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UPR, autophagy and mitochondria crosstalk underlies the ER stress response

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Abstract

Cellular stress, induced by external or internal cues, activates several well-orchestrated processes aimed at either restoring cellular homeostasis or committing to cell death. Those processes include the unfolded protein response (UPR), autophagy, hypoxia, and mitochondrial function, which are part of the global ER stress (ERS) response. When one of the ERS elements is impaired, as often occurs under pathological conditions, overall cellular homeostasis may be perturbed. Further, activation of the UPR could trigger changes in mitochondrial function or autophagy, which could modulate the UPR, exemplifying cross-talk processes. Among the numerous factors that control the magnitude or duration of these processes are ubiquitin ligases, which govern overall cellular stress outcomes. Here we summarize crosstalk among fundamental processes governing ERS responses.

Keywords

ER stress; UPR; ubiquitin; hypoxia; autophagy; mitochondria

ER stress, an overview

The endoplasmic reticulum (ER) is a complex, dynamic organelle whose functions include protein folding, Ca^{2+} storage, and lipid and carbohydrate metabolism. Diverse cellular stresses such as perturbations in Ca^{2+} homeostasis, redox imbalance, altered protein glycosylation, or protein folding defects cause unfolded or misfolded proteins to accumulate in the ER lumen, a condition known as ER stress (ERS). To guard against or respond to ERS, cells have an integrated signaling system to restore homeostasis and normal ER function. Fundamental pathways that constitute integral parts of this response include the unfolded protein response (UPR), ER-associated degradation (ERAD), autophagy, hypoxic signaling and mitochondrial biogenesis. Concerted activity of all of these processes determines the extent of ERS and thus governs whether cells will re-establish homeostasis or activate cell death programs.

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The UPR is a well-defined process that plays a critical role in restoring homeostasis following accumulation of potentially toxic misfolded proteins [1, 2]. The UPR is regulated by three ER membrane-embedded sensors—double-stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1)—that are activated by perturbed ER homeostasis. All three activate specialized transcriptional programs mediated by distinct transducers—ATF4 (for PERK), cleaved ATF6 (for ATF6), and spliced XBP1 (sXBP1; for IRE1). These factors directly activate transcription of chaperones or proteins functioning in redox homeostasis, protein secretion, lipid biosynthesis or cell death programs. A description of the UPR-mediated processes is provided in Box 1.

Box 1

The UPR as part of the cellular ERS

PERK, ATF6 and IRE1 have distinct cytosolic functions associated with the ability to activate respective transducers (ATF4, cleaved ATF6, and sXBP1, respectively) with a concomitant effect on protein translation, cellular metabolism and cell survival or cell death programs. For example, PERK inhibits general protein translation (via eIF2α phosphorylation) enabling dedicated translation of transcripts harboring an alternate open reading frame, including ATF4, a key transducer. ATF4 is also implicated in the induction of several ATG genes and, depending on the magnitude of stress stimuli, can activate cell death programs (in cooperation with CHOP) [1, 2]. IRE1 RNAse domain mediates splicing and activation of the transcription factor XBP-1, which induces expression of chaperones, ER-associated degradation (ERAD) components and proteins involved in lipogenesis. In addition, the IRE1 RNAse domain is also implicated in mRNA degradation by promoting mRNA decay and its kinase domain could be linked with other stress induced pathways such as JNK or NF-κB (CITE?). Upon endoplasmic reticulum stress (ERS), ATF6 translocates to the Golgi where it is cleaved by Site-1 protease (S1P) and Site-2 protease (S2P) to produce an active transcription factor that translocates to the nucleus and regulates genes such as CHOP, grp78 and ERAD components [59]. Furthermore, ATF6 activity stimulates mitochondrial biogenesis through its effect on PGC1α and related co-activators [42, 43]. Additionally, the UPR attenuates protein translocation to the ER (dependent on signal peptides) and enhances ERAD. Of note, some of these pathways can also be activated by other stress pathways independent of bona fide UPR components, including phosphorylation of eIF2α (by GCN2, PKR, or HRI) [60]. Also, non-canonical PERK and IRE signaling has been reported [61, 62]. Tissue- or cell type-dependent expression of UPR sensors also likely mediates specialized, lineage-dependent responses [63]. A simplified overview of molecular mechanisms of UPR signaling is provided in Figure I, and the reader is also directed to specific reviews in this area [1, 2].

UPR activity must be tightly regulated on several levels as current observations suggest that a high level or prolonged UPR signaling is associated with cell death, whereas more moderate or shorter UPR signaling enables restoration of homeostasis and cell survival (CITE?). For example, in yeast, under lower levels of stress, the ER chaperone grp78 reportedly regulates IRE1 activation–deactivation kinetics: IRE1 is only turned on once

stress levels reach a certain threshold, and grp78–IRE1 interaction sensitizes IRE1 to those lower levels. Once stress is relieved, grp78–IRE1 binding enhances IRE1 deactivation by favoring its de-oligomerization [64]. IRE1 also auto-regulates its own activity by two mechanisms: Hrd1, a XBP-1 transcriptional target, ubiquitylates IRE1, promoting its degradation [47], and the IRE1 RNAse domain degrades its own mRNA [65]. Overall, the idea is emerging that crosstalk among UPR components—exemplified by the effect of IRE1 on PERK, or ATF6 modulation of IRE1 signaling [66]—amplifies the UPR signal, which ultimately regulates cellular responses to stress stimuli.

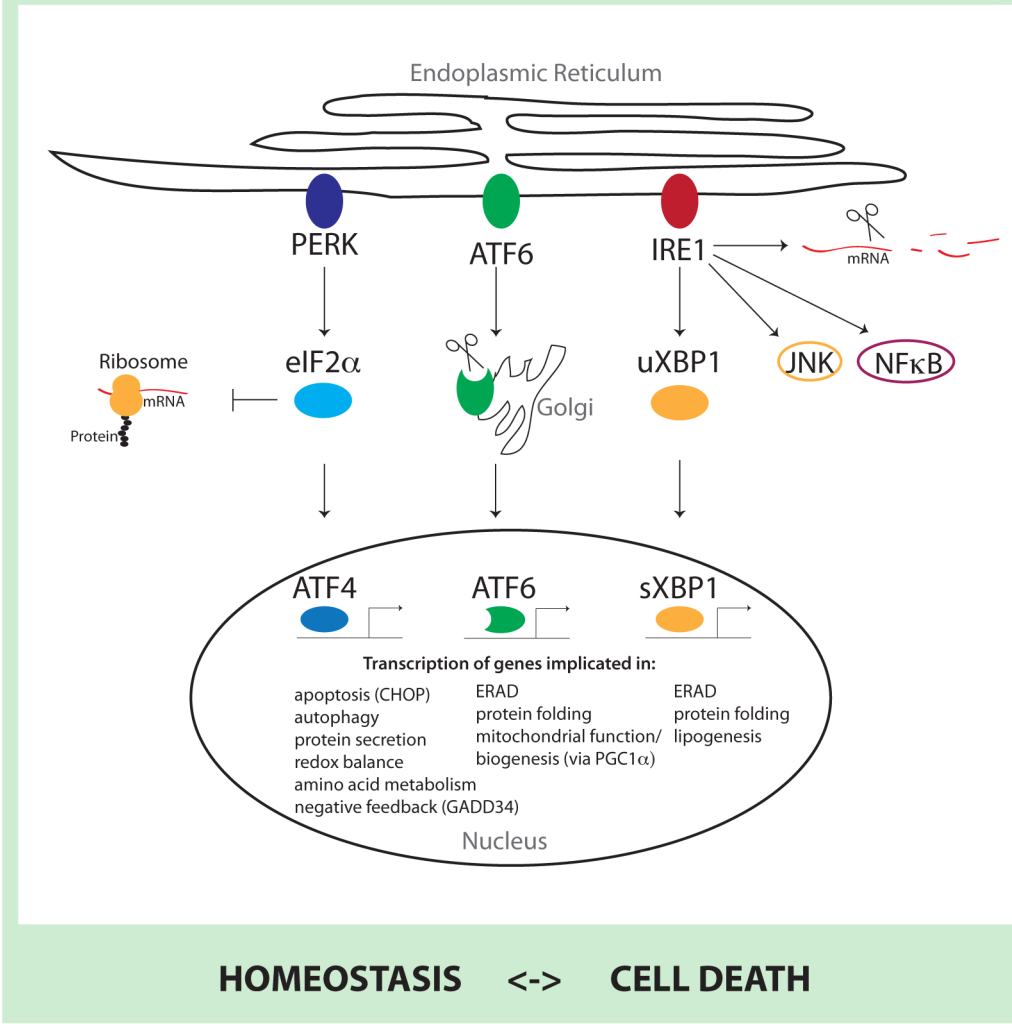

Figure I. Molecular mechanisms of UPR signaling

The three ERS sensors PERK, ATF6 and IRE1 activate a complex transcriptional cascade with distinct cytosolic functions. PERK phosphorylates eIF2α to decrease overall translation while increasing specific translation of genes, including ATF4. Upon ERS, ATF6 is translocated to and is processed at the golgi apparatus to create a highly active transcription factor. IRE1 decreases overall protein flux to the ER by enhancing mRNA degradation, activates other cellular pathways (such as JNK or NFκB) to counteract ERS and leads to the activation of the XBP1 transcription factor by splicing

the uXBP1 mRNA to create sXBP1 mRNA, which is more efficiently translated. All three transcription factors lead to the upregulation of chaperones, in addition to their respective specific targets (indicated in figure) to counteract ERS and restore homeostasis, or proceed to induce cell death pathways.
ATF: activating transcription factor; **eIF2α**: eukaryotic translation initiation factor 2 alpha; **IRE1**: inositol-requiring enzyme 1; **JNK**: c-Jun N-terminal kinase; **NFκB**: Nuclear Factor κB; **PERK**: double-stranded RNA-activated protein kinase (PKR)-like ER kinase; **u/sXBP1**: unspliced/spliced x-box binding protein 1.

The UPR is often associated with significant cellular threats stemming from altered environmental conditions (changes in nutrients or oxygen) or intracellular insult (carcinogens or oncogenic mutation), hence it is often closely linked to autophagy, hypoxia signaling, mitochondrial biogenesis or reactive oxygen species (ROS) responses. Given these interactions, it is not surprising that UPR dysfunction is implicated in many human diseases, including cancer, diabetes, neurodegeneration, ischemia, and infectious disease (reviewed in [3]). Accordingly, a better understanding of the complexity of the ERS response and the UPR could manifest novel therapeutic approaches.

Here, we review recent advances in our understanding of crosstalk between the UPR and mitochondrial function and autophagy, two fundamental processes associated with the cellular response to stress. In doing so, we emphasize the emerging roles played by E3 ubiquitin ligases in fine-tuning and regulating these processes.

The UPR and autophagy

Autophagy is a major catabolic process that delivers proteins, cytoplasmic components, and organelles to lysosomes for degradation and recycling. A well-orchestrated program including over 30 AuTophaGy-related (ATG) genes controls autophagy, which can be activated by nutrient starvation and subsequent inhibition of mechanistic target of rapamycin (mTOR) signaling [4], or by the UPR as aggregated misfolded proteins accumulate [5]. A link between autophagy and the UPR has been further substantiated by the demonstration that the PERK–eIF2α pathway is essential for autophagy induction after ERS (stimulated by tunicamycin treatment) [6]. Specifically, ATF4 and CHOP (C/EBP homologous protein, a transcription factor induced by ATF4, Box 1) were shown to transcriptionally regulate more than a dozen ATG genes [6]. In addition, IRE1 is also implicated in the activation of autophagy. TNF receptor-associated factor 2 (TRAF2)-dependent activation of IRE1 and c-Jun N-terminal kinase (JNK) reportedly results in Bcl-2 phosphorylation, enabling dissociation of Beclin-1 (an autophagy regulatory protein), activation of the Phosphoinositide-3-Kinase (PI3K) complex and autophagy [5]. Notably, it has been shown that following oxidative stress JNK contribution to the control of autophagy can be IRE1-independent [7].

Increased IRE1 activity and IRE1-dependent inflammation was observed in intestinal epithelial cells of ATG-knockout mice. The latter implies that dysregulated autophagy may also trigger the IRE1 activity with concomitant activation of the sXBP arm of the UPR,

thereby pointing to a plausible feedback mechanism in the control of UPR signaling [5, 8]. As there have been recent reviews on some molecular mechanisms linking ERS and the UPR to autophagy [5], we focus here on the bi-directional interaction between autophagy and ERS (Figure 1).

Autophagy and ERAD

In the canonical ERAD system, ubiquitylated un- or misfolded proteins are degraded by the ubiquitin-proteasome system (UPS) [9, 10]. However, activation of autophagy by UPR transducers also functions as a non-canonical ERAD pathway. Initially, autophagic degradation of unfolded proteins was viewed as a secondary response to ERS, engaged only when the canonical, proteasome-dependent pathway was overwhelmed by an excess of ubiquitylated proteins. Recent evidence suggests, however, that autophagy efficiently removes misfolded proteins that either form aggregates or, due to their structure, are not efficiently processed by the canonical ERAD machinery (such as mutant α1-antitrypsin or misfolded forms of the gonadotropin-releasing hormone receptor (GnRHR)) [11, 12]. Additionally, in select tissues, as shown for neuroblastoma cells, enhanced LC3 processing, a marker of autophagy, was observed, following UPR-inducing stimuli [13]. Likewise, phospho PERK-positive neurons derived from Alzheimer's disease patients showed elevated LC3 processing [13]. Although the role of autophagy in the clearance of misfolded proteins remains to be established, the possibility that certain tissue types benefit from autophagy as a process that serves to maintain homeostasis is intriguing and merits further studies.

Autophagy serves as a pro-survival mechanism under ERS conditions

As the UPR induces not only survival but also cell death signals, understanding the nature of the switch between cellular outcomes is of great importance, and is likely mediated by the fine-tuning of UPR signals. Cell death is largely associated with increased ATF4 and CHOP levels, whereby autophagy (which can also be regulated transcriptionally by ATF4 and CHOP) is recognized as a major pro-survival mechanism that counteracts excessive UPR-signaling [14]. Although most studies indicate that autophagy has a pro-survival function following ERS, a heightened degree/duration of stress can induce autophagy-dependent cell death mechanisms [15–17].

The role of autophagy in regulating cell survival is best illustrated by the response of tumor cells to chemotherapies. In some cases, activation of autophagy has been shown to confer tumor cell resistance and inhibit cell death, thereby antagonizing the effect of therapy [15]. For example, although treatment of glioma cells with Cyclosporine A induces ERS, autophagy (in an IRE1- and PERK-dependent manner), and apoptosis, the extent of cell death increases as autophagy is inhibited [18]. Altered expression/activity of key UPR components (i.e., IRE1, PERK and related transducers) has been reported in tumor cells. Those may account for autophagy-mediated resistance phenotypes, as exemplified by a report of UPR upregulation in melanoma cell lines following BRAF inhibitor (BRAFi) treatment, an effect accompanied by a PERK-dependent induction of autophagy, and inhibition of autophagy sensitized melanoma cells to BRAFi-induced cell death [19]. Likewise, pharmacological inhibition of autophagy augmented BRAFi-induced antitumor activity in a xenograft model [19]. Notably, another study reported that induction of

apoptosis following treatment with the BRAFi vemurafenib was associated with the ability of melanoma cells to induce ATF4 [20]. An emerging possibility, which remains to be confirmed, is that failure to activate the UPR is associated with resistance to therapy. This is attractive given the role of ATF4 in control of autophagy, thereby pointing to the link between UPR and autophagy as an underlying mechanism for tumor cell resistance mechanisms.

Autophagy and inflammation

The link between the UPR and autophagy can also explain protective mechanisms seen following inflammation of intestinal epithelial cells [8]. Loss of the IRE1 transducer, the transcription factor XBP-1, in cultured intestinal epithelial cells induces PERK- and p-eIF2α-dependent autophagy. This points to a plausible feedback mechanism and also indicates the need to balance the regulatory arms (sensors and their transducers) of the UPR. Consistent with this possibility is the observation that conditional knockout of XBP-1 in intestinal epithelial cells results in activation of autophagy in paneth cells, a secretory cell type important for the production of anti-microbial compounds, followed by enteritis, which is worsened when the ATG genes ATG7 or ATG16L1 are co-deleted. Both, $XBP1^{-/-}$ $ATG7^{-/-}$ and $XBP1^{-/-}$ $ATG16L1^{-/-}$ double-knockout mice exhibit a Crohn's disease-like inflammatory phenotype driven by IRE1-dependent nuclear factor (NF)-κB activation. Furthermore, ATG16L1 conditional knockout mice exhibit increased GRP78 (a chaperone that is induced by the UPR and is also recognized as a regulator of UPR activation) as well as eIF2α phosphorylation and JNK activation, culminating in IRE1 expression and increased XBP-1 splicing in intestinal crypts. These changes substantiate a link between autophagy and select arms of the UPR, which is sufficient to increase epithelial cell death. These conditions enhance the response to pro-inflammatory agents that further challenge the UPR. The latter finding suggests a negative feedback mechanism in which ERS induces autophagy, which in turn negatively regulates the UPR

Importantly, the finding that XBP-1 loss induces autophagy indicates that the UPR can also limit the degree of autophagy. Besides activation of PERK/ATF4 [8], XBP-1 loss was reported to regulates autophagy via the Forkhead box protein O1 (FOXO1), a transcription factor known to induce autophagy [21]. Under glutamine-starvation conditions, it was shown that after phosphorylation by ERK1/2 (extracellular-signal regulated kinase1/2) unspliced XBP-1 (uXBP1) can bind to FOXO1 and mediate its proteasomal degradation [22]. Notably, in overexpression studies, spliced XBP-1 was also demonstrated to bind and elicit degradation of FOXO1 [23]. While pointing to XBP-1 as a regulator of autophagy, further studies are needed to shed light on the molecular mechanisms underlying XBP-1 ability to regulate autophagy under physiological-relevant ERS conditions.

Overall, the UPR link with autophagy constitutes an important regulatory pathway that mediates pro-survival signals in response to misfolded proteins, chemotherapy, and inflammation. The degree of initial insult, often regarded as the degree of ERS or UPR activation, can determine the balance between pro- and anti-survival signals, in which autophagy may serve to either promote or attenuate ERS and UPR signals. Bona fide autophagy genes might affect ERS and the UPR independent of their role in autophagy, as

autophagy-independent processes are being described for autophagy-related genes. For example, the kinase ULK1 (unc-51 like autophagy activating kinase 1) was shown to affect microphtalmia-associated transcription factor (MITF) and melanogenesis independent of its role in autophagy and mTOR complex 1 (mTORC1) regulation [24]. Future studies are expected to illuminate this interesting regulatory cue.

A link between ERS, the UPR and autophagy may also explain how ER and possibly mitochondrial membranes serve as a source of autophagosomal membranes. Both omegasome sites in the ER and ER–Golgi contact areas provide a source for initiation of autophagosome membrane formation [25]. Furthermore, autophagosomes reportedly form at physical contact sites between the ER and the mitochondria (called mitochondrial associated membranes or MAM; Box 2). MAM disruption inhibits autophagosome formation [26]. Although the impact of these activities remains to be determined, they likely affect the rate of mitochondrial fusion and fission and ER signal integrity.

Box 2

ERS and mitochondria-associated ER membranes (MAMs)

MAMs are flexible, ER membrane-derived structures that form physical contact points between the ER and mitochondria. Mechanisms governing MAM formation are unclear, since most proteins enriched in MAMs (such as phosphatidylserine synthase, IP3R, VDAC1, calnexin, DRP1, ERO1, and PERK) appear not to serve a structural function but rather are associated with functions such as lipid biosynthesis, ER-mitochondria lipid or Ca^{2+} transfer, mitochondrial dynamics and bioenergetics, apoptosis, or autophagy [67, 68]. Most of these processes are also linked to ERS, and tethering of the two organelles is strengthened following ERS, supporting a key role for MAMs in ERS. MAM-mediated Ca^{2+} transfer from the ER to mitochondria may have either pro-survival or pro-death effects, perhaps contributing to enhanced mitochondrial ATP production to satisfy increased energy demands resulting from stress. Conversely, elevating mitochondrial Ca^{2+} may promote apoptosis. The ER is a source of autophagosome membrane components, and MAMs are directly implicated in autophagosome formation [68]. Autophagy may also control ER expansion through selective “ER-phagy”. Evidence suggests that MAMs serve as important hubs for mitochondrial fusion and fission. For example, ER-mitochondrial tethering contributes to the formation of constriction sites, a key step in Drp-1-mediated mitochondrial fission. Mfn-2 is an important regulator of mitochondrial fusion, and both Mfn-2 and PERK are critical to maintain the MAM. Thus, the fusion process is likely linked to the UPR [68].

The UPR link with mitochondrial function

Several regulatory components link the UPR with mitochondrial regulation and function. UPR transducers affect mitochondrial regulatory components, including ATF4, which was demonstrated to control expression of the ubiquitin ligase Parkin, a crucial regulator of mitochondria function and dynamics [27]. In turn, reciprocal activation is illustrated by the ability of Parkin to enhance select branches of the UPR signaling through the activation of

the sXBP1 pathway [28]. ATF6 is also associated with the activation of PCG1α (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha), a master regulator of mitochondrial biogenesis, thereby linking the UPR with metabolic gene programs [29].

Parkin exemplifies a regulatory node that underlies crosstalk between the UPR and mitochondria: while activated by ATF4, Parkin functions in mitochondrial dynamics [30, 31], bioenergetics [32], and mitophagy [33]. Furthermore, Parkin reportedly increases MAMs (see Box 2). By modulating the MAM, Parkin maintains ER-mitochondrial Ca^{2+} transfer and mitochondrial bioenergetics during ERS [32]. Although it is recognized that Parkin marks damaged or dysfunctional mitochondria for mitophagy, there is little evidence that this process is induced by ERS. Until recently, Parkin-mediated mitophagy upon accumulation of un- or misfolded proteins was reported only upon activation of the mitochondrial UPR [34] (a process that, like the endoplasmic UPR, communicates stress to the nucleus and increases expression of mitochondrial chaperones [35]). Nonetheless, ERS occurring as a result of cerebral ischemia has also been linked with mitophagy [36] and may protect cells from cell death signaling induced by ischemia/reperfusion-induced brain injury. Both mitophagy and cytoprotective effects are mediated by ATF4-dependent Parkin expression [36]. Consistent with these observations, a reduced level of UPR output under neuronal ischemic conditions was found to decrease infarct size and enhanced cell survival [37]. In these studies, reducing the expression of the Siah ubiquitin ligases, which are important in controlling the degree of UPR output (by stabilizing ATF4 levels) limited the degree of ATF4 and CHOP, and attenuated the induction of cell death programs under neuronal ischemic conditions [37] (see Box 3 for more details).

Box 3

UPR–HIF crosstalk

Cells respond to decreased oxygen levels by activating adaptive pathways including induction or stabilization of the transcription factors HIF1 (which consists of the oxygen-regulated subunit HIF1α and the constitutively expressed subunit HIF1α) and HIF2 and their transcriptional targets, which promote vascularization, glycolysis and survival. Under severe hypoxia other mechanisms, such as the UPR, are activated to antagonize stress, although all of these pathways synergize to activate common downstream targets.

During development, nutrient deprivation, hypoxia, and increased protein secretion activate the UPR. One UPR target is VEGF, which is activated independent of HIF1α [69]. Notably, endothelial cells respond to VEGF by upregulating the UPR, which supports VEGF-mediated angiogenesis [70]. In addition, anti-angiogenic Type I interferon signaling is inactivated via both VEGF and UPR-induced degradation of the interferon receptor [71, 72]. HIF1α heterodimerization with sXBP1, which has been demonstrated in breast cancer cells, exemplifies cooperation between the UPR and hypoxia, an activity that was implicated in maintenance of triple-negative breast cancer (TNBC). Consistent with earlier findings that HIF1α is hyperactivated in TNBC, sXBP1 reportedly co-localizes with HIF1α in TNBC tumor cells at distinct regulatory elements, where both regulate established HIF targets and maintain cancer stem cell activity [73].

The ubiquitin ligases Siah1/2 function in both hypoxia and UPR signaling. Siah stabilizes HIF1α by proteasomal degradation of PHD proteins, which destabilize HIF [74]. In addition, under ischemia Siah1/2 are transcriptionally upregulated by ATF4 or sXBP1. Siah-dependent PHD down-regulation increases ATF4 levels and activity, which are required to activate cell death programs under ischemia [37]. Siah2 contribution to UPR and hypoxic conditions also impacts its role in the control of mitochondrial dynamics [45].

Additional mechanisms link the ERS, UPR and mitochondrial function. One is regulation of mitochondrial fusion and fission processes by cues initiated by the ERS and UPR. Genetic inactivation of mitofusin-2 (Mfn-2), a key component of the mitochondrial fusion process, promotes mitochondrial swelling, Ca^{2+}-overload and reduced mitochondrial respiration [38]. Notably, PERK inhibition normalizes mitochondrial integrity in Mfn-2 mutant cells, identifying PERK as an important regulator of mitochondrial processes [38]. Accordingly, both Mfn-2 [39] and PERK [40] were shown to regulate MAM integrity. Although PERK function in control of mitochondrial dynamics may be independent of its function in the UPR (discussed in [41]), one cannot exclude UPR-dependent mechanisms. Consistent with the latter possibility is the observation that Mfn-2-deficient cells show hyperactivated UPR signaling and defective autophagy and apoptosis, which indicates a role of MAM integrity in UPR signaling [38].

The transcriptional co-regulator PGC1α, an important regulator of mitochondrial biogenesis and function, is also linked to the UPR in a tissue-specific manner. In skeletal muscle, PGC1α is upregulated upon ERS (induced by exercise) and then cooperates with ATF6 to mediate an adaptive response to stress [42]. In addition, in hepatoma cell lines ATF6 and PGC1α activity enhances expression of the estrogen-related receptor gamma (ERRγ), another regulator of mitochondrial function. In turn, ERRγ interacts with PGC1α to mediate transcription of ERR target genes. Interestingly, ATF6 has been identified as one ERRγ/PGC1α target, suggesting a feed forward mechanism whereby PGC1α mediates adaptation to stress [43].

An obvious function of UPR crosstalk with mitochondria is to control programmed cell death (reviewed in [14]). Small ubiquitin-like modifiers (SUMO) have been found to modulate ER–mitochondrial crosstalk and apoptosis through the deSUMOylating enzyme SENP3. ERS induces PERK- and cathepsin B-dependent degradation of SENP3, resulting in elevated levels of SUMOylated proteins. Among those is Drp-1, a key mediator of mitochondrial fission. DeSUMOylation of Drp-1 attenuates cytochrome c release and caspase-mediated cell death. Thus, Drp-1 SUMOylation constitutes an additional layer in the control of pro-survival signals from the ER to mitochondria [44]. Through their regulation of the adaptor protein AKAP121, which in turn controls the Drp1–Fis1 complex, the ubiquitin ligases Siah1/2 regulate mitochondrial fission under hypoxia conditions (known to induce ERS). The interplay between Siah1/2 control of fission complex and ATF4, which has also been associated with control of mitochondrial dynamics, is intriguing [45] (Box 3).

The UPR influences mitochondrial function on several levels: it induces mitophagy to clear stress-damaged mitochondria, regulates mitochondrial bioenergetics by influencing the MAM, and promotes loss of mitochondrial membrane potential. Parkin coordinates crosstalk between these activities; likewise, the ubiquitin ligases Siah1/2 function in the crosstalk between the UPR and mitochondrial functions under ischemic conditions [37].

Ubiquitin ligases fine-tune the ERS response

The observation that ubiquitin ligases regulate crosstalk between the UPR, autophagy and mitochondria suggests that they play a more dominant role within the ERS response than foreseen.

Ubiquitin ligases are central for the ERAD process (reviewed in [9, 10]) where they ubiquitylate misfolded proteins, tagging them for proteasomal degradation or removal by autophagy. However, emerging evidence suggests that E3 ubiquitin ligases contribute to ERS response through mechanisms beyond their role in ERAD. Exemplified by studies with Parkin and Siah1/2, ubiquitin ligases spatially and temporally regulate factors that fine-tune the magnitude and duration of UPR, autophagy or mitochondrial function, and thus, impact overall cellular stress outcome (Table 1). Mechanistically, SUMO and ubiquitin E3 ligases either regulate the abundance of major UPR proteins by targeting them for proteasomal degradation [44, 46–51], or modulate activity of UPR components by direct modification or modification of upstream regulators; for example, through modulation of XBP-1 transcriptional activity by SUMOylation [52, 53] or of ATF4 activity via prolyl hydroxylase 1/3 (PHD1/3)-mediated hydroxylation [37].

Other E3 ligases that function in ERS or autophagy (such as RNF5 [54]) or are relevant to mitochondrial function (like MITOL [55]) could also function in crosstalk between these processes. The recent finding that ubiquitin itself can be phosphorylated [56–58] serves to link two important signaling pathways and adds another level of complexity to mechanisms governing various cellular processes, including the ERS response.

Concluding remarks

Under normal growth conditions, concerted action of ERS components is required to maintain cellular homeostasis following external stimuli. Imbalance in these regulatory components, as often seen in pathological conditions, presents a challenge to develop therapies to restore such homeostasis.

Here, we point to the link between UPR-activating conditions, (i.e. hypoxia, ischemia, inflammation, and disruption of the ER-mitochondria contact sites) and the fine balance of UPR regulatory arms in defining the cellular outcomes including autophagy and cell death. We point to the importance of transcriptional and post-transcriptional programs, which dictate the duration and magnitude of signals, and thereby fine-tune the UPR.

From a therapeutic perspective, it will be of great importance to understand how the UPR could be pharmacologically manipulated to favor pro-death or pro-survival signaling. Understanding crosstalk among the different elements of the UPR as well as how all these

activities are linked with mitochondrial function, hypoxia and autophagy should provide new treatment options for a variety of pathologies, including neurodegenerative disorders, heart disease, diabetes, inflammatory diseases and cancer.

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Highlights

- Autophagy and UPR interaction underlies ER stress response outcomes
- ER stress modulates mitochondrial biogenesis and function
- Ubiquitin ligases fine-tune the ER stress response

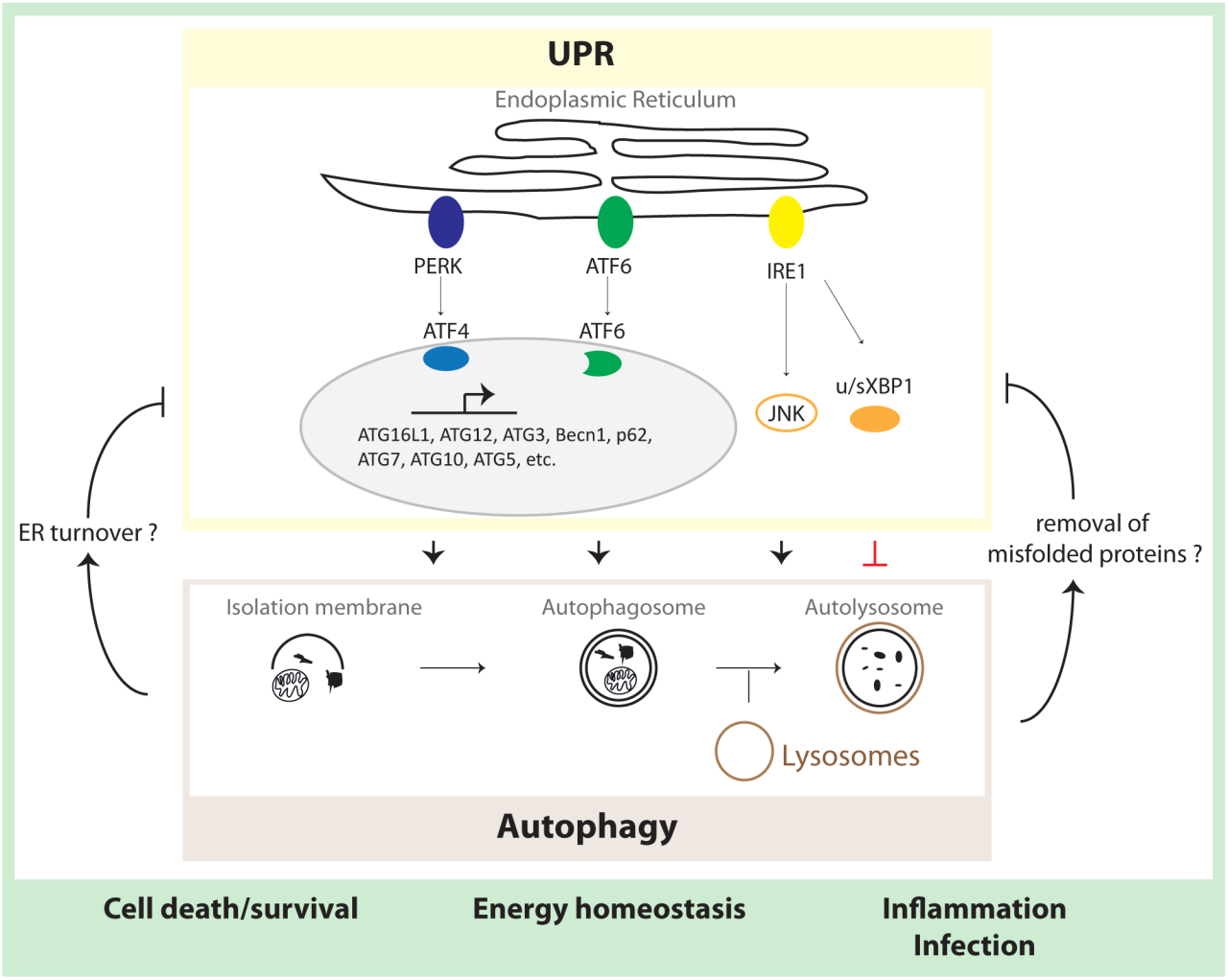

Figure 1. UPR crosstalk with autophagy

Autophagy is initiated by encapsulation of cytoplasmic components (proteins and organelles) within isolation membranes to form autophagosomes. These structures eventually fuse with lysosomes, and the cargo is degraded. Activation of the PERK–eIF2α–ATF4 pathway upregulates expression of a large set of autophagy genes. While IRE1 signaling has been implicated in promoting autophagy (via JNK-mediated signaling), it was also shown to elicit negative regulation of autophagy. Functionally, autophagy promotes cell survival, increases energy supply and mediates innate immune responses. Loss of autophagy genes induces the UPR, indicative of a negative feedback mechanism. Autophagy may decrease cellular stress levels by removal of ER membranes, which contain UPR sensors, or decrease the amplitude of stress by clearing aberrant proteins from the ER.

ATF: activating transcription factor; **ATG**: autophagy-related gene; **Becn1**: Beclin 1; **eIF2**α: eukaryotic translation initiation factor 2; **IRE1**: inositol-requiring enzyme 1; **JNK**: c-Jun N-terminal kinase; **NF-κB**: Nuclear Factor kappa B; **PERK**: double-stranded RNA-activated protein kinase (PKR)-like ER kinase; **UPR**: unfolded protein response; **u/sXBP1**: unspliced/spliced x-box binding protein 1.

Table 1

Ubiquitin ligases implicated in the ERS response

E3 ligase	Target	Functions in	Ref
Hrd1	Unfolded proteins IRE1, OASIS, BBF2H7, ATF6	ERAD IRE1-, ATF6 signaling	[9, 10, 46, 47, 49]
gp78	Unfolded proteins	ERAD	[9, 10]
Chip	Pael-R, CFTR	ERAD	[74, 75]
TMEM129	MHC class I	ERAD	[76, 77]
RNF5	CFTR, HERP, JAMP	ERAD	[78–80]
RNF185	CFTR	ERAD	[81]
TRC8	MHC class I, uXBP1	ERAD, IRE1 signaling	[82, 83]
Parkin	Pael-R,	ERAD, mitophagy, ER- mitochondria tethering	[32, 34, 36, 84, 85]
TRIM13	CD3d, p62, caspase-8	ERAD, autophagy, apoptosis	[17, 86, 87]
cIAP	CHOP	apoptosis	[51]
P300	CHOP	apoptosis	[50]
RNF186	BNip1	apoptosis	[88]
Siah1/2	PHD1/3	ATF4-signaling, apoptosis	[37]
Smurf1	WFS1	ATF6 signaling	[48]