XBP-1 and the UPRosome: Mastering Secretory Cell Function

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Abstract: A unique feature of secretory cells is the proliferation of the endoplasmic reticulum (ER) and Golgi network. The high demand imposed by protein synthesis and folding in secretory cells constitutes an endogenous source of stress due to the accumulation of toxic misfolded intermediaries. Adaptation to ER stress is mediated by the activation of a complex signal transduction pathway known as the unfolded protein response (UPR). Evidence from genetic manipulation of the UPR supports the notion that components of the pathway are essential to sustain the function of secretory cells. Expression of the UPR transcriptional factor X-Box binding protein-1 (XBP-1) is essential for the proper function of plasma B cells and exocrine cells of pancreas and salivary glands. Active XBP-1 is generated by the direct processing of its mRNA by the ER stress sensor IRE1α through the formation of a complex signaling platform at the ER membrane, here termed as *UPRosome*. XBP-1 controls the expression of genes involved in almost every aspect of the secretory pathway including folding, protein quality control, ER translocation, glycosylation, and remodeling of ER and Golgi structure. In this article we discuss recent advances in understanding the mechanisms through which XBP-1 controls secretory cell function and the possible involvement of this pathway in disease conditions related to irreversible ER damage. Current therapeutic strategies to modulate ER function are also discussed.

INTRODUCTION

Secretory cells require a developed endoplasmic reticulum (ER) for their proper function. The need for efficient protein folding in secretory cells constitutes a source of stress initiated by the presence of large amounts of misfolded proteins that are normally generated during the protein maturation process. Therefore, a specialized secretory activity is translated into a constant perturbation, constituting a threat to the life of a cell. Adaptation to protein misfolding stress is mediated by the activation of a complex signaling pathway known as the unfolded protein response (UPR). Genetic manipulation of the UPR supports the notion that components of the pathway are essential to sustain the function of secretory cells. In this review we discuss recent evidence behind an emerging concept wherein the phenotype of a secretory cell is maintained through constant feedback between the demands of protein synthesis and protein folding and the activation of stress responses that globally impact ER and Golgi function to maintain homeostasis.

THE UPR: ADAPTIVE MECHANISMS TO PROTEIN FOLDING STRESS

A number of conditions interfere with oxidative protein folding at the ER lumen [1], inciting the accumulation of unfolded or misfolded intermediates, a cellular condition referred to as "ER stress". ER stress is triggered by alteration of protein maturation, expression of certain mutant proteins, decreased chaperone function, abnormal ER calcium content and many other conditions [2]. To alleviate ER stress, cells activate the UPR. The UPR transmits information about protein folding status in the ER lumen to the cytoplasm and nucleus in order to increase the protein folding capacity. In doing so, UPR activation enforces global changes in expression of proteins related to nearly every aspect of the secretory pathway. Microarray experiments have demonstrated that activation of the UPR affects genes related to protein entry into the ER (*i.e. Sec61*), folding (*i.e. Grp78* and *ERdj4*), glycosylation, ER-associated degradation (*i.e. EDEM*), and vesicular trafficking (reviewed in [1,3]). Increasing attention has been given to the regulation of the UPR based on substantial evidence for the involvement of ER stress in many diseases, including neurodegenerative conditions [4], cancer [5] and diabetes [6], hence offering new therapeutic targets to treat these diseases.

The UPR was first characterized in yeast where a single signaling pathway mediated by a type I transmembrane ER protein known as IRE1 (inositol-requiring transmembrane kinase/endonuclease) governs the response to ER stress [7-10]. In higher eukaryotes, the UPR is more complex as it is mediated by at least three distinct UPR signaling pathways initiated by the sensors IRE1α, PERK (PKR-like ER kinase), and ATF6 (activating transcription factor 6) (Fig. **1A**). Activated PERK inhibits protein translation in to the ER through the inactivation of the initiation factor eIF2α, alleviating ER stress by decreasing the overload of misfolded proteins [1]. PERK controls the expression of ATF4 (activating transcription factor 4)[1], a transcription factor that induces the expression of UPR genes that function in amino acid and redox metabolism, including *chop/gadd153* and *gadd34* [11-13]. A second UPR pathway is initiated by ATF6α and ATF6β, a

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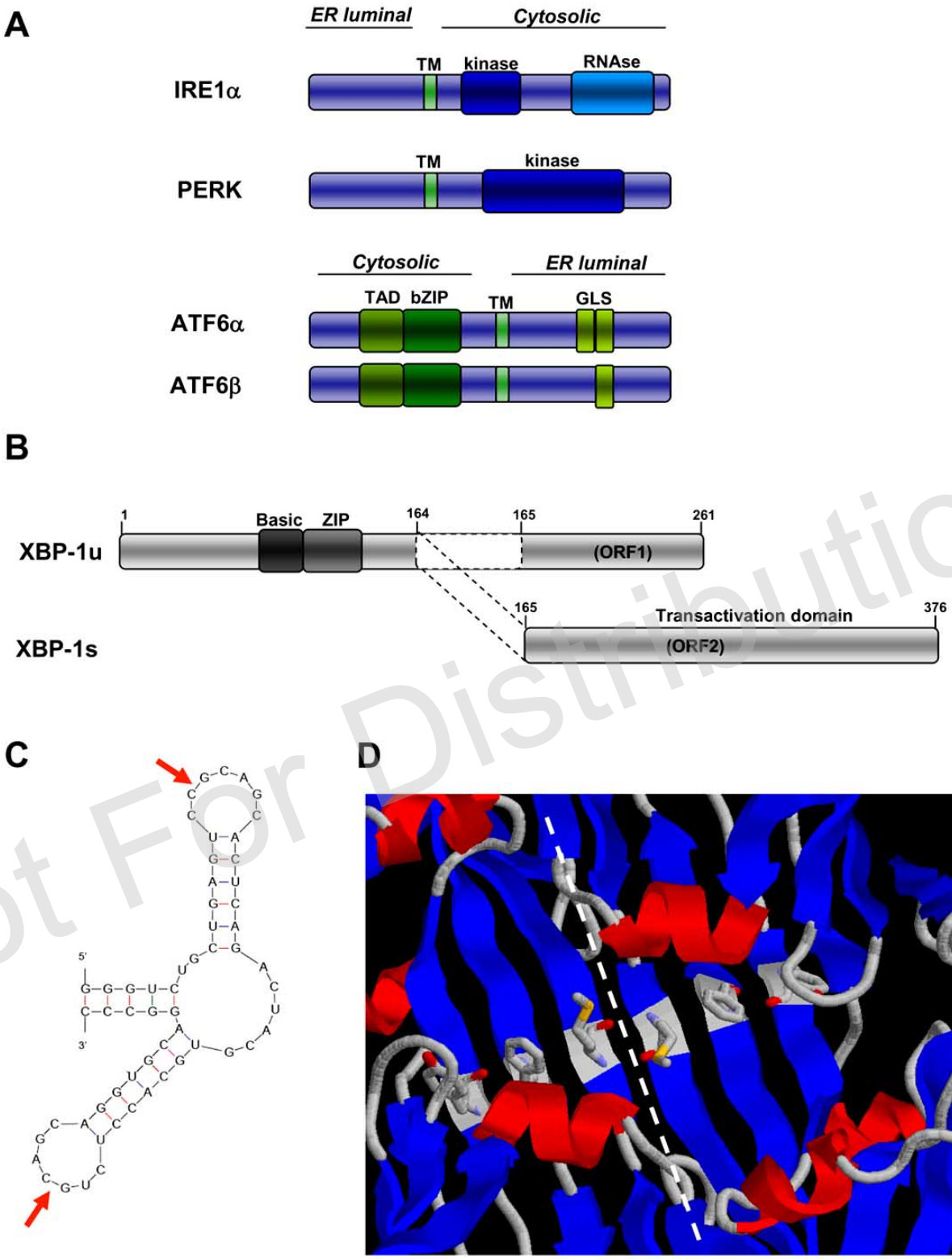

Fig. (1). Essential components of the UPR. (**A**) *ER stress sensors*. Inositol requiring kinase 1 (IRE1α), protein kinase-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6). Abbreviations are bZIP, basic leucine zipper; GLS, Golgi localization sequences; TAD, transcriptional activation domain; and TM, transmembrane domain. (**B**) *XBP-1 splicing*. Schematic representation of the unspliced and spliced forms of XBP1 (XBP-1u and XBP-1s, respectively). Numbers indicate amino acid positions with the initiation methionine set at 1. ORF1 and ORF2 for the C-terminal domain as well as the basic and leucine zipper (ZIP) domains are indicated. (**C**) *XBP-1 mRNA splicing site*. Secondary structure of the *XBP-1* mRNA splicing site. Symmetric structures are depicted. IRE1α cleaving sites are indicated with red arrows. (**D**) *yeast IRE1 partial structure*. Ribbon representation looking into the IRE1 ER luminal domain groove (MHC-I like structure), displaying residues important for its ER stress sensing activity (Tyr301, Phe2085 and Met229). Dimer interface is indicated with a white line. This structural domain is proposed to bind misfolded proteins to stabilize the dimeric or oligomeric conformation of IRE1.

type II ER transmembrane protein whose cytosolic domain encodes a bZIP transcriptional factor [14,15]. Upon ER stress induction, ATF6 is processed at the Golgi, releasing its cytoplasmic domain which acts as a transcriptional activator [16]. Interestingly, this branch of the UPR has gained more complexity since recent studies identified a series of ATF6 homologues that are modulated by ER stress in specific tissues including OASIS [17], CREBH [18], LUMAN/CREB3 [19], CREB4 [20], and BBF2H7 [21]. All of these ATF6-related bZip factors are processed at the Golgi in a similar way as ATF6, but their function in the UPR is not completely understood. Interestingly, Mori's group recently generated ATF6α- and ATF6β-knockout mice, which developed normally [22]. However, double knockout mice are embryonic lethal, similar to the phenotype of XBP-1 or IRE1α deficient mice. Analysis of cells deficient in ATF6α revealed that it is solely responsible for transcriptional induction of some ER chaperones and that ATF6α heterodimerizes with XBP1 for the induction of ER-associated degradation components.

IRE1α is the most evolutionarily conserved branch of the UPR. Nevertheless, little is known about the regulation of IRE1α activity. IRE1α is a Ser/Thr protein kinase and endoribonuclease that, upon activation, initiates the unconventional splicing of the mRNA encoding the transcriptional factor X-Box-binding protein 1 (XBP-1) [16,23,24]. In mammalian cells, a 26 nucleotide intron of *xbp-1* mRNA is spliced out by activated IRE1α, leading to a shift in the codon reading frame. Translation of the new reading frame results in the conversion of XBP-1 from an unspliced form of 267 amino acids to a spliced form of 371 amino acids that comprises the original N-terminal DNA binding domain plus an additional, potent trans-activation domain in the C terminus (Fig. **1B**,**C**).

Spliced XBP-1 (XBP-1s) controls the upregulation of a broad spectrum of UPR-related genes involved in protein folding, redox metabolism, ER-associated degradation and protein quality control (see below) [25]. A genome-wide search for substrates of the ribonuclease activity of IRE1α revealed only XBP-1 mRNA; no additional substrates were identified [26]. In insect cells, active IRE1α was recently proposed to control the degradation of mRNAs encoding certain ER proteins that were predicted to be difficult to fold [27]. Although the mechanism underlying these observations is not clear, this novel function of IRE1α is well suited to be another type of UPR response that selectively decreases the production of proteins that challenge the ER at the level of folding under stress conditions.

THE COMPLEXITY OF IREα SIGNALING: *THE UPRosome*

A key finding in the UPR field was the discovery of a possible mechanism underlying IRE1α activation. It was originally proposed that, under normal conditions, the chaperone BiP binds to IRE1α through its ER luminal domain, maintaining the protein in an inactive monomeric state [28,29]. In ER stressed cells, BiP is preferentially bound to misfolded proteins, thereby releasing IRE1α to multimerize and autophosphorylate its cytosolic domain. This phosphorylation event triggers the activation of the RNase activity, initiating *xbp-1* mRNA splicing and UPR responses. This model suggested a direct mechanism by which IRE1α senses the accumulation of misfolded proteins at the ER lumen. However, the role of BiP in regulating IRE1α activation has been questioned, given that mutations in the possible BiP binding site of IRE1α do not alter its ability to sense ER stress in yeast [30]. Recently, new insights into the mechanism of IRE1α activation were proposed when Peter Walter and Randall Kaufman's groups independently solved the structure of the ER luminal domain of yeast and human IRE1. Walter speculated that misfolded proteins may directly bind to the N-terminal region of IRE1, facilitating its oligomerization through a binding motif similar to an MHC-like groove (Fig. **1D**) [31,32]. Thus, misfolded proteins may be directly recognized by IRE1α. Recent *in vitro* studies consolidated both models for IRE1α activation. It was proposed that during the activation process, BiP first dissociates from IRE1 leading to its cluster formation. In the second step, direct interaction of unfolded proteins with the stress sensing domain orients the protein into an active signaling cluster [33]. It may also be possible that an unknown "sensor protein" exists at the ER which becomes easily misfolded under ER stress conditions, that then specifically binds to this MHC-like groove in IRE1 promoting its dimerization.

The levels of IRE1α signaling are controlled by the ER-located Protein-tyrosine phosphatase 1B (PTP-1B) [34]. The absence of PTP-1B caused impaired XBP-1 splicing and expression of XBP-1 target genes that did not affect other ER stress sensors such as PERK. In addition, IRE1α signaling is instigated by the expression of some pro-apoptotic BCL-2 family members such as BAX and BAK through direct binding [35]. Indirect evidence also indicates that IRE1α is inhibited by the ER located anti-apoptotic protein BAX-inhibitor-1 [36,37]. Finally, another stage of regulation of IRE1α signaling has been proposed at the level of XBP-1. Under normal conditions the non-processed form of XBP-1 is rapidly degraded by the proteasome [23]. However, under prolonged or chronic ER stress, unspliced XBP-1 accumulates in the cytosol heterodimerizing with XBP-1s. This protein complex inhibits the translocation of XBP-1s to the nucleus promoting its ubiquitination and degradation [38]. This interesting mechanism may constitute a feedback loop to inactivate the UPR in cells irreversibly damaged.

The cytosolic domain of activated IRE1α binds to the adaptor protein TRAF2 (TNFR-associated factor 2), triggering the activation of the Apoptosis Signal-regulating Kinase 1 (ASK1) and cJun-N terminal kinase (JNK) pathway [39,40]. In addition, activated IRE1α modulates the activation of the ERK [41] and NF-κB pathways [42] through the binding of the adaptor proteins Nck and a complex between inhibitor NF-κB kinase (IKK)/TRAF2 respectively. However, the function of these UPR signaling branches is not well understood. In this context, activation of JNK by IRE1α has been proposed to activate apoptosis under irreversible ER stress in analogous fashion to what has been described for the TNF receptor [43], but the mechanism linking these two phenomena are not clear [40]. Interestingly, a new function for IRE1α was recently proposed through activation of JNK whereby it controls levels of autophagy [44,45]. Autophagy is a survival pathway classically linked to adaptation to nutrient starvation. Conversely, in cells undergoing ER stress, autophagy may serve as a mechanism to eliminate damaged organelles and proteins [46-48].

In murine cells, activation of the ER-resident caspase-12 has been suggested to be linked to the UPR pathway through an interaction with TRAF2 and possibly with active IRE1α [49], but a complex between procaspase-12/TRAF2/IRE1α has not been described. In addition, the contribution of caspase-12 to ER stress-mediated apoptosis is actively debated [50,51]. Based on the studies described in this section, we envision a model where a complex signaling platform is formed at the level of IRE1α activation. This complex, here termed the *UPRosome* (or UPRosome-1) initiates multiple responses by the differential binding of adaptor proteins and modulators to IRE1α (Fig. **2**).

XBP-1 FUNCTION: LESSONS FROM *IN VIVO* MANIPULATION OF THE UPR

XBP-1 is a member of the CREB/ATF family of transcription factors and was first isolated in our laboratory through its ability to bind a cyclic AMP response element (CRE) sequence in the gene encoding the MHC class II molecule DRα [52]. In adult tissues XBP-1 mRNA is expressed ubiquitously, but in fetal tissues it is expressed preferentially in exocrine glands, osteoblasts, chondroblasts and liver. XBP-1 is essential for the differentiation of hepatocytes, as *XBP-1* deficient embryos die *in utero* from severe liver hypoplasia and a resulting fatal anemia [53].

The first insights about the function of XBP-1 *in vivo* came from studies in the immune system. XBP-1 is highly expressed in myeloma cell lines, in LPS-stimulated splenic B cells, and in plasma cells *in vivo*. The function of XBP-1 in lymphoid cells was originally addressed in a recombination-activating gene-2 (RAG-2) complementation system to generate mice in which XBP-1 is deleted only in lymphocytes. These chimeric mice developed normally, allowing the discovery of the essential function of XBP-1 in plasma-cell development [54]. XBP-1-deficient B cells are markedly defective in antibody secretion *in vivo* in response to antigenic challenge. This activity was shown to be directly dependent on XBP-1 splicing in stimulated B cells [55]. Signals involved in plasma cell differentiation, specifically interleukin-4, control the transcription of XBP-1, whereas its post-transcriptional processing is dependent on synthesis of immunoglobulins during B cell differentiation [55]. XBP-1

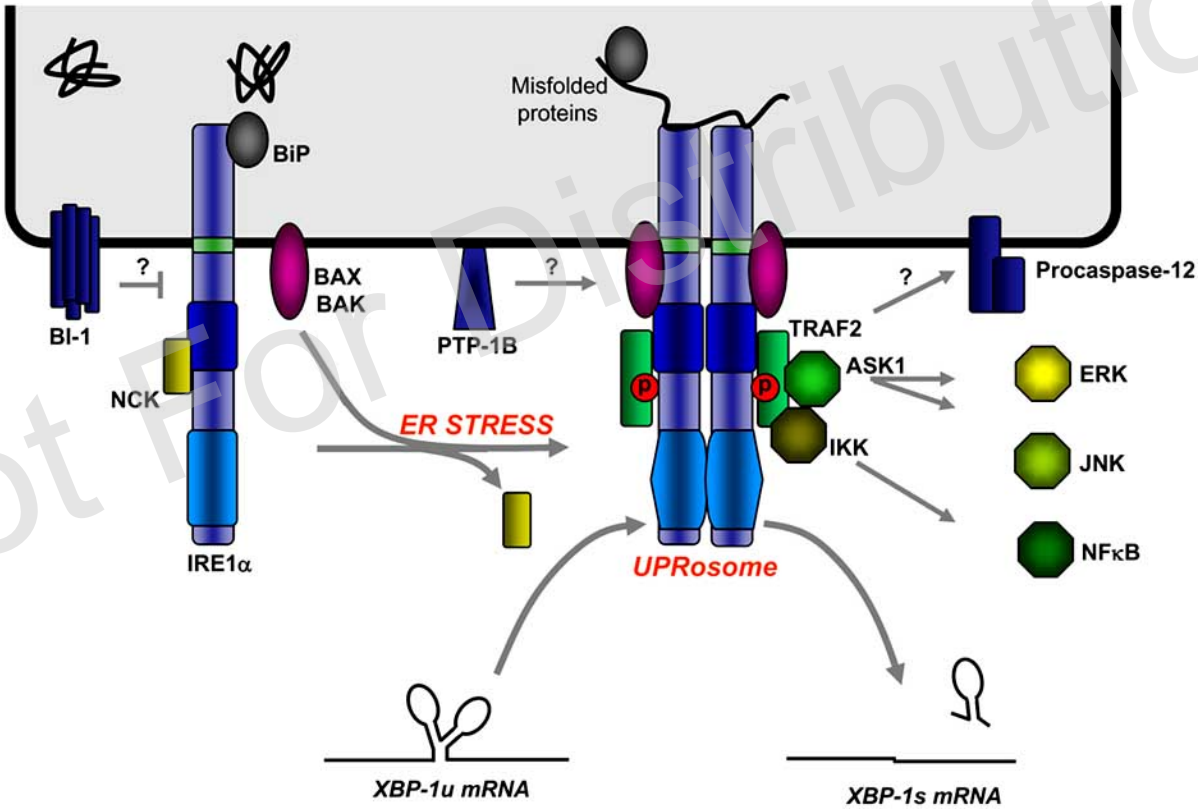

Fig. (2). The UPRosome. Under non-stress conditions IRE1α is maintained in an inactive monomeric state through its binding to the ER chaperone BiP. During a stress response, BiP binds preferentially to unfolded/misfolded proteins in the ER lumen, releasing its partial inhibitory effect over IRE1α. Interaction of the ER luminal domain of IRE1α with unfolded proteins induces its dimerization and autophosphorylation, leading to activation of the RNAse domain and binding of adaptor proteins. The endoribonuclease activity of IRE1α mediates the processing of the mRNA of *xbp-1*. IRE1α signaling is initiated by the formation of a complex protein platform at the ER membrane, the *UPRosome* (or UPRosome-1), where multiple factors modulate the activity of IRE1α and the initiation of a variety of signaling responses. Activation of IRE1α requires the binding of accessory proteins, such as BAX and BAK and may be inhibited by anti-apoptotic proteins such as BI-1. Active IRE1α interacts with TRAF2, initiating the activation of the ASK1/JNK pathway. Sequestration of IKK by IRE1α/TRAF2 induces NF-κB signaling and TNF-alpha production. Inactive IRE1α interacts with the adaptor protein Nck, which is released upon ER stress, leading to the activation of the ERK pathway. The ER located phosphatase PTP-1B is required for IRE1α activation, however its binding to IRE1α has not been determined. Finally, IRE1α may regulate the processing of pro-caspase-12 (in murine cells), through the binding of TRAF2.

is involved in controlling the production of interleukin-6, a cytokine that is essential for plasma cell survival. These findings provided the first link between the UPR and secretory cell function. Using a similar experimental system, the role of IRE1α was corroborated in plasma B cells as an upstream regulator [56]. An additional function for IRE1α was proposed in early B cell development where it is required for immunoglobulin gene rearrangement and production of B cell receptors [56].

The embryonic lethality of XBP-1$^{-/-}$ mice made studying its role impossible in adult mice. To circumvent the lethal liver phenotype of XBP-1$^{-/-}$ mice, we targeted an XBP-1 transgene back to liver using a liver-specific promoter [57]. XBP-1$^{-/-}$;Liv^{XBP1} mice lacking XBP-1 in all organs except the liver died shortly after birth from a severe impairment in the production of pancreatic digestive enzymes leading to hypoglycemia and death. At the cellular level expansion of the ER was severely impaired in pancreatic exocrine cells, resulting in a complete disorganization of the ER network. In addition, the number of zymogen granules was drastically decreased in XBP-1$^{-/-}$;Liv^{XBP1} mice concomitant with decreased α-trypsin and α-amylase production [57]. These events correlated with decreased expression of certain ER chaperones in the affected organs. A similar phenotype was observed to a lesser degree in salivary gland acinar cells lacking XBP-1. Taken together with the requirement for XBP-1 in plasma cell differentiation, our findings suggested that XBP-1 is essential for the development of highly secretory exocrine cells. Finally, in order to address the specific role of XBP-1 in different organs in the adult, we recently generated a XBP-1 conditional knockout mouse model [58], and characterized the function of XBP-1 in some pathological conditions affecting the nervous system. We are currently investigating the effects of the specific deletion of XBP-1 in the pancreas, liver, and diverse immune cell types.

The phenotypes caused by defects in the PERK/eIF2α and IRE1α/XBP-1 pathways are disparate, indicating some divergence in their functions. For example, UPR-mediated translational control through eIF2α phosphorylation is not required for B lymphocyte maturation and/or plasma cell differentiation [56]. Similarly, PERK knockout mice do not show any deficiency in B cell function [59]. Interestingly, PERK deficiency leads to abnormalities in the exocrine pancreas with decreased secretion of digestive enzymes, distended ER and increased apoptosis of acinar cells [60,61], although the phenotype is modest as compared to XBP-1$^{-/-}$;LivXBP1 mice. Instead, PERK or eIF2α deficiency causes progressive loss of pancreatic islet β-cells and impaired bone formation, indicating a function for the PERK pathway in β-cells and osteoblasts rather than other secretory cells [60-62]. In contrast, XBP-1$^{-/-}$;LivXBP-1 mice do not show drastic changes at birth in the endocrine pancreas, reflected in normal levels of insulin and insulin-containing granules in β-cells. The differences in the requirement for PERK and IRE1α pathways could be ascribed to differences in the mode of activation and/or the downstream target genes. Although PERK and IRE1α both share functionally similar luminal sensing domains and both are activated in cells treated *in vitro* with ER stress inducers, they clearly are selectively activated *in vivo*. This may be related to different *UPRosome* formation through specific binding of adaptor and regulatory proteins. The PERK/eIF2α pathway activates a broad range of target genes, which is not surprising given that various cellular stresses converge upon the regulation of eIF2α activity. In contrast, as we will describe in the next section, XBP-1 target genes largely increase the folding capacity of the ER, trigger ER/Golgi biogenesis and improve the quality control system.

Finally, a new function of XBP-1 was recently described in dendritic cells (DCs) [63]. Purified DCs show high levels of basal *XBP-1* mRNA splicing in the unstimulated state. Lymphoid chimeras lacking XBP-1 possessed decreased numbers of both conventional and plasmacytoid DCs with reduced survival both at baseline and in response to toll-like receptor signaling. Overexpression of XBP-1 in hematopoietic progenitors rescued and enhanced DC development suggesting a role of XBP-1 in early stages of their development [63].

XBP-1 DEPENDENT TRANSCRIPTIONAL REPROGRAMMING

Previous work has demonstrated that XBP-1, as well as its yeast homolog HAC1p, regulate ER stress-induced genes that promote folding, degradation of misfolded ER proteins through the ERAD pathway and proteins involved in the translocation of proteins into the ER. The first XBP-1s target genes in mammalian cells were defined in murine embryonic fibroblasts (MEFs) through microarray analysis [25]. These genes included (i) chaperones such as *p58^{IPK}*, *ERdj4*, *HEDJ* and protein disulfide isomerase *PDI-P5*; (ii) the ERAD related gene *EDEM*; and (iii) genes involved in glycosylation such as *RAMP4* [25]. The induction of other important UPR genes such as *Grp78/BiP*, *Grp94* and *CHOP* were not strictly dependent on XBP-1s expression, indicting a compartmentalization of UPR target genes according to each UPR signaling branch.

A systematic analysis of gene expression profiling in plasma cells expanded the role of XBP-1 to the secretory function and biogenesis of the ER/Golgi apparatus [64]. A remarkably consistent set of XBP-1 target genes emerged from studies of *xbp1*-deficient mouse B cells and ectopic XBP-1s expression, many of which encode proteins that function in essentially every stage of the secretory process. For example, new genes were identified such as proteins involved in the targeting of proteins to the ER (*i.e. srp9*, *srp54*, and *rpn1*), translocation of proteins across the ER membrane (*i.e. sec61a* and *sec61g*), cleavage of signal peptide, folding of ER proteins (*i.e. dnaJb9*, *erp70*, *grp58* and *pdir*), degradation of misfolded ER proteins by the ERAD pathway (*i.e. sel1h*), protein glycosylation (*i.e. gcs1*, *man1b1* and *fut8*), ER-Golgi vesicular trafficking (*i.e. cope*, *sec24C*, *and vdp*), endosomal trafficking (*rabac*1), and targeting of secretory vesicles to the plasma membrane (*arhq/tc10*) [64].

In a neuronal cell line, gene expression profile analysis identified *WFS-1*, a gene responsible for Wolfram syndrome [65]. We recently corroborated this finding in primary neuronal cultures from an XBP-1 conditional knockout mouse model and identified *Grp58*, *PDI* and *Herp* as additional targets in neurons [110]. Interestingly classical XBP-1s target genes such as *ERdj4*, *EDEM* and *Sec61* were not altered in XBP-1 deficient neurons [110], suggesting that XBP-1-dependent responses may vary depending on the cell type. Using a genome-wide promoter binding assay approach, a

recent study described a regulatory circuit governed by XBP-1 in skeletal muscle and secretory cells. As expected, a core group of genes involved in constitutive maintenance of ER function in all cell types tested was identified [66]. In addition, this approach identified a cadre of unexpected targets that may link XBP-1 to neurodegenerative and myodegenerative diseases, as well as to DNA damage and repair pathways. Interestingly, the critical regulator of differentiation Mist1 was shown to be an important XBP-1 target, providing a possible explanation for developmental defects associated with XBP1 deficiency [66]. In summary, the emerging view indicates that 1) XBP-1s expression affects broad categories of genes involved in almost every aspect of ER physiology and 2) the defined pool of regulated genes may vary in the context of specific stimuli and cell types.

XBP-1 AND ORGANELLE BIOGENESIS

Based on the high impact of XBP-1s expression on secretory pathway gene expression, Shaffer *et al* analyzed the rate of organelle biogenesis in XBP-1s overexpressing B-cell lines [64]. XBP-1s triggered an increase of ER and Golgi mass, a phenomena classically linked to plasma B cell maturation. Similarly, IRE1α deficient MEFs show impaired expansion of the ER and Golgi under ER stress conditions [35]. The effects on organelle size were also observed when lysosomes and mitochondrial content was analyzed [64], consistent with a general increase in cell size after the ectopic expression of XBP-1s or during plasma cell maturation. In agreement with these *in vitro* observations, the failure of exocrine pancreas function of XBP-1-deficient animals correlates with a drastic decrease in the amount of ER and the disorganization of the ER network *in vivo* (Fig. 3) [57].

It has been difficult to identify the mechanisms involved in ER expansion at the molecular level since the genes identified so far as XBP-1s targets in secretory cells do not include genes encoding enzymes required for endomembrane phospholipid biosynthesis, with the exception of *Chkb*, which catalyzes the first step in the biosynthesis of phosphatidylcholine in mammals [64]. Interestingly, enforced expression of XBP1s is sufficient to induce synthesis of phosphatidylcholine, the primary phospholipid of the ER membrane [67]. Cells overexpressing XBP1s exhibit elevated levels of membrane phospholipids and enhanced activity of phosphatidylcholine biosynthesis. Another report indicated that these effects are due to an increase on the level and synthesis of choline cytidylyltransferase (CCT) (Fig. **3B**) [68]. In addition, increased synthesis of phosphatidylcholine alone in CCT-transduced cells is sufficient to induce partial expansion of rough ER, suggesting that XBP-1 orchestrates ER biogenesis by a coordinate regulation of phospholipid biosynthesis and expression of ER proteins [68]. Consistent with a physiological role for ER expansion in secretory cells, similar observations were presented in LPS stimulated B cells [69]. Therefore, the IRE1α-XBP1 pathway may regulate the synthesis of phospholipids according to cellular needs for ER function initiated by a high demand for protein synthesis and secretion (ER stress). This activity in organelle biogenesis may be related to an indirect effect on gene expression based on the lack of direct mechanistic data available despite extensive searches for XBP-1 target genes. It remains to be determined how XBP-1 affects the remodeling of ER structure and whether or not the synthesis of other components of the ER membrane is regulated by the UPR (Fig. **3B**).

XBP-1 AND PATHOLOGICAL CONDITIONS: A GENERAL OVERVIEW WITH THERAPEUTIC PERSPECTIVES

Genetic manipulation of the UPR has illustrated the fundamental role of this adaptive response in the physiology of many cell types. Secretory cells depend on the UPR for a robust rate of synthesis, folding and secretion of proteins. As discussed, growing evidence suggests that a dynamic regulation occurs at the organelle level where high rates of expression of certain secreted proteins constitute a source of protein folding stress, activating the UPR and, as a consequence, many fundamental aspects of ER function are affected. Along this line of reasoning, the need for an efficient secretory pathway may "epigenetically" shape organelle structure and folding capacity through the UPR, and possibly not through a predetermined genetic program.

Based on the strict correspondence between secretion and ER stress, impairment of UPR function has been proposed to be a causal factor in diseases such as diabetes, where stimulation of insulin secretion depends on UPR activation [6]. In addition, a role for UPR signaling in insulin resistance has been proposed at the level of the liver [70]. In animal models, obesity induces ER stress, playing a central role in the development of insulin resistance and diabetes by triggering JNK activity *via* IRE1α and inhibition of insulin receptor signaling [70]. Mice deficient only in one *xbp-1* allele showed an increased susceptibility to develop insulin resistance [70]. On the other hand the UPR has been shown to participate in other pathologies, such as cancer. Two independent groups demonstrated that XBP-1 or PERK deficient cells are impaired in their ability to form solid tumors [71,72], possibly related to a role of the UPR in survival under hypoxic conditions. Recently it was shown that Eμ-driven XBP-1s transgenic mice develop multiple myeloma characterized by elevated serum inmunoglobulin and skin alterations. This was accompanied at late time points by bone lytic lesions and subendothelial immunoglobulin deposition [73], correlating with aberrant expression of known human multiple myeloma-related genes.

Accumulating evidence suggests that ER stress is particularly relevant to a variety of neurological disorders involving the misfolding and deposition of abnormal protein aggregates in the brain [74], including Alzheimer's disease [75,76], Parkinson's disease [77,78], Amyotrophic lateral sclerosis [79,80], Prion-related disorders [81-83] and many others. Engagement of the IRE1α pathway occurs in models of brain ischemia injury [84,85], brain trauma [86], behavioral stress [87,88], retinal cell death [89], spinal cord injury [90] and trauma [91], brain viral infections [92], Parkinson's disease [93] and Amyotrophic lateral sclerosis [79,80]. Thus, activation of the UPR/IRE1α pathway may be a primary response against neurodegeneration. The first insights about the function of XBP-1 in the nervous system came from genetic studies of human patients affected with bipolar disorders [94,95]. A polymorphism in the XBP-1 promoter was

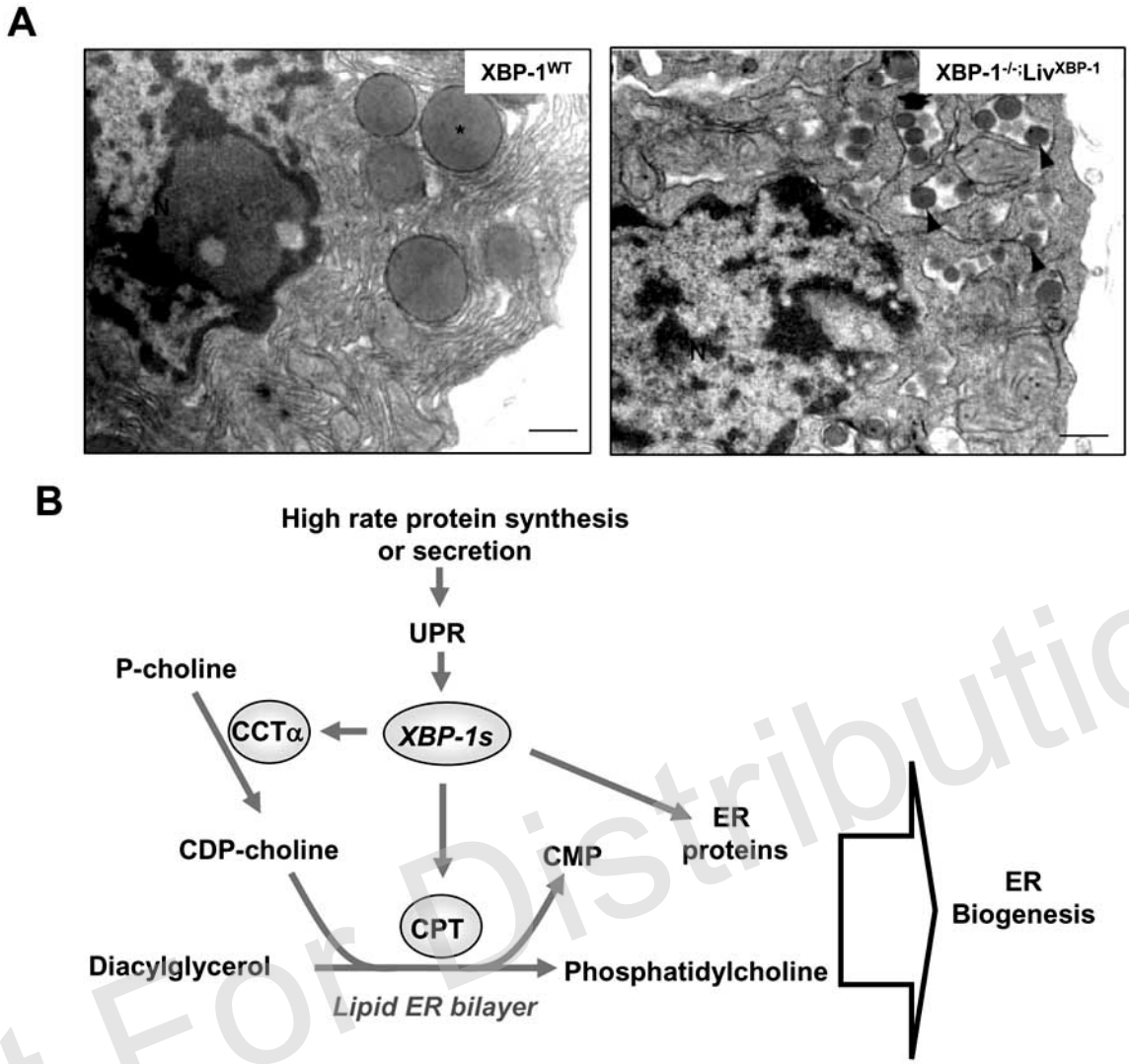

Fig. (3). XBP-1 and ER biogenesis. (**A**) Ultrastructure of pancreatic acini reveals defects in the production of zymogen granules and the structure of the ER. Electron micrographs of sections of pancreatic acini cells from XBP-1^{WT} and XBP-$1^{-/-}$;Liv^{XBP1} mice. While pancreatic cells from XBP-1^{WT} mice have abundant apically located membrane bound zymogen granules, pancreatic acinar cells of the XBP-$1^{-/-}$;Liv^{XBP1} mice have only a few, small apical granules as well as immature granule precursors located inside the endoplasmic reticulum lumen. In addition, both a reduction in and a complete disorganization of the ER network is observed in XBP-$1^{-/-}$;Liv^{XBP1} mice. Electron microscopy pictures were obtained from Lee *et al*. 2005. The EMBO Journal (24): 4368-4380. Permission obtained from The EMBO Journal Copy Rights. (**B**) *Lipid metabolism and XBP-1*. XBP-1s may induce ER biogenesis by both transcriptional and translational regulation. XBP-1s-mediated transcriptional regulation includes increased expression of many secretory pathways. XBP-1s also upregulates the activity of lipid metabolic proteins, such as cholinephosphotransferase (CPT) and choline cytidylyltransferase (CCT). These proteins are key enzymes in phosphatidylcholine biosynthesis and thus directly affect the production of organelle membranes.

shown to be a risk factor for this illness in the Japanese population, that may be related to changes in XBP-1 expression and ER stress responsiveness as suggested *in vitro*. An additional study also suggested an association between sensitivity to lithium treatment in Japanese patients affected with bipolar disorder and the XBP-1 promoter polymorphism [96,97]. This polymorphism was also genetically linked to particular personality types in females [98,99] and the occurrence of schizophrenia in the Japanese population [100,101]. Expression of XBP-1 in *C. elegans* is increased during neuronal development and is a crucial factor for the assembly and secretion of the glutamate receptor [102]. Based on these studies, it is possible to speculate that the pharmacological manipulation of the UPR may have therapeutic benefits. However, we recently described that ablation of XBP-1 in the brain does not affect Prion misfolding and pathogenesis *in vivo* [58]. A complex scenario may operate *in vivo* where different UPR signaling branches can provide functional redundancy to compensate for a loss of one pathway. Recent reports indicate that it is feasible to develop pharmacological strategies to decrease ER stress and improve protein folding in a disease context [103]. Synthetic drugs called chemical chaperones, including 4-phenyl butyric acid (4-PBA) and trimethylamine N-oxide dihydrate, are a group of low molecular weight compounds known to stabilize protein conformation, improve ER folding capacity, and facilitate the

trafficking of mutant proteins (review in [104]). Likewise, endogenous bile acid derivatives, such tauroursodeoxycholic acid (TUDCA), can also modulate ER function [105]. Treatment of obese and diabetic mice with chemical chaperones resulted in normalization of hyperglycemia, restoration of systemic insulin sensitivity, resolution of fatty liver disease, and enhancement of insulin action in liver, muscle, and adipose tissues. 4-PBA has been recently shown to prolong survival in an ALS mouse model, and it is now in clinical trials for ALS [106]. 4-PBA can reverse the misfolding of mutant proteins involved in cystic fibrosis, α1-anitrypsin deficiency and juvenile parkinsonism [107,108]. Moreover, 4-PBA has protective effects against ER stress-induced neuronal cell death in models of cerebral ischemic injury [109]. TUDCA administration reduced striatal neuronal apoptosis, as well as the size of intranuclear huntingtin inclusions in different Huntington's disease models [110]. Furthermore, locomotor and sensorimotor deficits were significantly improved with the treatment [110]. The ability of chemical chaperones to alleviate ER stress demonstrates the feasibility of targeting organelle function for therapeutic gain.

Although the possible use of chemical chaperones in a disease context is promising, the effect of these compounds is not specific and affects the general protein folding process. Consequently, potential side effects associated with their use are difficult to predict. In this regard, it is not yet clear if a decrease in accumulation of misfolded proteins or a decrease in UPR signaling is mediating the protective effect observed in the aforementioned disease models. Specific agonists or inhibitors targeted to components of the UPR may display greater efficacy with lower risk of adverse events in specific disease conditions. It will be essential to first define positive or negative effects of such compounds on disease conditions using genetic manipulation of the UPR. In addition, the molecular mechanisms by which the transition between adaptive processes and apoptosis occurs under chronic ER stress are not well understood. In the context of autoimmunity or cancer, inhibition of XBP-1 function may have therapeutic benefits, whereas activation of the UPR may protect against conditions involved in neurodegeneration or diabetes.

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