Inositol Requiring Enzyme (IRE), a multiplayer in sensing endoplasmic reticulum stress
Zhixin Zhou, Qian Wang and Marek Michalak

Department of Biochemistry, University of Alberta, Edmonton, Canada

ABSTRACT
The endoplasmic reticulum (ER) can sense a wide variety of external and internal perturbations and responds by mounting stress coping responses, such as the unfolded protein response (UPR). The UPR is composed of three stress sensors, namely IRE1α, PERK, and ATF6 that are activated to re-establish ER homeostasis. IRE1α represents the most ancient branch of the UPR affecting many cellular processes in plant and animal cells. IRE1α is a type I transmembrane protein with kinase/nuclease activities in response to ER stress. Both the ER luminal and cytosolic IRE1α interactomes have been identified revealing a multifunctional role of the ER stress sensor. IRE1α is also associated with organelar membrane contacts to promote rapid communication between intracellular organelles under stress conditions.

Introduction
Responses to stress are an integral part of an organism’s physiology and biology. To deal with stress cells have evolved various mechanisms; the success or failure of these mechanisms depends to a large extent on the nature and duration of the stress. The endoplasmic reticulum (ER) is a large, dynamic organelle and one of the largest components of the cellular reticular network (CRN) (Michalak and Agellon 2018; Wang et al. 2019). The ER plays many vital roles in the cell including Ca<sup>2+</sup> storage, protein synthesis, folding and post-translational modification, phospholipid and steroid synthesis, and stress responses (Schroder and Kaufman 2005; Schroder 2008; Lam and Galiane 2013; Schwarz and Blower 2016; Wang and Kaufman 2016). The ER continuously communicates with other components of the CRN including the Golgi apparatus, nucleus, and mitochondria; mediates lipid synthesis, Ca<sup>2+</sup> and inflammatory signaling, and transcriptional regulation (Phillips and Voeltz 2016; Lombardi and Elrod 2017). Not surprisingly, disruption of ER function caused by intrinsic and extrinsic factors culminates in ER stress, with the ER initiating a coping response [e.g. unfolded protein response (UPR)], to mitigate the stress (Groenendyk, Sreenivasah, Kim, et al. 2010; Walter and Ron 2011; Kraskiewicz and FitzGerald 2012; Chen and Brandizzi 2013; Groenendyk et al. 2013; Grootjans et al. 2016; Wang and Kaufman 2016; Hetz and Papa 2018; Gonzalez-Quiroz et al. 2020; Hetz et al. 2020; Urra et al. 2020). The ER, therefore, is an important component of CRN that allows cells to adjust to a wide variety of conditions. The UPR pathway can sense disturbance in protein folding in the ER and involves distinct components designed to re-establish the protein synthetic machinery, including translational attenuation, transcriptional activation of genes encoding chaperones and components of the ER-associated degradation (ERAD), and activation of apoptotic and autophagy pathways (Kraskiewicz and FitzGerald 2012; Groenendyk et al. 2013; Grootjans et al. 2016; Gonzalez-Quiroz et al. 2020; Urra et al. 2020; Wang and Kaufman 2016; Hetz and Papa 2018).

There are three integral ER membrane proteins, stress sensors, and signal transducers: the ER kinase dsRNA-activated protein kinase-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1) that in combination with the ER molecular chaperone immunoglobulin binding protein (BiP), they comprise the UPR response to ER stress (Groenendyk et al. 2013; Hetz and Papa 2018). BiP interacts with IRE1α, PERK, and ATF6 but upon stress, BiP is sequestered away from the stress sensors, allowing activation of the UPR pathways (Demay et al. 2014; Yukimoto et al. 2021; Groenendyk, Sreenivasah, Kim, et al. 2010;
Many excellent reviews have been published on the UPR signaling (Groenendyk, Sreenivasaiah, Kim, et al. 2010; Walter and Ron 2011; Chen and Brandizzi 2013; Groenendyk et al. 2013; Grootjans et al. 2016; Gonzalez-Quiroz et al. 2020; Hetz et al. 2020; Urra et al. 2020).

IRE1α, the most ancient branch of the UPR affects many cellular processes in plant and animal cells (Groenendyk, Sreenivasaiah, Kim, et al. 2010; Groenendyk et al. 2013). Here we focus on selected aspects of the IRE1α structure, function, and regulation. Recent work also places IRE1α signaling as an important factor in physiology and pathology of the cardiovascular system (Groenendyk, Sreenivasaiah, Kim do, et al. 2010; Groenendyk et al. 2013; Glembotski 2014; Groenendyk et al. 2016; Arrieta et al. 2017; Groenendyk et al. 2020).

**IRE1, the gene, and the protein**

The IRE1 gene was originally identified by complementation of a yeast mutant auxotrophic for inositol and subsequently characterized as a serine/threonine protein kinase required for myo-inositol synthesis (Nikawa and Yamashita 1992). Since then, IRE1α has been identified as a component of the UPR signaling pathway important for sensing and responding to ER stress in a variety of eukaryotic organisms (Chen and Brandizzi 2013; Grootjans et al. 2016; Gonzalez-Quiroz et al. 2020; Urra et al. 2020; Li and Howell 2021; Siwecka et al. 2021). In mammals, there are two homologs of IRE1, IRE1α, and IRE1β encoded by two genes, *ERN1* and *ERN2*, respectively (Figure 1). IRE1α is expressed in all cells, whereas IRE1β is expressed predominantly in the intestinal epithelium (Zhou et al. 2006). IRE1β is restrictively expressed in the gut and IRE1β knockout mice are viable (Tirasophon et al. 2000; Tsuru et al. 2013).

Interestingly, whole-body IRE1α deficiency in mice is embryonic lethal at E9.5-11.5 in mice due to placental malformation (Iwawaki et al. 2009). However, whole-body gene knockout of the *Xbp1* gene, which encodes the transcription factor induced by the ‘canonical’ activation of IRE1α signaling, is embryonic lethal at E12.5-14.5 due to impaired hepatocyte development and hepatic hypoplasia (Reimold et al. 2000). The observed delay in the onset of lethality exhibited by whole-body XBP1-deficient mice relative to the whole-body IRE1α-deficient mice supports the notion that IRE1α is involved in regulating functions in addition to those associated with XBP1 splicing.

Both IRE1 homologs are type I transmembrane proteins with kinase/nuclease activities triggered by oligomerization of IRE1α in response to ER stress (Tirasophon et al. 2000; Li et al. 2010). IRE1α contains an N-terminal ER luminal domain responsible for stress sensing and C-terminal kinase and endoribonuclease domain in the cytosol involved in splicing of XBP1 mRNA and in regulated IRE1-dependent decay (RIDD) (Figures 1 and 2). The luminal domain of the mammalian IRE1α crystallizes as a dimer with an overall architecture similar to the yeast protein (Zhou et al. 2006). A monomer of the luminal domain of IRE1α is composed of unique protein fold of a triangular-shaped β-sheet clusters, which provide a dimerization interface stabilized by hydrogen bonds and hydrophobic interactions (Zhou et al. 2006). Dimerization of the IRE1α luminal domain initiates autophosphorylation of the IRE1α cytosolic domain leading to activation of RNase activity (Zhou et al. 2006; Li et al. 2010). Moreover, dimerization of IRE1α creates a shared central groove that resembles a major histocompatibility complex-like fold allowing for peptide binding. This suggests that IRE1α can interact

**Figure 1.** The IRE1α gene and protein. Human IRE1α encoded by the *ERN1* gene, consists of 22 exons and 93,390 bases. The IRE1α protein consists of signal peptide, N-terminal luminal domain (NLD), a signal helix transmembrane domain, and cytoplasmic region containing kinase and RNase activity.
with peptides primarily composed of basic and hydrophobic residues that mimic misfolded proteins in the ER (Zhou et al. 2006; Gardner and Walter 2011). Mutation of amino acid residues within the groove prevents IRE1α interaction with peptides in vitro (Gardner and Walter 2011) and leads to impaired IRE1α signaling (Credle et al. 2005; Gardner and Walter 2011). Crystal structure of the cytoplasmic domains of IRE1α in the face-to-face (kinase active site points toward the active site of the opposite molecule) or back-to-back orientations provide important information for a mechanistic understanding of the function of IRE1α (Lee et al. 2008; Ali et al. 2011; Adams et al. 2019). These different orientations of the cytoplasmic domain may represent dynamic interactions between kinase and RNase activities of IRE1α to support its oligomerization and stress-induced signaling (Tirasophon et al. 2000; Korennykh et al. 2009; Itzhak et al. 2014).

Activation of RNase function of IRE1α requires dimerization-dependent intermolecular autophosphorylation
(Tirasophon et al. 2000; Itzhak et al. 2014; Prischi et al. 2014). Mutations of IRE1α phosphorylation site reduce RNase splicing activity towards XBP1 mRNA (Prischi et al. 2014). Five amino acid residues within the RNase domain (D847, K907, G923, D927, and Y932) have been identified as essential for RNase activity but not kinase activity, and these mutations prevent activation of IRE1α (Tirasophon et al. 2000). These observations established an intrinsic mechanistic requirement for activation of IRE1α through the oligomerization of its kinase and RNase domains (Korennykh et al. 2009; Itzhak et al. 2014).

**The many functions of IRE1α**

In response to ER stress, the luminal domain of IRE1α dimerizes/oligomerizes, and initiates trans-autophosphorylation of its cytosolic domain inducing a conformational change that leads to activation of IRE1α RNase activity located in the cytoplasmic domain (Liu et al. 2000; Zhou et al. 2006). RNase activity of IRE1α catalyzes the excision of 26 nucleotides within the mRNA encoding XBP1 transcription factor. This unconventional splicing event causes a frameshift resulting in a generation of a longer, stable, and activate transcription factor known as spliced XBP1 (XBP1s) (Yoshida et al. 2000; Calfon et al. 2002). XBP1s binds to a specific promoter element, known as the ER stress element and unfolded protein response element, and turns on expression of genes encoding proteins that modulate protein folding and, secretion, ERAD, protein translocation into the ER and lipid synthesis (Yoshida et al. 2001; Yamamoto et al. 2004). In addition, IRE1α can cleave multiple mRNA targets with consensus sequences and secondary structures that are similar to the XBP1 mRNA, via RIDD (Maurel et al. 2014). RIDD degrades RNAs, including mRNA encoding ER and cytosolic localized proteins, ribosomal RNA, and microRNAs, involved in many cellular functions such as energy metabolism, inflammation, and apoptosis (Maurel et al. 2014). Activation of RIDD preserves ER homeostasis or induces cell death but how IRE1α switches between cytoprotective to cytotoxic RIDD is not known (Lerner et al. 2012; Upton et al. 2012; Maurel et al. 2014). Among the three UPR signaling branches, IRE1α is the major trigger in ER stress-induced apoptosis, whereas PERK and ATF6 are dispensable in activation of apoptosis during prolonged ER stress (Upton et al. 2012). Sulfonation of IRE1α inhibits its signaling and activates p38/Nrf2 antioxidant responses under oxidative stress conditions (Hourihan et al. 2016).

IRE1α interacts with ER-associated inositol-1,4,5-triphosphate receptor/Ca²⁺ channel (IP₃R) and affects IP₃R intracellular distribution and channel activity involved in the formation of functional ER-mitochondria contacts to transport of Ca²⁺ from the ER to the mitochondria (Agellon and Michalak 2019; Carreras-Sureda et al. 2019). Recently, two pools of IRE1α were identified in skeletal muscle and cardiomyocytes; one associated with junctional sarcoplasmic reticulum (SR) responsible for regulation of muscle excitation-contraction coupling and another in the ER-like perinuclear localized membrane system (Wang et al. 2019). |Junctional SR is enriched with the ryanodine receptor/Ca²⁺ channel (RyR) and calsequestrin, a Ca²⁺ binding muscle-specific protein (Wang and Michalak 2020). The RyR, at the junctional SR, is localized to membrane contacts enriched in L-type Ca²⁺ channel of the T-system, an invagination of the plasma membrane (Barone et al. 2015). Both RyR and L-type Ca²⁺ channel are critical for the regulation of Ca²⁺ released from the SR to trigger muscle contraction (Barone et al. 2015). As IRE1α is localized near both Ca²⁺ channels in muscle cells (Wang Q et al. 2019), it is tempting to speculate that IRE1α influences Ca²⁺ channel(s) function and, consequently, excitation-contraction coupling of muscle cells (Agellon and Michalak 2019). Interestingly, calsequestrin binds to IRE1α at the junctional SR preventing its oligomerization and splicing of the XBP1 mRNA (Wang et al. 2019) suggesting that IRE1α at the junctional SR represents different functions of the stress sensor. A role of IRE1α in the regulation of cellular Ca²⁺ signaling remains to be established.

**IRE1α interactome in the lumen of the ER**

In the lumen of the ER, there are a number of multifunctional residents and integral membrane proteins that support many of the ER cellular functions including protein synthesis and post-translational modification, Ca²⁺ buffering/binding and signaling, the synthesis of lipids and steroids, regulation of gene expression, and energy metabolism (Benyair et al. 2011; Braakman and Bulleid 2011; Stutzmann and Mattson 2011). These proteins have access to the N-terminal luminal domain of IRE1α and some of them interact with IRE1α to influence its ability to detect or respond to ER stress (Table 1).

**BIP**

BiP, one of the most abundant ER-resident chaperones, was the first identified modulator of the IRE1α luminal domain (Bertolotti et al. 2000; Okamura et al. 2000). BiP interacts with ER luminal domain of IRE1α and prevents its dimerization and UPR signaling (Table 1). BiP also binds to the luminal domain of PERK and ATF6 under resting conditions and dissociates from PERK
Table 1. IRE1α interacting proteins in the lumen of the ER/SR. In the lumen of the ER IRE1α forms functional complexes with proteins involved in ER Ca^{2+} signaling, protein syntheses, folding, and post-translational modification.

| Protein | Function of interactors | Site of interaction with IRE1α | Impact on IRE1α function |
|---------|-------------------------|-------------------------------|--------------------------|
| BIP/GRP78, COX-2 (Gardner and Walter 2002) | Immunglobulin binding protein | A loop region proximal to the membrane | Binding to IRE1α under unstressed condition<br>Key component of IRE1α ER stress sensing |
| Casq1 and Casq2 (Wang et al. 2019) | Overexpressed misfolded proteins in the ER | Peptide binding groove, center of core IRE1α luminal domain | Activates IRE1α by increasing its oligomerization<br>Increases IRE1α phosphorylation and XBP1 splicing<br>Forms a dynamic feedback loop with ER Ca^{2+} and miR-322 for IRE1α regulation |
| PDIA6 (Eletto et al. 2014; Groenendyk et al. 2014) | Protein disulfide isomerase A6 | Cys^{109}, Cys^{148}, and Cys^{332} in IRE1α ER luminal domain | Enhances ER stress-mediated autophosphorylation and oligomerization of IRE1α<br>Contributes to tumor resistance to ER stress<br>Interacts with IRE1α monomers<br>Stabilizes IRE1α at mitochondria-ER-associated membrane (MAM) under ER stress<br>Stabilizes BIP interaction with IRE1α to inhibit ER stress-induced IRE1α activation and apoptosis |

and ATF6 under ER stress (Bertolotti et al. 2000; Shen et al. 2002). These observations indicate that BIP is a common negative regulator of UPR by binding to the luminal regions of ER stress sensors (IRE1α, PERK, and ATF6) to maintain them in an inactive state.

Dissociation of BIP from IRE1α triggers activation of IRE1α to mediate UPR responses (Bertolotti et al. 2000; Okamura et al. 2000; Kimata et al. 2004). BIP dissociation from IRE1α may be mediated by interaction between BIP and misfolded proteins to sequester BIP away from IRE1α (Kopp et al. 2018; Adams et al. 2019). Alternative mechanisms have been put forward for IRE1α activation indicating that BIP dissociation may not be the sole criterion needed for activation of the IRE1α (Kimata et al. 2007; Oikawa et al. 2007; Pincus et al. 2010). For example, IRE1α may also be regulated by direct binding of unfolded protein (Gardner and Walter 2011; Amin-Wetzel et al. 2019), change in membrane lipid composition (Promlek et al. 2011), AMPylation of BIP [affected by ER Ca^{2+} (Veyron et al. 2019)], cooperation between BIP and ERδj4 (Amin-Wetzel et al. 2019), or yet unidentified factor(s).

**PDIA6**

PDIA6, an ER-resident oxidoreductase, was identified as a regulator of IRE1α activity in response to depletion of the ER Ca^{2+} store (Eletto et al. 2014; Groenendyk et al. 2014). PDIA6 interacts with the luminal domain of IRE1α in a cysteine-dependent manner to enhance IRE1α activity (Table 1). Interestingly, PDIA6 does not substantially affect the activity of the PERK pathway that mediates responses to ER stress, suggesting that each arm of the UPR may be responsive to different components of the ER lumen. Importantly, ER store Ca^{2+} depletion and activation of store-operated Ca^{2+} entry reduces the abundance of the microRNA miR-322, which regulates PDIA6 mRNA stability and consequently IRE1α activity (Groenendyk et al. 2014). This is the first documented case for ER luminal Ca^{2+} together with PDIA6, IRE1α, and miR-322 functioning in a dynamic feedback loop regulating the UPR (Groenendyk et al. 2014).

**HSP47**

HSP47 is an ER-resident foldase that belongs to the family of heat shock proteins and functions as a specific carrier for different types of collagens. It assists the transport of triple-helix procollagen from ER lumen to the cis-Golgi (Nagata 1996; Nagata Kazuhiro and Hoshokawa 1996). Upon ER stress, HSP47 associates with the ER luminal domain of IRE1α reduces binding of BIP to IRE1α, promotes IRE1α dimerization/oligomerization.
and activates IRE1α-mediated UPR (Sepulveda et al. 2018). Importantly, HSP47 enhances the UPR upon ER stress specifically via the IRE1α signaling branch. Interestingly, overexpression or knockdown of HSP47 does not alter PERK and ATF6-mediated UPR signaling indicating HSP47 specificity for IRE1α (Sepulveda et al. 2018). In the heart transient activation of IRE1α results in severe fibrosis (Groenendyk et al. 2016). It is likely that HSP47-
dependent activation of IRE1α plays a role in pathogenesis of cardiac fibrosis (Groenendyk et al. 2016).

**COX-2**

Cyclosporine is an inhibitor of a Ca²⁺-dependent phosphatase, calcineurin, and it is widely used as an immunosuppressant drug (Azzi et al. 2013). Cyclosporine binds to cyclooxygenase-2 (COX-2) and chronic exposure to cyclosporine causes nephrotoxicity and organ damage. COX-2, an inducible cyclooxygenase that drives inflammation, interacts with the ER luminal domain of IRE1α and enhances its XBP1 splicing (Groenendyk et al. 2018). Cyclosporine triggers activation of IRE1α through binding to COX-2, which forms a complex with IRE1α (Groenendyk, Paskevicius, Urra, et al. 2018). Cyclosporine associates to COX-2 resulting in enhanced COX-2 enzymatic activity that is required for IRE1α activation. This offers a novel mechanism for cyclosporine-induced IRE1α signaling (Groenendyk, Paskevicius, Urra, et al. 2018).

**Calsequestrin**

Calsequestrin (skeletal muscle and cardiac calsequestrin PDIAB1 and PDIAB2, respectively), another PDI-like family of protein, is a muscle-specific Ca²⁺ binding and storage protein in the SR (Costello et al. 1986; Wang S et al. 1998; Eisner et al. 2017). Recently, we discovered that both skeletal muscle and cardiac calsequestrin bind to the IRE1α luminal domain in the SR where it modulates IRE1α activity (Wang Q et al. 2019). Association between calsequestrin and IRE1α prevents IRE1α dimerization/oligomerization, an initiation step in IRE1α activation, making calsequestrin a muscle-specific modulator of IRE1α (Wang Q et al. 2019).

Taken together, these findings reveal crucial role of the ER/SR luminal proteins in providing multiple level of regulation of stress sensing and stress responses.

**IRE1α interacting partners in the cytosol**

Most studies on regulation of the IRE1α signaling pathway have focussed on the cytoplasmic regulators of IRE1α activity (Table 2). While the ER luminal domain of IRE1α is important in stress sensing, IRE1α activation is tightly controlled by a number of proteins interacting with its cytoplasmic domain including phosphatases, kinases, apoptosis-related proteins, and the cytoskeleton (Table 2) (Hetz 2012; Chen and Brandizzi 2013; Groenendyk et al. 2013; Riaz et al. 2020). IRE1α cytosolic domain interacting proteins enhance or inhibit IRE1α RNase activity, or act as a scaffold and recruit other proteins to activate apoptosis signaling (Table 2) (Hetz and Glimcher 2009; Chen and Brandizzi 2013). For example, the cytosolic domain of oligomerized IRE1α binds to the adapter protein TNFR-associated factor 2 (TRAF2), triggering activation of the apoptosis signal-regulating kinase 1 (ASK1) and c-Jun-N-terminal kinase (JNK) pathway (Urano et al. 2000; Nishitoh et al. 2002). IRE1α-TRAF2 also promotes NF-κB in a TNFR1-dependent manner and is dependent on the autocrine production of TNFα. Phosphorylated JNK stimulates the cytochrome c-mediated apoptotic pathway by phosphorylating different members of the Bcl-2 family of proteins (Tournier et al. 2000; Lei and Davis 2003).

**Summary points**

- Structural studies revealed mechanistic requirements for IRE1α activation.
- IRE1α is found in membrane contact sites where it regulates organellar communication.
- ER luminal proteins responsible for ER Ca²⁺ signaling, protein synthesis, folding and modification interact with IRE1α to regulate its functions.
- In the cytosol IRE1α is regulated by molecules involved in cellular metabolism, apoptosis and signaling.

**Acknowledgements**

This work was supported by the Canadian Institutes of Health Research grant PS-153325 and the Natural Sciences and Engineering Research Council of Canada and a generous donation from the Kenneth and Sheelagh McCourt family. Z.Z. was supported by a China Council Scholarship.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This work was supported by the Canadian Institutes of Health Research [Grant Number PS-153325] and the Natural Sciences and Engineering Research Council of Canada [Grant Number RGPIN-2019-04908].

**ORCID**

Marek Michalak http://orcid.org/0000-0002-9343-9084

**References**

Adams CJ, Kopp MC, Larburu N, Nowak PR, Ali MMU. 2019. Structure and Molecular mechanism of ER stress signaling
by the unfolded protein response signal activator IRE1. Front Mol Biosci. 6:11.

Agellon LB, Michalak M. 2019. Avoiding raising the IRE of IRE1α. Cell Calcium. 83:102056.

Ali MM, Bagratuni T, Davenport EL, Nowak PR, Silva-Santisteban MC, Hardcastle A, McAndrews C, Rowlands MG, Morgan GJ, Aherne W, et al. 2011. Structure of the IRE1 autophosphorylation complex and implications for the unfolded protein response. EMBO J. 30(5):894–905.

Amin-Wetzel N, Neidhardt L, Yan Y, Mayer MP, Ron D. 2019. Avoiding raising the IRE of IRE1

Costello B, Chadwick C, Saito A, Chu A, Maurer A, Fleischer S. 1986. Characterization of the junctional face membrane from terminal cisternae of sarcoplasmic reticulum. J Cell Biol. 103(3):741–753.

Credle JJ, Finer-Moore JS, Papa FR, Stroud RM, Walter P. 2005. On the mechanism of sensing unfolded protein in the endoplasmic reticulum. Proc Natl Acad Sci U S A. 102(52):18773–18781.

Crespo Y, Pechochon J, Szuplewska S, Mignotte B, Gauzer S. 2014. The PERK pathway independently triggers apoptosis and a Rac1/slrp/JNK/Dilp8 signaling favoring tissue homoeostasis in a chronic ER stress drosophila model. Cell Death Dis. 5:e1452.

Eisner DA, Caldwell JL, Kistamas K, Trafford AW. 2017. Calcium and excitation-contraction coupling in the heart. Circ Res. 121(2):181–195.

Elette D, Elett D, Dersh D, Gidleivitz T, Argon Y. 2014. Protein disulfide isomerase A6 controls the decay of IRE1α signaling via disulfide-dependent association. Mol Cell. 53(4):562–576.

Gao B, Lee S-M, Chen A, Zhang J, Zhang DD, Kannan K, Ortmann RA, Fang D. 2008. Synoviolin promotes IRE1 ubiquitination and degradation in synovial fibroblasts from mice with collagen-induced arthritis. EMBO Rep. 9(5):480–485.

Gardner BM, Walter P. 2011. Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. Science. 333(6051):1891–1894.

Glembotski CC. 2014. Roles for ATF6 and the sarco/endoplasmic reticulum protein quality control system in the heart. J Mol Cell Cardiol. 71:11–15.

Gonzalez-Quiroz M, Blondel A, Sagredo A, Hetz C, Chevet E, Peudeux R. 2020. When endoplasmic reticulum proteostasis meets the DNA damage response. Trends Cell Biol. 30 (11):881–891.

Groendyk J, Agellon LB, Michalak M. 2013. Coping with endoplasmic reticulum stress in the cardiovascular system. Annu Rev Phys. 75:49–67.

Groendyk J, Lee D, Jung J, Dyck JR, Lopaschuk GD, Agellon LB, Michalak M. 2016. Inhibition of the unfolded protein response mechanism prevents cardiac fibrosis. PLoS One. 11(7):e0159682.

Groendyk J, Paskevicius T, Urra H, Viricel C, Wang K, Barakat K, Hetz C, Kurgan L, Agellon LB, Michalak M. 2018. Cyclosporine A binding to COX-2 reveals a novel signaling pathway that activates IRE1α unfolded protein response sensor. Sci Rep. 8:16678.

Groendyk J, Peng Z, Dudek E, Fan X, Mizainty MJ, Dufey E, Urra H, Sepulveda D, Rojas-Rivera D, Lim Y, Kim DH. 2014. Interplay between the oxidoreductase PDXA6 and microRNA-322 controls the response to disrupted endoplasmic reticulum calcium homeostasis. Sci Signal. 7(329):ra54.

Groendyk J, Sreenivasaiah PK, Kim do H, Agellon LB, Michalak M. 2010. Biology of endoplasmic reticulum stress in the heart. Circ Res. 107(10):1185–1197.

Groendyk J, Wang Q, Wagg C, Lee D, Robinson A, Barr A, Light PE, Lopaschuk GD, Agellon LB, Michalak M. 2020. Selective enhancement of cardiomyocyte efficiency results in a pericard heart condition. PLoS one. 15(8):e0236457.

Chen Y, Brandizzi F. 2013. IRE1α: ER stress sensor and cell fate executor. Trends Cell Biol. 23(11):547–555.

Chen L, Xu S, Liu L, Wen X, Xu Y, Che J, Teng J. 2014. Cab45S inhibits the ER stress-induced IRE1-JNK pathway and apoptosis via GRP78/Bip. Cell Death Dis. 8:e1219.

Costello B, Chadwick C, Saito A, Chu A, Maurer A, Fleischer S. 1986. Characterization of the junctional face membrane from terminal cisternae of sarcoplasmic reticulum. J Cell Biol. 103(3):741–753.

Demay Y, Pechochon J, Szuplewska S, Mignotte B, Gauzer S. 2014. The PERK pathway independently triggers apoptosis and a Rac1/slrp/JNK/Dilp8 signaling favoring tissue homeostasis in a chronic ER stress drosophila model. Cell Death Dis. 5:e1452.

Eisner DA, Caldwell JL, Kistamas K, Trafford AW. 2017. Calcium and excitation-contraction coupling in the heart. Circ Res. 121(2):181–195.

Elette D, Elett D, Dersh D, Gidleivitz T, Argon Y. 2014. Protein disulfide isomerase A6 controls the decay of IRE1α signaling via disulfide-dependent association. Mol Cell. 53(4):562–576.

Gao B, Lee S-M, Chen A, Zhang J, Zhang DD, Kannan K, Ortmann RA, Fang D. 2008. Synoviolin promotes IRE1 ubiquitination and degradation in synovial fibroblasts from mice with collagen-induced arthritis. EMBO Rep. 9(5):480–485.

Gardner BM, Walter P. 2011. Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. Science. 333(6051):1891–1894.

Glembotski CC. 2014. Roles for ATF6 and the sarco/endoplasmic reticulum protein quality control system in the heart. J Mol Cell Cardiol. 71:11–15.

Gonzalez-Quiroz M, Blondel A, Sagredo A, Hetz C, Chevet E, Peudeux R. 2020. When endoplasmic reticulum proteostasis meets the DNA damage response. Trends Cell Biol. 30 (11):881–891.

Groendyk J, Agellon LB, Michalak M. 2013. Coping with endoplasmic reticulum stress in the cardiovascular system. Annu Rev Phys. 75:49–67.

Groendyk J, Lee D, Jung J, Dyck JR, Lopaschuk GD, Agellon LB, Michalak M. 2016. Inhibition of the unfolded protein response mechanism prevents cardiac fibrosis. PLoS One. 11(7):e0159682.

Groendyk J, Paskevicius T, Urra H, Viricel C, Wang K, Barakat K, Hetz C, Kurgan L, Agellon LB, Michalak M. 2018. Cyclosporine A binding to COX-2 reveals a novel signaling pathway that activates IRE1α unfolded protein response sensor. Sci Rep. 8:16678.

Groendyk J, Peng Z, Dudek E, Fan X, Mizainty MJ, Dufey E, Urra H, Sepulveda D, Rojas-Rivera D, Lim Y, Kim DH. 2014. Interplay between the oxidoreductase PDXA6 and microRNA-322 controls the response to disrupted endoplasmic reticulum calcium homeostasis. Sci Signal. 7(329):ra54.
Grootjans J, Kaser A, Kaufman RJ, Blumberg RS. 2016. The unfolded protein response in immunity and inflammation. Nat Rev Immunol. 16(8):469–484.

Gu F, Nguyen DT, Stuble M, Dubé N, Tremblay ML, Chevet E. 2004. Protein-tyrosine phosphatase 1B potentiates IRE1 signaling during endoplasmic reticulum stress. J Biol Chem. 279(48):49689–49693.

Guo J, Polymenis M. 2006. DCR2 targets IRE1 and downregulates the unfolded protein response in Saccharomyces cerevisiae. EMBO Rep. 7:1124–1127.

Gupta S, Deepti A, Deegan S, Lisbona F, Hetz C, Samali A. 2010. HSP72 Protects cells from ER stress-induced apoptosis via enhancement of IRE1α-XBP1 signaling through a physical interaction. PLoS Biol. 8(7):e1000410.

He Y, Beatty A, Han X, Ji Y, Ma X, Adelstein RS, Yates III JR, Iwawaki T, Akai R, Yamanaka S, Kohno K. 2009. Function of α-reticulum protein required for the PERK- and IRE1- mediated unfolded protein response in Drosophila. EMBO Rep. 7:1124–1127.

Hetz C. 2012. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol. 13(2):89–102.

Hetz C, Glomcher LH. 2009. Fine-tuning of the unfolded protein response: assembling the IRE1α interactome. Mol Cell. 35 (5):551–561.

Hetz C, Papa FR. 2018. The unfolded protein response and Cell fate control. Mol Cell. 69(2):169–186.

Hetz C, Zhang K, Kaufman RJ. 2020. Mechanisms, regulation and functions of the unfolded protein response. Nat Rev Mol Cell Biol. 21(8):421–438.

Hetz C, Bernasconi P, Fisher J, Lee AH, Bassik MC, Antonsson B, Brandt GS, Iwakoshi NN, Schinzel A, Glomcher LH, et al. 2006. Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1α. Science. 312:572–576.

Hourihan JM, Moronetti Mazzeo LE, Fernández-Cárdenas LP, Blackwell TK. 2016. Cysteine sulfenylation directs IRE-1 to activate the SKN-1/Nrf2 antioxidant response. Mol Cell. 63 (4):553–566.

Itzhak D, Bright M, McAndrew P, Mirza A, Newbatt Y, Srover J, Widya M, Thompson A, Morgan G, Collins I, Davies F. 2014. Multiple autophosphorylations significantly enhance the endoribonuclease activity of human inositol requiring enzyme 1α. BMC Biochem. 15:3.

Iwakawa T, Akai R, Yamanaka S, Kohno K. 2009. Function of α-reticulum protein required for the PERK- and IRE1-mediated unfolded protein response in Drosophila. EMBO Rep. 7:1124–1127.

Jwa M, Chang P. 2012. PARP16 is a tail-anchored endoplasmic reticulum stress sensor IRE1 involving its cluster formation and interaction upon endoplasmic reticulum stress. J Cell Biol. 199(6):1141–1152.

Korennykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, Shokat KM, Stroud RM, Walter P. 2009. The unfolded protein response signals through high-order assembly of IRE1. Nature. 457(7230):687–693.

Kratzkevicz H, FitzGerald U. 2012. InterFERing with endoplasmic reticulum stress. Trends Pharmacol Sci. 33(2):53–63.

Lam AK, Galione A. 2013. The endoplasmic reticulum and junctional membrane communication during calcium signaling. Biochim Biophys Acta. 1833:2542–2559.

Lee KP, Dey M, Neculai D, Cao C, Dever TE, Sicheri F. 2008. Structure of the dual enzyme Ire1 reveals the basis for catalysis and regulation in nonconventional RNA splicing. Cell. 132(1):89–100.

Lei K, Davis RJ. 2003. JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. Proc Natl Acad Sci U S A. 100(5):2432–2437.

Lerner AG, Upton J-P, Praveen PVK, Ghosh R, Nakagawa Y, Igbaria A, Shen S, Nguyen V, Backes BJ, Heiman M, et al. 2012. IRE1α induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. Cell Metab. 16 (2):250–264.

Li H, Korennykh AV, Behrmann SL, Walter P. 2010. Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering. Proc Natl Acad Sci U S A. 107 (37):16113–16118.

Li Z, Howell SH. 2021. Review: The two faces of IRE1 and their role in protecting plants from stress. Plant Sci. 303:110758.

Lisbona F, Rojas-Rivera D, Thielen P, Zamorano S, Todd D, Martinon F, Glavic A, Kres C, Lin JH, Walter P, et al. 2009. BAX inhibitor-1 is a negative regulator of the ER stress sensor IRE1α. Mol Cell. 33:679–691.

Liu CY, Schroder M, Kaufman RJ. 2000. Ligand-independent dimerization activates the stress response kinase IRE1 and PERK in the lumen of the endoplasmic reticulum. J Biol Chem. 275(32):24881–24885.

Liu D, Liu X, Zhou T, Yao W, Zhao J, Zheng Z, Jiang W, Wang F, Alkionbore FO, Hill DL, Emmett N. 2016. IRE1–RACK1 axis orchestrates ER stress preconditioning-elicted cytoprotection from ischemia/reperfusion injury in liver. J Mol Cell Biol. 8(2):144–156.

Liu J, Wang Y, Song L, Zeng L, Yi W, Liu T, Chen H, Wang M, Ju Z, Cong Y-S. 2017. A critical role of DDRGK1 in endoplasmic reticulum homoeostasis via regulation of IRE1α stability. Nat Commun. 8(1):14186.

Lombardi AA, Elrod JW. 2017. Mediating ER-mitochondrial cross-talk. Science. 358(6363):591–592.

Luo D, He Y, Zhang H, Yu L, Chen H, Xu Z, Tang S, Urano F, Min W. 2008. AIP1 is critical in transducing IRE1-mediated endoplasmic reticulum stress response. J Biol Chem. 283 (18):11905–11912.

Marcu Monica G, Doyle M, Bertolotti A, Ron D, Hendershot L, Neckers L. 2002. Heat shock protein 90 Modulates the stress response kinases of the endoplasmic reticulum stress sensor IRE1. J Biol Chem. 277(25):22488–22497.

Mauro M, Chevet E, Tavernier J, Gerlo S. 2014. Getting RIDD of RNA: IRE1 in cell fate regulation. Trends Biochem Sci. 39 (5):245–254.

Michalak M, Agellon LB. 2018. Stress coping strategies in the heart: an integrated view. Front Cardiovasc Med. 5:168.

Mori T, Hayashi T, Hayashi E, Su TP. 2013. Sigma-1 receptor chaperone at the ER-mitochondrion interface mediates the
mitochondrion-ER-nucleus signaling for cellular survival. PLoS One. 8(10):e76941.
Morita S, Villalta SA, Feldman HC, Register AC, Rosenthal W, Hoffmann-Petersen IT, Meh dizadeh M, Ghosh R, Wang L, Colon-Negron K, et al. 2017. Targeting ABL-IRE1α signaling spares ER-stressed pancreatic β cells to reverse autoimmune diabetes. Cell Metab. 25(4):883–897. e888.
Nagata K. 1996. Hsp47: a collagen-specific molecular chaperone. Trends Biochem Sci. 21(1):22–26.
Nagata K, Hosokawa N. 1996. Regulation and function of collagen-specific molecular chaperone, HSP47. Cell Struct. Funct. 21(5):425–430.
Nguyen DT, Kebache S, Fazel A, Wong HN, Jenn S, Emadali A, Lee EH, Bergeron JJ, Kaufman RJ, Larose L, Chevet E. 2004. Nck-dependent activation of extracellular signal-regulated kinase-1 and regulation of Cell survival during endoplasmic reticulum stress. Mol Biol Cell. 15(9):4248–4260.
Nikawa J, Yamashita S. 1992. IRE1 encodes a putative protein kinase containing a membrane-spanning domain and is required for inositol phototrophy in saccharomyces cerevisiae. Mol Microbiol. 6(11):1441–1446.
Nishitoh H, Matsuzawa A, Tobiume K, Saegusa K, Takeda K, Inoue K, Hori S, Kakizuka A, Ichijo H. 2002. ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. Genes Dev. 16(11):1345–1355.
Okawa D, Kimata Y, Kohno K. 2007. Self-association and BiP dissociation are not sufficient for activation of the ER stress sensor Ire1. J Cell Sci. 120(Pt 9):1681–1688.
Okamura K, Kimata Y, Higashio H, Tsuru A, Kohno K. 2000. Dissociation of Kar2p/Bip from an ER sensory molecule, Ire1p, triggers the unfolded protein response in yeast. Biochem Biophys Res Commun. 279(2):445–450.
Oono K, Yoneda T, Manabe T, Yamagishi S, Matsuda S, Hitomi J, Miyata S, Mizuno T, Imaizumi K, Katayama T, Toyama M. 2004. JAB1 participates in unfolded protein responses by association and dissociation with IRE1. Neuromusc Int. 45(5):765–772.
Phillips MJ, Voeltz GK. 2016. Structure and function of ER membrane contact sites with other organelles. Nat Rev Mol Cell Biol. 17(2):69–82.
Pincus D, Chevalier MW, Aragon T, van Anken E, Vidal SE, El-Samad H, Walter P. 2010. BiP binding to the ER-stress sensor Ire1 tunes the homeostatic behavior of the unfolded protein response. PLoS Biol. 8(7):e1000415.
Pinkaew D, Chattopadhyay A, King MD, Chunhacha P, Liu Z, Stevenson HL, Chen Y, Sinthujaren P, McDougall OM, Fujise K. 2017. Fortilin binds IRE1α and prevents ER stress from signaling apoptotic cell death. Nat Commun. 8(1):18.
Plumb R, Zhang Z-R, Appathurai S, Mariappan M. 2015. A functional link between the co-translational protein translocation pathway and the UPR. eLife. 4:e07426.
Prisci F, Nowak PR, Carrara M, Ali MMU. 2014. Phosphoregulation of Ire1 RNase splicing activity. Nat Commun. 5(1):3554.
Promlek T, Ishiwata-Kimata Y, Shido M, Sakuramoto M, Kohno K, Kimata Y. 2011. Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways. Mol Biol Cell. 22(18):3520–3532.
Qiu Y, Mao T, Zhang Y, Shao M, You J, Ding Q, Chen Y, Wu D, Xie D, Lin X, et al. 2010. A crucial role for RACK1 in the regulation of glucose-stimulated IRE1α activation in pancreatic beta cells. Sci Signal. 3(19).
Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, Ron D. 2000. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science. 287(5453):664–666.

Urra H, Pihan P, Hetz C. 2020. The UPRosome - decoding novel biological outputs of IRE1α function. J Cell Sci. 133:15.

Urra H, Henriquez DR, Cánovas J, Villarroel-Campos D, Carreras-Sureda A, Pulgar E, Molina E, Hazari YM, Limia CM, Alvarez-Rojas S, et al. 2018. IRE1α governs cytoskeleton remodelling and cell migration through a direct interaction with filamin A. Nat Cell Biol. 20:942–953.

Veyron S, Oliva G, Rolando M, Buchrieser C, Peyroche G, Cherfils J. 2019. A Ca2+-regulated deAMPylation switch in human and bacterial FIC proteins. Nat Commun. 10(1):1142.

Walter P, Ron D. 2011. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 334 (6059):1081–1086.

Wang M, Kaufman RJ. 2016. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature. 529 (7586):326–335.

Wang Q, Groenendyk J, Paskevicius T, Qin W, Kor KC, Liu Y, Hiess F, Knollmann BC, Chen SW, Tang J, Chen XZ. 2019. Two pools of IRE1α in cardiac and skeletal muscle cells. FASEB J. 33(8):8892–8904.

Wang Q, Michalak M. 2020. Calsequestrin. structure, function and evolution. Cell Calcium. 90:102242.

Wang S, Trumble WR, Liao H, Wesson CR, Dunker AK, Kang C. 1998. Crystal structure of calsequestrin from rabbit skeletal muscle sarcoplasmic reticulum. Nat Struct Biol. 5(6):476–483.

Yamamoto K, Yoshida H, Kokame K, Kaufman RJ, Mori K. 2004. Differential contributions of ATF6 and XBP1 to the activation of endoplasmic reticulum stress-responsive cis-acting elements ERSE, UPRE and ERSE-II. J Biochem. 136(3):343–350.

Yoneda T, Imaizumi K, Oono K, Yui D, Gomi F, Katajama T, Tohyama M. 2001. Activation of caspase-12, an endoplasmic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. J Biol Chem. 276:13935–13940.

Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. 2001. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell. 107(7):881–891.

Yukimoto A, Watanabe T, Sunago K, Nakamura Y, Tanaka T, Koizumi Y, Yoshida O, Tokumoto Y, Hirooka M, Abe M, Hiasa Y. 2021. The long noncoding RNA of RMRP is downregulated by PERK, which induces apoptosis in hepatocellular carcinoma cells. Sci Rep. 11(1):7926.

Zhou J, Liu CY, Back SH, Clark RL, Peisach D, Xu Z, Kaufman RJ. 2006. The crystal structure of human IRE1 luminal domain reveals a conserved dimerization interface required for activation of the unfolded protein response. Proc Natl Acad Sci U S A. 103(39):14343–14348.