Molecular Cell
Review

The Unfolded Protein Response and Cell Fate Control

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https://doi.org/10.1016/j.molcel.2017.06.017

The secretory capacity of a cell is constantly challenged by physiological demands and pathological perturbations. To adjust and match the protein-folding capacity of the endoplasmic reticulum (ER) to changing secretory needs, cells employ a dynamic intracellular signaling pathway known as the unfolded protein response (UPR). Homeostatic activation of the UPR enforces adaptive programs that modulate and augment key aspects of the entire secretory pathway, whereas maladaptive UPR outputs trigger apoptosis. Here, we discuss recent advances into how the UPR integrates information about the intensity and duration of ER stress stimuli in order to control cell fate. These findings are timely and significant because they inform an evolving mechanistic understanding of a wide variety of human diseases, including diabetes mellitus, neurodegeneration, and cancer, thus opening up the potential for new therapeutic modalities to treat these diverse diseases.

Introduction

The endoplasmic reticulum (ER) is a network of branching tubules and flattened sacs that governs the synthesis, folding, and processing of over a third of all cellular proteins. Most proteins destined for residence in the ER, plasma membrane, Golgi apparatus, and lysosomes are translated on ER membrane-bound ribosomes while being injected into the ER lumen. Similarly, most proteins that are ultimately secreted from the cell begin their journey in the ER. Proteins targeted for the ER have a signal peptide sequence that directs these proteins to the ER membrane while still attached to the ribosome; the signal peptide is removed by a protease, typically before translation of the polypeptide is completed. Once in the ER lumen, proteins are folded into their unique three-dimensional shapes as they concomitantly undergo various post-translational modifications, including glycosylation and disulfide bond formation. These processes are catalyzed by an ER-resident protein-folding and modification machinery comprising a network of chaperones, glycosylating enzymes, and oxido-reductases (Sevier and Kaiser, 2002). The ionic and electronic milieu of the ER is optimally suited for the operation of these protein-folding activities. Compared to the cytosol, the ER maintains a much higher calcium concentration and a more oxidizing redox potential (van Anken and Braakman, 2005). Cells expend extensive amounts of energy to maintain the unique environment and functions of the ER. Chaperones bind and assist folding of secretory proteins by preventing aggregation of these client proteins during structural maturation. In many cases, folding and structural maturation of proteins also involves the covalent addition and trimming of sugars. Together, these enzymatic processes ensure that secretory proteins are properly folded, modified, and assembled into multi-protein complexes in the ER before they traffic farther downstream in the secretory pathway.

Despite the function of these protein-folding machines, the success rate for proper folding is often quite low for many client proteins of the secretory pathway. Incompletely folded proteins are not tolerated by the cell and are removed by stringent quality control systems. Through a process called ER-associated degradation (ERAD), unfolded proteins are returned to the cytosol for subsequent ubiquitylation and degradation by the 26S proteasome (Smith et al., 2011). The folding capacity within the ER varies greatly among cells types. Cells with the potential to secrete high protein loads are able to do so because they contain a large and well-developed ER. For example, each β cell of the endocrine pancreas is capable of synthesizing and secreting up to one million molecules of insulin per minute; in insulin-resistant states, this enormous protein synthetic load becomes even greater. Moreover, plasma cells can secrete their own weight in antibodies every day.

Despite the robustness of the ER, cells often work near the limits of their secretory capacity. A wide range of cellular disturbances can disrupt the efficiency of protein folding in the ER and

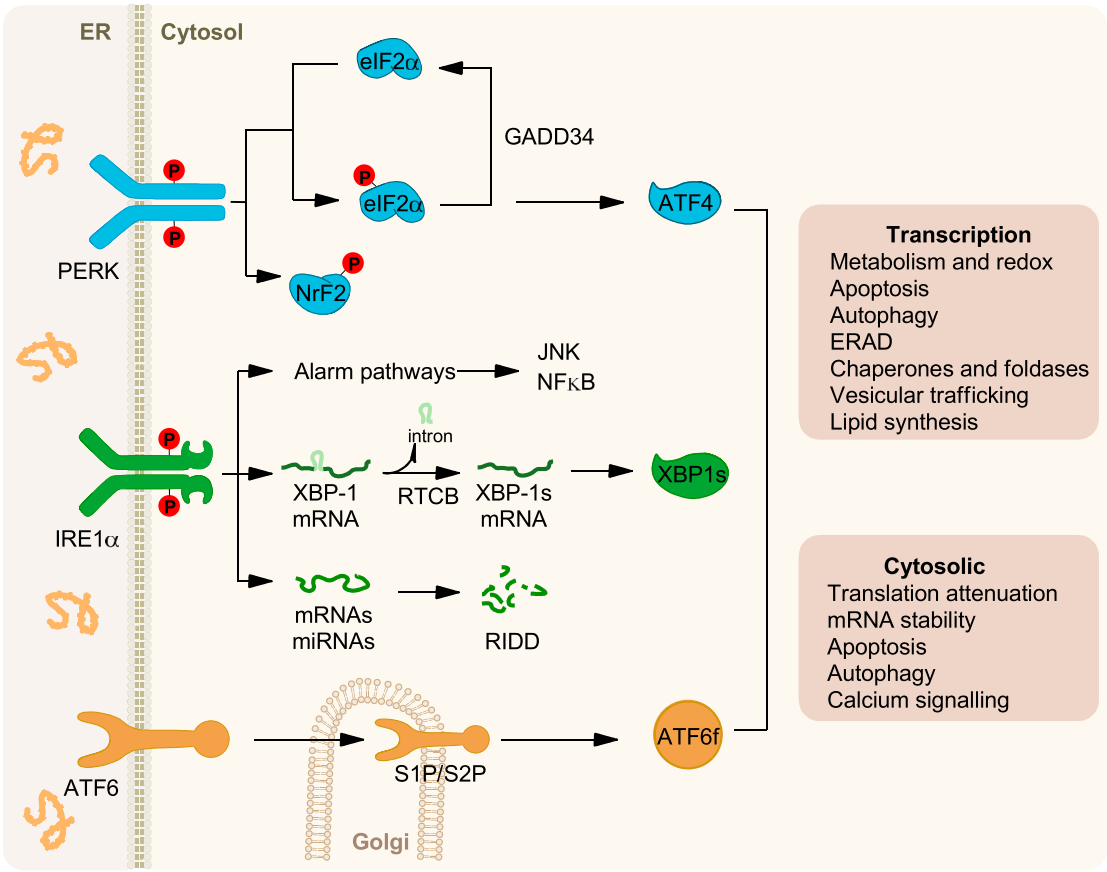

Figure 1. The Three Arms of the UPR
All three ER stress sensors (PERK, IRE1α, and ATF6) initially activate signaling events that increase protein-folding capacity and reduce protein load on the ER. These transcriptional and translational outputs tend to re-establish protein-folding homeostasis in the ER and promote cell survival.

lead to the accumulation of misfolded proteins within this organelle—a state known as "ER stress" (Walter and Ron, 2011). Conditions provoking ER stress include nutrient deprivation, hypoxia, point mutations in secreted proteins that stabilize intermediate folding forms or cause aggregation, and loss of calcium homeostasis, which has detrimental effects on ER-resident, calcium-dependent chaperones. In the case of pancreatic β cells, ER stress can occur from the inability to fold the increased levels of (pro)insulin intermediates needed to maintain blood glucose. In other cells, such as neurons, the chronic expression of folding-defective secretory proteins leads to unsustainable demands on the protein-folding machinery, causing chronic ER stress. Therefore, cells have evolved a sophisticated surveillance system to sense and respond to ER stress before it becomes a threat to their survival.

To ensure that the protein-folding capacity is in balance with protein-folding demand, cells constantly monitor the amount of misfolded proteins in the ER lumen. When misfolded proteins accumulate above a critical threshold, this sets in motion a signal transduction pathway called the unfolded protein response (UPR) within the cell to restore homeostasis. In this Review, we discuss recent advances in the mechanisms of UPR signaling, its regulation, and its relevance to pathological conditions.

Proximal UPR Signaling Components

The communication between the ER and the nucleus in cells experiencing the stress of unfolded protein accumulation in the early secretory pathway was reported in mammals for the first time in 1988 (Kozutsumi et al., 1988). This phenomenon was then characterized genetically in *S. cerevisiae*, leading to the identification of a linear signaling pathway governed by the ER stress transducer Inositol-requiring protein-1 (Ire1p) and a downstream transcription factor known as Hac1 (homologous to ATF/CREB1) (Cox and Walter, 1996; Mori et al., 1993). In baker's yeast, ER stress results in the upregulation of a large cluster of genes involved in protein folding, quality control, and secretion. In vertebrates, the UPR has evolved toward the establishment of a complex network of interconnected signaling pathways initiated by the stimulation of three signal transducers located in the ER known as IRE1 (α and β), activating transcription factor-6—ATF6—(α and β) and protein kinase RNA (PKR)-like ER kinase (PERK) (Figure 1) (Wang and Kaufman, 2016).

Activation of the UPR triggers two temporally distinct cellular events to mitigate protein misfolding: an initial reaction to reduce protein synthesis and enhance degradation of misfolded proteins and a second wave of transcriptional upregulation of hundreds of target genes involved in global proteostasis control

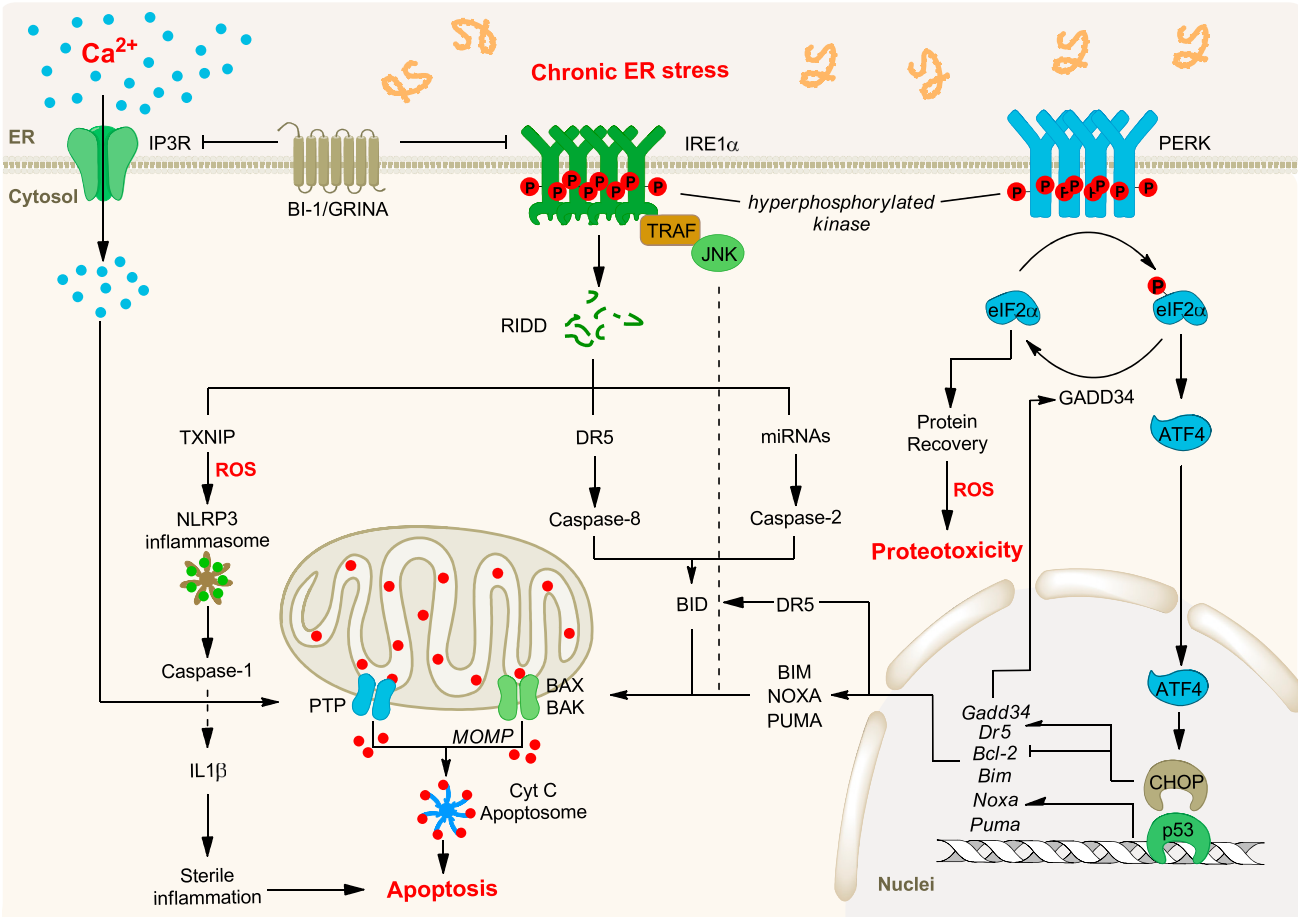

Figure 2. ER Stress-Induced Apoptosis Pathways
When exposed to chronically high levels of ER stress, PERK and IRE1α both drive multiple signaling outputs that lead to cell dysfunction, activation of the inflammasome, and apoptosis. A key step in the regulation of apoptosis is the crosstalk between the ER and mitochondria through transcriptional and post-translational modifications of members of the BCL-2 family of proteins. In addition, calcium release from the ER and exacerbated protein synthesis and ROS production may influence the induction of apoptosis.

(Figure 1). PERK is a type I transmembrane kinase that under ER stress conditions oligomerizes and *trans*-autophosphorylates, inhibiting general protein translation through the phosphorylation of eukaryotic translation initiator factor-2 (eIF2α) at serine 51 (Walter and Ron, 2011). This event reduces the overload of proteins entering the ER of a stressed cell but also allows the selective translation of the mRNA encoding the transcription factor ATF4, thus contributing to reinforcement of an antioxidant response, enhancement of the folding capacity of the ER, and upregulation of macroautophagy (Harding et al., 2000, 2003). Under chronic ER stress, sustained ATF4 expression contributes to induction of apoptosis (Figure 2; see next section). Importantly, eIF2α phosphorylation represents a convergence point of different stress pathways known as the "integrated stress response," which is governed by specific kinases that are activated by inflammation, viral infections, nutrient deprivation, and heme deficiency (Pakos-Zebrucka et al., 2016).

IRE1α initiates the most conserved UPR signaling branch. IRE1α—the widely expressed IRE1 paralog—is a type I transmembrane protein containing a serine/threonine kinase and an endoribonuclease (RNase) domain on its cytosolic face. In response to unfolded protein accumulation in the ER, IRE1α's lumenal domain self-associates, causing IRE1α to dimerize and *trans*-autophosphorylate, thereby inducing a conformational change that activates its RNase domain to catalyze excision of a 26-nt intron within the XBP1 mRNA (Figure 1) (Calfon et al., 2002; Shen et al., 2001; Yoshida et al., 2001). This unconventional splicing event shifts the open reading frame of the mRNA to generate a stable and active transcription factor known as XBP1s (Figure 1). The scope of XBP1 target genes may vary depending on the tissue context and stimuli since XBP1s has the ability to interact with other transcription factors by forming heterodimers (Hetz, 2012). In cellular models of ER stress, XBP1s controls the expression of genes encoding factors that modulate protein folding, secretion, ERAD, protein translocation into the ER, and lipid synthesis (Acosta-Alvear et al., 2007; Lee et al., 2003). The RNase domain of IRE1α also regulates the stability of multiple RNAs through a direct endonucleolytic cleavage reaction involving specific sequences and secondary structure in a process known as "regulated IRE1-dependent decay," or

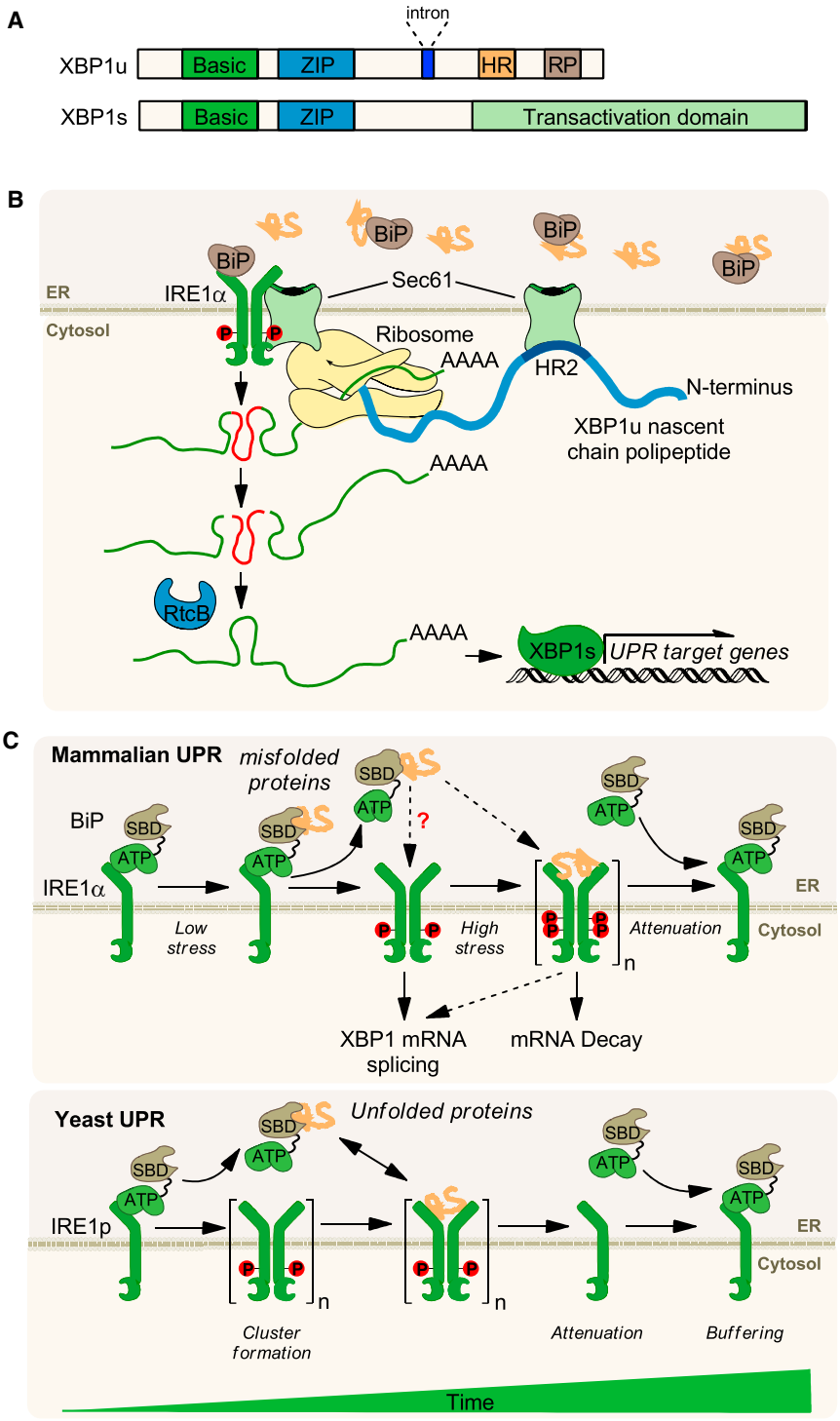

Figure 3. Regulation of IRE1α Activation and XBP1 mRNA Splicing

(A) Primary structure of XBP1u and XBP1s. The hydrophobic region (HR) and the ribosome pausing (RP) sequence are highlighted, in addition to the domains involved in transcriptional regulation.

(B) As the unspliced XBP1 mRNA is translated in mammals, a HR on the nascent peptide targets the translated XBP1 mRNA to the ER membrane, enhancing its processing by active IRE1α, in a reaction that is completed by the RctB ligase. XBP1 mRNA targeting to the ER membrane depends on the physical interaction between IRE1α and the Sec61 translocon.

(C) Top: in mammalian cells, IRE1α is maintained in a repressed state through an association with the ATPase domain of BiP (ATP). Upon ER stress, misfolded proteins dock to the substrate binding domain (SBD) of BiP, triggering the dissociation from IRE1 through this allosteric regulation. This event triggers IRE1α dimerization and autotransphosphorylation, inducing a conformational change on the RNase domain that allows its activation. IRE1α may also bind misfolded proteins to induce its oligomerization. Bottom: in yeast cells, a direct recognition model has been also proposed where unfolded proteins bind directly to the luminal domains of IRE1p, facilitating the assembly of highly ordered IRE1p clusters exemplified by the parenthesis and "n" IRE1p units). This may orient the cytosolic region of the dimer to form the ribonuclease active site and generation of an mRNA docking region. BiP dissociation from IRE1p may play an indirect role in unfolded-peptide loading. During the attenuation phase, BiP may operate as a buffer to sequester inactive IRE1p.

RIDD (Hollien and Weissman, 2006). This additional output of IRE1α has been associated with the degradation of myriad ER-localized and cytosolic mRNAs, ribosomal RNA, and microRNAs and has an important biological function in the control of glucose metabolism, inflammation, and apoptosis (Maurel et al., 2014). The kinetics of RIDD versus XBP1 mRNA splicing are distinct, suggesting the existence of differential regulatory mechanisms of the RNase activity or in the delivery of RNA substrates to IRE1α. Although the molecular basis of this phenomenon is still unclear and debated, this switch may depend on the differential oligomeric state of IRE1α (Bouchecareilh et al., 2011; Ghosh et al., 2014; Han et al., 2009; Tam et al., 2014).

The efficiency of XBP1 mRNA splicing is regulated at different levels. For example, the delivery of its mRNA to the ER membrane is mediated by the transient expression of the unspliced form of XBP1 (XBP1u). Although XBP1u is highly unstable and is rapidly degraded by the 26S proteasome, during its translation, the nascent chain docks the ribosome to the ER membrane through a highly conserved hydrophobic domain (Kanda et al., 2016; Yanagitani et al., 2009) that, together with a translation pausing sequence (Yanagitani et al., 2011) (Figure 3A), allows the efficient processing of XBP1 mRNA in the cytosol (Figure 3B). In addition, the selective targeting of the XBP1u mRNA to the ER membrane is mediated by a direct interaction of IRE1α with the Sec61 translocon (Plumb et al.,

2015). Interestingly, The XBP1u mRNA is delivered to the IRE1/Sec61 complex by docking of its nascent protein to the classical signal-recognition particle (SRP) pathway through the hydrophobic domain (Figure 3B). After cleavage of XBP1 mRNA, the tRNA ligase RtcB completes the cytosolic splicing event, leading to the expression of XBP1s (Jurkin et al., 2014; Kosmaczewski et al., 2014; Lu et al., 2014b).

ATF6α is also an ER transmembrane protein that contains a bZIP transcription factor on its cytosolic domain. Under ER stress, ATF6 translocates to the Golgi apparatus, where it is cleaved by the proteases S1P and S2P, thereby releasing its cytosolic domain (ATF6f) (Haze et al., 1999). ATF6f causes upregulation of a select set of UPR genes, including those that reinforce the ERAD pathway (Yamamoto et al., 2007). Interestingly, ATF6f exhibits crosstalk with XBP1s by forming heterodimers, which may drive specific gene expression programs (Shoulders et al., 2013). Overall, the UPR's transcriptional programs operate as a complex signaling network that enforces multiple outputs to restore ER proteostasis and preserve cellular function.

The mechanisms underlying the detection or sensing of misfolded proteins in the ER lumen is still poorly defined. In mammals, the ER chaperone BiP/Grp78 is fundamental for the activation of the three main sensors (Figure 3C). Under resting conditions, BiP associates with the ER luminal domain of PERK and IRE1α via its ATPase domain, maintaining them in an inactive monomeric state (Bertolotti et al., 2000; Carrara et al., 2015). Upon ER stress, BiP binds to misfolded proteins through its substrate binding domain, operating as an allosteric regulator to release the sensors and allow their dimerization (Carrara et al., 2015). While this model has been reconstituted *in vitro*, the involvement of BiP binding for the activation of IRE1α and PERK still relies on correlative evidence, and only one study has shown that ablation of the BiP binding site on IRE1 enhances its basal activation status (Oikawa et al., 2009) (Figure 3C). ATF6 also interacts with BiP at the ER, masking a binding site to coat protein-II (COP-II) vesicles (Shen et al., 2002). Thus, release of BiP under ER stress allows ATF6 translocation to the Golgi for further processing and activation. In yeast, a direct recognition model has been proposed, where IRE1 binds misfolded proteins as ligands through a groove similar to the major histocompatibility complex (MHC) I as demonstrated through structural and biochemical analysis (Gardner and Walter, 2011). In yeast, BiP/Kar2 may participate in setting the threshold for IRE1 activation/inactivation (Pincus et al., 2010) in addition to disassembling large IRE1 clusters (Ishiwata-Kimata et al., 2013) (Figure 2C). The direct recognition model for mammalian IRE1α has been debated because the recombinant lumenal domain of IRE1α does not bind misfolded proteins *in vitro* and the crystal structure of this region might not be compatible with the binding of a peptide to the groove because of space constrains (Kimata and Kohno, 2011). However, a recent study suggested that unfolded proteins may bind to IRE1α, inducing allosteric changes that trigger its oligomerization (Karagöz et al., 2017). Thus, in mammals, the activation of ER stress sensors may involve BiP, in addition to the binding of misfolded proteins to engage an optimal UPR response. Other ER chaperones also modulate the UPR. For example, the disulfide isomerase PDIA6 binds and controls the attenuation of IRE1α and PERK signaling, but not ATF6. At the molecular level, PDIA6 associates with IRE1, possibly through specific cysteines in its lumenal domain (Eletto et al., 2014; Groenendyk et al., 2014). Also, PDIA5 selectively regulates ATF6 activation (Higa et al., 2014), consistent with the fact that lumenal disulfide bonds in ATF6 modulate its translocation to the Golgi apparatus. Taken together, these findings suggest that a complex network of ER chaperones and foldases underlie the surveillance mechanism of ER proteostasis, connecting this sensing process with signal transduction events to enforce adaptive programs.

Chronic ER Stress and Apoptosis

If the adaptive responses fail to restore protein-folding homeostasis, UPR signaling continues to persist and eventually morphs into alternate signaling programs called the "terminal UPR" that ultimately promote apoptosis (Figure 2) (Shore et al., 2011). Ample evidence supports that the two UPR kinases, PERK and IRE1α, engage a distinct set of pro-apoptotic outputs that contribute to cell degeneration and death if ER stress cannot be resolved. For example, while a temporary pause in protein translation due to eIF2α phosphorylation can be beneficial by reducing secretory load, a protracted block in translation from sustained PERK signaling is incompatible with survival (Urra et al., 2013). Moreover, PERK hyperactivation can upregulate the CHOP/GADD153 transcription factor, which inhibits expression of the gene encoding anti-apoptotic BCL-2 to hasten cell death, in addition to enhancing the expression of pro-apoptotic BCL-2 members such as BIM, as well as improving protein synthesis and oxidative stress (Urra et al., 2013). Similarly, when hyperactivated by chronic ER stress, phosphorylated IRE1α assembles into high-order oligomers, allowing its RNase to acquire affinity for RIDD substrates and causing massive endonucleolytic decay of hundreds of these ER-localized mRNAs, which depletes ER cargo and protein-folding components to further worsen ER stress at later time points (Han et al., 2009; Hollien and Weissman, 2006). While the aforementioned studies also demonstrated that intermediate states are available to either PERK or IRE1α leading to divergent cell-fate outcomes, IRE1α oligomerization under irremediably high levels of ER stress has been shown to induce activation or upregulation of a number of pro-inflammatory and pro-apoptotic proteins. For example, when hyperactivated, IRE1α's RNase also reduces the levels of select microRNAs (possibly through directly cleaving their precursors at the ER membrane) that normally repress pro-apoptotic targets, such as pro-oxidant protein TXNIP (thioredoxin-interacting protein), leading to their rapid upregulation (Lerner et al., 2012; Upton et al., 2012). Increased TXNIP protein levels then activate the NLRP3 inflammasome and its caspase-1-dependent pro-death pathway, leading to sterile inflammation and pyroptotic cell death (Lerner et al., 2012) (Figure 2). Finally, sustained IRE1α activity may serve as an activation platform for apoptosis signal-regulating kinase 1 (A SK1) and its downstream target c-Jun NH2-terminal kinase (JNK) (Nishitoh et al., 2002; Urano et al., 2000), a process that may regulate apoptosis under unmitigated stress.

Many of the pro-death signals from the UPR sensors ultimately converge on the intrinsic apoptotic pathway, which is triggered when certain mitochondrial proteins, such as cytochrome *c*

and Smac/Diablo, are forcibly released into the cytoplasm where they lead to activation of downstream effector caspases (e.g., caspase-3) (Wang and Youle, 2009). The BCL-2 family, a large class of both pro- and anti-death proteins, governs this "intrinsic" apoptotic pathway, including ER stress, by regulating the integrity of the outer mitochondrial membrane. The intrinsic mitochondrial apoptotic pathway is engaged when cell injury leads to the expression and/or post-translational activation of one or more BH3-only proteins, a structurally diverse collection of pro-death proteins that contain a short alpha helix known as the BCL-2 homology 3 (BH3) domain necessary for cell death. At least four distinct BH3-only proteins (i.e., BID, BIM, NOXA, PUMA) become activated in the terminal UPR (Pihán et al., 2017). Each of these BH3-only proteins is activated by ER stress in a unique way. BIM, for example, is transcriptionally upregulated by PERK and its protein product is stabilized in response to ER stress (Puthalakath et al., 2007). Once activated, BH3-only proteins antagonize mitochondrial-protective proteins (e.g., BCL-2, BCL-X_L) or directly trigger the multidomain pro-apoptotic BAX and BAK proteins to permeabilize the outer mitochondrial membrane by a process referred to as MOMP (mitochondrial outer membrane permeabilization). The RIDD activity of IRE1α also controls the mRNA levels of caspase-2 by degrading a specific miRNA, resulting in the activation of BID to trigger MOMP (Upton et al., 2012). However, the role of caspase-2 in ER stress signaling is still debated (Sandow et al., 2014). Although it was reported that CHOP upregulates the death receptor DR5 to induce apoptosis via initiator caspase-8 (Lu et al., 2014a), a new report suggested that this pathway may not control cell death under ER stress (Glab et al., 2017). BOK is a poorly studied multi-domain BCL-2 family member. Recent studies identified BOK at the ER membrane, where it contributes to apoptosis in response to ER stress (Carpio et al., 2015). Under normal conditions, BOK is continually degraded by the proteasome through ERAD, whereas it is stabilized to induce MOMP when the UPR is altered (Llambi et al., 2016). Finally, necroptosis may also participate in cell death under ER stress (Estornes et al., 2014), a process classically linked to abnormal inflammation. Overall, the regulation of ER stress-induced apoptosis is not governed by a singular pathway and is instead the result of the interaction of many different mechanisms that may be even cell type specific (reviewed in Urra et al., 2013).

The UPRosome, Multiple Regulatory Checkpoints

The control of the temporal behavior of UPR signaling is fundamental in determining the fate of a cell under ER stress. Although the mechanisms explaining the transition from adaptive to a terminal pro-apoptotic UPR are not well established, several models have been proposed to explain how information about the intensity and duration of the stress stimuli is integrated. The ER lumenal domains of PERK and IRE1 are structurally similar and functionally interchangeable; however, their temporal behavior differs depending on the experimental system (Hetz, 2012). Under conditions of mild ER stress, activation of XBP1 mRNA splicing is transient, leading to its attenuation after prolonged stimulation, which may sensitize cells to undergo apoptosis (Lin et al., 2007). In contrast, the activity of RIDD under increased IRE1α oligomerization appears sustained over time, contributing to cell death (Ghosh et al., 2014). In the case of PERK, the levels of eIF2α phosphorylation are negatively controlled by two phosphatase complexes, the ER stress-inducible PPP1R15A/GADD34 (a target of ATF4/CHOP) form and the constitutive phosphatase PPP1R15B/CReP (Hetz et al., 2013).

Initially, the field viewed UPR signaling as a direct and linear transduction of ER stress levels. However, recent findings have indicated that the three major UPR sensors are tightly regulated through post-translational modifications and the binding of co-factors. For instance, the concept of the UPRosome was proposed to envision IRE1α as a scaffold where many components assemble dynamically to regulate its signaling outputs (Hetz and Glimcher, 2009). The UPRosome may also serve as a platform to enable the crosstalk between the UPR and other signaling pathways operating as a scaffold (Figure 4A). For example, the binding of IRE1 to TRAF2 activates the JNK and autophagy pathways (Castillo et al., 2011), whereas its interaction with the adaptor proteins Nck engages nuclear factor κB (NF-κB) (Nguyên et al., 2004). Several factors physically associate with IRE1α at basal levels or by an ER stress-dependent manner, modulating specific molecular events involved on IRE1α signaling. Interactions with Nonmuscle myosin-IIB (NMIIB) and the actin cytoskeleton contribute to stabilizing IRE1α clusters for optimal activation (He et al., 2012). Similarly, the ABL kinase family members—c-Abl and Arg—scaffold and rheostatically hyperactivate IRE1α RNase at the ER membrane to promote apoptosis (Morita et al., 2017). Binding to N-MYC Interactor (NMI) specifically enhances JNK activation without affecting XBP1 mRNA splicing levels (Brozzi et al., 2014). The kinetics and amplitude of IRE1α signaling are also controlled by the binding of positive and negative regulators (Figure 4A), highlighting several proteins previously linked to apoptosis, including members of the BCL-2 family (Hetz et al., 2006). The BH3-only proteins BIM and PUMA selectively enhance the sustained signaling of IRE1α during the attenuation phase possibly in complex with BCL-2 (Rodriguez et al., 2012). In contrast, the ER-located protein BAX inhibitor-1 (BI-1) interacts with the cytosolic domain of IRE1α accelerating its attenuation after prolonged ER stress (Lisbona et al., 2009). Other proteins, such as Fortilin, were recently shown to inhibit IRE1α signaling through a direct interaction, increasing cell death resistant under ER stress (Pinkaew et al., 2017).

Several post-translational modifications are reported at the level of IRE1 and PERK (Figure 4A). IRE1 phosphorylation by protein kinase A (PKA) can engage its RNase domain in the absence of ER stress (Mao et al., 2011), whereas its dephosphorylation could be mediated by PP2A (Qiu et al., 2010) and the ER phosphatase PP2Ce (Lu et al., 2013). PARP16 has been also shown to ADP-rybosylate IRE1 and PERK, enhancing their activities even in the absence of ER stress (Jwa and Chang, 2012). Besides, S-nitrosylation of a specific cysteine of IRE1 inhibits its RNase activity, whereas it can enhance PERK signaling (Nakato et al., 2015). The E3 ligase CHIP induces IRE1α ubiquitination at Lys(545) and Lys(828), selectively affecting IRE1α phosphorylation and TRAF2 binding/JNK activation (Zhu et al., 2014). Finally, the control of IRE1α stability is also relevant to adjust the UPR. DDRGK1 is a critical component of the ubiquitin-fold modifier 1 (Ufm1) system, which was recently shown to regulate the stability of IRE1α (Liu et al., 2017). In addition, IRE1α levels are set by

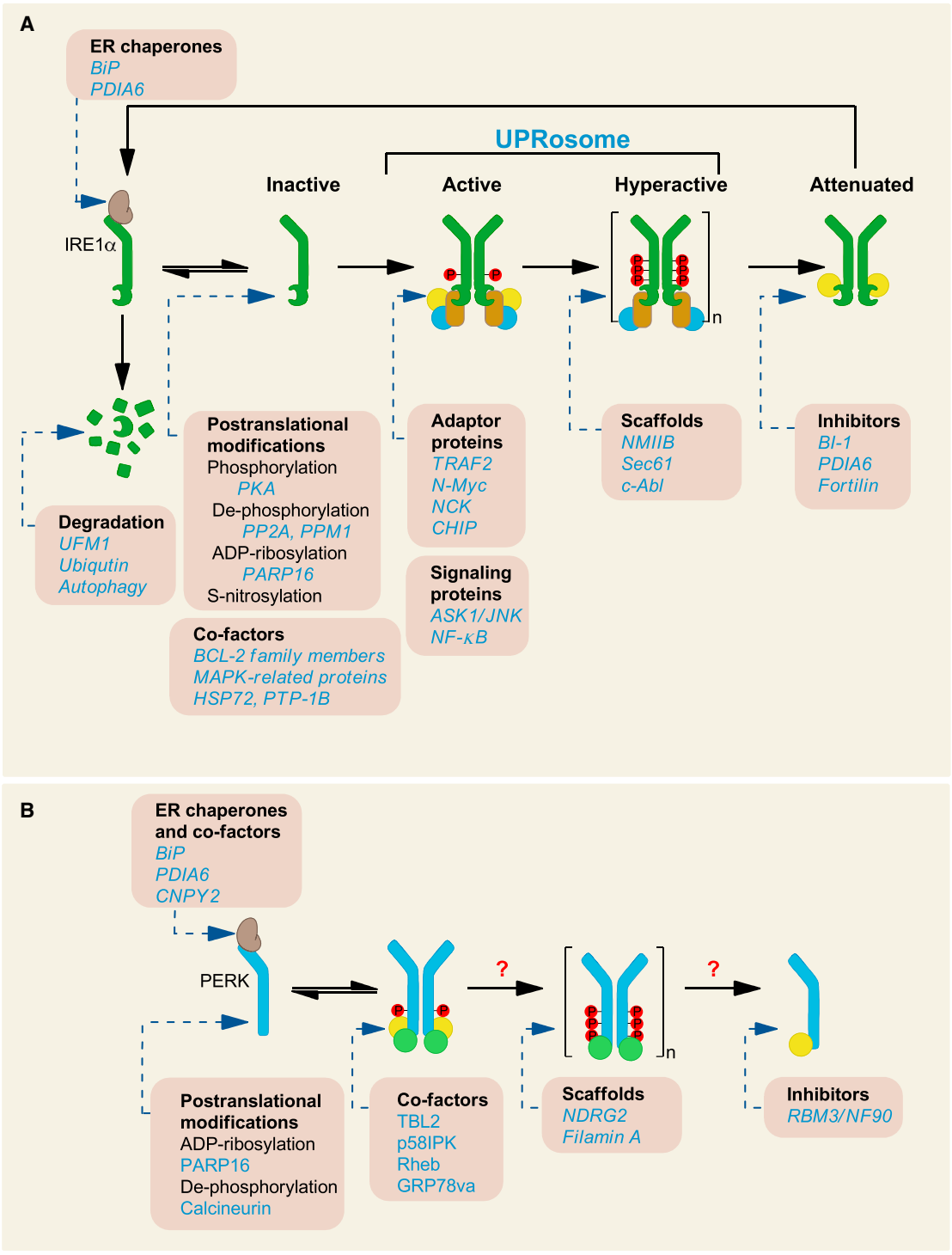

Figure 4. The UPR, Multiple Regulatory Checkpoints

Fine-tuning IRE1 (A) and PERK (B) signaling. The activity of UPR stress sensors is regulated through the binding of co-factors and post-translational modifications that modulate the amplitude of their downstream signaling and the kinetics of activation and attenuation, in addition to their protein stability. In addition, the binding of specific factors may regulate the assembly and stabilization of large clusters. The assembling of distinct UPRosomes may also mediate the crosstalk of the UPR with other signaling pathways and the control of novel stress-independent functions.

an interaction with the selective autophagy receptor optineurin (Tschurtschenthaler et al., 2017). In addition, another study suggested that IRE1α levels are also controlled by the ERAD pathway, a process that unexpectedly depends on BiP (Sun et al., 2015). It was proposed that, under ER stress, BiP is released and IRE1α is stabilized (Figure 2C). Thus, multiple checkpoints regulate IRE1α signaling through distinct protein-protein interactions, setting the threshold of stress for its activation.

IRE1α signaling is also tuned at the level of XBP1 by post-translational modifications, including ubiquitination, acetylation, and phosphorylation (Hetz et al., 2015; Liu et al., 2016). XBP1u also operates as a negative regulator of XBP1s and ATF6f by forming a complex that is degraded by the proteasome (Oda et al., 2006; Yoshida et al., 2009). Interestingly, XBP1u has been proposed to be one of the most unstable proteins in the proteome (Tirosh et al., 2006). XBP1u turnover is mediated in part by the generation of a type II transmembrane domain, allowing its degradation by the ERAD pathway and processing by the signal peptide peptidase (SPP) (Chen et al., 2014a).

Although less explored, PERK (Figure 4B) and ATF6 are also differentially regulated. The activity of PERK is modulated by binding to p58IK and a cytosolic variant of BiP known as Grp78va (Ni et al., 2009; Yan et al., 2002). The ER transmembrane protein Transducin b-like2 (TBL2) associates with phosphorylated PERK in addition to eIF2α, reducing protein translation (van Huizen et al., 2003). Similarly, N-myc downstream-regulated gene-2 (NDRG2), canopy homolog 2 (CNPY2), and the small GTPase Rheb can bind to PERK to enhance its signaling (Hong et al., 2017; Tyagi et al., 2015; Zhang et al., 2017). Another study suggested that the cold-inducible RNA-binding protein 3 (RBM3) inhibits PERK signaling through an association with nuclear factor 90 (NF90) (Zhu et al., 2016). Interestingly, a recent screen revealed an interaction between PERK and the actin crosslinking enzyme Filamin A, which mediates the assembly of ER and plasma membrane contact sires (van Vliet et al., 2017). The dephosphorylation of eIF2α is controlled by interactions with γ-actin and its phosphatases (Chambers et al., 2015; Chen et al., 2015). The function of ATF6 is also regulated by phosphorylation, proteasome-mediated degradation, glycosylation, and reduction of luminal cysteines (Hetz et al., 2015). Overall, these selected findings illustrate the dynamic and complex nature of UPR signaling, where several checkpoints are established to determine downstream signaling responses. However, it is important to highlight the fact that most of the data discussed in this section rely on single studies, and the generality of those observations needs to be further validated. Systematic interactome screens are still needed to define the composition of the UPRosome and the dynamic nature of its assembling under ER stress conditions in different cell types. We predict that distinct UPRosome complexes may exist at the level of the three major UPR stress sensors.

The UPR in Physiology and Disease

The generation of genetically modified mice for proximal UPR components has revealed multiple activities of the pathway in different organs. Due to the fundamental role of the UPR in controlling protein folding, abnormal levels of ER stress have been associated with a variety of pathologies involving specialized secretory cells, in addition to diseases linked to protein misfolding and aggregation (Oakes and Papa, 2015), highlighting cancer, autoimmunity, diabetes, obesity, and neurodegeneration. Overall, despite the fact that the field has relied on the use of pleiotropic pharmacological strategies to perturb ER function, since the beginning of the year 2000, accumulating evidence supports a fundamental role of the UPR in sustaining the activity of specialized secretory cells. In fact, the biological function of XBP1 was discovered from studies in the immunology field before it was linked to IRE1α and the UPR. XBP1 deficiency in plasma B cells has a profound effect on their differentiation into secretory cells, ablating the secretion of immunoglobulins and the morphological changes associated with their stimulation (Reimold et al., 2001). Although it was speculated that the stress generated by expressing large amounts of antibodies was the signal to activate the IRE1α/XBP1 pathway and transit into a new homeostatic state, experiments in cells lacking immunoglobulin revealed that the establishment of a differentiation program by XBP1 was initiated via signaling through the B cell receptor (Hu et al., 2009). Genetic ablation of XBP1 expression has pleiotropic effects in secretory organs, impacting activity of the endocrine and exocrine pancreas, in addition to salivary glands (for this section, see specific articles in Cornejo et al., 2013). Studies to identify the universe of XBP1s target genes uncovered a direct link with a central controller of cell differentiation, the transcription factor MIST1 (Acosta-Alvear et al., 2007), impacting gastric zymogenic cell physiology (Huh et al., 2010).

XBP1-deficient animals die during embryogenesis due to severe liver dysfunction, resulting in anemia. In adult mice, XBP1 and IRE1α have distinct roles in controlling lipid synthesis and cholesterol metabolism. Although XBP1 has been widely studied on a variety of tissues, the generation of IRE1α-null mice revealed unanticipated findings. Reconstitution of IRE1 in the placenta fully bypassed the embryonic lethality of the full knockout mice (Iwawaki et al., 2009). Unexpectedly, using this strategy, a viable animal was generated with overall minor defects in secretory organs, observing partial reduction in plasma immunoglobulin levels and subtle changes in blood glucose levels (Iwawaki et al., 2010). The authors speculated that XBP1-dependent/IRE1α-independent functions might exist since the phenotypes of XBP1- and IRE1α-deficient tissues do not fully match. Since IRE1α has additional activities (i.e., RIDD, JNK, NF-κB control), assessing its functional relevance *in vivo* is challenging. In fact, recent advances in the field also suggest that part of the phenotypes described in XBP1-deficient animals are due to an overactivation of RIDD (Dufey et al., 2014), reflecting the complex regulatory feedbacks underlying UPR signaling.

Many other studies have revealed important functions of the UPR in the control of immune cell function and differentiation, including dendritic cells and macrophages, where XBP1 regulates various aspects of their biology (Bettigole and Glimcher, 2015). XBP1 was also shown to control intestinal epithelial cell function, where its deficiency results in chronic inflammation resembling Crown disease (Kaser et al., 2008). In the case of ATF6 alpha, few data are available about its physiological role. ATF6 alpha and beta have related function as illustrated by the

fact that double deficient animals are embryonic lethal (Yamamoto et al., 2007). ATF6 alpha deficiency in mice leads to altered retinal function, which is consistent with the recent discovery of ATF6 mutations in human achromatopsia (Kohl et al., 2015). Many other activities of the UPR have been described in organ physiology and are reviewed elsewhere (Cornejo et al., 2013; Wang and Kaufman, 2016).

IRE1α was identified as the fifth-most mutated kinase in cancer, with the reported mutations leading to hypomorphic variants in which the RNase retains XBP1 mRNA splicing ability while disabling RIDD (Chevet et al., 2015; Ghosh et al., 2014). Genetic and pharmacological inhibition of PERK or IRE1 signaling demonstrated their essential activity in driving tumor growth. Beyond providing a selective capacity for malignant cells in solid tumors, recent findings suggest that the UPR may affect all the cardinal hallmarks of cancer, including angiogenesis, metastasis, genome stability, inflammation, and drug resistance (Urra et al., 2016). Interestingly, XBP1 was shown to physically interact with hypoxia-inducing factor 1 (HIF1), contributing to tumor angiogenesis (Chen et al., 2014b). XBP1 may also accelerate the progression of ovarian carcinoma by attenuating anti-tumor responses associated with altered lipid metabolism (Cubillos-Ruiz et al., 2015).

Extensive studies have implicated maladaptive UPR signaling in experimental and common forms of diabetes mellitus. A myriad of rodent models of ER stress-induced β cell degeneration have proven the principle that premature β cell loss from dysregulated UPR signaling is a causative insult leading to diabetes (Papa, 2012; Wang and Kaufman, 2016). Studying these rare diseases' forms may inform the understanding of pathophysiology in common human diabetic syndromes (i.e., types 1 and 2). Indeed, dysregulation of PERK and IRE1α signaling has critical consequences for β cell survival. A striking example of UPR dysregulation in diabetes is evidenced in PERK-deficient animals (Delépine et al., 2000; Harding et al., 2000). PERK and XBP1 knockout mice also develop pancreatic exocrine insufficiency and exhibit growth defects early in life, digesting global defects in different types of professional secretory cells. Interestingly, a rare human diabetic syndrome caused by *PERK* gene mutations (called Wolcott-Rallison syndrome) is a phenocopy of the mouse *PERK* gene knockout. The genetic removal of *Xbp1* in β cells triggers IRE1 hyperactivation, resulting in cell degeneration and hyperglycemia (Lee et al., 2011).

The accumulation of abnormal protein aggregates is a hallmark of several neurodegenerative diseases, including Alzheimer, Parkinson, and Huntington's diseases and amyotrophic lateral sclerosis (ALS), among other conditions. The activity of the UPR has been extensively studied in most of these diseases, including human postmortem tissue, animal models, and induced pluripotent stem cell (iPSC)-derived human neurons (Smith and Mallucci, 2016). Overall, a direct correlation has been established between the progression of the diseases, histopathological alterations, protein aggregation, and the occurrence of ER stress. Targeting selective UPR components has revealed a complex and unexpected involvement of the pathway in neurodegeneration (Hetz and Saxena, 2017). Three major UPR outputs may contribute to brain disease: (1) proteostasis enhancement by reducing ER stress levels (adaptive programs), (2) a pro-degenerative pathway triggering neuronal loss, and (3) impairment of synaptic function through protein translational repression. In the context of basal brain function, XBP1 has a relevant contribution to synaptic plasticity, improving learning and memory-related processes by *trans*-activating brain-derived growth factor (BDNF) expression (Martínez et al., 2016). Because of their large capacity to synthetize myelin sheets, oligodendrocytes and Schwann cells are the major cell types of the nervous system where the UPR may have physiological relevance (Lin and Popko, 2009). In fact, studies using multiple sclerosis and spinal cord injury models indicated that UPR signaling operates as a barrier to avoid the detrimental consequences of chronic ER stress. Recent advances in the field led to the identification of selective compounds that block specific components of the UPR with outstanding therapeutic effects in disease models. For example, inhibition of PERK or IRE1α with small molecules impairs tumor growth, whereas targeting PERK or eIF2α phosphorylation has positive consequences in the progression of several neurodegenerative diseases (Hetz et al., 2013; Maly and Papa, 2014). In addition, IRE1α inhibitors can delay the progression of, or even reverse, diabetes in diverse rodent models, including those caused by proinsulin misfolding ("Akita") or autoimmune islet infiltration (non-obese diabetic [NOD] mouse) (Morita et al., 2017). Chronic ER stress has been also linked to fibrosis in different tissues, which involves the abnormal accumulation of collagens (Lenna and Trojanowska, 2012). Remarkably, recent studies using medaka fish have demonstrated that the main source of physiological ER stress when the UPR is impaired is the misfolding and deposition of collagens (Ishikawa et al., 2013; Ishikawa et al., 2017). This observation is consistent with the fact that collagens are the main cargo of the secretory pathway, involving a highly complex folding process. Overall, increasing evidence supports the involvement of the UPR in many diseases, playing distinct roles depending on the disease type and the signaling branch engaged.

Concluding Remarks

The UPR is a quality control pathway with binarily opposite cell-fate outputs. In response to ER stress, the UPR initially engages adaptive outputs that decrease the load of unfolded proteins and buttress the capacity of the secretory pathway in an effort to restore ER homeostasis. However, under irremediable ER stress, the UPR in mammals assembles into a signaling platform that transduces pro-inflammatory and pro-death signals to hasten cell demise. Both outputs can be reconciled as beneficial to multi-cellular organisms during evolution; however, in our modern era, chronic disease may dysregulate UPR circuits leading to cell degeneration. Importantly, UPR signal transduction into specific outputs is tightly regulated by the assembly of specific UPRosome complexes that fine-tune the amplitude and kinetics of UPR signals and mediate the crosstalk with other stress pathways. As we have detailed, cell injury from chronic ER stress is emerging as central to the pathophysiology of a wide range of prevalent human diseases. Thus, recent advances in our understanding of how the UPR switches from life to death signaling may lead to new strategies to combat these ER stress-associated diseases. The identification of novel small-molecule

modulators of the IRE1α and PERK kinases (Maly and Papa, 2014), as well as more recently for ATF6 (Gallagher et al., 2016; Plate et al., 2016), offers interesting opportunities to dissect the contribution of these signaling proteins to pathogenesis in myriad of diseases. As such, future studies will (1) be enabled by optimizing compounds directed toward these master UPR regulators to determine whether the pathway is a viable target for disease modification and (2) further lead compound optimization to serve as starting points for first-in-class series of UPR-modulatory drugs. Finally, combinatorial therapies to modulate ER proteostasis may emerge in the future as a useful means to ameliorate the critical downstream terminal signaling events that are linked to cell demise in myriad human diseases.

ACKNOWLEDGMENTS

We thank Dr. Hery Urra for figure designs and conceptualization. This work was funded by FONDECYT no. 1140549, FONDAP program 15150012, Millennium Institute P09-015-F, and European Commission R&D MSCA-RISE #734749 (C.H.). We are also thankful for the support from the Michael J. Fox Foundation for Parkinson's Research – Target Validation grant no. 9277, FONDEF ID16I10223, FONDEF D11E1007; U.S. Office of Naval Research-Global (ONR-G) N62909-16-1-2003; U.S. Air Force Office of Scientific Research FA9550-16-1-0384, ALSRP Therapeutic Idea Award AL150111; the Muscular Dystrophy Association 382453; and CONICYT-Brazil 441921/2016-7 (C.H.). This work was also supported by NIH RO1DK080955 (F.P.) and RO1 DK100623 (F.P.), Burroughs Wellcome Fund (F.P.), Juvenile Diabetes Research Foundation (JDRF) #2-SRA-2016-234-S-N (F.P.), and Breakthrough Therapeutics Initiative from Leona M. and Harry B. Helmsley Charitable Trust (F.P.).

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