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Emerging functions of the unfolded protein response in immunity

Sophie Janssens[1,2,3], **Bali Pulendran**[4], and **Bart N. Lambrecht**[1,2,3,5]

[1]Unit Immunoregulation and Mucosal Immunology, VIB Inflammation Research Centre, Belgium [2]GROUP-ID Consortium, Ghent University and University Hospital, Belgium [3]Department of Respiratory Medicine, Ghent University, Belgium [4]Emory Vaccine Center, Yerkes National Primate Center, Department of Pathology, Emory University [5]Department of Pulmonary Medicine, Erasmus MC, Rotterdam, The Netherlands

Abstract

The unfolded protein response (UPR) has traditionally been viewed as an adaptive response triggered upon accumulation of unfolded proteins in the endoplasmic reticulum (ER), aimed at restoring ER function. The UPR can also be an anticipatory response that is activated well before the disruption of protein homeostasis. UPR signaling intersects at many levels with the innate and adaptive immune response. In some immune cell types like dendritic cells and B cells, particular UPR sensors appear constitutively active in the absence of traditional UPR gene program induction, necessary for antigen presentation and immunoglobulin synthesis. The UPR also influences Toll-like receptor signaling and NF-κB activation, and some pathogens subvert the UPR. This review summarizes these emerging non-canonical functions of the UPR in immunity.

The endoplasmic reticulum (ER) is the production and folding factory of secreted and transmembrane proteins of the cell. It is well adapted for this function and performs complex protein modifications, such as glycosylation and disulfide bond formation[1]. Quality control ensures that only properly folded proteins exit the ER via the secretory pathway, while improperly folded proteins exit the ER through ER-associated degradation (ERAD) or via autophagy[2]. Depending on the physiological and environmental demand, the protein production rate in the ER can increase very rapidly[3]. An imbalance between the folding load of nascent proteins entering the ER and the capacity of the ER to handle this load causes ER stress. This is detected by three sensors that face the ER lumen: inositol-requiring enzyme 1 (IRE1 also known as ERN1, ER to nucleus signaling 1), protein kinase R-like ER kinase (PERK) and activating transcription factor 6 (ATF6) (Fig. 1). They trigger the unfolded protein response (UPR), an adaptive response aimed at restoring protein-folding homeostasis by three main mechanisms: transient reduction in protein translation[4, 5, 6]; increase in the folding capacity and ERAD[7]; and initiation of programmed cell death, when ER stress cannot be resolved[8].

Correspondence: Bart N. Lambrecht, MD, PhD, VIB Inflammation Research Center, Ghent University, Technologiepark 927, B-9052 Zwijnaarde, Belgium, Phone: +32-9-33213607, Fax: +32-9-2217673, bart.lambrecht@dmbr.vib-ugent.be.

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IRE1α is a bifunctional type I transmembrane protein that harbors a serine/threonine kinase domain and a unique ribonuclease (RNAse) domain in its cytoplasmic domain. The luminal domain senses unfolded proteins and is responsible for high-order oligomerization of IRE1α. Oligomerization activates the RNase domain, which cleaves *XBP1* (X-box binding protein) mRNA at two discrete stem loop structures through an unconventional cytoplasmic splicing reaction[9, 10]. The resulting fragments are ligated by an as yet unidentified – at least in mammals – RNA ligase, yielding an active transcription factor of the bZIP family, XBP-1s[9, 10]. XBP-1s plays a major role in the induction of genes involved in lipid biosynthesis, ERAD and chaperone production[11, 12, 13, 14]. In *Saccharomyces cerevisiae*, the *XBP1* homolog *HAC1* mRNA is the only known substrate of IRE1. In fission yeast (*S. pombe*) and metazoans however, the IRE1 has more promiscuous endonuclease activity, cleaving many mRNAs that are localized to the ER membrane[6, 15]. IRE1 dependent decay of mRNAs (termed RIDD) presumably represents another mechanism for the attenuation of protein translation[5]. Upon oligomerization, IRE1α autophosphorylates via the activity of its kinase domain. The physiological significance of this is unclear, as only nucleotide binding and not phosphotransfer appears to be required for RNase activity[16], although phoshorylation may drive further oligomerization[17]. Alternatively, phosphorylated sites in IRE1α might serve as docking sites for TRAF2 and enable ER stress-induced JNK activation[18]. Mammals have a second isoform of IRE1[19]. Unlike IRE1α, IRE1β does not cleave XBP1 mRNA and its expression is restricted to epithelial cells lining the gut and lung[20, 21, 22], where it may control RIDD[22, 23, 24].

The ATF6 sensor is a type II transmembrane protein with its carboxy-terminus facing the ER lumen and a bZIP transcription factor in its amino-terminus[25, 26] (Fig. 1). In response to ER stress, ATF6 is transported via COPII vesicles to the Golgi apparatus where it undergoes regulated intramembrane proteolysis by sequential cleavage by site 1 and site 2 proteases (S1P and S2P) in a manner reminiscent of SREBP transcription factor activation[27]. This releases the amino-terminal transcription factor fragment pATF6-N that moves to the nucleus and directs expression of UPR genes involved in ER membrane expansion, the ERAD pathway and folding[26, 28, 29]. Several other bZIP transcription family members, such as OASIS or CREB3, are related to ATF6 and are also cleaved by regulated intramembrane proteolysis[30]. Some of them such as CREBH, do not trigger genes that enhance capacity of the ER but rather trigger acute phase proteins, representing an intriguing link between ER stress and inflammation[31].

Finally, PERK, is another type I transmembrane protein kinase that shares about 20% similarity in its luminal domain with IRE1[4, 32]. Unlike IRE1, PERK has a well-established cytoplasmic kinase substrate, the eukaryotic initiation factor 2 alpha (eIF2α)(Figure 1)[33]. Phosphorylation of eIF2α leads to an inhibition of the guanine exchange factor eIF2B that recycles eIF2 to its active GTP bound form. As such, there is a delay in ternary complex formation and a strong reduction in cap-dependent translation, which is thought to be essential for cell survival in conditions of ER stress[34, 35]. A few transcripts with short upstream open reading frames (uORFs) in their 5’ UTR escape inhibition of translation under conditions of high eIF2a phosphorylation and mediate a PERK-dependent transcriptional response[33]. ATF4 is a bZIP transcription factor induced in this manner.

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ATF4 directs the expression of CHOP, a proapoptotic factor[33] and GADD34, a negative feedback regulator of eIF2α phosphorylation[36]. Additionally, ATF4 coordinates a gene program needed for amino acid metabolism, glutathione biosynthesis and resistance to oxidative stress[37]. The latter pathway was discovered based on its homology to the amino acid starvation control response in yeast ([38] and Box 1).

Detection of stress and activation of the UPR

Facing the ER lumen with their sensor domains are PERK, ATF6 and IRE1 which can sense ER stress in different ways. Originally, it was proposed that ER stress was perceived as a drop in the amount of free BiP (binding immunoglobulin protein, also known as Grp78 or Hspa5), the most abundant ER chaperone[43, 44]. BiP binds all three sensors but detaches upon ER stress to assist the folding of nascent proteins[43, 44]. Consistent with this model, release of BiP would be sufficient to allow formation of high molecular mass complexes of activated PERK and IRE1[43]. However, there were a few limitations to this model[45, 46] and alternatively, it was suggested that unfolded proteins bind IRE1 directly. Crystallization of yeast IRE1 revealed the presence of a peptide-binding groove in the luminal domain of active oligomerized IRE1 that was very reminiscent of the one present in MHC-molecules[47]. Binding of unfolded proteins to the inactive conformation triggered a conformational change in the sensor domain, opening the peptide groove and inducing oligomerization[16, 48]. In this latter model, it is thought that the function of BiP is to keep PERK and IRE1 in a free, monomeric state (reviewed in[16]). The crystal structure of the PERK luminal domain has not been solved yet, but based on structural and functional homology with IRE1, PERK could also be activated by higher order oligomerization[49]. How ATF6 senses unfolded proteins remains as yet unknown. BiP dissociates from ATF6 upon ER stress[44] by an active regulatory mechanism, and this leads to a conformational change from an oligomeric to monomeric form[50].

The UPR as a part of normal cellular physiology

While most of the signaling cascades of the UPR have been unraveled in conditions of ER stress - defined as an excess of client load to folding capacity – most likely this does not represent the prime function of the UPR[51]. Especially in vertebrates with complex secretory functions, the UPR provides flexibility to ensure the multitude of ER functions across a wide range of physiological demands. If the UPR would be launched only after misfolded proteins accumulate in the ER, this would be a highly inefficient system, causing unnecessary impairment of the ER before homeostasis is restored. An anticipatory response would be more desirable, especially when high ER client load handling is part of normal physiology[51]. In some immune cells, activation of the UPR is part of the normal differentiation program of a cell. The differentiation of antigen triggered B cells into antibody-producing plasma cells requires the expansion of the ER membrane, a response previously assumed to be triggered by the accumulation of unfolded heavy chains[52]. However, more recent data showed that the activation of the UPR occurs before the onset of Ig chain synthesis[53] and even in the (engineered) absence of Ig molecules altogether[54]. Reporter gene studies *in vivo* showed constitutive activation of the IRE1 pathway in specific cell types, like $CD8\alpha^+$ dendritic cells and developing B and T cells without any sign of

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activation of any of the other UPR cascades[55, 56]. Similarly, activation of Toll-like receptors (TLR) impinges on UPR signaling cascades[57, 58]. This most likely reflects an anticipatory response of the cell to prepare the ER to combat infection. It is at present still unclear how IRE1 or other UPR sensors are activated in these conditions, however perturbations in the composition of the ER lipid bilayer have been demonstrated to directly trigger IRE1 and PERK independently of their luminal sensor domain[59]. In addition, the flavonoid component quercetin activates IRE1 by binding to a pocket at the dimer interface of the RNase domain[60]. Thus, alternative ways to trigger ER stress sensors exist. These act independently of defects in protein folding homeostasis.

The UPR shows conserved functions in immune responses

As IRE1 is the only UPR sensor in yeast, and HAC1 its only known target, it was long assumed that the most ancient function of the UPR was a transcriptional response intended to increase ER folding capacity. However, further data challenged this concept. In most fungi, the IRE1-HAC1 branch is conserved (Fig. 2)[61]. However in *S. pombe*, no *HAC1* gene could be found and activation of IRE1 mainly leads to RIDD. RIDD may thus be the ancestral function of IRE1[15]. In pathogenic fungi, a fully competent UPR is linked to pathogen virulence, facilitating secretion of toxic compounds or mediating adaptation of the fungus to a host microenvironment that triggers a UPR[62].

In plants, the ATF6 and IRE1-XBP1 branch of the UPR appear to be conserved, while PERK is absent. In *A. thaliana*, the transcription factor bZIP60 is spliced by one of two IRE1 homologs at two stem-loop structures, which leads to the removal of a transmembrane domain and the nuclear localization of bZIP60[63]. Two ATF6 like factors, bZIP28 and bZIP17 are cleaved by S1P and S2P in response to ER stress and mediate the induction of BiP[64]. In addition, ER stress-induced downregulation of mRNAs has been observed[65], and this RIDD-like mechanism was suggested to compensate for the lack of translational regulation by PERK. Intriguingly, in plants the UPR is not only activated in response to increased ER client protein load (e.g. during seed development), but also in response to abiotic stress, like heat shock or salt stress. In addition, IRE1 and bZIP60 is activated in response to plant pathogens and is required for antibacterial defense[66, 67]. Also in plants, the UPR response is anticipatory. During plant pathogen responses, foldases and chaperones are produced before defensive hydrolases are secreted[68].

In *Caenorhabditis elegans*, the three branches of the UPR are conserved[69]. The IRE1-XBP-1 branch is specifically triggered by p38 homolog pmk in response to infection and is essential for larval survival in response to pathogens[70]. XBP-1 did not control the infection itself but rather was needed to prevent the detrimental effect of an overwhelming innate immune response on ER fitness. This suggests an evolutionarily-conserved role of the IRE1-XBP-1 arm to mediate protection against ER toxicity caused by inflammatory pathways[71].

While the different branches of the UPR became fully established at the metazoan level only in the face of increasing complexity in the secretory functions of the ER, the integrated stress response is much older[34, 61]. Translational control as a proper response to nutrient deprivation was already established in yeast[34], and most of the UPR sensors developed from

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more ancient metabolic regulators. As such, the PERK sensor is closely related to the GCN2 and GCN4 systems in unicellular eukaryotes and the regulated intramembrane proteolysis of ATF6-like factors is very similar to the system in which SREBP transcription factors control cholesterol metabolism[27, 37].

Intriguingly, the integrated stress response and the UPR appear closely intertwined with host immune responses. Several microarray studies have shown a marked, though partial, overlap between genes induced by the UPR or ISR and genes induced by microbial infection or stimulation with TLR ligands[72, 73]. Compelling data on the activation of the ISR by bacterial[72] or viral[74] infection showed that metabolic responses controlling translation and autophagy most likely represent an ancient host defense response against invasive pathogens, that might predate the development of more dedicated pattern recognition receptors belonging to the NLR or TLR family[72, 75].

The IRE-1-XBP-1 branch in differentiation of immune cells

In cells that have mainly a secretory function it is no surprise that genetic deletion of UPR sensors or their signaling intermediates also affects the cellular differentiation process. The analysis of PERK deficient mice revealed a crucial role for the UPR in exocrine pancreas acinar cells, endocrine pancreas insulin producing β-cells, chondrocytes and osteoclasts[76, 77]. XBP-1 plays a similar regulatory role in the development of exocrine pancreas acinar cells and intestinal Paneth cells[78, 79]. In the immune system, XBP-1 is essential in the terminal differentiation of B cells to plasma cells. It controls the expansion of the ER and its secretory function to enable the massive increase in immunoglobulin synthesis[80,81]. In B cells, the absence of XBP-1 led to hyperactivation of IRE-1, triggering RIDD and decay of secretory immunoglobulin μ mRNA, further curtailing IgM responses[82]. XBP-1 turned out to be downstream of Blimp-1 and IRF4, and was proposed to be the central hub in the physiological UPR of plasma cells[11].

Further studies with RAG-2 blastocyst complementation systems showed that XBP-1 is also needed for the development and survival of other immune cell types, such as splenic conventional and plasmacytoid dendritic cells (cDCs and pDCs respectively)[83]. By making use of an IRE-1 activity reporter mouse (termed ERAI[84]), one study could confirm the constitutive but subset-specific activation of the IRE1 branch in CD8α^+ cDCs[55]. Splenic macrophages and NK cells also activate the IRE1-XBP-branch at baseline, while naive T and B cells, monocytes and neutrophils do not show any basal IRE1 activity[55]. The physiological role of IRE1-XBP-1 in certain DC populations at baseline is still unclear. Spontaneous UPR could be linked to the capacity of pDCs to produce type I interferon[83]. In CD8α^+ cDCs, it could be related to antigen cross-presentation, as well as to an adaptive strategy to combat infection[55].

The UPR triggers inflammation

Defects in protein folding, either environmentally induced or caused by genetic defects in individual branches of the UPR, spontaneously induce an inflammatory phenotype. This has been described particularly in models of inflammatory bowel disease (IBD)[71], metabolic disease[85] or lung respiratory disease[86]. Loss of XBP-1 in intestinal epithelial or Paneth cells

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leads to enteritis, associated with strongly increased nuclear factor κB (NF-κB) activation. This is dependent on the hyperactivation of IRE-1 (and consequent RIDD), rather than on the loss of XBP-1[71]. Interestingly, polymorphisms in both *XBP1* and *AGR2*, a protein disulfide isomerase needed for mucin folding, have been found in Crohn's disease and Ulcerative Colitis patients, suggesting that unresolved ER stress due to improper functioning of major UPR branches could indeed contribute to the inflammatory pathology typically observed in IBD[87]. This and many other studies, especially in diabetes[85, 88], have led to the paradigm that dysregulated UPR signaling underlies chronic low-grade inflammation[89].

The UPR intersects at various levels with inflammatory pathways, such as reactive oxygen species (ROS) production, and the activation of NF-κB, c-Jun N-terminal kinase (JNK), and IRF3 (Fig, 3 and Fig. 4)[90]. Activation of PERK and concomitant translational inhibition leads to a disequilibrium in the ratio of the short-lived IκB protein (inhibitor of NF-κB) to the longer-lived NF-κB protein, resulting in activation of NF-κB, independent of IκB phosphorylation[91]. On the other hand, IRE-1 directly triggers IκB kinase and as such IκB phosphorylation, in a TNF receptor associated factor (TRAF)-2 dependent manner[92, 93], while ATF6 activates NF-κB via AKT phosphorylation[94]. IRE-1 also mediates phosphorylation of JNK in a TRAF2-ASK dependent pathway[18], which was linked to insulin receptor substrate (IRS)-1 phosphorylation and the development of insulin resistance in type II diabetes[95]. ER stress and lipids also trigger the eIF2α-kinase PKR that coordinates activation of JNK and IRS-1 phosphorylation in a complex termed the 'metaflammasome'[85, 96]. Also other innate immune pathways leading to IRF3 activation become activated in response to ER stress[97].

There is limited evidence that the UPR by itself is sufficient to trigger production of inflammatory mediators such as interleukin-6 (IL-6), IL-8 or the proangiogenic factor VEGF. This appears highly cell type specific and can be mainly observed in cancer cells, epithelial or endothelial cells[98, 99, 100]. In DCs, the induction of IL-6 by tunicamycin was observed at the transcriptional but not at the protein level (Tavernier *et al*., unpublished data). Increased production of IL-1β upon UPR activation has been suggested to rely on IRE-1α mediated degradation of the micro RNA miR-17, causing the stabilization of thioredoxin interacting protein (TXNIP), and activation of the NLRP3 inflammasome[101].

Several reports have shown that cytokine secretion by stimulation of the UPR requires a second signal[57, 102]. Concomitant activation of the UPR clearly amplifies the cytokine response induced by several TLR ligands[57, 73, 103, 104, 105]. According to the specific stimulus, this synergism depends on the IRE1-XBP-1s axis[57], CHOP[103] or the ATF4-GADD34-axis[73]. Synergism can be regulated at the transcriptional level, entailed by binding of XBP-1s[106] or CHOP[103] to particular cytokine promoters, but can also be regulated at the translational level[73].

ER stress can also make cells refractory to inflammatory stimuli[107, 108]. For example it was proposed proposed that NF-κB activation resulting from ER stress occurs in a biphasic manner, with an early positive feedback loop and a later negative feedback loop[109]. Inhibition of NF-κB mediated signaling was shown to depend on TRAF2 downregulation[90] and on induction of C/EBP-β by ER stress[110], which acts as a transcriptional repressor of

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cytokine expression. This was confirmed in a recent study showing C/EBP-β dependent downregulation of IL-4 and IL-13 signaling upon ER stress[111]. Also other inflammatory pathways can be downregulated by UPR signaling components. ATF4 inhibits type I IFN expression, by interfering with TBK-IKKε-mediated phosphorylation of IRF7[112].

Pathogens trigger the UPR

Both in plants and in *C.elegans*, infection is sufficient to trigger rigorous activation of UPR pathways[66, 70]. Also in mammals, several viruses, invasive bacteria and parasites elicit ER stress responses[113, 114]. Many pathogens interfere with the function of the ER as part of their infectious life cycle. Viruses like hepatitis C virus (HCV), poliovirus, human cytomegalovirus or herpes simplex virus strictly depend on the ER for assembly and budding of virion particles[112]. For successful replication of bacteria like *Brucella* or *Legionella pneumophila* and parasites like *Toxoplasma* inside the host cell, extensive interactions are required between the ER and the membranous compartments in which these pathogens reside[114]. Some bacterial toxins, like Shiga toxin, are retrogradely transported to the ER and as such interfere with ER homeostasis[114]. Unfolding of the A1 subunit of cholera toxin in the ER activates IRE1 and triggers the RIDD pathway, leading to the accumulation of small single stranded RNA products that launch a RIG-I dependent immune response[115] (see Box 2).

As some pathogens hijack the ER during their life cycle for assembly or exit, it is easy to understand how they activate the UPR. However, in other settings the mechanism is less obvious. *Listeria monocytogenes* has been found to induce ER expansion prior to host entry[116], while HCV activates the UPR independent of viral replication. A direct interaction between HCV viral hydrophobic non-structural proteins and IRE-1 was proposed to trigger activation of the IRE1-XBP-1 arm[117]. Finally, stimulation of TLR2 and TLR4 by bacterial ligands in macrophages has been demonstrated to trigger specifically the IRE1-XBP-1 arm by a TRAF6 and NADPH oxidase 2 (NOX2) -dependent mechanism[57].

Pathogen associated molecular patterns often activate a specific arm of the UPR, while actively suppressing the other arms[118, 119, 120]. TLR stimulation in macrophages induces XBP-1 splicing, but inhibits PERK or ATF6 activation[57]. Furthermore, LPS induces no canonical XBP-1s gene signature, but specifically triggers XBP-1s for optimal cytokine secretion[57]. This has also been observed in the case of HCV, CMV or West Nile virus infection[117, 121, 122]. TLR signaling suppresses the ATF4-CHOP branch by enhancing eIF2B guanine exchange activity and as such counteracting the inhibitory effects of phosphorylated eIF2α on ternary complex formation and protein translation[58, 123]. This TLR effect occurs via the adaptor molecule TRIF and allows for uninterrupted protein synthesis in response to pathogen infection in innate immune cells[123]. As a consequence, LPS pretreatment also prevented ER stress-induced CHOP expression and hence apoptosis, while GADD34 expression remained unaffected[123]. This was confirmed in other studies[124], although the mechanism is likely to be more complex. In DCs for example, stimulation with lipopolysaccharide (LPS) does induce CHOP expression[103], while stimulation with the TLR3 ligand poly(I:C) leads to upregulation of CHOP mRNA, but not protein[73]. The timing of LPS or poly(I:C) addition to DC cultures and UPR treatment determines whether CHOP

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is induced or not. Pretreatment with microbial stimuli appears to block subsequent UPR signals, while post-treatment with microbial stimuli leads to synergistic enhancement of the UPR response and CHOP expression, without causing cell death (Tavernier *et al*., unpublished data). This suggests that there are multiple mechanisms to modulate the UPR during immune responses. Induction of ATF4, ATF3, CHOP or GADD34 by microbial triggers can also occur in a PERK-independent manner, via TRIF, PKR or RIG-like receptor-MAVS pathways[125]. Furthermore, activation of these molecules downstream of the UPR or downstream of microbial stimulation yields a completely different functional outcome. Hence, pIC poly(I:C)-induced GADD34 is not sufficient to relieve the block on translational inhibition in immune cells, but rather plays a role in selective induction of IFN[73]. All these studies point towards an intimate link between pathogen detection, translation regulation and the UPR or ISR. It has been proposed that the term "microbial stress response" should be used to define these stress pathways. They are activated by pathogens, hijack some of the main components of the UPR for slightly adapted functions, without fully activating all arms of the UPR[125].

Subversion of the UPR by viruses

There are several reasons why viruses would benefit from triggering the UPR. An increase in folding capacity and chaperones could sustain viral replication. Activation of lipid biosynthesis pathways through the UPR could help the formation of membrane associated replication complexes[113]. However, the UPR can also have adverse effects on viral propagation. Both the PERK dependent block in protein translation, the IRE-1-RIDD dependent degradation of glycoprotein-encoding mRNAs, the induction of IFN and the degradation of nascent viral proteins by ERAD pathways are likely to have a negative impact on viral replication[113]. Emerging evidence indicates that viruses selectively modulate specific branches of the UPR to maximally benefit from the UPR while circumventing the detrimental effects. Hepatitis C virus (HCV) triggers the UPR, which in turn activates the autophagic pathway that promotes viral replication[126]. The cytomegalovirus protein M50 specifically constrains IRE1-dependent ERAD pathways by binding to and degrading IRE1[121]. On the other hand, the IRE1 pathway is specifically activated by Japanese encephalitis virus (JEV) and influenza to support viral replication[119, 127]. In the case of JEV, the beneficial effect of IRE1 was found to depend on activation of the RIDD pathway[127]. RIDD led to cleavage of host transcripts, without any effect on the JEV RNA whereas inhibition of RIDD activity led to a reduction in JEV viral protein translation by an unknown mechanism[127]. In contrast, IRE1-dependent RIDD has been shown to degrade Respiratory syncytial virus (RSV) mRNAs and as such IRE1 activation blocks RSV replication[118]. How RIDD activity and target selection is regulated is far from clear (Box 2).

ER stress pathways and antigen presentation during vaccination

There is a generally unappreciated link between activation of the UPR and ISR pathways in DCs and antigen presentation to CD4 and CD8 T cells. XBP1 binds to a cyclic AMP-response element (CRE)-like sequence in the genes encoding MHC class II HlA-DPB and HlA- DRA[135]. Also H2-M2, a murine MHC class Ib gene with as yet unknown function, appears to be dependent on XBP-1 expression[55]. Several studies have shown that ER stress

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interferes with MHC-I surface expression, most likely linked with a defect in antigen supply due to an inhibition in protein translation[136, 137]. For example, activation of RIDD, but not loss of XBP-1s, interferes with the crosspresentation ability of CD8α DCs due to the degradation of several components in the crosspresentation machinery, notably tapasin[55]. Since CD8α DCs show high constitutive IRE1 endonuclease activity, it is possible that RIDD represents an alternative mechanism to prevent constitutive presentation of autoantigens and as such avoid autoimmunity. Phosphorylation of the PERK-eIF2a branch favors the translation of so-called cryptic antigens, initiated by CUG rather than AUG-codons[138]. As such, ER stress might change the peptide repertoire presented by MHC molecules, an area that certainly deserves further attention.

Finally, studies using systems biology approaches in humans have revealed that the live attenuated yellow fever vaccine 17D induced the expression of GCN2 in peripheral blood mononuclear cells within a few days after vaccination. This expression strongly correlates with the magnitude of the later $CD8^+$ T cell response to the vaccine[139]. Subsequent mechanistic studies using GCN2 deficient mice demonstrated a critical role for virus-induced GCN2 activation in programming DCs to initiate autophagy and enhance antigen presentation to both $CD4^+$ and $CD8^+$ T cells[74]. These results reveal an unappreciated link between virus-induced ISR in DCs and the adaptive immune response. Furthermore, these results suggest that vaccine adjuvants that activate GCN2 in DCs may be efficient at inducing enhanced antigen presentation to T cells. Induction of the ISR or UPR might be predictive of good vaccine responses in humans. Consistent with this notion, the same vaccinology study showed that in human subjects vaccinated against influenza, the early expression of XBP-1 and other genes related to the UPR are robust biomarkers for the later occurrence of protective antibody titers[139]. In cancer, immunogenic cell death is favorable for anti-tumor responses and is accompanied by expression of the ER chaperone calreticulin on the cell surface of dying cells. The expression of calreticulin was shown to depend on PERK[140].

Concluding remarks

The UPR is more than just an adaptive response to accumulation of unfolded proteins in the ER. In some immune cells, like plasma cells and DCs, as well as in barrier epithelial cells that are increasingly implicated in the regulation of the mucosal immune response, parts of the UPR and the ISR seem to be crucial for normal cellular differentiation and function. In the coming years, there will be a need to explore in greater detail how the UPR, or selective arms of this response, are activated as part of this normal physiology. It is possible that lineage-specific transcription factors drive the expression of certain UPR sensors, but this needs more study. Alternatively, some specialized cellular functions like crosspresentation require poorly studied cell biological processes, like the fusion of ER components with phagosomes. This might also lead to exploitation or triggering of certain UPR pathways like ERAD, or expansion of ER membranes or ER to Golgi transport. The degradation of RNA by IRE1 (RIDD) is emerging not only as a novel means of gene regulation in conditions of ER stress, but also could constitute an ancient pattern recognition pathway that senses ER resident pathogens and triggers immunity via the RIG-I pathway. It will be crucial to understand when exactly this pathway gets triggered and how pathogens subvert it.

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Activation of innate immune receptors like TLRs or RIG-I receptors intersect with the UPR signaling pathways. We need to understand better if and how this represents a cellular adaptation to prepare the cell for secretion of cytokines, inflammatory mediators or other defense mechanisms. Given the fact that genome-wide studies of genetic risk have identified key UPR regulators to be associated with the risk of asthma, IBD and diabetes, we also need to understand in much greater detail how alterations in the UPR lead to chronic inflammatory disease, and whether these UPR related polymorphisms mainly affect inflammatory or structural cells. Ultimately, interfering with the UPR might constitute a novel way of promoting immunity or alternatively to circumvent chronic immune activation. Much more research is needed before we can exploit this new knowledge to the design of better vaccines or forms of immunotherapy.

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Box 1: The integrated stress response (ISR)

In mammalia four eIF2α kinases, termed general control nonderepressible (GCN) 2, heme-regulated eIF2α kinase (HRI), protein kinase R (PKR) and PERK jointly constitute the integrated stress response, an ancient stress response that is highly conserved from yeast to mammals[39, 40]. It is known to modulate protein biosynthesis by integrating various types of stress signals, including ER stress, amino acid deprivation, infection with double stranded RNA viruses, and oxidative stress[41]. These diverse signals activate specific stress kinases, resulting in the phosphorylation of serine 51 of the alpha subunit of the eukaryotic initiation factor 2 (eIF2), which has GTPase activity. This modification changes the capacity of eIF2α to be recharged by the nucleotide exchange factor eIF2B, which subsequently leads to a reduction in the availability of active initiation complexes, and thus attenuates translation. In addition, the expression of proteins responsible for damage repair is increased, whereas translation of constitutively expressed mRNA is shut down by redirection of these mRNAs from polysomes to discrete cytoplasmic foci known as stress granules (SGs) for transient storage[42]. Defects in the ISR are associated with several diseases including those caused by viral infections, diabetes and Alzheimer's disease[39, 40].

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Box 2: RIDD - an ancient IRE1 function?

The RNAse domain of *D. melanogaster* IRE-1α was found to have a more promiscuous function than just splicing of HAC1 or XBP1[6]. Upon prolonged ER stress, IRE1 degrades diverse ER-localized mRNAs via RIDD. Though this function of IRE1 is now widely accepted and has been recognized in many different phyla, its exact physiological role and its regulation remain largely unclear[128, 129]. The free 5' and 3' ends of the RIDD-derived mRNAs become substrates for cellular exoribonucleases and as such are targeted for degradation[23]. It is still enigmatic what determines recognition by the IRE-1 endonuclease domain, but based on all validated substrates to-date a consensus sequence is emerging that shows similarity with the cleavage site in the stem loop structure of XBP-1 and that is highly conserved[128]. IRE-1 also degrades several miRNAs, and as such indirectly affects the expression of many hundreds of mRNAs[130].

The physiological roles for RIDD have mainly been uncovered in genetic models of XBP-1 deficiency that lead in most - but not all - cells to strong activation of the RIDD pathway[55, 131, 132]. RIDD has a cytoprotective function in pancreatic beta cells in where it reduces the secretory load by degrading proinsulin[131], or in liver cells by mediating protection from acetaminophen toxicity through the degradation mRNAs encoding cytochrome P450 enzymes[132]. In other systems, RIDD appears to play a cytotoxic role and it has been proposed that in conditions of unmitigated ER stress, RIDD triggers apoptosis[17, 130, 133]. In macrophages cholera toxin triggers the RIDD pathway upon unfolding of the A1 chain[115]. This leads to a rapid activation of the RIG-I pathway and a strong inflammatory cytokine response means IRE1α is the first pathogen receptor found surveying the lumen of the ER[115]. Further, the RNA helicase SKIV2L normally metabolizes intracellular RNA ligands generated upon activation of the UPR and as such limits activation of RIG-I[134]. In patients with genetic deficiencies in these RNA-metabolizing enzymes, a chronic inappropriate antiviral response would be triggered leading to gradual immune-mediated destruction of secretory cells with high constitutive UPR.

IRE-1β is typically expressed in epithelial cells lining mucosal interfaces, such as bronchi or the colon[86]. Its predominant RIDD activity might endow this molecule with an enhanced capacity to exert immune surveillance and control the interface between the host and many trillions of microbes present in the colon or airways[115].

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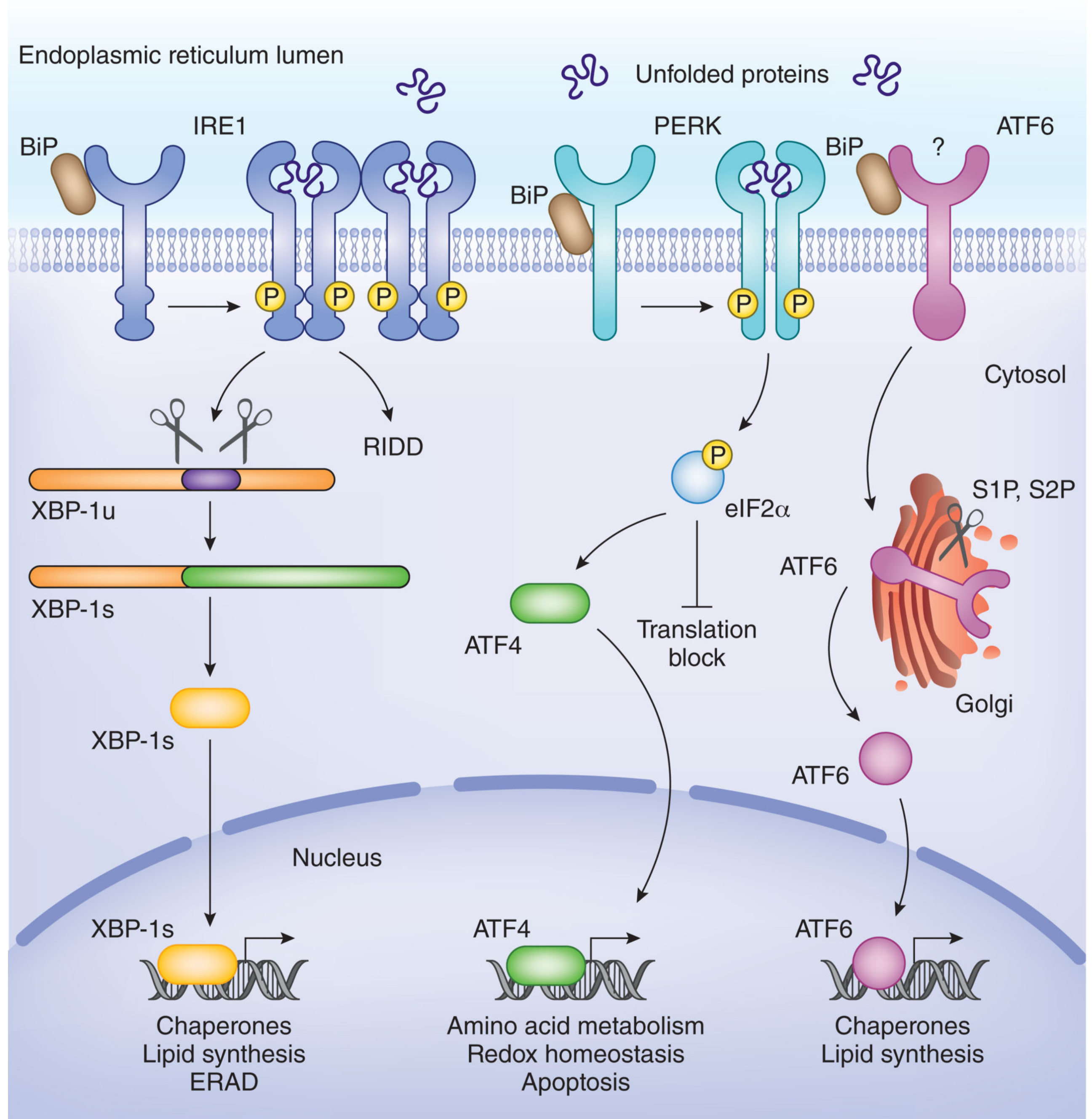

Figure 1. Three different sensors jointly coordinate the UPR in mammals

IRE1 and PERK are activated by oligomerization and transphosphorylation upon binding of unfolded proteins and release of the chaperone BiP. The endonuclease domain of IRE1 cleaves XBP-1 mRNA in an unconventional splicing reaction to generate XBP-1s mRNA, and the resulting product encodes a transcription factor of the bZIP family. In addition the endonuclease activity of IRE-1 also contributes to Regulated IRE-1 dependent decay (RIDD), the degradation of mRNAs that are recruited at ribosomes at the ER. PERK is a transmembrane kinase that phosphorylates eukaryotic initiation factor 2 alpha (eIF2α). This

leads to a transient inhibition of protein translation. Some mRNAs that encode small upstream open reading frames (uORFs) in their 5’ UTR escape the translation stop, the most prominent being the transcription factor ATF4. ATF4 in turn triggers expression of CHOP, GADD34 and additional factors important for amino acid metabolism and redox control. ATF6 is activated upon release of BiP and translocated to the Golgi where it undergoes sequential cleavage and removal of its luminal domain. The remaining transactivation domain of ATF6 moves to the nucleus and coordinates expression of genes involved in chaperone pathways or lipid biosynthesis.

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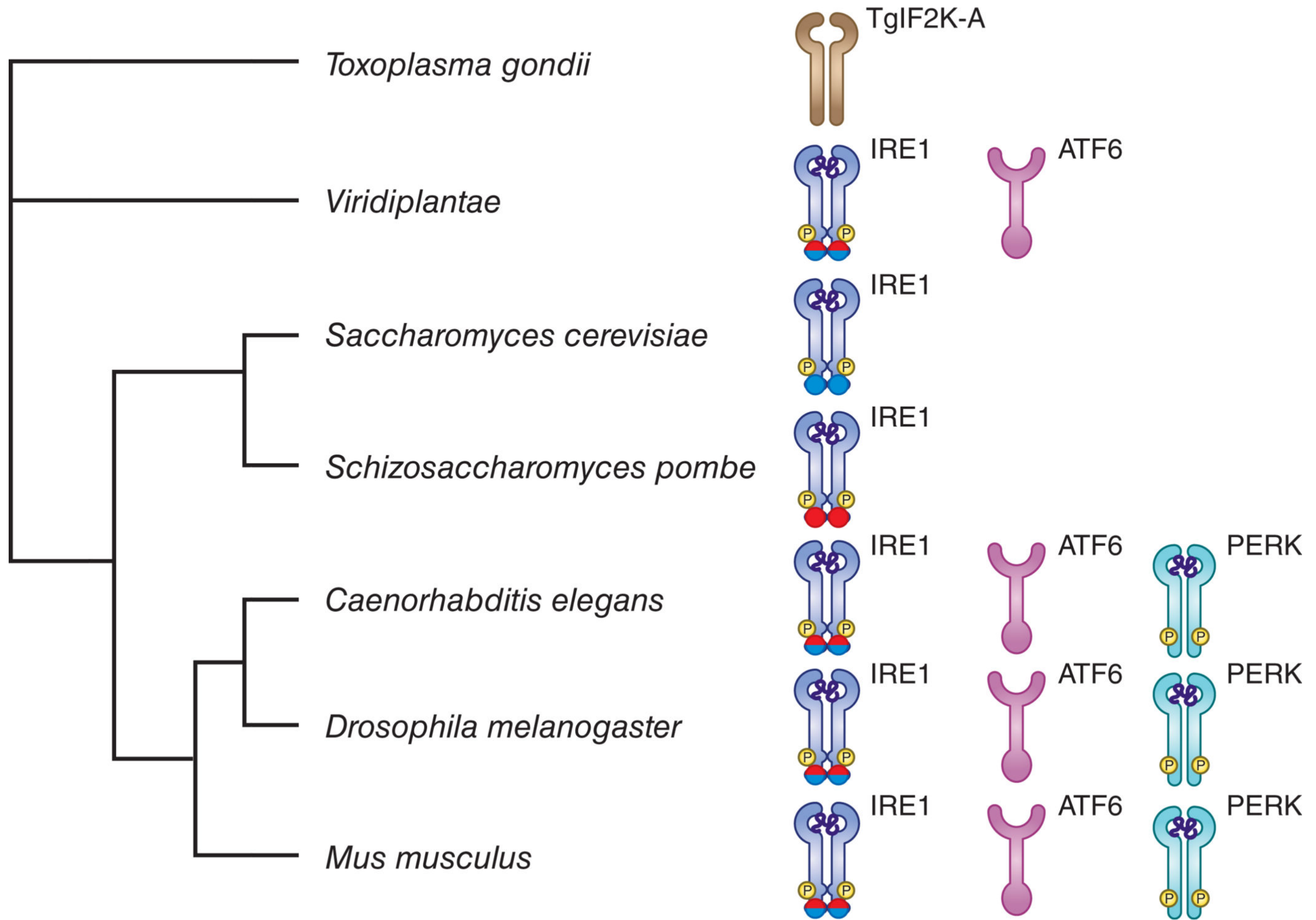

Figure 2. Evolution of the UPR shows conserved functions in immune responses

Eukaryotic phylogenetic tree of the main species mentioned in the text. In *S. pombe* and *S. cerevisiae* only the IRE1 branch is present. In *S. pombe* the IRE-1 endonuclease is involved in RIDD (red color in endonuclease domain), in *S. cerevisiae* the IRE1 endonuclease is needed for splicing of the yeast XBP-1 homolog HAC-1 (blue color in endonuclease domain). In most fungi, a fully competent UPR is required for pathogen virulence. In protozoa, no recognizable orthologs of IRE1 and XBP1 can be found, however, some of them express an ER-based transmembrane kinase, called TgIF2K-A, that has the ability to phosphorylate eIF2α in response to ER stress, and exert some degree of translational control[141]. In plants, 2 branches of the UPR, ATF6 and IRE1, are represented. The plant UPR is also activated in response to pathogens and is needed for proper antibacterial defense. In *C.elegans*, the three branches of the UPR are found and mediate protection against overwhelming hyperinflammatory reactions. In mice and humans the three branches of the UPR are fully established and interact at different levels with inflammatory pathways.

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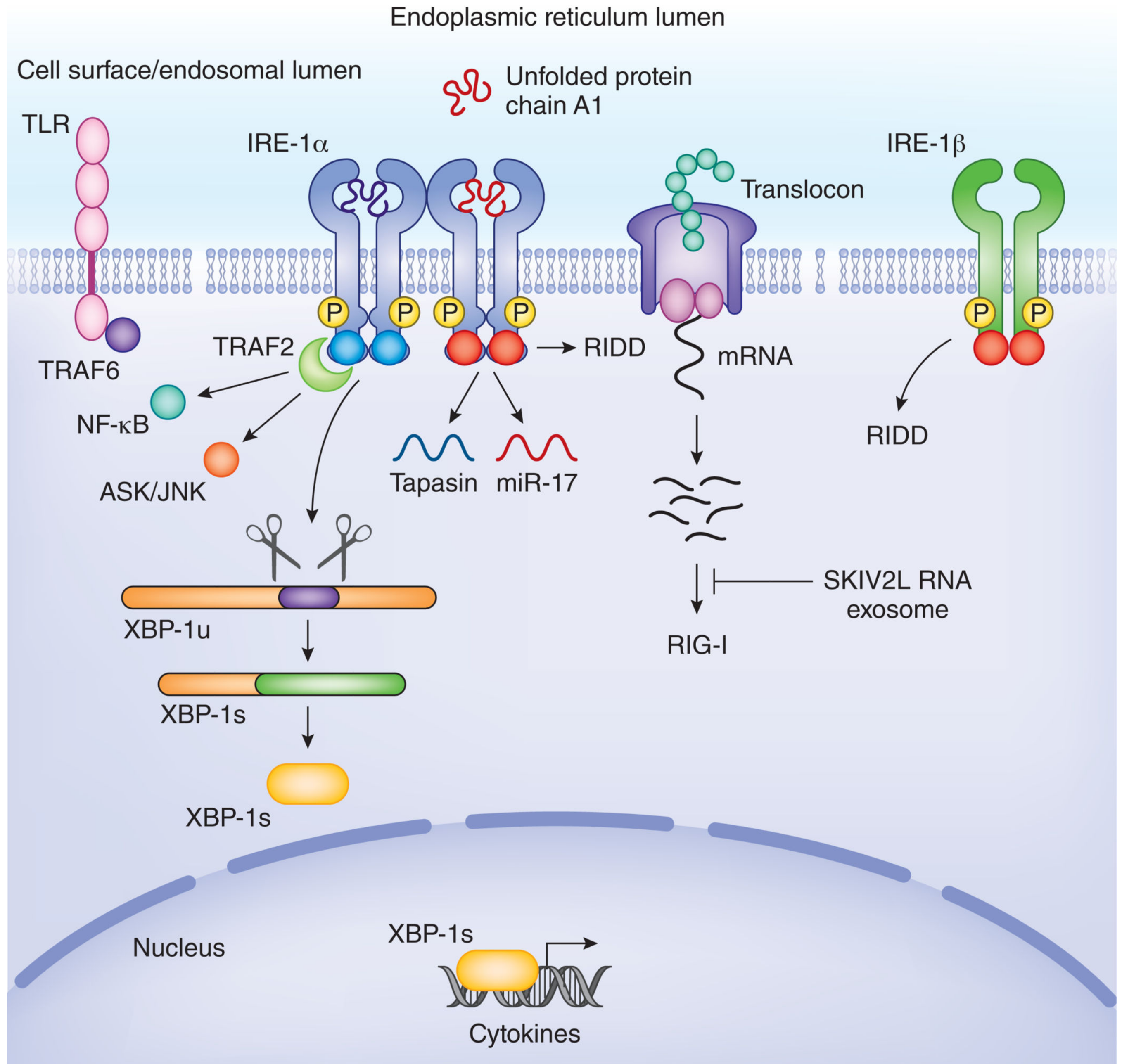

Figure 3. IRE1 intersects with inflammatory pathways

TLRs stimulate IRE1 mediated XBP-1 splicing in a TRAF6 and NOX2 dependent manner. XBP-1s binds at the promoter of several cytokines and is needed for optimal cytokine expression. Phosphorylated IRE1 interacts via TRAF2 with the IKK complex and with ASK/JNK. In this way, IRE1 controls activation of NF-κB and AP-1, 2 major inflammatory transcription factors. IRE1 also serves as a host defense receptor, surveying the lumen of the ER for pathogens. In response to cholera toxin, IRE1 is activated and starts degrading mRNAs at the translocon complex. This generates several short RNA stretches that ligate RIG-I and trigger IFN-α via RIG-I. These RNA species can in turn be degraded by the SKIV2L exosome to prevent inappropriate inflammatory responses. RIDD also leads to

degradation of specific mRNAs like that encoding tapasin as well as microRNAs such as miR-17. Degradation of tapasin and other components of the MHC-I loading machinery interfere with antigen presentation. Degradation of miR17, leads to degradation of TXNIP and subsequent stabilization of the NLRP3 inflammasome which causes enhanced IL-1β release. In addition to IRE-1α, a second isoform called IRE-1β is specifically expressed in epithelial cells lining mucosal interfaces, such as bronchi or the colon. Its RIDD function appears to predominate its capacity to splice XBP-1. This might endow IRE-1β with an enhanced capacity to exert immune surveillance and control the interface between the host and microbes present in the colon or airways.

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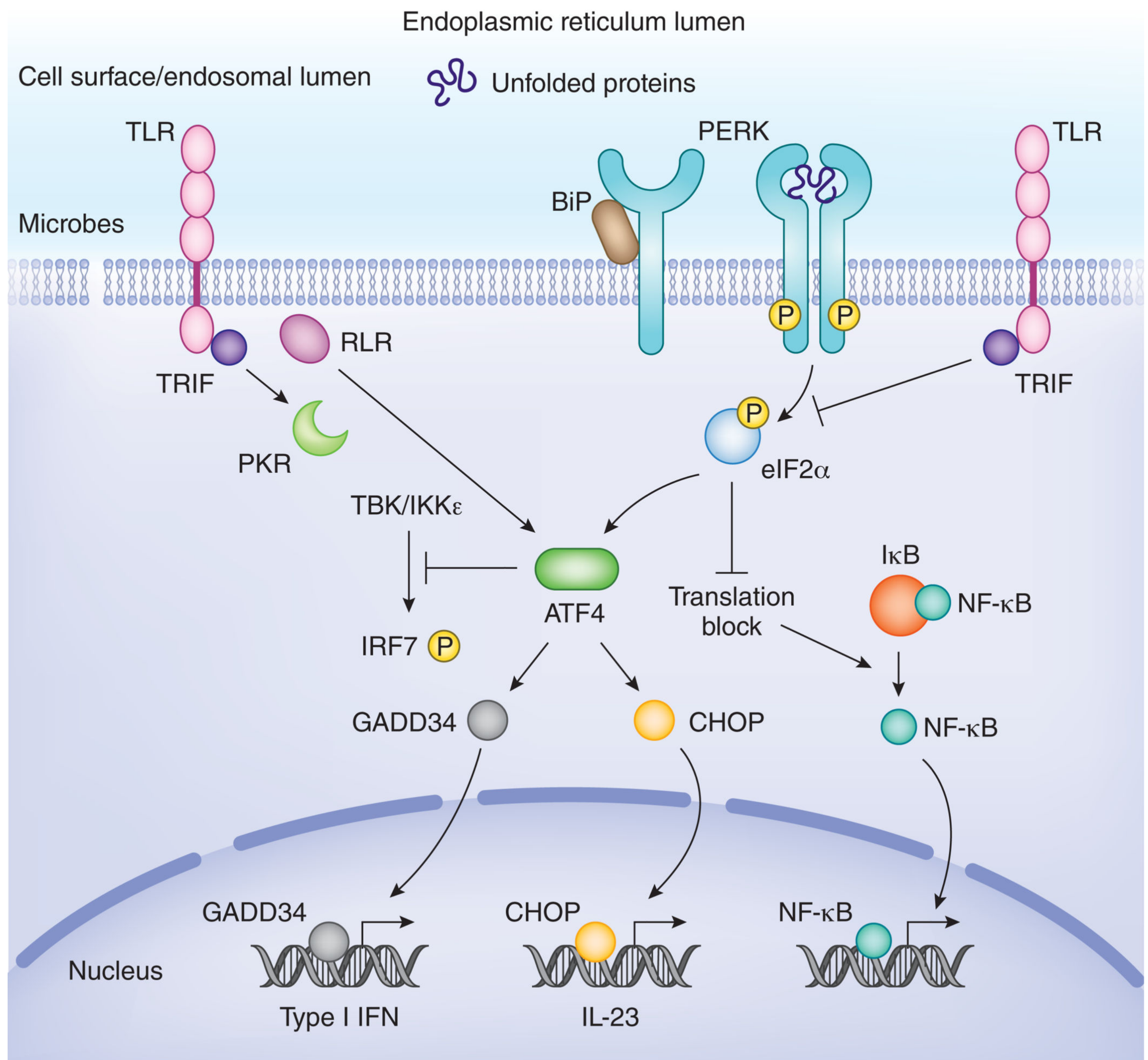

Figure 4. Pathways downstream of PERK are tightly controlled by inflammatory signals

PERK-mediated translational inhibition leads to a shutdown of IκB *de novo* synthesis and as such leads to activation of NF-κB. ATF4 is involved in IFN and IL-23 cytokine expression via activation of GADD34 and CHOP respectively. On the other hand, it has also been reported that ATF4 interferes with TBK-IKKε mediated phosphorylation of IRF7 and IFN production in embryonic fibroblasts. TLRs tightly control the ATF4-CHOP branch and prevent induction of CHOP in macrophages, in a TRIF-dependent manner. In general, several microbial stress signals also lead to activation of ATF4, ATF3, CHOP or GADD34 in a PERK-independent, but TRIF, PKR or RLR-MAVS dependent manner. This microbial stress response hijacks components of the UPR, but has a different functional outcome.