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REVIEW

The UPRosome – decoding novel biological outputs of IRE1α function

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ABSTRACT

Different perturbations alter the function of the endoplasmic reticulum (ER), resulting in the accumulation of misfolded proteins in its lumen, a condition termed ER stress. To restore ER proteostasis, a highly conserved pathway is engaged, known as the unfolded protein response (UPR), triggering adaptive programs or apoptosis of terminally damaged cells. IRE1α (also known as ERN1), the most conserved UPR sensor, mediates the activation of responses to determine cell fate under ER stress. The complexity of IRE1α regulation and its signaling outputs is mediated in part by the assembly of a dynamic multi-protein complex, named the UPRosome, that regulates IRE1α activity and the crosstalk with other pathways. We discuss several studies identifying components of the UPRosome that have illuminated novel functions in cell death, autophagy, DNA damage, energy metabolism and cytoskeleton dynamics. Here, we provide a theoretical analysis to assess the biological significance of the UPRosome and present the results of a systematic bioinformatics analysis of the available IRE1α interactome data sets followed by functional enrichment clustering. This *in silico* approach decoded that IRE1α also interacts with proteins involved in the cell cycle, transport, differentiation, response to viral infection and immune response. Thus, defining the spectrum of IRE1α-binding partners will reveal novel signaling outputs and the relevance of the pathway to human diseases.

KEY WORDS: IRE1α, UPRosome, ER stress, Cell fate

Introduction

The endoplasmic reticulum (ER) is a highly dynamic and complex membranous network, responsible for a variety of crucial cellular functions, including protein synthesis and folding, and intracellular Ca^{2+} storage (Schwarz and Blower, 2016). A complex network of chaperones, foldases and cofactors, in addition to specific ionic and redox requirements, tightly control protein folding and quality within the ER lumen (Dubnikov et al., 2017). However, a significant amount of newly synthetized proteins do not reach their proper folding state and are delivered to the proteasome by the ER-associated degradation (ERAD) machinery (Hwang and Qi, 2018). Altered ER function can lead to the abnormal accumulation of unfolded or misfolded proteins, a condition known as 'ER stress' (Walter and Ron, 2011). ER stress triggers a series of adaptive mechanisms collectively known as the unfolded protein response (UPR) (Hetz, 2012; Walter and Ron, 2011). UPR signaling results in transcriptional and translational responses in order to increase the protein folding capacity of the cell and restore proteostasis (Oakes and Papa, 2015). If the UPR is unable to cope with protein misfolding stress, the pathway activates self-destruction programs to eliminate damaged cells by apoptosis (Urra et al., 2013).

Abnormal levels of ER stress are implicated in a variety of human diseases, including cancer, metabolic disorders, inflammation and neurodegenerative diseases (Wang and Kaufman, 2016). However, novel biological functions of the UPR beyond its traditional role in protein homeostasis are currently emerging. The UPR consists of three arms and the most conserved branch is initiated by the stress sensor inositol-requiring enzyme 1α (IRE1α; also known as ERN1) (Walter and Ron, 2011). The activation status of IRE1α signaling is regulated by the assembly of a multiprotein platform at the ER membrane, which we have previously termed the UPRosome (Hetz and Glimcher, 2009). The UPRosome also controls the crosstalk between the UPR and other stress pathways through the binding of adapter and signaling proteins and, in addition, might mediate non-canonical functions of the UPR (Hetz et al., 2020).

Here, we review all available protein–protein interaction studies to discuss emerging roles of the UPR in the control of cell function in addition to highlight novel regulatory aspects of IRE1α. We also present a new global analysis of available interactome data sets to speculate about possible novel functions of IRE1α in normal physiology and disease.

ER stress and the UPR

Under normal conditions, specialized secretory cells (i.e. pancreatic β-cells, B cell lymphocytes and salivary glands) require an active UPR to cope with the high demand for folded proteins, which generates abnormal levels of misfolded or unfolded intermediates. In addition, a number of conditions, such as hypoxia, nutrient deprivation, mutations in secretory cargoes and loss of Ca^{2+}, redox or lipid homeostasis, can also result in altered ER protein homeostasis or 'proteostasis' (Walter and Ron, 2011). In the past 20 years, chronic ER stress and overactivation of the UPR have been proposed as a relevant contributor to the development of several diseases, including cancer, diabetes, neurodegeneration and inflammatory disorders, among others (Wang and Kaufman, 2016). Activation of the UPR reprograms the transcription of hundreds of genes involved in different aspects of the secretory pathway including the translocation of proteins into the ER, protein folding, glycosylation, redox metabolism, protein quality control, translation, ERAD and lipid biogenesis, among others (Hetz, 2012). If chronic ER stress results in irreversible cellular damage, UPR signaling switches from adaptive to pro-apoptotic programs through the engagement of several cell death mechanisms (Tabas and Ron, 2011; Urra et al., 2013).

The UPR is initiated by three types of ER transmembrane proteins that act as ER stress sensors and transducers, including IRE1α and IRE1β (ERN2), activating transcription factor 6 (ATF6; also known as ATF6α) and ATF6β (also known as

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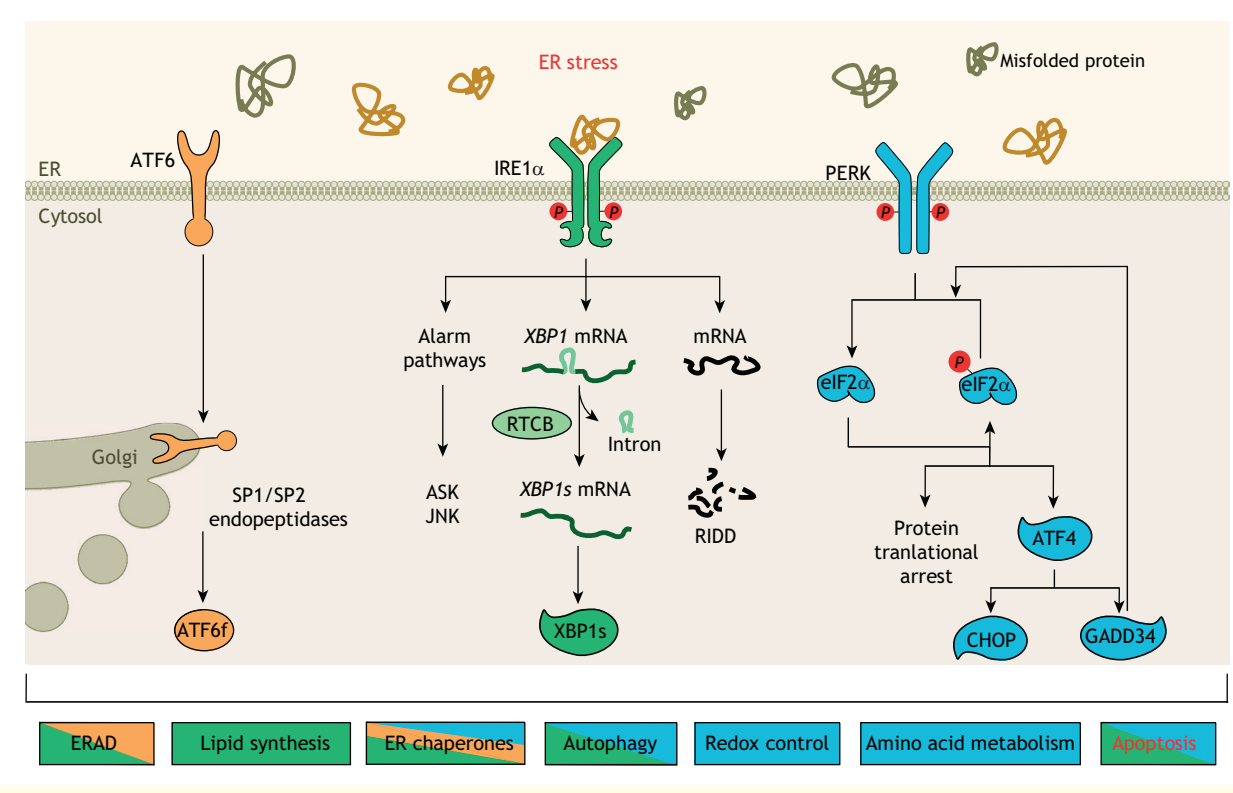
ATF6B), and protein kinase RNA (PKR)-like ER kinase (PERK; also known as EIF2AK3) (Box 1). These sensors act in concert to regulate downstream transcription factors that engage adaptive or pro-apoptotic programs depending of the extent of cellular damage (Box 1).

The most conserved pathway of the UPR is initiated by the serine/threonine protein kinase IRE1α. Under ER stress, IRE1α dimerizes and auto-transphosphorylates, favoring a conformational change that activates its endoribonuclease domain (Karagöz et al., 2019). Active IRE1α catalyzes the excision of a 26-nucleotide intron in the mRNA encoding the transcriptional factor X-box binding protein-1 (XBP1) (Walter and Ron, 2011), which then is ligated by the tRNA ligase RTCB (Jurkin et al., 2014; Kosmaczewski et al., 2014; Lu et al., 2014). This processing event leads to a shift in the open reading frame of the *XBP1* mRNA, generating a new C-terminal domain sequence (Calfon et al., 2002; Lee et al., 2002; Yoshida et al., 2001). The resulting spliced XBP1 protein (XBP1s) acts as a potent transcription factor that controls the upregulation of several UPR genes involved in protein folding, protein entry into the ER and ERAD (Hetz et al., 2011). In addition, the RNase domain of IRE1α can mediate the direct cleavage of multiple RNAs, in a process known as regulated IRE1α-dependent decay (RIDD) (Hollien et al., 2009; Hollien and Weissman, 2006). RIDD involves the cleavage of conserved RNA sequences that contain a specific secondary structure similar to the hairpins present in the mRNA of XBP1 (Maurel et al., 2014). The binding of IRE1α to the adaptor protein TNFR-associated factor 2 (TRAF2) can engage the activation of other signaling pathways, such as MAPK pathways, regulating cell death and autophagy (Nishitoh et al., 2002; Urano et al., 2000) (see figure in Box 1). Active IRE1α molecules can be found as dimers and oligomers, which might determine the regulation of *XBP1* mRNA splicing and RIDD (Bouchecareilh et al., 2011; Ghosh et al., 2014; Wang et al., 2012). Nevertheless, the molecular mechanism underlying the selectivity for mRNA substrates is still controversial and poorly understood. In summary, the UPR represents a network of signaling pathways that orchestrate adaptive responses to ER stress on a dynamic and regulated manner.

Box 1. The unfolded protein response

The UPR is controlled by three ER transmembrane proteins named IRE1α, PERK and ATF6 (see figure). PERK is a type I protein kinase, which under ER stress phosphorylates the eukaryotic translation initiator factor-2 (eIF2α) at serine 51, resulting in the inhibition of general protein translation (Harding et al., 2000b). In addition, this event simultaneously triggers the selective translation of mRNAs that contain inhibitory upstream open reading frames (uORFs) within their 5′-untranslated region (UTR) such as the activating transcription factor 4 (ATF4) (Vattem and Wek, 2004). ATF4 controls the expression of genes involved in the antioxidant response, folding capacity, amino acid metabolism and autophagy (Harding et al., 2000a). Upon chronic ER stress, ATF4 upregulates the transcription factor CCAAT-enhancer-binding protein homologous protein (CHOP; also known as GADD153), resulting in the activation of pro-apoptotic programs (Tabas and Ron, 2011; Urra et al., 2013). For instance, CHOP induces the expression of the phosphatase subunit (GADD34 or PP1C) that results in eIF2α de-phosphorylation, thereby restoring protein synthesis and resulting in oxidative stress and proteotoxicity (Han et al., 2013; Marciniak et al., 2004). ATF6 is an ER transmembrane protein that contains a bZIP transcription factor in its cytosolic domain. Upon ER stress, ATF6 is cleaved in the Golgi apparatus by the site-1 and site-2 proteases (S1P and S2P), resulting in the release of its cytosolic region (ATF6f) (Haze et al., 1999). ATF6f then acts as a potent transcription factor, regulating many UPR target genes that are related to ERAD and protein folding (Adachi et al., 2008; Yamamoto et al., 2007).



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IRE1α stress-sensing mechanism and regulation

For almost 20 years, it has been assumed that the chaperone BiP (also known as Grp78 and HSPA5) is the main mediator involved in the activation of all three UPR stress transducers (Bertolotti et al., 2000; Shen et al., 2002). However, accumulating evidence suggests that additional regulators and modes of activation exist. In yeast, Kar2 (a homolog of BiP) interacts with the luminal domain of IRE1p, keeping it in a monomeric and inactive form (Okamura et al., 2000). Interestingly, the direct binding of misfolded proteins to a MHC-like groove present in the luminal domain of IRE1p has been proposed to trigger its activation (Credle et al., 2005; Gardner and Walter, 2011).

For mammalian IRE1α, the mechanisms of activation are still under debate and three different models have been proposed (for extensive reviews see Adams et al., 2019; Karagöz et al., 2019; Preissler and Ron, 2019). Historically, it has been proposed that IRE1α monomers are prone to spontaneously forming dimers and become activated, a process inhibited by a physical interaction with BiP (Bertolotti et al., 2000). In this model, BiP binding destabilize IRE1α dimers, resulting in their inactivation, whereas, under ER stress, BiP preferentially binds unfolded proteins, releasing IRE1α, which then spontaneously activates (Amin-Wetzel et al., 2019, 2017). Alternatively, BiP has been suggested to act as a sensor where the binding of misfolded proteins to its substrate-binding domain (SBD) transduce signals to IRE1α through its ATPase domain (Carrara et al., 2015; Kopp et al., 2018). Once BiP is released from the IRE1α luminal domain, it interacts with substrates to assist the folding of non-native proteins at the ER (Adams et al., 2019). A recent report also reinforced the idea of a crucial role for BiP in the activation and clustering of IRE1α (Ricci et al., 2019) (Fig. 1). As for its yeast homolog, mammalian IRE1α also contains an MHC-like groove at the interface facing the ER lumen; however, the volume and positioning of this groove, as resolved in the crystal structure, was not compatible with peptide binding (Zhou et al., 2006). In contrast, *in vitro* studies have demonstrated that misfolded proteins can bind to the luminal domain of IRE1α, inducing a structural rearrangement that facilitates its dimerization (Karagöz et al., 2017) (Fig. 1). However, the experimental systems used in these studies are exclusively based on *in vitro* approaches and further work is needed to demonstrate the so-called 'direct recognition model'. Besides, other studies have failed to detect the binding of misfolded proteins to the luminal domain of IRE1α (Oikawa et al., 2009). Interestingly, structural and functional analysis of the luminal domain of PERK suggest that it is also able to bind to misfolded proteins (Dalton et al., 2018 preprint; Wang et al., 2018b).

Recent studies have also suggested a new concept whereby IRE1α signaling is coupled to the function of a network of ER luminal chaperones. For example, the binding of BiP to IRE1α is mediated by the co-chaperone ERdj4 (also known as DNAJB9), functioning as a recycling loop to keep IRE1α in a monomeric state (Amin-Wetzel et al., 2017) (Fig. 1). In addition, using an interactome screen followed by functional validation we recently identified HSP47 (also known as SERPINH1), a collagen-specific chaperone, as a new IRE1α-binding partner that facilitates the dissociation of BiP from its luminal domain. HSP47 binding to IRE1α promotes its activation (Sepulveda et al., 2018) (Fig. 1).

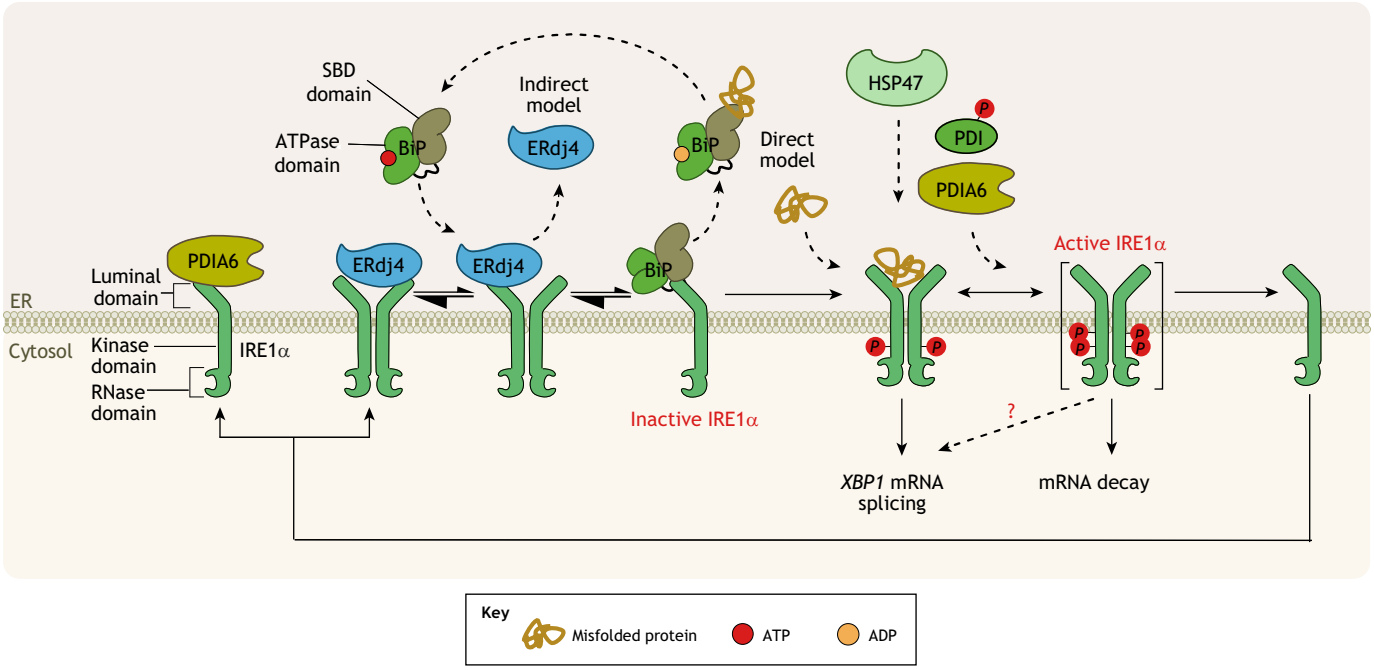

Fig. 1. Sensing of ER stress by mammalian IRE1α. IRE1α is maintained in a repressed and monomeric state through an association with the ATPase domain of BiP (brighter green) through a recycling mechanism that involves ERdj4. ERdj4 binds to the IRE1α luminal domain, which leads to BiP recruitment. The interaction between ERdj4 and the substrate-binding domain (SBD) of BiP (in darker green) results in the activation of the ATPase domain of BiP and the dissociation or ERdj4, thus BiP–ERdj4-mediated repressing mechanism is crucial for maintaining IRE1α in a monomeric and inactive form. Then, BiP is released from the IRE1α luminal domain allowing the interaction with misfolded proteins. Upon ER stress, misfolded proteins dock to the SBD of BiP, triggering the dissociation from IRE1α through allosteric regulation (indirect model). In addition, misfolded proteins interact directly with the luminal domain of IRE1α, promoting IRE1α dimerization (direct model). These events induce the auto-transphosphorylation of IRE1α, which induces a conformational change in its RNase domain that allows its activation. Besides the BiP- and ERdj4-mediated repressing mechanism, IRE1α activation may be regulated by other ER chaperons, such as HSP47 and PDIA6, which can further boost IRE1α signaling, or by PDIA1 (also known as PDI) that can inhibit IRE1α activity. HSP47 can bind to and displace BiP from the luminal domain of IRE1α, thereby allowing the binding of misfolded proteins. During the attenuation phase, BiP and ERdj4 may operate as a buffer to sequester inactive IRE1α.

Since collagens are the most abundant cargo of the secretory pathway, these findings suggest that the demand for collagen production is tightly coupled to the activation of the UPR, possibly as a mechanism to improve the secretory capacity of the cell (Rojas-Rivera et al., 2018). Furthermore, depending on the experimental setting, the disulfide isomerase PDIA6 attenuates or enhances IRE1α signaling through an interaction with specific oxidized cysteine residues in the ER lumen (Eletto et al., 2016, 2014; Groenendyk et al., 2014). Recently, it has been demonstrated that phosphorylated PDI (also known as PDIA1) directly interacts with the IRE1α luminal domain, reducing its activity under ER stress (Yu et al., 2020) (Fig. 1). Thus, these few studies suggest that multiple ER chaperones are coupled to the IRE1α activation and signaling process to adjust the folding capacity of the cell.

Accumulating evidence indicates that IRE1α is also activated by lipid bilayer stress. Structural and functional studies have revealed that the IRE1α transmembrane domain is indispensable for its activation under lipid bilayer stress (Kono et al., 2017), while the luminal domain is not required (Volmer et al., 2013). A similar mechanism has also been proposed for ATF6 activation under these stress conditions (Tam et al., 2018). In addition to the transmembrane domain of IRE1α, an adjacent amphipathic helix is required to sense lipid stress (Halbleib et al., 2017). Thus, the UPR-sensing mechanisms might be coupled to other homeostatic fluctuations that are beyond its function in protein folding stress.

The UPRosome – fine-tuning ER stress signaling

Although all three UPR stress sensors are simultaneously activated under ER stress, studying the composition of the UPRosome has revealed a new paradigm whereby the kinetics and amplitude of their individual responses can be regulated through the selective binding of specific cofactors. The concept of the UPRosome suggests the establishment of a dynamic multi-protein platform in which IRE1α associates with other components to regulate its signaling and crosstalk with other pathways (Hetz and Glimcher, 2009). Thus, UPR signaling intensity does not represent a direct measurement of stress levels since the amplitude and kinetics of IRE1α downstream responses can be tuned by several positive and negative regulatory steps.

Because most of the studies characterizing IRE1α-binding partners have only been performed once, or the characterizations use non-equivalent experimental conditions or were validated at different levels, here, we have compared the experimental conditions used in different studies to identify regulators of IRE1α signaling. Almost 95% of the interactors described in the literature were defined using overexpression systems; however, 48% of them were also validated at the level of endogenous proteins or in cell-free systems with purified components to detect direct interactions. In addition, in many studies, the possible effects of ER stress on the formation of protein complexes were tested.

As shown in Table 1, many of the IRE1α-binding partners have been described only once, with no follow-up studies confirming these interactions. Thus, readers must be aware of the differences between widely studied and confirmed interactors (i.e. BiP, PDIs and filamin A) and proteins that have been assessed only once. To this end we have summarized all the available data about IRE1α interactors (Table 1).

IRE1α has been described as a low-abundance protein in HeLa cells (Kulak et al., 2014), questioning the existence of multiple protein complexes or its function as a scaffold protein. The most probable scenario is that the interaction of IRE1α with UPRosome components is highly dynamic and transient (a 'hit and run' model), modulated by signaling events initiated by ER stress, or the interactions could depend on subpools of IRE1α segregated by their oligomerization and phosphorylation state (or other post-translational modifications). Supporting this notion, it was recently demonstrated that IRE1α cluster formation is highly dynamic, with changes within minutes upon ER stress (Belyy et al., 2020). An alternative hypothesis is that IRE1α interactions occurred in subdomains within the ER membrane where some UPRosome components are enriched. This is supported by recent findings indicating the enrichment of IRE1α and PERK in contacts between mitochondria and ER (Carreras-Sureda et al., 2019; Hayashi and Su, 2007; Mori et al., 2013) (see below). In addition, interactions of IRE1α with the translocon (Acosta-Alvear et al., 2018; Plumb et al., 2015; Sundaram et al., 2017) and the ERAD machinery (Yanagitani et al., 2009, 2011) have been reported, supporting the idea that there are distinct ER subdomains containing specific IRE1α-containing protein complexes. Moreover, IRE1α interacts with many proteins through adaptors that can physically bind to other signaling proteins. In the next three sections, we discuss the identification of different components of the UPRosome and their significance to the regulation and function of the UPR. We divide the discussion into three different categories: inhibitors, positive modulators and post-translational modifiers (see also Table 1 and Fig. 2).

Cytosolic IRE1α inhibitors

We previously identified Bax inhibitor-1 (BI-1, also known as TMBIM6) as the first negative regulator of IRE1α signaling that associated with its cytosolic domain (Lisbona et al., 2009). This interaction was also shown to be important to regulate IRE1α function in models of ischemia, diabetes and liver steatosis (Bailly-Maitre et al., 2010, 2006; Lebeaupin et al., 2018). Another negative regulator of IRE1α signaling is fortilin (also known as TPT1), which can directly interact and inhibit both the kinase and RNase domains, protecting cells from ER stress-induced apoptosis (Pinkaew et al., 2017). Fortilin preferentially binds to phosphorylated and active IRE1α, modulating the attenuation phase under prolonged ER stress (Pinkaew et al., 2017). A recent report showed that the BH3-only protein BID also negatively regulates the RNase activity of IRE1α (Bashir and Majid Fazili, 2019 preprint). A common feature of the negative regulators of IRE1α is that they block its sustained activation, disabling the capacity to engage apoptotic programs under chronic ER stress (Fig. 2A).

Positive modulators of IRE1α

The enhancement of IRE1α signaling by positive regulators is highly complex as these proteins can control several steps of IRE1α activation and signaling (Hetz et al., 2020) (Fig. 2B). The first identified enhancer of IRE1α signaling was the protein tyrosine phosphatase 1B (PTP-1B; also known as PTPN1) (Gu et al., 2004). PTP-1B promotes *XBP1* mRNA splicing and JNK activation under ER stress, but not PERK signaling (Gu et al., 2004). However, a physical interaction between IRE1α and PTP-1B has not been reported.

We identified the first direct enhancers of IRE1α signaling as the pro-apoptotic BCL-2 family members BAX and BAK (also known as BAK1), two crucial components of the cell death machinery (Hetz et al., 2006a). BAX and BAK locate to the ER and fine-tune IRE1α activation and the amplitude of its signaling under ER stress. Similarly, two other BCL-2 family members, the BH3-only proteins BIM (BCL2L11) and PUMA (BBC3), have been described to repress the attenuation phase of IRE1α to sustain its signaling (Rodriguez et al., 2012). Thus, one possible scenario is that BAX and/or BAK might facilitate IRE1α dimer formation in the activation phase,

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Table 1. Possible components of the UPRosome

		Experiment description							
				Detection			Interaction		
Protein	Methods used	Endo-genous	Over expressed	*In vitro*	*In vivo*	Cell type	ER stress	Others	References
Activity modifiers									
Positive modifiers									
ABL1	IP	✕	✓	✕	✕	INS-1	–	–	Morita et al., 2017
BAK	PD-IP	✓	✓	✓	✕	MEF	▲	–	Hetz et al., 2006
BAX	PD-IP	✓	✓	✓	✕	MEF	▲	–	Hetz et al., 2006
PUMA	IPMS-PD-Y2H	✓	✓	✓	✕	MEF/HEK	▲	–	Rodriguez et al., 2012
BIM	IPMS-PD-Y2H	✓	✓	✓	✕	MEF/HEK	▲	–	Rodriguez et al., 2012
DAB2IP	IP	✓	✓	✓	✕	EC	▲	–	Luo et al., 2008
ER PTC	PAR-CLIP-IPMS	–	–	–	–	HEK	▲	415	Acosta-Alvear et al., 2018
HSP47	IPMS-PLA-IP-PD-MST	✓	✓	✓	✕	MEF/HEK	▲	8	Sepulveda et al., 2018
HSP90AA1	IP	✓	✓	✕	✕	COS-7/INS-1	=	–	Marcu et al., 2002; Ota and Wang, 2012
HSP72	IP-PD	✕	✓	✓	✕	PC12	=	–	Gupta et al., 2010
JAB1	Y2H-IP-PD-CL	✕	✓	✕	✕	HEK	▼	–	Oono et al., 2004
NMIIB	IPMS-IP	✕	✓	✕	✕	HEK	▲	–	He et al., 2012
NMI	MAPPIT-IP	✕	✓	✕	✕	HEK	–	5	Brozzi et al., 2014
PRKCSH	IP-PD	✓	✕	✓	✕	LO2	▲	–	Shin et al., 2019
PTP1B	N.T.	–	–	–	✕	MEF	–	–	Gu *et al*., 2004
RIPK1	IP	✕	✓	✕	✕	HEK	–	–	Estornes et al., 2014
RNF13	IP	✕	✓	✕	✕	HEK	–	–	Arshad et al., 2013
RNH1	IPMS-PLA	✓	–	✕	✕	HK-2	▲	–	Tavernier et al., 2018
RTCB	IP	✓	✓	✓	✕	HEK	–	–	Jurkin et al., 2014; Kosmaczewski et al., 2014
Sec61	IPMS-IP	✕	✓	✓	✕	HEK	=	–	Plumb et al., 2015; Sundaram et al., 2017
TAOK3	Y2H-IP	✕	✓	✕	✕	HEK	▲	–	Yoneda et al., 2001
TRAF2-6	IP	✓	✓	✕	✕	PM HEK	–	3	Nishitoh et al., 2002
UBD	MAPPIT	✓	–	✕	✕	HEK βH1 cells	–	5	Brozzi et al., 2016
YIPF5	IP	✓	–	✕	✕	HeLa	–	5	Taguchi et al., 2015
Inhibitors									
BI-1	IP-PD	✓	✓	✓	✕	MEF HEK	=	–	Lisbona et al., 2009
BiD	PD-Y2H	✕	✓	✓	✕	HEK	–	–	Bashir and Majid Fazili, 2019
Fortilin	PLA-IP-BLI	✓	-	✓	✕	PC3	▲	–	Pinkaew et al., 2017
BiP	IP-PD-MST	✓	✓	✓	✕	AT42J HEK	▼	–	Bertolotti et al., 2000 and many others
PDIA6	IP/MST	✓	✓	✓	✕	HEK	▼	–	Eletto et al., 2014; Groenendyk et al., 2014
PDI	IP-PD	✓	✓	✓	✕	HepG2	▲	–	Yu et al., 2020
PTM modifiers									
Positive modulators									
CHIP	IPMS	✓	✓	✕	✕	HEK	–	4	Zhu et al., 2014
PARP16	IP	✕	✓	✕	✕	Hela	=	–	Jwa and Chang, 2012
PSEN1	IP	✕	✓	✕	✕	SK-N-SH	–	–	Katayama et al., 1999
PKA	N.T.	–	–	–	–	Hepatocytes	–	–	Mao et al., 2011
Inhibitors									
Caspases	N.T.	–	–	–	–	KMS11/OPM2	–	–	Shemorry et al., 2019
PPM1l	IPMS	✕	✓	✕	✕	INS-1	▲	6	Lu et al., 2013
RACK1	IP	✓	-	✕	✕	INS-1 HEPG2	▲	–	Qiu et al., 2010
RPAP2	IP	✕	✓	✕	✕	OVCAR8	–	–	Chang et al., 2018
SEL1L	IP	✕	✓	✕	✕	HEK	▼	OS9	Sun et al., 2015
MITOL	IP-PD	✓	✓	✕	✕	HEK	–	–	Takeda et al., 2019
Protein stability									
DDRGK1	IP	✕	✓	✕	✕	HEK	▲	UFM1	Liu et al., 2017
OPTN	IP	✓	–	✕	✓	MODE HT29	=	–	Tschurtschenthaler et al., 2017
SIGMAR1	IP	✕	✓	✓	✕	CHO	–	–	Mori et al., 2013
SYVN1	IP	✓	✓	✕	✕	HEK HCT116	▲	p53	Gao et al., 2008; Sun et al., 2015
USP14	Y2H-IP	✓	✓	✕	✕	MEF HEK PC	▼	–	Nagai et al., 2009
Novel functions									
FLNA	Y2H-IP-PD-CL	✓	✓	✓	✕	MEF HEK	▲	70	Urra et al., 2018; Tavernier et al., 2018; Augusto et al., 2020
ITPR1–ITPR3	IP-PLA-PD	✕	✓	✓	✕	MEF HEK	–	–	Carreras-Sureda et al., 2019

The table indicates different IRE1α-binding partners identified with different approaches. Methods used indicates the technique used to determine the interaction including immunoprecipitation (IP), pull-down (PD), immunoprecipitation followed by mass spectrometry (IPMS), yeast two hybrid (Y2H), proximity ligation assay (PLA), mammalian protein–protein interaction trap (MAPPIT), microscale thermophoresis (MST), bio-Layer interferometry (BLI), photoactivatable ribonucleoside enhanced cross-linking and immunoprecipitation (PAR-CLIP) and co-localization (CL). N.T., not tested; PTM, post-translational modifications; ER PTC, ER protein targeting components; ✕, no positive interaction detected; ✓, interaction detected; ▲, upregulation; ▼, downregulation; =, no changes observed. The number of the other IRE1α-binding proteins described is also indicated (Others) and these are listed in Table S1.

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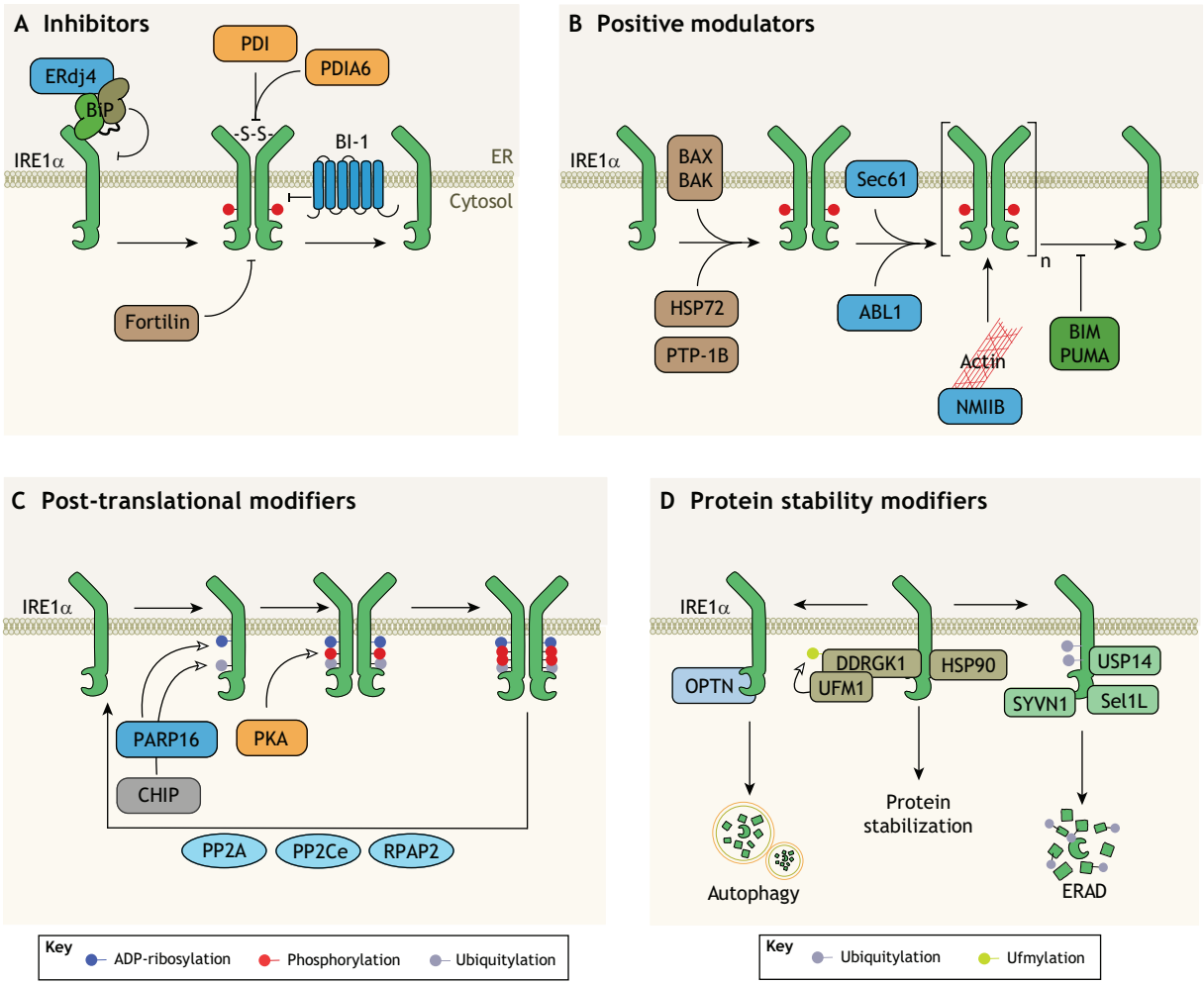

Fig. 2. Function of UPRosome components in IRE1α signaling. The activity of IRE1α is fine-tuned by several proteins that interact with it, forming a complex multiprotein platform referred to as the UPRosome. IRE1α-interacting proteins can be divided into several groups as shown in A–D. (A) Inhibitors of IRE1α signaling. Inhibitors such as BI-1 and fortilin, specifically decrease IRE1α signaling. In addition, PDIA6 and PDI can also inhibit IRE1α activity by interacting and regulating the oxidation status of IRE1α. (B) Positive regulators of IRE1α signaling. Several components of the BCL-2 family can control the balance between monomers and oligomers, such as BAX, BAK, BIM and PUMA. Sec61, ABL1 and NMIIB have also been described has specific inducers of IRE1α oligomers. In addition, components, such as HSP72 and PTP-1B, can specifically induce IRE1α activation upon ER stress. (C) Post-translational modifiers that affect the activation status of IRE1α. Besides IRE1α auto-transphosphorylation, PKA can also induce IRE1α phosphorylation at serine 752, inducing its activity. The balance between phosphorylated and total IRE1α is tightly regulated by several phosphatases, including RPAP2, PP2A and PP2Ce. In addition, IRE1α can also be ADP-ribosylated (PARP16) or ubiquitylated (CHIP), regulating the amplitude of activation. (D) Protein stability modifiers that alter IRE1α protein levels. Several proteins, such as HSP90 and UFM1, can regulate IRE1α protein stability. In addition, IRE1α can be targeted to autophagy through OPTN or degraded through ERAD by USP14, Sel1 L or SYVN1.

whereas BH3-only proteins stabilize IRE1α dimers and/or oligomers during the attenuation phase after prolonged ER stress.

IRE1α oligomerization can be also regulated by specific factors. For instance, the non-muscle myosin heavy chain IIB (NMIIB) protein stabilizes the formation of IRE1α oligomers (He et al., 2012). In agreement with this finding, drugs that destabilize the actin cytoskeleton decrease IRE1α activation and cluster formation in mammalian and yeast cells (He et al., 2012; Ishiwata-Kimata et al., 2013). Remarkably, ER stress induction leads to the phosphorylation and activation of two important regulators of the actin cytoskeleton, the myosin regulatory light chain (RLC), a crucial activator of NMIIB (He et al., 2012), and filamin A (Urra et al., 2018), supporting the idea of an actin cytoskeleton crosstalk with the UPR. It has been also described that the tyrosine protein kinase ABL1 interacts with IRE1α and stabilizes the formation of oligomers, which may favor IRE1α RNase activation towards RIDD (Dufey et al., 2020; Morita et al., 2017). Finally, Yip1A (also known as YIPF5) regulates IRE1α auto-transphosphorylation and the formation of oligomers (Taguchi et al., 2015).

Sec61, a core component of the translocon, forms a complex with IRE1α at the ER membrane, recruiting the unspliced XBP1 (XBP1u) through a pseudo-transmembrane domain present in the nascent protein chain (Plumb et al., 2015). The close proximity between XBP1u and the IRE1α–Sec61 complex is crucial to optimize the *XBP1* mRNA splicing process (Plumb et al., 2015). Other studies have suggested that the interaction between Sec61 and IRE1α maintains the balance between dimers and oligomers (Sundaram et al., 2017). In addition, this protein complex was also found in an unbiased interactome, showing that IRE1α binds to several proteins and ribosomal RNAs that are part of the signal recognition particle (SRP) and the ribosome (Acosta-Alvear et al., 2018), indicating that IRE1α is in close proximity with the ER protein translocation and targeting machineries. Recently, the protein kinase C substrate 80K-H (PRKCSH) was shown to physically interact with IRE1α, promoting

its phosphorylation and oligomerization under ER stress (Shin et al., 2019). Taken together, this evidence indicates that different factors tune the balance between IRE1α dimers and oligomers to determine the amplitude and kinetics of UPR signaling.

Post-translational modifiers of IRE1α signaling

Auto-transphosphorylation of IRE1α is a key event required for the activation of the RNase domain. Several phosphorylation sites have been identified in the cytosolic domain of human IRE1α, including serine residues 551 and 562 in the linker region, serine residues 724, 726 and 729 in the kinase activation loop, and threonine 973 in the C-terminal RNase domain (Prischi et al., 2014), whereas the phosphorylation in the serine residues located in the kinase domain are crucial for IRE1α RNase activation (Prischi et al., 2014). Homologous serine phosphorylation sites have also been described in yeast (Armstrong et al., 2017). Interestingly, the phosphorylation at serine 729 has been associated with the selective increase in RIDD activity in XBP1-deficient B cells (Tang et al., 2018).

Protein kinase A (PKA) has been suggested to directly phosphorylate serine 724 to enhance IRE1α signaling after fasting or glucagon stimulation (Mao et al., 2011). Similarly, in the brain, the activation of IRE1α by brain-derived growth factor (BDNF) (Martínez et al., 2016) is also mediated by PKA activation downstream of its receptor (Saito et al., 2018). Three phosphatases dephosphorylate IRE1α in different contexts. For instance, the association of the receptor for activated C kinase 1 (RACK1) to IRE1α recruits the phosphatase PP2A to the complex after glucose stimulation to sustain UPR signaling (Qiu et al., 2010). In addition, the ER-located phosphatase PP2Ce (also known as PPM1L) dephosphorylates IRE1α in the context of metabolic lipid control (Lu et al., 2013). Interestingly, in a siRNA screen targeting 273 human phosphatases, the RNA polymerase II-associated protein 2 (RPAP2) was found to de-phosphorylate IRE1α (Chang et al., 2018). Hence, the regulation of the phosphorylation status of IRE1α is another key step fine tuning its signaling.

Additional post-translational modifications also modulate IRE1α signaling (Fig. 2C). IRE1α can be ADP-ribosylated under ER stress by the poly(ADP-ribose) polymerase 16 (PARP16), an event that is required for their full activation (Jwa and Chang, 2012). In addition, ubiquitylation of IRE1α at lysine residues 545 and 828 by the E3 ligase CHIP (also known as STUB1) regulates the phosphorylation status of IRE1α (Zhu et al., 2014). The E3 ligase MITOL (also known as MARCHF5) ubiquitylates IRE1α at lysine 481, inhibiting its signaling under prolonged ER stress (Takeda et al., 2019). S-nitrosylation of IRE1α at two conserved cysteine residues located in the RNase domain also attenuates its activity under metabolic stress and neurodegeneration (Nakato et al., 2015; Wang et al., 2018a; Yang et al., 2015).

Another important step modulating IRE1α signaling is the control of its stability (Fig. 2D). The E3 ubiquitin ligase synoviolin (SYVN1) ubiquitylates IRE1α to induce its degradation (Gao et al., 2008; Sun et al., 2015). Interestingly, ubiquitin D (UBD) can associate with IRE1α, impacting JNK signaling (Brozzi et al., 2016). Furthermore, DDRGK domain containing 1 (DDRGK1), a component of the ubiquitin-fold modifier 1 (Ufm1) system, interacts with and regulates IRE1α protein stability by inducing its ufmylation through a non-covalent modification (Liu et al., 2017). IRE1α can also be targeted to ERAD by USP14 (Nagai et al., 2009), or to the autophagy pathway by optineurin (Tschurtschenthaler et al., 2017). In addition, HSP90 can stabilize IRE1α by interacting with its cytosolic domain (Marcu et al., 2002; Ota and Wang, 2012). Interestingly, IRE1α can also be cleaved by caspases in some cancer models, resulting in the generation of a fragment that can inhibit cell death (Shemorry et al., 2019). Altogether, these reports suggest that many different factors tune IRE1α signaling at multiple levels.

Finally, although the UPRosome concept was originally conceived exclusively for IRE1α regulation (Hetz and Glimcher, 2009), recent studies have shown that ATF6 and PERK can be also modulated by specific interacting proteins (reviewed in Hetz et al., 2020). Thus, the concept of the UPRosome could be extended to PERK and ATF6.

IRE1α as a signaling platform – novel physiological outputs

Most of the studies in the field have addressed the biological function of IRE1α in the control of transcription and translation through XBP1s or RIDD, impacting multiple cellular processes (reviewed in Oakes and Papa, 2015; Wang and Kaufman, 2016). However, recent findings have pointed to novel functions executed through alternative signaling pathways (Fig. 3).

Two recent examples from our group have revealed that IRE1α influences cell physiology by acting as a scaffold protein, independently of its enzymatic activities (see below) (Carreras-Sureda et al., 2019; Urra et al., 2018). To further decode possible new IRE1α functions, here, we performed an extensive analysis of almost 40 IRE1α-binding partners reported in the literature (Table 1), followed by a gene ontology (GO) analysis and functional clustering. In addition, we also included other IRE1α-binding proteins presented in data sets available from different public interactome screenings (Acosta-Alvear et al., 2018; Urra et al., 2018) (Table S1). This comparison revealed eight common UPRosome components in at least two data sets (Fig. 3A). Furthermore, our GO analyses of all the 495 proteins identified showed that almost all significant associations fall into 20 different biological processes (Table S2). This functional analysis can be further minimized to five major clusters of proteins involved in cell cycle, cytoskeleton regulation, protein targeting, ribosome biogenesis and protein translation (Table S2). In the next sections, we discuss available evidence that suggests the involvement of IRE1a in different biological processes by IRE1α through the interaction with specific factors.

IRE1α and cell death programs

A number of studies support a direct molecular connection between IRE1α and several signaling pathways regulating cell death. For instance, binding of IRE1α to SH2/SH3-containing adaptor protein Nck can modulate p38 MAPK family and ERK1 (MAPK3) signaling, impacting cell survival (Nguyên et al., 2004). In addition, IRE1α can enhance the activation of NF-κB by interacting with TRAF2 and the inhibitor of nuclear factor κβ kinase (IKK), promoting cell death (Hu et al., 2006). Remarkably, the IRE1α–TRAF2 interaction also triggers the activation of the apoptosis signal-regulating kinase 1 (ASK1) and Jun-N terminal kinase (JNK) pathway (Nishitoh et al., 2002; Urano et al., 2000). The IRE1α–MAPK signaling branch is also modulated by other components of the UPRosome, such as AIP1 (Luo et al., 2008), RNF13 (Arshad et al., 2013), UBD (Brozzi et al., 2016), BI-1 (Castillo et al., 2011) and N-Myc interactor (Brozzi et al., 2014). However, the mechanisms of JNK activation downstream of IRE1α and its consequences for cell fate under ER stress are not completely understood.

Our GO enrichment analyses and functional clustering revealed a group of IRE1α-binding partners that are involved in apoptosis, including pro-apoptotic proteins of the BCL-2 family (BAX, BAK, BIM and PUMA), α-catenin and BI-1 (Fig. 3B). We speculate that this association may represent a molecular interface to monitor alterations in proteostasis and engage cell death programs when cell damage is deemed irreversible.

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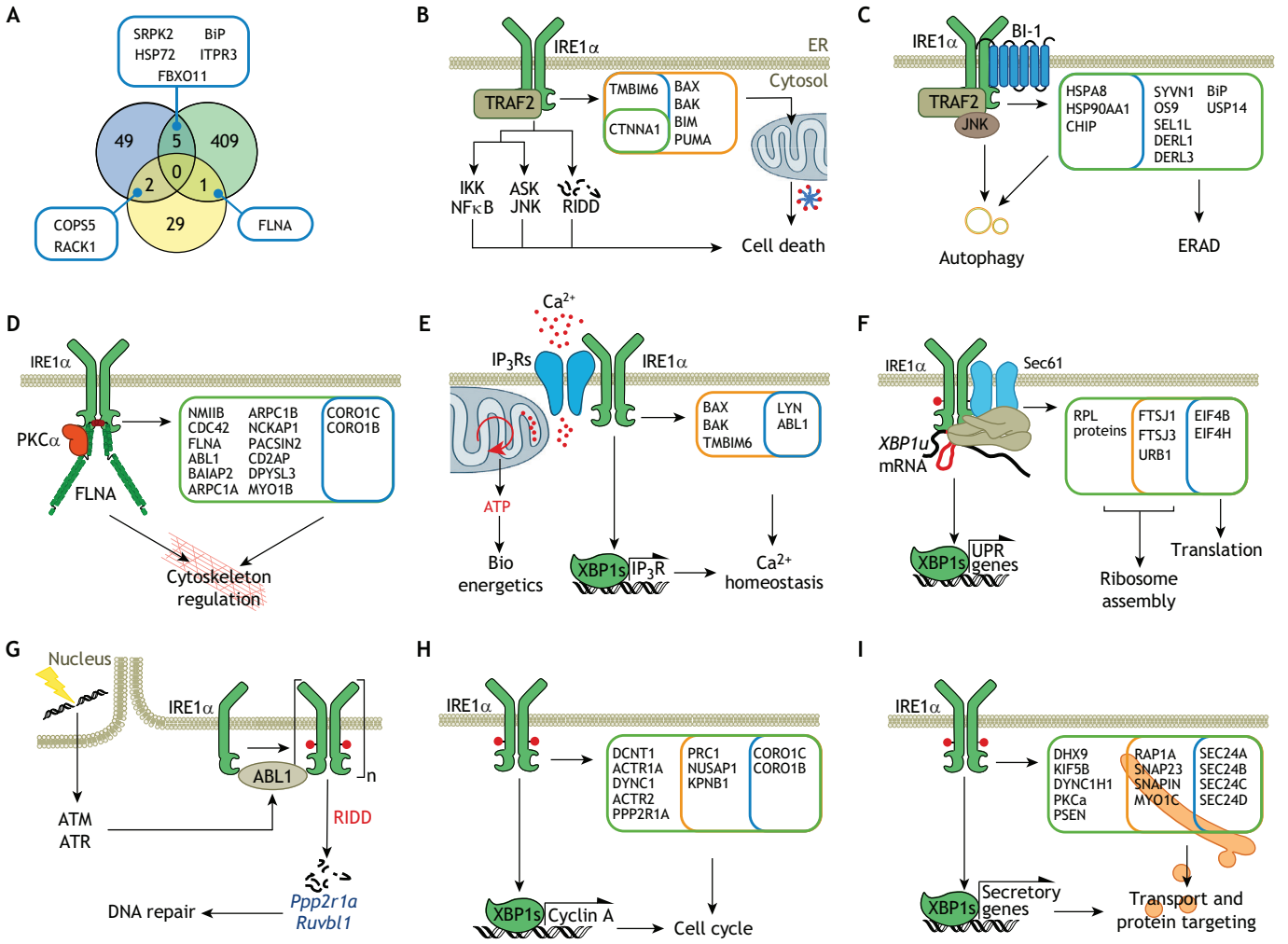

Fig. 3. Possible novel outputs of the IRE1α UPRosome. (A) Venn diagram comparing the composition of all UPRosome proteins described in global references listed in Table 1 (56 proteins; blue circle), and two available screens using either yeast two-hybrid [32 proteins (index score >4); yellow circle; Urra et al., 2018] and immunoprecipitation followed by mass-spectrometry analysis [405 proteins (P>0.01); green circle; Acosta-Alvear et al., 2018]. Gene ontology (GO) analysis and functional clustering of all IRE1α-binding proteins was performed (see Table S2). (B) Cell death. IRE1α regulates the cell death process through multiple mechanisms that involve NFκB, JNK and RIDD. Our GO analysis also indicates that IRE1α interacts with clusters of proteins involved in cell death such as components of the BCL-2 family (BAX, BAK, BIM and PUMA), and BI-1 (TMBIM6) or α-catenein (CTNNA1), which can also be crucial components of the cell death machinery. (C) Protein degradation pathways. IRE1α associates with TRAF2 and BI-1/TMBIM6 to regulate JNK activation and autophagy. Our GO functional clustering indicates that IRE1α can also physically interact with components of the chaperone-mediated autophagy (HSPA8, HSP90AA1 and CHIP) or components of the ERAD pathway, among them SYVN1, USP14, the DERL1 family and SEL1L. Also, IRE1α interacts with OS9 and BiP, which also have been described to target proteins for degradation. (D) Cytoskeleton regulation. We have previously described that IRE1α regulates actin cytoskeleton through FLNA interaction. Here, we found that IRE1α also interacts with a cluster of proteins that are part of or regulate the cytoskeleton such as CORO1B and CORO1C, ARPC1A and ARPC1B, NMIIB, CDC42 and MYO1B. In addition, IRE1α binds to other proteins that can indirectly regulate the cytoskeleton, such as ABL1, BAIAP2, NCKAP1 and DPSYSL3. (E) Ca^{2+} homeostasis. IRE1α location at mitochondria-associated membranes (MAMs) regulates the docking of the IP3Rs (ITPR1, ITPR2 or ITPR3) at this subcompartment, promoting Ca^{2+} release from the ER. This mechanism enhances the transfer of Ca^{2+} to the mitochondrial matrix, promoting ATP production to tune bioenergetics. Our GO analysis reveals that IRE1α could affect this process by interacting with other proteins such as BAX, BAX and BI-1/TMBIM6 or through ABL1 and LYN. (F) Translation and ribosome function. IRE1α interacts with SEC61, keeping it in close proximity to all ribosome components. We postulate that IRE1α can interact with several ribosomal proteins (RPL proteins) and ribosome assembly proteins such as FTSJ1–FTSJ3 and URB1. In addition, IRE1α can bind to proteins involved in translation such as EIF4B–EIF4H. (G) DNA damage response. We recently described that upon DNA damage, IRE1α is specifically activated to induce the degradation of mRNAs (*Ppp2r1a* and *Ruvbl1*) through regulated IRE1α-dependent decay (RIDD), sustaining DNA repair and promoting survival. The molecular mechanism involves that downstream of ATM and ATR; ABL1 interacts with IRE1α promoting its oligomerization and RIDD. (H) Cell cycle control. It is known that IRE1α can regulate cyclin A expression through XBP1s promoting cell cycle progression. Our GO analysis reveals that IRE1α specifically binds to components that regulate cytokinesis such as CORO1B and CORO1C, PRC1, NUSAP1 and KPNB1. Also, IRE1α interacts with several proteins that can indirectly regulate cell cycle such as DCNT1, ACTR1A and PPP2R1A. (I) Vesicle transport and protein targeting. IRE1α can regulate the expression of secretory genes through XBP1s. We also indicate that IRE1α can affect vesicle transport through several other proteins such as the SEC24 family or components of vesicle formation and transport, such as SNAP23, SNAPIN and MYO1C. In addition, this process can be further modulated by other proteins including KIF5B, PKA, PSEN and DHX9. See Table S1 for the full names of proteins included here.

IRE1α and protein degradation pathways

One of the outputs of UPR activation under ER stress is the engagement of the autophagy and ERAD pathways, contributing to stress mitigation (Rashid et al., 2015). The interplay between autophagy and the UPR affects the progression of several human diseases, such as neurodegenerative diseases (Hetz et al., 2009;

Vidal et al., 2012), cancer (Hart et al., 2012) and auditory loss (Kishino et al., 2017). Interestingly, the formation of the IRE1α–TRAF2 complex can also induce autophagy in a JNK-dependent manner (Castillo et al., 2011). Indeed, JNK activation mediates the phosphorylation of BCL-2 (Pattingre et al., 2009); this disrupts the Beclin-1–BCL-2 complex, facilitating phagosome initiation and autophagy (Pattingre et al., 2005). The IRE1α–TRAF2 interaction can be further modulated by BI-1, displacing TRAF2 from the complex, blocking autophagy flux (Castillo et al., 2011).

Our GO analysis indicates that IRE1α interacts with three proteins involved in targeting substrates to chaperone-mediated autophagy (CMA) – HSPA8, HSP90AA1 and STUB1 (Kaushik et al., 2011). IRE1α also associates with factors involved in ERAD, including SYVN1, a crucial E3 ubiquitin-protein ligase of the ERAD pathway, DERL1 and DERL3, proteins regulating the retrotranslocation of misfolded proteins to the cytosol, and the proteasome-associated deubiquitylase USP14, which controls the ubiquitylation status of proteasome-targeted proteins (Fig. 3C). Hence, IRE1α interacts with components of autophagy and ERAD machineries, suggesting that IRE1α could be involved in the regulation of these pathways.

Regulation of the actin cytoskeleton

Using a yeast two-hybrid screen, we recently identified almost 70 candidate proteins that might directly bind to the cytosolic region of IRE1α, where filamin A was the strongest hit (Urra et al., 2018). Filamin A was also validated as a strong IRE1α interactor in a second unbiased screen (Tavernier et al., 2018) and, in addition, in a recent study (Augusto et al., 2020). Filamin A is an actin-filament-crosslinking protein that regulates cytoskeleton dynamics, impacting different processes, such as cell migration, adhesion and mechanotransduction (Kim and McCulloch, 2011; Lynch and Sheetz, 2011). We showed that IRE1α acts as a scaffold to recruit filamin A to the ER membrane and facilitate its phosphorylation to modulate cytoskeleton dynamics (Urra et al., 2018; Fig. 3D). In this model, PKCα mediates the phosphorylation of many filamin A proteins at serine 2152 in an IRE1α-dependent manner (Urra et al., 2018).

The interaction between IRE1α and filamin A requires a proline-rich domain located at the distal C-terminal region of IRE1α (Urra et al., 2018). Interestingly, disruption of this domain does not affect *XBP1* mRNA splicing, whereas it selectively inhibits the regulation of the actin cytoskeleton, fully separating both cellular functions (Urra et al., 2018). *In vivo* studies demonstrated that IRE1α ablation decreases cortical neuronal migration in the mouse brain (Urra et al., 2018). Since filamin A mutations in humans are the main cause of periventricular nodular heterotopia (PVNH), a genetic disorder characterized by abnormal neuronal migration (Fox et al., 1998; Sarkisian et al., 2008), we speculate that IRE1α loss of function might contribute to PVNH as reported for other filamin A regulators (Sarkisian et al., 2008; Zhou et al., 2010). Recently, it has been reported that the IRE1α and FLNA interaction is also implicated in the migration of cells infected with intracellular parasites (Augusto et al., 2020).

According to our GO analysis, IRE1α interacts with other actin-binding proteins such as CORO1B and CORO1C, which are involved in actin remodeling and lamellipodia formation in the migrating leading edge (Cai et al., 2007) and in ER-associated endosome fission (Hoyer et al., 2018) (Fig. 3D). In addition, we found an important cluster of regulators of actin and microtubule dynamics, suggesting that IRE1α may affect cytoskeleton remodeling through several mechanisms.

Reprograming cell metabolism by IRE1α through Ca^{2+} signaling

IRE1α and PERK have been reported to be enriched at the ER–mitochondrial contact sites (termed mitochondria-associated membranes; MAMs) (Carreras-Sureda et al., 2019; Mori et al., 2013; van Vliet et al., 2017). It was suggested that the specific localization of UPR stress sensors at this ER membrane subdomain set the threshold for UPR activation (Mori et al., 2013). However, the actual role of the IRE1α pool located at MAMs in mitochondrial physiology was not defined until very recently. Under ER stress, the inositol-1,4,5-trisphosphate receptors (IP_3Rs) trigger the exit of Ca^{2+} from the ER to the cytosol, regulating autophagy and apoptosis (Kiviluoto et al., 2013). ER Ca^{2+} release is pivotal to tune mitochondrial function to produce ATP, because different enzymes of the tricarboxylic acid cycle (TCA) are Ca^{2+} dependent (Giorgi et al., 2018). The ER-to-mitochondria Ca^{2+} transfer process requires local microdomains of high Ca^{2+} concentrations to be efficient (Csordás et al., 2010; Giacomello et al., 2010). We recently reported a novel function of IRE1α in sustaining ER–mitochondrial communication and bioenergetics by demonstrating that IRE1α regulates the distribution of IP_3Rs at MAMs through a direct interaction (Carreras-Sureda et al., 2019). IRE1α localization at MAMs recruits and docks IP_3Rs, improving mitochondria calcium transfer to burst energy metabolism. In addition, besides IP_3R localization, IRE1α expression alters the protein composition of MAMs (Fig. 3E). The GO analysis also suggested that IRE1α interacts with other proteins that can regulate calcium homeostasis, such as BAX, BAK and BI-1 (Fig. 3E).

Ribosomes and the translocation machinery

As discussed above, the *XBP1u* mRNA is recruited to the ER by the formation of a complex between IRE1α and Sec61 (Plumb et al., 2015). However, the mechanisms explaining how RIDD substrates are made available to the ER membrane are still unknown. Using photoactivatable ribonucleoside crosslinking and immunoprecipitation (PAR-CLIP), it was shown that IRE1α is part of a large complex containing almost 400 different RNAs, including mRNAs, ncRNA and rRNA (Acosta-Alvear et al., 2018). Furthermore, IRE1α interacts with the exposed surface of translating ribosomes, and with components of the SRP and translocon (Acosta-Alvear et al., 2018). Therefore, IRE1α could function as a scaffold to recruit and maintain the protein targeting and translation machineries in close proximity under ER stress conditions (Acosta-Alvear et al., 2018) (Fig. 3F). Our analysis suggests that IRE1α could not only regulate mRNA translation by interacting with several ribosomal proteins, but also through the regulation of ribosome biogenesis or their assembly since IRE1α interacts with FTSJ3 and URB1 (Acosta-Alvear et al., 2018), two important regulators of ribosomal biogenesis (Morello et al., 2011). Other IRE1α-binding proteins are involved in the translation machinery for rRNA, ncRNA and mRNAs, suggesting that IRE1α could also regulate translation through the EIF4 complex. Hence, IRE1α associates with ribosome and translocon components; however, the functional consequences of these interactions need to be defined.

DNA damage response

Although it was not found in our dataset analysis, we recently reported that ABL1, a previously characterized IRE1α-interacting protein (Morita et al., 2017), induces the selective activation of IRE1α under genotoxic stress to promote DNA repair programs. Upon DNA damage, ABL1 interacts with IRE1α, selectively activating RIDD in the absence of *XBP1* mRNA splicing (Fig. 3G).

This activity results in the degradation of *Ppp2r1a* and *Ruvbl1* mRNAs, two factors that are involved in the DNA damage response (DDR) by regulating the de-phosphorylation of checkpoint kinase 1 and 2 (CHK1/2; also known as CHEK1/2) and histone 2AX (H2AX) (Chowdhury et al., 2005; Jha et al., 2008). The specific activation of IRE1α by genotoxic stress promotes DNA repair and cell cycle progression, resulting in cell survival. This function is conserved in evolution as demonstrated in fly and mouse models of DNA damage (Dufey et al., 2020). Other studies have also linked the DDR with the UPR, because XBP1u controls the levels of p53, a central regulator of the DNA damage response, through its ubiquitylation and degradation (Huang et al., 2017). In addition, ER stress in tumor cells sensitizes cells to death induced by DNA-damaging agents (e.g. chemotherapy and radiotherapy) (Bobrovnikova-Marjon et al., 2010; Weatherbee et al., 2016; Yamamori et al., 2013). These studies suggest that the UPR functionally interacts with pathways that maintain genome stability.

In addition to the above examples, our GO enrichment analyses identified some interesting clusters of proteins involved in additional functions, including cell cycle control and protein transport (see Box 2 and Fig. 3H,I). Taken together, the analysis of the available interactome data sets reinforce the notion that the UPRosome is implicated in several biological processes that need to be further studied.

Concluding remarks

The UPR is a central signal transduction pathway that acts to restore ER function and proteostasis, and engages a series of adaptive responses or triggers apoptosis under acute or sustained ER stress. The mechanisms that explain this transition from adaptive to pro-death are not completely defined, but are essential to understanding how cell fate is determined under ER stress. One possible explanation may involve the dynamic nature of the UPRosome as a mechanism to regulate IRE1α signaling outputs. BiP is the most validated IRE1α-binding partner and has a fundamental role in adjusting the capacity of a cell to trigger the UPR. Importantly, several single studies suggest that UPR activation, and the threshold of ER stress to induce IRE1α activation and attenuation, is regulated by other ER chaperones and foldases including HSP47, PDIA6 and PDIA1. We speculate that these observations represent the tip of the iceberg, suggesting that the ER stress surveillance process is broadly coupled to the folding machinery involving a complex network of chaperones.

After the proposition of the UPRosome concept more than 10 years ago (Hetz and Glimcher, 2009), many new IRE1α-binding partners have been identified that modulate its activity, conformation and stability. As stated in Table 1, many of these interactions were determined using artificial overexpression systems and validated only once. Although the number of IRE1α-binding partners is quite large (~47 proteins), with dozens of laboratories confirming the concept of the UPRosome, readers must be cautious when interpreting these data sets since further independent validation is needed. Despite the fact some of these interactors were defined in single studies, several binders were properly validated using different complementary approaches. Here, we provide a new layer of analysis by performing a broad bioinformatics assessment using most data sets available, highlighting clusters of proteins sharing similar functions, suggesting novel functions of the UPR through protein–protein interactions and eliminating the bias of one-time interactors or reports.

Overall, available studies assessing the nature of the IRE1α interactome have revealed unexpected biological functions of the pathway in diverse processes such as autophagy, cell migration and cytoskeleton dynamics, protein translation and energy metabolism. Some of these emerging roles are mediated by non-canonical signaling modes independent of the canonical IRE1α RNase domain, where IRE1α operates as a scaffold that recruits proteins involved in multiple cellular processes. Thus, we propose that a comprehensive analysis of the IRE1α interactome is required to understand the global impact of the pathway to cell physiology and disease. We expect that, depending on the cell type and stimuli analyzed, distinct UPRosomes are assembled in a dynamic manner. There might be even different IRE1α-containing complexes in different ER compartments, as we proposed for the MAM subdomains. Since IRE1α function and dysfunction has been extensively linked to different human diseases, strategies to fine tune the composition and activity of the UPRosome may have important therapeutic applications.

Box 2. Deciphering novel physiological functions of IRE1α

Our GO enrichment analyses and functional clustering of IRE1α-binding partners identified some interesting clusters of proteins involved in different functions including cell cycle control and cytokinesis (Fig. 3H). IRE1α interacts with CORO1B and CORO1C, two coronins that together with the Arp2/3 complex control actin rearrangement and cell cycle progression (Molinie et al., 2019). PRC1 and NUSAP1, two important players involved in cytokinesis regulation and cancer progression, were also identified (Li et al., 2018). In addition, our analysis also revealed a cluster of proteins regulating vesicle transport and protein targeting (Fig. 3I). For example, distinct isoforms of Sec24 are observed that are part of the coat protein complex II (COPII), which is involved in the selection of cargo proteins and the formation of transport vesicles at the ER (Mancias and Goldberg, 2007). In addition, proteins involved in vesicle transport and fusion, like SNAP23 and SNAPIN, are also detected in IRE1α-containing protein complexes (van Niel et al., 2018). Several molecular motors involved in mRNA trafficking, such as KIF5B and FXR2 (Hirokawa, 2006), are detected in IRE1α-containing protein complexes that are crucial for axonal transport, promoting axonal growth cone and dendrite morphogenesis (Fig. 3I). IRE1α can also interact with proteins involved in cell differentiation, including the myeloid, hematopoietic and osteoblastic lineage (Table S2). In addition, IRE1α associates with crucial components involved in viral transcription, replication and defense (Table S2). Viral infection induces ER stress (Verchot, 2016); however, the actual impact of IRE1α on viral infection and replication is poorly defined. Our analysis reinforces the idea that IRE1α interacts with partners involved in immune responses including antigen processing and presentation via MHC class I, cytokine production and secretion, and phagocytosis. This is in agreement with functional studies indicating that IRE1α can regulate some of these processes by mechanism involving RIDD or XBP1s (for an extensive review, see Osorio et al., 2018).

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Competing interests

The authors declare no competing or financial interests.

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Supplementary information

Supplementary information available online at
http://jcs.biologists.org/lookup/doi/10.1242/jcs.218107.supplemental

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Table S1: IRE1α-binding partners analyzed by gene ontology and functional clustering. IRE1α binding partners described in published studies (56 proteins), and two available screen using Yeast-two hybrid (32 proteins) and Immunoprecipitation followed by Mass-spectrometry (405 proteins). For the Yeast-two hybrid screen, only proteins that show a yeast growing index >4 were included (Urra et al., 2018). In the case of IP-MS screen all proteins that have a P value > 0,01 in the raw data were included to the analysis (Acosta-Alvear et al., 2018). IRE1α-binding proteins described in other reports are also shown

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Table S2: Gene ontology analysis of IRE1α-binding partners. All IRE1α interacting proteins described in Supplementary table 1 were analyzed using the gene ontology resource platform (PANTHER-based software (http://www.pantherdb.org/)) in order to perform GO Enrichment Analysis. A full list of all biological functions of GO is provided with the respective fold enrichment, P value and FDR.

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