depolarizations reflect focal sarcoplasmic reticular calcium release in single rat cardiomyocytes. J Cell Biol *142*, 975-988. 10.1083/jcb.142.4.975.
128. Huser, J., and Blatter, L.A. (1999). Fluctuations in mitochondrial membrane potential caused by repetitive gating of the permeability transition pore. Biochem J *343 Pt 2*, 311-317.
129. Feng, G., Liu, B., Hou, T., Wang, X., and Cheng, H. (2017). Mitochondrial Flashes: Elemental Signaling Events in Eukaryotic Cells. Handb Exp Pharmacol *240*, 403-422. 10.1007/164_2016_129.
130. Szalai, G., Krishnamurthy, R., and Hajnoczky, G. (1999). Apoptosis driven by IP(3)-linked mitochondrial calcium signals. EMBO J *18*, 6349-6361. 10.1093/emboj/18.22.6349.
131. Halestrap, A.P., and Richardson, A.P. (2015). The mitochondrial permeability transition: a current perspective on its identity and role in ischaemia/reperfusion injury. J Mol Cell Cardiol *78*, 129-141. 10.1016/j.yjmcc.2014.08.018.
132. Jonas, E.A., Porter, G.A., and Alavian, K.N. (2014). Bcl-xL in neuroprotection and plasticity. Front Physiol *5*, 355. 10.3389/fphys.2014.00355.
133. Karch, J., and Molkentin, J.D. (2015). Regulated necrotic cell death: the passive aggressive side of Bax and Bak. Circ Res *116*, 1800-1809. 10.1161/CIRCRESAHA.116.305421.
134. Bhola, P.D., and Letai, A. (2016). Mitochondria-Judges and Executioners of Cell Death Sentences. Mol Cell *61*, 695-704. 10.1016/j.molcel.2016.02.019.
135. Melo, E.P., Lopes, C., Gollwitzer, P., Lortz, S., Lenzen, S., Mehmeti, I., Kaminski, C.F., Ron, D., and Avezov, E. (2017). TriPer, an optical probe tuned to the endoplasmic reticulum tracks changes in luminal H(2)O(2). BMC Biol *15*, 24. 10.1186/s12915-017-0367-5.

136. Scorrano, L., De Matteis, M.A., Emr, S., Giordano, F., Hajnoczky, G., Kornmann, B., Lackner, L.L., Levine, T.P., Pellegrini, L., Reinisch, K., et al. (2019). Coming together to define membrane contact sites. Nat Commun *10*, 1287. 10.1038/s41467-019-09253-3.
137. Cali, T., Bayer, E.M., Eden, E.R., Hajnoczky, G., Kornmann, B., Lackner, L., Liou, J., Reinisch, K., Rhee, H.W., Rizzuto, R., et al. (2025). Key challenges and recommendations for defining organelle membrane contact sites. Nat Rev Mol Cell Biol *26*, 776-796. 10.1038/s41580-025-00864-x.
138. Nichtova, Z., Fernandez-Sanz, C., De La Fuente, S., Yuan, Y., Hurst, S., Lanvermann, S., Tsai, H.Y., Weaver, D., Baggett, A., Thompson, C., et al. (2023). Enhanced Mitochondria-SR Tethering Triggers Adaptive Cardiac Muscle Remodeling. Circ Res *132*, e171-e187. 10.1161/CIRCRESAHA.122.321833.
139. Wang, T., Liu, S., Ke, Y., Ali, S., Wang, R., Hong, T., Liu, Z., Ma, G., Lan, T.H., Wang, F., et al. (2025). Repurposing salicylic acid as a versatile inducer of proximity. Nat Chem Biol *21*, 1444-1456. 10.1038/s41589-025-01918-z.
140. Woods, J.J., and Wilson, J.J. (2020). Inhibitors of the mitochondrial calcium uniporter for the treatment of disease. Curr Opin Chem Biol *55*, 9-18. 10.1016/j.cbpa.2019.11.006.
141. Marta, K., Hasan, P., Rodriguez-Prados, M., Paillard, M., and Hajnoczky, G. (2021). Pharmacological inhibition of the mitochondrial Ca(2+) uniporter: Relevance for pathophysiology and human therapy. J Mol Cell Cardiol *151*, 135-144. 10.1016/j.yjmcc.2020.09.014.
142. De Mario, A., Tosatto, A., Hill, J.M., Kriston-Vizi, J., Ketteler, R., Vecellio Reane, D., Cortopassi, G., Szabadkai, G., Rizzuto, R., and Mammucari, C. (2021). Identification and functional validation of FDA-approved positive and negative modulators of the mitochondrial calcium uniporter. Cell Rep *35*, 109275. 10.1016/j.celrep.2021.109275.
143. Di Marco, G., Vallese, F., Jourde, B., Bergsdorf, C., Sturlese, M., De Mario, A., Techer-Etienne, V., Haasen, D., Oberhauser, B., Schleeger, S., et al. (2020). A High-Throughput Screening Identifies MICU1 Targeting Compounds. Cell Rep *30*, 2321-2331 e2326. 10.1016/j.celrep.2020.01.081.
144. Germani, S., Van Ho, A.T., Cherubini, A., Varone, E., Chernorudskiy, A., Renna, G.M., Fumagalli, S., Gobbi, M., Lucchetti, J., Bolis, M., et al. (2024). SEPN1-related myopathy depends on the oxidoreductase ERO1A and is druggable with the chemical chaperone TUDCA. Cell Rep Med, 101439. 10.1016/j.xcrm.2024.101439.
145. Varone, E., Retini, M., Cherubini, A., Chernorudskiy, A., Marrazza, A., Guidarelli, A., Cagnotto, A., Beeg, M., Gobbi, M., Fumagalli, S., et al. (2025). Small molecule-mediated inhibition of the oxidoreductase ERO1A restrains aggressive breast cancer by impairing VEGF and PD-L1 in the tumor microenvironment. Cell Death Dis *16*, 105. 10.1038/s41419-025-07426-1.
146. Retini, M., Cherubini, A., Marrazza, A., Varone, E., Germani, S., Renna, G.M., Recchia, A., Guidarelli, A., Fumagalli, S., Grasselli, C., et al. (2025). Pyrazolone-based ERO1 inhibitors in ERO1-driven Triple-Negative Breast Cancer and SEPN1-Related Myopathy: Structure-activity relationship and therapeutic potential. Pharmacol Res, 108037. 10.1016/j.phrs.2025.108037.
147. Zito, E., Guarrera, L., and Janssen-Heininger, Y.M.W. (2023). Fingerprint of the oxido-reductase ERO1: A protein disulfide bond producer and supporter of cancer. Biochim Biophys Acta Rev Cancer *1879*, 189027. 10.1016/j.bbcan.2023.189027.
148. Blais, J.D., Chin, K.T., Zito, E., Zhang, Y., Heldman, N., Harding, H.P., Fass, D., Thorpe, C., and Ron, D. (2010). A small molecule inhibitor of endoplasmic reticulum oxidation 1 (ERO1) with selectively reversible thiol reactivity. J Biol Chem *285*, 20993-21003. M110.126599 [pii] 10.1074/jbc.M110.126599.
149. Lee, C.S., Hanna, A.D., Wang, H., Dagnino-Acosta, A., Joshi, A.D., Knoblauch, M., Xia, Y., Georgiou, D.K., Xu, J., Long, C., et al. (2017). A chemical chaperone improves muscle function in mice with a RyR1 mutation. Nat Commun *8*, 14659. 10.1038/ncomms14659.

150. Ozcan, U., Yilmaz, E., Ozcan, L., Furuhashi, M., Vaillancourt, E., Smith, R.O., Gorgun, C.Z., and Hotamisligil, G.S. (2006). Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science *313*, 1137-1140. 10.1126/science.1128294.
151. Rashid, T., Nemazanyy, I., Paolini, C., Tatsuta, T., Crespin, P., de Villeneuve, D., Brodesser, S., Benit, P., Rustin, P., Baraibar, M.A., et al. (2019). Lipin1 deficiency causes sarcoplasmic reticulum stress and chaperone-responsive myopathy. EMBO J *38*. 10.15252/embj.201899576.

Figures

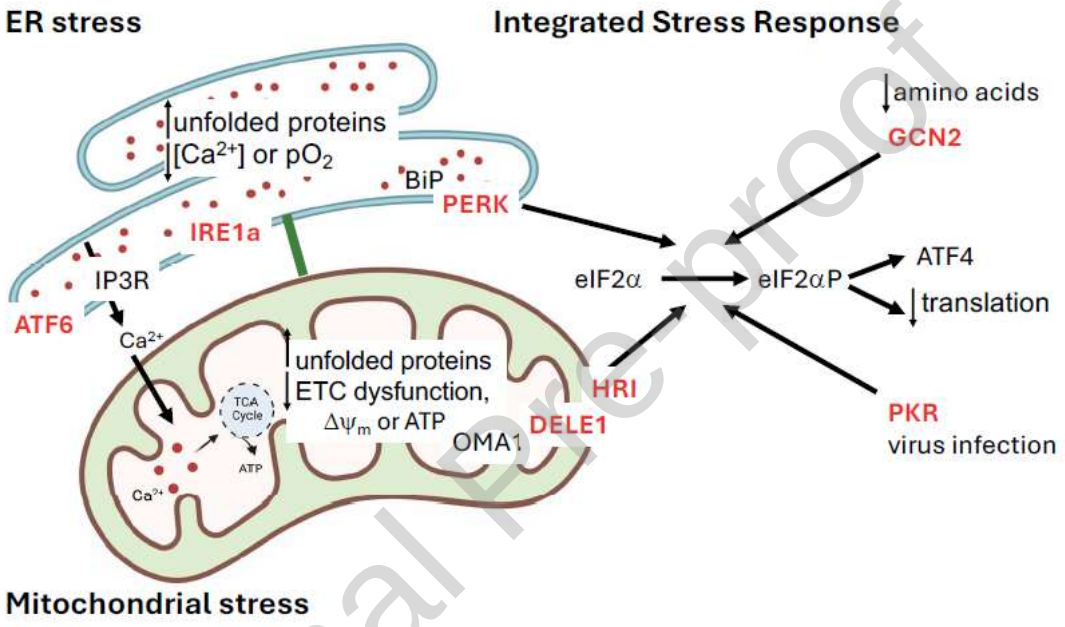


Figure 1 Interconnected cellular stress surveillance networks. ER stress, triggered by the accumulation of unfolded proteins or alterations in ER Ca^{2+} homeostasis and oxygen availability, activates the unfolded protein response (UPR) through the ATF6, IRE1α, and PERK pathways. Mitochondrial stress, resulting from impaired electron transport chain (ETC) function, protein misfolding, loss of membrane potential (ΔΨm), or ATP depletion, activates the OMA1–DELE1–HRI signaling pathway. Both PERK and HRI converge on eIF2α phosphorylation, leading to activation of ATF4 and translational attenuation as part of the ISR. Additional ISR inputs include GCN2, activated by amino acid deprivation, and PKR, activated during viral infection. The green line indicates endoplasmic reticulum–mitochondria contact sites (ERMCs), highlighting the physical interface that enables communication between the ER and mitochondria, including Ca^{2+} exchange and stress signaling. Please, note that UPR mechanisms downstream of the three major sensors, with impact on ERMC, are shown in Figure 3.

Journal Pre-proof

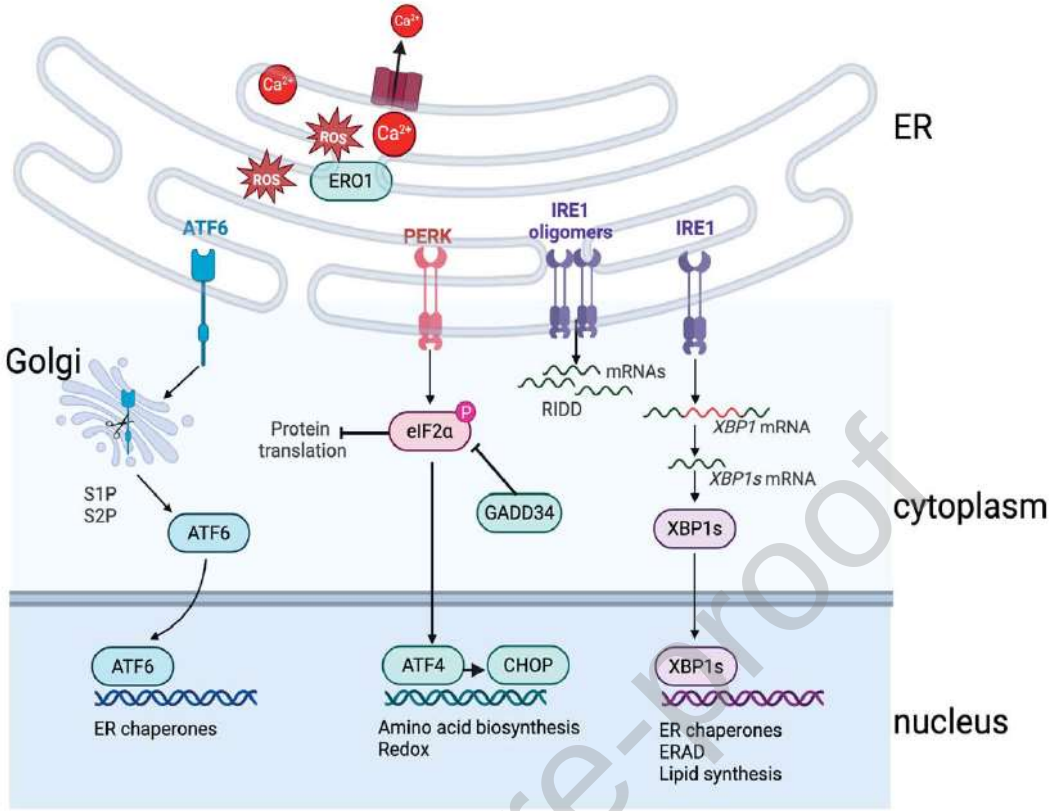


Figure 2 From ER stress to adaptive and maladaptive UPR. Accumulation of unfolded proteins, reactive oxygen species (ROS), or disturbances in ER Ca^{2+} homeostasis activate the three canonical UPR sensors: ATF6, PERK, and IRE1. Following activation, ATF6 translocates to the Golgi apparatus, where sequential cleavage by S1P and S2P releases the active transcription factor that enters the nucleus to induce genes encoding ER chaperones. PERK phosphorylates eIF2α, resulting in transient attenuation of global protein translation while promoting selective translation of ATF4, which induces genes involved in amino acid metabolism, redox homeostasis, and the pro-apoptotic transcription factor CHOP. GADD34 provides negative feedback by promoting eIF2α dephosphorylation. IRE1 oligomerization activates its endoribonuclease activity, leading to regulated IRE1-dependent decay (RIDD) of selected mRNAs and unconventional splicing of XBP1 mRNA to generate the active transcription factor XBP1s, which induces genes involved in ER protein folding, ER-associated degradation (ERAD), and lipid biosynthesis. CHOP upregulates ERO1, which promotes oxidative protein folding, increases ROS production, and alters ER Ca^{2+} homeostasis, thereby amplifying ER stress and contributing to cellular dysfunction. The red transmembrane channel depicted in the ER membrane represents an ER Ca^{2+} release channel.

Journal Pre-proof

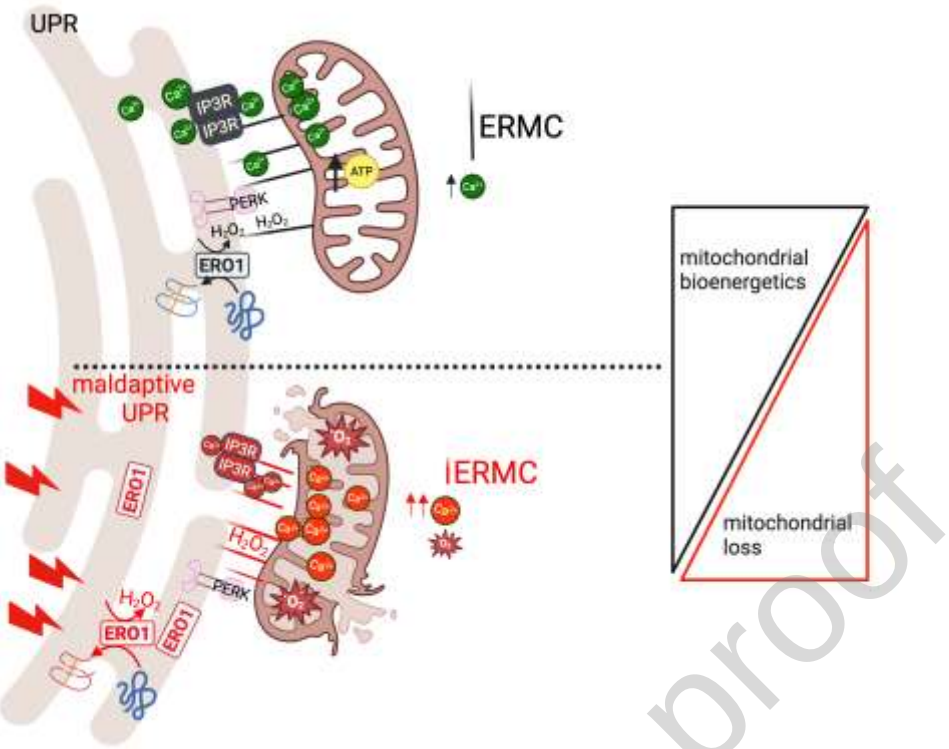


Figure 3 Involvement of ER-mitochondrial communication in adaptive and maladaptive UPR. Under physiological conditions (top), ERMCs facilitate controlled Ca^{2+} transfer through IP3Rs to mitochondria, supporting ATP production and mitochondrial bioenergetics. During prolonged or maladaptive UPR activation (bottom), CHOP-dependent upregulation of ERO1 promotes its association with PERK, tightening ER–ERMCs. This enhanced coupling, together with ERO1-derived H_2O_2, promotes oxidative activation of IP3Rs, increasing ER-to-mitochondria Ca^{2+} transfer. Sustained mitochondrial Ca^{2+} overload results in excessive ROS production, mitochondrial dysfunction, and loss of mitochondrial integrity. The diagram on the right illustrates the balance between mitochondrial bioenergetics and mitochondrial damage, highlighting how persistent UPR activation shifts ERMC signaling from adaptive bioenergetic support toward mitochondrial dysfunction and cell injury.

Conflict of Interest

The authors declare no competing interests.

Data Availability

No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Highlights

- UPR signaling involves remodelling of ERMCs, recruiting mitochondria to both adaptive and maladaptive stress response.

- Sustained CHOP–ERO1–IP_3R activation engages ER Ca^{2+} release to cause mitochondrial Ca^{2+} overload and loss of fitness.

- Bidirectional ROS/H_2O_2 exchange between ER and mitochondria establishes a feed-forward redox–Ca^{2+} loop that amplifies mitochondrial damage.

- Tightening of ERMCs, H_2O_2 nanodomains, mitochondrial Ca^{2+} overload and permeability transition may promote cell death and disease progression.

Journal Pre-proof