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Editors

Coordinating Organismal Physiology Through the Unfolded Protein Response

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Preface

The endoplasmic reticulum (ER) is a central stress-sensing platform responsible for regulating diverse biologic functions including protein secretion, lipid synthesis, and cellular metabolism. As such, genetic, environmental, and aging-related insults that challenge ER function (i.e., ER stress) can profoundly influence cellular physiology and contribute to the pathogenesis of diverse diseases. To protect cells against ER stress, eukaryotes evolved a complex, integrated stress-responsive signaling pathway called the Unfolded Protein Response (UPR). While initially identified in yeast as a single signaling pathway, the eukaryotic UPR is a complex signaling network consisting of three integrated signaling pathways activated downstream of the ER stress-sensing transmembrane proteins IRE1, PERK, and ATF6. In response to ER stress, these sensors are activated through diverse mechanisms, resulting in a transient attenuation of new protein synthesis and the activation of stress-responsive transcription factors such as XBP1s (activated downstream of IRE1), ATF4 (activated downstream of PERK), and ATF6 (the active transcription factor cleaved off of full length ATF6). Through these mechanisms, the UPR functions to alleviate ER stress and adapt cellular physiology to protect against a given environmental, genetic, or aging-related insult. However, if activation of these pathways prove insufficient to alleviate a severe or chronic ER insult, the UPR promotes pro-apoptotic signaling to induce cell death. Through this combined adaptive and pro-apoptotic activity, the UPR has a critical role in both regulating cellular physiology and dictating cell fate in response ER stress.

Due to its role in coordinating cellular physiology and fate in response to pathologic insults, UPR signaling is inextricably linked to the regulation of diverse biologic functions including secretory proteostasis maintenance, immune cell development, and cellular and organismal metabolism. In addition, alterations in UPR signaling are implicated in the onset and pathogenesis of diverse diseases including cancer, diabetes, and neurodegenerative disorders. The central importance of UPR signaling in regulating both health and disease has led to significant interest in targeting specific aspects of the UPR to intervene in diverse diseases. As we continue to learn more about the UPR in the context of health and disease, we will gain additional understanding into how to best target UPR signaling in the context

of a given disease and better appreciate the overall implications of how activating or inhibiting selective UPR signaling pathways influence global organismal physiology.

In this volume, we include chapters from experts in diverse aspects of UPR signaling. The first five chapters are designed to highlight unique roles of UPR signaling in the regulation of different aspects of cellular and organismal physiology such as secretory proteostasis, cell–cell signaling, immune cell development, and metabolism. These chapters provide a broad background in the different mechanisms by which UPR signaling can influence biological functions. In the final three chapters, we describe the contributions of altered UPR signaling in etiologically diverse diseases such as cancer, neurodegenerative disease, and cardiovascular disorders to directly demonstrate the critical role for UPR signaling during different types of pathologic insults. While a comprehensive description of the UPR and all of its functions could fill many volumes, the chapters included in this volume are designed to provide a background in understanding how the UPR can influence diverse physiologic functions in the context of health and disease. Our goal in putting this together was to incorporate diverse aspects of UPR signaling to provide the reader the necessary resources to help them understand the importance of UPR signaling in the context of their own interests and systems. We are confident that readers will enjoy the diverse chapters included this volume and hope that this work will continue to spur new interest in understanding the molecular mechanisms by which the UPR influences cellular and organismal biology.

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Contents

Adapting Secretory Proteostasis and Function Through the Unfolded Protein Response

Madeline Y. Wong, Andrew S. DiChiara, Patreece H. Suen, Kenny Chen, Ngoc-Duc Doan and Matthew D. Shoulders

Abstract Cells address challenges to protein folding in the secretory pathway by engaging endoplasmic reticulum (ER)-localized protective mechanisms that are collectively termed the unfolded protein response (UPR). By the action of the transmembrane signal transducers IRE1, PERK, and ATF6, the UPR induces networks of genes whose products alleviate the burden of protein misfolding. The UPR also plays instructive roles in cell differentiation and development, aids in the response to pathogens, and coordinates the output of professional secretory cells. These functions add to and move beyond the UPR's classical role in addressing proteotoxic stress. Thus, the UPR is not just a reaction to protein misfolding, but also a fundamental driving force in physiology and pathology. Recent efforts have yielded a suite of chemical genetic methods and small molecule modulators that now provide researchers with both stress-dependent and -independent control of UPR activity. Such tools provide new opportunities to perturb the UPR and thereby study mechanisms for maintaining proteostasis in the secretory pathway. Numerous observations now hint at the therapeutic potential of UPR modulation for diseases related to the misfolding and aggregation of ER client proteins. Growing evidence also indicates the promise of targeting ER proteostasis nodes downstream of the UPR. Here, we review selected advances in these areas, providing a resource to inform ongoing studies of secretory proteostasis and function as they relate to the UPR.

Contents

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1 Introduction

The endoplasmic reticulum (ER) is responsible for secretory proteostasis, involving the coordinated folding, processing, quality control, and trafficking of ~1/3 of the proteome. Protein folding is a highly complex and error-prone process, requiring a delicate balance between function and risk of aggregation in crowded biological microenvironments where total protein concentrations can range from 100 to 400 mg/mL (Gershenson et al. 2014; Hartl et al. 2011). ER clients, which include secreted, membrane, and lysosomal proteins, face additional challenges, including unique posttranslational modifications (e.g., N-glycosylation) that require specialized cellular machinery (Aebi 2013), oxidative folding processes associated with selective disulfide bond formation (Tu and Weissman 2004), and both spatial and temporal restraints on the completion of folding, modification, assembly, and transport steps. Cells account for this complexity via a diverse array of folding (Hartl et al. 2011) and quality control mechanisms (Smith et al. 2011a), some of which are only recently coming to light. The resulting balance of protein synthesis, folding, and recycling is essential for health. Dysregulated proteostasis in the secretory pathway underpins a diverse array of diseases.

Maintaining secretory proteostasis requires the ability to dynamically respond to challenges such as protein misfolding, often by large-scale remodeling of the ER and the ER proteostasis environment (Walter and Ron 2011). The unfolded protein response (UPR; Fig. 1) is the central stress response pathway involved. The three arms of the metazoan UPR are controlled by the signal transducers IRE1, PERK, and ATF6 (Cox et al. 1993; Harding et al. 1999; Haze et al. 1999; Tirasophon et al. 1998). Activation of these ER transmembrane proteins induces a transcriptional response mediated by three transcription factors, XBP1s (Calfon et al. 2002; Yoshida et al. 2001), ATF4 (Harding et al. 2000; Vattem and Wek 2004), and ATF6f (ATF6-fragment) (Adachi et al. 2008). This coordinated transcriptional response alleviates the burden of protein misfolding in the secretory pathway by upregulating ER chaperone, quality control, and secretion mechanisms (Adachi et al. 2008; Harding et al. 2000; Shoulders et al. 2013b). UPR activation also

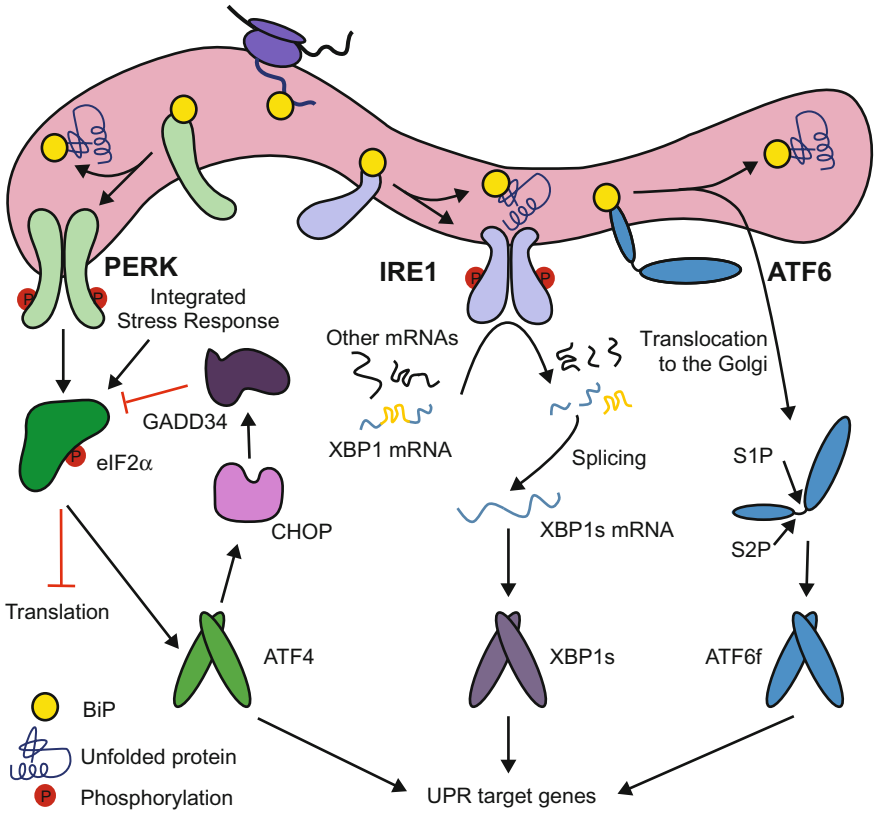

Fig. 1 The unfolded protein response (UPR). Accumulation of misfolding proteins in the endoplasmic reticulum (ER) activates the transmembrane protein UPR signal transducers PERK, IRE1, and ATF6. Dimerization and auto-phosphorylation of PERK and IRE1, or trafficking to the Golgi and subsequent proteolytic processing of ATF6, result in the production of the UPR transcription factors by enhancing translation of ATF4, cleaving and splicing *XBP1* mRNA to yield *XBP1s*, and releasing ATF6f from the Golgi membrane. These transcription factors proceed to the nucleus and remodel the ER proteostasis environment by upregulating chaperones, quality control components, and other UPR target genes to maintain or recover secretory proteostasis. PERK can globally reduce the nascent protein load on the ER via phosphorylation of eIF2α, a pathway that can be similarly induced by the integrated stress response. The RNase domain of activated IRE1 degrades several ER-targeted transcripts and may play a related role

inhibits protein translation to lower the net nascent protein load on the ER, a process mediated primarily by PERK activation and subsequent phosphorylation of eIF2α (Harding et al. 1999), but also influenced by the selective degradation of ER-directed mRNA transcripts by IRE1 (Hollien et al. 2009; Moore and Hollien 2015). If proteostasis cannot be restored, pro-apoptotic mechanisms within the UPR lead to programmed cell death in part through induction of the transcription factor CHOP downstream of PERK.

While extensive research has yielded a relatively well-defined picture of the UPR, the discovery of new regulatory mechanisms continues to shape our understanding of how the UPR relates to ER homeostasis. The ER not only functions as a protein-folding factory, but also participates in calcium storage and lipid biosynthesis (Fu et al. 2011). Along these lines, a recent study highlighted the capacity of IRE1's membrane-spanning domain to activate the protein in response to lipid perturbation even when the luminal protein misfolding stress-sensing domain is deleted using CRISPR/Cas9 (Kono et al. 2017). Moreover, the ER is involved in cellular responses to oxidative stress, metabolic imbalance, and pathogen invasion (Malhotra and Kaufman 2007; Roy et al. 2006; Volmer and Ron 2015). Each of these processes is modulated by the UPR. Thus, despite its name, the UPR is not simply a reaction to protein misfolding, but is instead a fundamental driving force for physiology and pathology. The central roles of the UPR in health and disease have catalyzed the development of methods to modulate the UPR, with the goal of better understanding key regulatory axes and identifying opportunities to influence phenotypic outcomes. Below, we review our current picture of the metazoan UPR in the context of secretory proteostasis, from its connections to health and disease (Sect. 2), to methods for selectively perturbing the UPR and their potential applications in disease therapy (Sect. 3), to efforts to target downstream nodes in ER proteostasis (Sect. 4).

2 The UPR in Health and Disease

Key functional nodes within the ER proteostasis network include chaperones, quality control mechanisms, posttranslational modifiers, and trafficking pathways (Fig. 2). Each of these nodes is dynamically regulated by the UPR to match proteostatic capacity to demand, thereby maintaining balanced levels of protein folding and quality control both during normal cellular function and under stressful conditions.

2.1 Development, Professional Secretory Cells, and Immunity

The UPR plays critical roles during development that have been demonstrated in several model systems (Coelho et al. 2013; Shen et al. 2001). In particular, UPR activation appears to upregulate ER-resident chaperones and signaling pathways, which work collectively to relieve stress and regulate development in differentiating cells (Dalton et al. 2013; Laguesse et al. 2015; Reimold et al. 2001). For example, upon B-cell differentiation into plasma cells, the ER undergoes extensive XBP1-driven expansion (Reimold et al. 2001; Shaffer et al. 2004), in part to

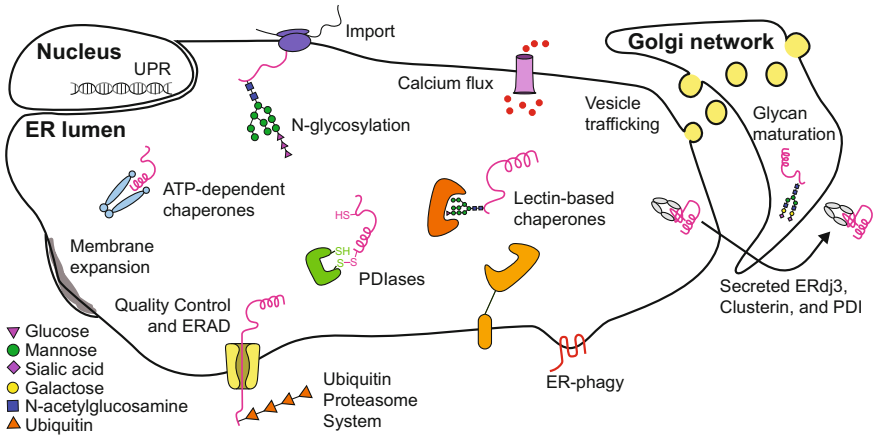

Fig. 2 Representative nodes in the secretory proteostasis network. Diverse proteins and pathways collectively modulate folding, secretion, quality control, and/or degradation of ER clients. ATP-dependent chaperones and PDIases assist in the folding of client proteins, as do lectin-based chaperones such as calnexin and calreticulin. Terminally misfolded proteins are typically cleared by ER-associated degradation (ERAD) via the ubiquitin-proteasome system. ER-phagy can serve as a counterpart to membrane expansion mechanisms, reducing organelle size to regulate ER proteostasis. Meanwhile, calcium flux, vesicle trafficking, and UPR-mediated changes in the chaperone:client balance, import, disulfide bond formation, and N-glycosylation of nascent polypeptides help to maintain or create favorable folding conditions and buffer ER protein-folding capacity. A handful of chaperones, including ERdj3, can accompany proteins to the extracellular space

accommodate high levels of antibody synthesis. B-Cell differentiation in vitro also induces the UPR-regulated proteins XBP1s, BiP, and Grp94. Notably, the process occurs without expression of CHOP or inhibition of protein translation, suggesting that a physiologic UPR need not involve all three UPR arms, in contrast to the case of attenuating acute ER stress-induced protein misfolding. Moreover, induction of XBP1, BiP, and Grp94 transcripts apparently occurs prior to any significant protein-folding load on the ER, suggesting further differences between developmental and stress-associated signaling pathways (Gass et al. 2002). Other professional secretory cells, including pancreatic β-cells, hepatic cells, and osteoblasts, also must sustain high rates of ER client protein synthesis, folding, and secretion, and thus rely on the UPR and its downstream signaling mechanisms for survival and function.

Other work highlights roles of the UPR in cellular responses to pathogen invasion. Binding of unfolded cholera toxin A subunit induces IRE1α ribonuclease activity, but not the canonical UPR involving PERK and ATF6 (Cho et al. 2013). The fragments of endogenous mRNA produced by IRE1α prompt RIG-I to activate NF-κB and interferon signaling. Other work indicates that Toll-like receptors (TLRs) in macrophages promote splicing of XBP1 to optimize the production of proinflammatory cytokines (Martinon et al. 2010), although XBP1s can also be essential in protecting against the effects of prolonged inflammation (Adolph et al.

2013; Richardson et al. 2010). Viral pathogens are also capable of hijacking the UPR to promote proliferation in host cells. For example, IRE1 activity is critical for the replication of at least some strains of the influenza virus (Hassan et al. 2012). In contrast, HSV1 suppresses both PERK and IRE1 signaling: glycoprotein B interacts with the luminal domain of PERK to block kinase activation, the late viral protein γ1 34.5 recruits PP1α to dephosphorylate eIF2α, and the UL41 protein acts as an endoribonuclease to degrade XBP1 mRNA (Zhang et al. 2017). Similarly, recent studies of *Legionella pneumophila*, the organism responsible for Legionnaires' disease, show that the pathogen forestalls a typical ER stress response by repressing translation of a subset of UPR-associated genes to prevent host-cell apoptosis that would otherwise be induced (Hempstead and Isberg 2015; Treacy-Abarca and Mukherjee 2015). The relevant bacterial effector proteins may serve as springboards for biomimetic approaches to modulate UPR pathways.

2.2 *Emerging Functions of the UPR*

Beyond established roles in development and immunity, new functions for and consequences of the UPR continue to emerge. ER recycling via ER-phagy is critical for ER homeostasis (Bernales et al. 2006), and several constituent biochemical pathways were recently mapped (Fumagalli et al. 2016; Khaminets et al. 2015). A possible role for the IRE1-XBP1s arm of the UPR in inducing such ER-phagy may exist (Margariti et al. 2013). By reducing organelle size and/or disposing of dysfunctional ER regions, UPR induction of selective ER-phagy could serve as a counterpart to membrane expansion mechanisms for resolving ER stress (Schuck et al. 2009).

The discovery that the IRE1-XBP1s axis of the UPR is responsible for cell non-autonomous UPR activation (Taylor and Dillin 2013) is also intriguing. Such cell-to-cell communication of stress is likely to have important biological consequences that merit further investigation. ATF6 activation was recently shown to increase not just expression but also secretion of ERdj3, an ER-localized Hsp40 co-chaperone (Genereux et al. 2015). The consequent co-secretion of ERdj3 with misfolded client proteins may be protective for the origin cell or ameliorate harmful protein aggregation in the extracellular milieu. Stress-induced ERdj3 secretion thus provides a mechanism by which the UPR can modify not just ER but also extracellular proteostasis.

Emerging functions of the UPR described above focus on direct modulation of protein folding and production. In addition to these mechanisms, a role for the ER in regulating the extent of protein posttranslational modifications has emerged. For example, two groups showed that the UPR can modulate hexosamine biosynthesis to promote ER client clearance and prolong life in the face of chronic protein misfolding stress (Denzel et al. 2014; Wang et al. 2014). These studies suggest that UPR

activation, and especially the IRE1-XBP1s arm of the UPR, may enhance the extent of client protein N- and O-glycosylation and/or modify oligosaccharyltransferase efficiency. While the consequences require further investigation, N-glycosylation promotes both ER client folding, by providing access to the lectin-based chaperone machinery, and the identification of misfolded proteins for ER-associated degradation (ERAD) via the lectin-based quality control machinery, providing a potential rationale for IRE1-XBP1s enhanced N-glycosylation (Ruiz-Canada et al. 2009).

Surprisingly, the UPR can also remodel the actual molecular architecture of N-glycans added to ER client proteins by modulating their biosynthesis (Dewal et al. 2015). Stress-independent activation of XBP1s changes transcript levels of N-glycan modifying enzymes, leading to altered mature glycan structures on model secreted N-glycoproteins. More work is required to establish the biological relevance and consequences of this phenomenon. However, this newly established connection between N-glycan signatures and the UPR suggests that the UPR may unexpectedly influence processes such as cell–cell interactions, cell–matrix interactions, and trans-cellular communication by actually modifying the molecular structure of secreted ER clients (Dewal et al. 2015).

2.3 Dysregulated ER Proteostasis and Disease

When proteostasis networks function properly, cells maximize production of properly folded, functional proteins. Meanwhile, quality control mechanisms ensure that only folded proteins are transported to their final locations, while production of misfolded and aggregated proteins is minimized (Fig. 3a). The UPR regulates this process by sensing the accumulation of misfolded proteins, whether due to genetic mutations or adverse physiological conditions, and remodeling the ER proteostasis network to resolve emerging problems.

Chronically dysregulated ER proteostasis, unresolved by the UPR, leads to diverse protein misfolding and aggregation-related diseases. For many mutations that destabilize or prevent the folding of a protein, the UPR may in principle have the potential to resolve the proteostatic defect—if it is activated. However, just one mutant protein misfolding in a background of thousands of well-behaved proteins may not always be sufficient to trigger a protective UPR. In other cases, the ER may be overwhelmed by high concentrations of an aggregating mutant protein, leading to chronic ER stress and cellular apoptosis. In either scenario, pharmacologic perturbation of the UPR could be therapeutically useful. Moreover, many cancer cells rely on constitutive activation of pro-survival pathways (in particular the IRE1-XBP1s arm) within the UPR (Chen et al. 2014b; Jamora et al. 1996; Mimura et al. 2012). This observation suggests that UPR inhibition could also prove valuable for diseases that do not stem directly from protein misfolding.