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ROS signaling and ER stress in cardiovascular disease

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ABSTRACT

The endoplasmic reticulum (ER) produces the vast majority of all proteins secreted into the extracellular space, including hormones and cytokines, as well as cell surface receptors and other proteins which interact with the environment. Accordingly, this organelle controls essentially all vital links to a cell's external milieu, responding to systemic metabolic, inflammatory, endocrine, and mechanical stimuli. The central role the ER plays in meeting protein synthetic and quality control requirements in the face of such demands is matched by an extensive and versatile ER stress response signaling network. ROS mediate several critical aspects of this response. Nox4, an ER resident capable of producing ROS, acts as a proximal signaling intermediate to transduce ER stress-related conditions to the unfolded protein response, a homeostatic corrective mechanism. However, chronic ER stress caused by unrelenting internal or external demands produces a secondary rise in ROS, generally resulting in cell death. Sorting out the involvement of ROS at different levels of the ER stress response in specific cell types is key to understanding the molecular basis for chronic diseases such as atherosclerosis, hypertension, and diabetes. Here, we provide an overview of ER stress signaling with an emphasis on the role of ROS.

Introduction

Even at repose, the human body is constantly subjected to a variety of biochemical and biomechanical stresses. Thus, a complex and interlocked series of cellular response mechanisms has evolved to cope with the daily stresses of life. Not surprisingly, the molecular basis for a number of diseases such as atherosclerosis, diabetes, and neurodegeneration can be traced to the failure of these response pathways to rectify such stresses. Interestingly, the ER, best known as a protein synthesis organelle, is now recognized as a principal organizer of cellular stress response pathways, and is consequently the focus of much work in these chronic diseases. This functional arrangement in part stems from the sensitivity of de novo protein translation, folding, and post-translational modifications to optimal intracellular conditions; therefore, the ER reacts to any perturbations which compromise the integrity of this process. It has also become clear that the different arms of the ER stress pathways can differentially respond to specific cellular stressors independent of protein misfolding, increasing the biological versatility of ER-based pathways in stress response.

A unique characteristic of the ER is its luminal redox tension. Since the vast majority of proteins translated within the ER lumen are destined for secretion or to span the plasma membrane and extend into the extracellular space, the ER must post-translationally modify proteins to function outside of the cell. Thus, the ER interior mimics the relatively oxidized extracellular environment and promotes crosslinking of sulfhydryl groups to form disulfides that would not survive the reduced cytosol. Accurate titration and strict compartmentalization of the oxidized intraluminal environment renders the ER sensitive to perturbations in overall cellular redox status, so the ER responds rapidly to both energetic and redox stresses. Perhaps related to this, the production of ROS has become an essential part of the ER stress response. While seemingly counterproductive, such spatiotemporally-directed ROS production is now recognized as a specific signal transduction mechanism for many cellular functions. This response raises several key questions such as how do we, and in fact, how does the ER, distinguish such ROS signaling from oxidative stress, and how does it distinguish each of these from normal oxidation reactions within the ER? More importantly, which ER mechanisms go awry in cardiovascular and metabolic diseases, and how do ROS affect disease progression?

The unfolded protein response

ER stress is generally defined as the accumulation of misfolded client proteins within the ER lumen, which itself is difficult to measure and quantify. ER stress is therefore most commonly inferred by activation of the unfolded protein response (UPR). At the outset, it should be recognized that using the UPR as a surrogate measure of ER stress has significant limitations, especially when various arms of the UPR are asymmetrically activated or disabled. In addition, interventions which attenuate the UPR do not necessarily reduce ER stress, and specific

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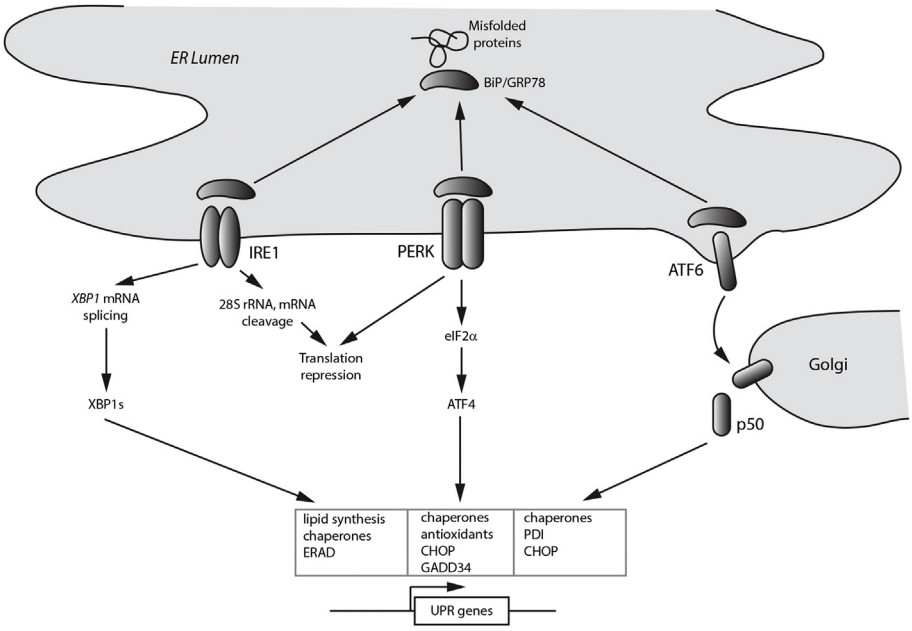

Fig. 1. *Overview of the unfolded protein response.* Schematic shows titration of the chaperone BiP/Grp78 from the three main ER sensors IRE1 (inositol requiring enzyme 1), PERK (PKR-like endoplasmic reticulum kinase), and ATF6 (activating transcription factor 6) by misfolded proteins. The IRE1 pathway, through production of the alternatively translated product XBP1s, induces genes involved in lipid biosynthesis, ER-associated protein degradation (ERAD), and chaperones, to promote ER biogenesis, protein refolding, and disposal of damaged proteins. The PERK pathway, largely through induction of the transcription factor ATF4, additionally induces antioxidant-related genes and the phosphatase cofactor GADD34, which dephosphorylates eIF2α and deactivates the unfolded protein response (UPR). Release of ATF6 from BiP exposes Golgi localization signals, and ATF6 subsequently undergoes regulated intramembrane proteolysis to release a 50 kDa active transcription factor that targets genes involved in ER biogenesis and protein refolding.

pathways associated with ER stress can be coopted by other stress-related stimuli. Thus, different arms of the UPR may move in parallel to or independent of ER stress itself. However, a basic understanding of the UPR is required to study ER stress and its role in human disease.

Under normal conditions, the intraluminal domains of the three canonical UPR sensors PERK (PKR-like eukaryotic initiating factor α kinase), ATF6 (activating transcription factor-6), and IRE1 (inositol requiring enzyme 1) are capped by the chaperone BiP/GRP78 and rendered inactive. Only properly folded proteins are allowed to enter ER exit vesicles and leave the ER; consequently, when excessive synthetic demands or suboptimal processing conditions prevail, misfolded client proteins accumulate in the ER and titrate BiP away from these sensors, signaling ER stress. The dissociation of BiP allows oligomerization of PERK and IRE1 and translocation of ATF6 to the Golgi, collectively initiating the classical tripartite UPR (Fig. 1). Mammals express ten known ER stress sensors: IRE1α, IRE1β, PERK, ATF6α, ATF6β, and five other ATF6-like ER membrane-bound transcription factor proproteins (OASIS, BBF2H7, Luman, CREBH, and Tisp40). The large number of stress sensors reflects the evolutionary importance of ER stress and complexity of the cellular response.

The oldest branch of the UPR is IRE1, a serine/threonine kinase conserved from yeast to humans. Upon release of BiP, IRE1 homodimerizes and transphosphorylates, revealing its C-terminal endoribonuclease domain. Yeast Ire1 appears to be capable of directly binding misfolded proteins as well as BiP (Kimata et al., 2004), and its autophosphorylation is dispensable for mRNA splicing but may delay signal termination (Chawla et al., 2011). The IRE1 endoribonuclease domain specifically recognizes and cleaves a 26-base fragment from the *XBP1* mRNA transcript (*HAC1* in yeast), resulting in an altered translational product, XBP1s. XBP1s in turn acts as a transcription factor which recognizes ER stress-response elements in the promoter regions of genes encoding chaperones such as BiP and proteins involved in ER biogenesis, secretory function, and the degradation of damaged proteins. The latter function primarily entails the exportation of misfolded proteins from the ER into the cytosol, recognition and ubiquitination of these proteins, and proteasomal degradation, in total the process of ERAD (ER-associated protein degradation). IRE1 is thought to cleave additional mRNAs targeted to the ER as well as the 28S ribosomal subunit, thus decreasing global protein translation (Iwawaki et al., 2001; Hollien and Weissman, 2006). Interestingly, TRAF2 is also recruited to IRE1 through JIK (JNK inhibitory kinase), triggering activation of the ASK1/JNK pathway. The highly conserved IRE1 sensor thus links ER stress with other stress response pathways, a recurrent paradigm in ER biology.

Like IRE1, PERK is an ER resident type I transmembrane serine/threonine kinase that homodimerizes and autophosphorylates upon release of intraluminal BiP. Phosphorylation of its key substrate, the translation initiation factor eIF2α, converts it into a competitor of eIF2B which broadly suppresses translation to alleviate protein folding and processing demands within the ER. Simultaneously, eIF2α phosphorylation also selectively increases translation of the transcription factor ATF4, which transactivates amino acid transporter and redox control genes (Harding et al., 2000). Because of its latter activity, the PERK pathway is most closely tied to oxidative stress and signaling, as discussed below. ATF4 also upregulates GADD34, a stress response phosphatase cofactor that promotes dephosphorylation eIF2α and translational recovery (Fig. 1).

ATF6 represents the third family of ER stress sensors. While mammals express seven ATF6 paralogs, ATF6α and ATF6β are best studied. Dissociation of BiP from ATF6 exposes two Golgi localization signals, causing ATF6 translocation to the Golgi. Notably, reduction of disulfides on the luminal surface of ATF6, normally held in an oxidatively cross-linked state, is also required for Golgi translocation (Nadanaka et al., 2007). Thus ATF6 can directly respond to abnormally reducing conditions within the ER, which is thought to be a unifying biochemical feature of ER stress (Merksamer et al., 2008). Following Golgi translocation, ATF6 is cleaved by regulated intramembrane proteolysis (RIP) by the Golgi resident proteases S1P and S2P to release its 50 kDa cytosolic domain as a functioning bZIP transcription factor. The targets of this factor include genes which promote ER maintenance, protein folding, and post-translational modification such as the chaperones BiP and Grp94, and cysteine cross-linker PDI (protein disulfide-isomerase) (Fig. 1). Of the three pathways, ATF6 target genes are perhaps the most focused on maintenance of ER homeostasis.

Intersection of the UPR with other stress pathways

An important concept uncovered in recent years has been the appreciation that different sensors and effector pathways of the UPR respond differentially to a variety of perturbations and cellular stresses which do not directly compromise protein quality; thus, the constituents of the “unfolded protein response” are not necessarily