This Review is part of a thematic series on **Endoplasmic Reticulum Stress and Cardiac Diseases**, which includes the following articles:
What Is the Role of ER Stress in the Heart? Introduction and Series Overview [*Circ Res.* 2010;107:15–18]
The Role of Endoplasmic Reticulum Stress in the Progression of Atherosclerosis [*Circ Res.* 2010;107:839–850]
Endoplasmic Reticulum Stress As a Therapeutic Target in Cardiovascular Disease [*Circ Res.* 2010;107:1071–1082]

Biology of Endoplasmic Reticulum Stress in the Heart

Interrelationship Between Cardiac Hypertrophy, Heart Failure, and Chronic Kidney Disease—Endoplasmic Reticulum

Masafumi Kitakaze, Guest Editor

Biology of Endoplasmic Reticulum Stress in the Heart

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Abstract: **The endoplasmic reticulum (ER) is a multifunctional intracellular organelle supporting many processes required by virtually every mammalian cell, including cardiomyocytes. It performs diverse functions, including protein synthesis, translocation across the membrane, integration into the membrane, folding, posttranslational modification including *N*-linked glycosylation, and synthesis of phospholipids and steroids on the cytoplasmic side of the ER membrane, and regulation of Ca^{2+} homeostasis. Perturbation of ER-associated functions results in ER stress via the activation of complex cytoplasmic and nuclear signaling pathways, collectively termed the unfolded protein response (UPR) (also known as misfolded protein response), leading to upregulation of expression of ER resident chaperones, inhibition of protein synthesis and activation of protein degradation. The UPR has been associated with numerous human pathologies, and it may play an important role in the pathophysiology of the heart. ER stress responses, ER Ca^{2+} buffering, and protein and lipid turnover impact many cardiac functions, including energy metabolism, cardiogenesis, ischemic/reperfusion, cardiomyopathies, and heart failure. ER proteins and ER stress-associated pathways may play a role in the development of novel UPR-targeted therapies for cardiovascular diseases. (*Circ Res*. 2010;107:1185-1197.)**

Key Words: endoplasmic reticulum stress ■ misfolded protein response ■ autophagy ■ endoplasmic reticulum–associated degradation ■ cardiac disease

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The endoplasmic reticulum (ER) is a centrally located, multifunctional, and multiprocess intracellular organelle supporting many mechanisms required by virtually every mammalian cell, including cardiomyocytes. The membrane performs a remarkable number of diverse functions, including protein synthesis, translocation across the membrane, integration into the membrane, folding, posttranslational modification including *N*-linked glycosylation, and synthesis of phospholipids and steroids on the cytoplasmic side of the ER membrane, and regulation of Ca^{2+} homeostasis.[1] Development and maintenance of optimally functioning ER membrane is essential for virtually all cellular activities, from intracellular signaling to control of transcriptional pathways; ion fluxes to control of energy metabolism; protein synthesis to multisubunit assembly; and lipid synthesis to transcriptional regulation of steroid metabolism. One of the major advantages of the centrally located ER network for the cell is the ability to control the composition and the dynamics of the ER luminal environment in an extracellular environment-independent way. Furthermore, the ER is not an isolated organelle because it has developed sophisticated mechanisms of communication with many other cellular compartments,

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Non-standard Abbreviations and Acronyms	
AMPK	AMP-activated protein kinase
ATF	activating transcription factor
CHOP	C/EBP homologous protein
CRE	cAMP-response element
ER	endoplasmic reticulum
ERAD	endoplasmic reticulum–associated degradation
ERp	endoplasmic reticulum protein
ERSE	endoplasmic reticulum stress element
GRP	glucose-regulated protein
IRE	inositol-requiring kinase
JNK	c-Jun amino-terminal kinase
PDI	protein disulfide isomerase
PERK	dsRNA-activated protein kinase–like endoplasmic reticulum kinase
SR	sarcoplasmic reticulum
SREBP	sterol response element binding protein
UPR	unfolded protein response
UPRE	unfolded protein response element
XBP	X-box binding protein

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especially mitochondria,[2] the plasma membrane,[3] and the nucleus.[4] Perturbation of any of the major functions of ER (protein folding, lipid and sterol synthesis, and Ca^{2+} homeostasis) results in ER stress via the activation of complex cytoplasmic and nuclear signaling pathways collectively termed the unfolded protein response (UPR) (also known as misfolded protein response; Figure 1).[4] The UPR has been associated with numerous human pathologies[4] and may play an important role in the pathophysiology of the heart.[5] The aim of this review is to focus on the biology of ER stress and its role in cardiac physiology and pathology.

Endoplasmic and Sarcoplasmic Reticulum in the Heart

In the heart muscle cell, sarcoplasmic reticulum (SR) is recognized as an intracellular organelle specializing in the regulation of Ca^{2+} fluxes and control of the excitation–contraction coupling in the heart. For many decades, SR has been considered a unique name for a muscle-specific specialized form of the ER. Is the SR just a label reflecting specialized functional features of muscle ER, or is there a functional muscle ER separate from a traditional SR membrane? These questions remain unanswered. The existence of a functional ER compartment in cardiac muscle was proposed almost a decade ago,[6] but only recently has existence of a functional ER compartment received significant attention in the cardiovascular community. Molecular studies on ER function and proteins indicate that ER-associated functions are critical in cardiac physiology and pathology.[7,8] The ER has also developed sophisticated mechanisms operating in the heart to support ER-stress responses and ER-dependent intracellular signaling. The UPR triggers an adaptive response to reestablish ER homeostasis by coordinating reduction in the quantity of protein expressed, with increased production

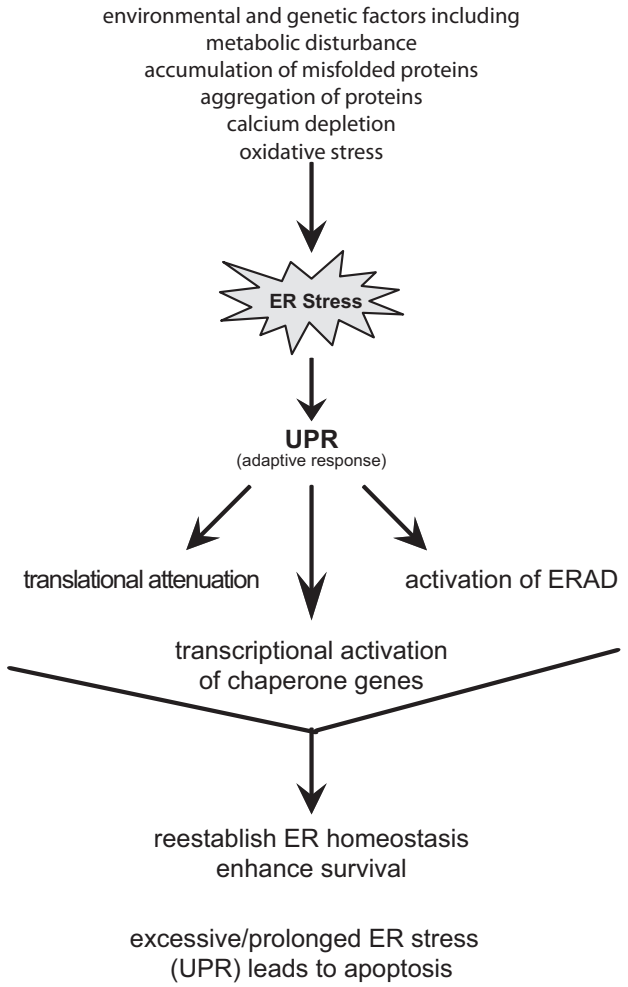

Figure 1. ER stress and the UPR. Schematic representation of ER stress (UPR) signaling pathway. Activation of ER stress (UPR) results in protein translational attenuation, transcriptional activation of chaperone and folding enzyme genes, and activation of ERAD to rectify the accumulation of misfolded protein. The goal of the UPR is to limit cellular damage by adapting to the situation causing the ER stress by reestablishing ER homeostasis and enhancing survival. Prolonged UPR may lead to apoptosis.

of chaperones to handle accumulation of misfolded protein, as well as promotion of ER-associated degradation to remove misfolded proteins (Figure 1).[4] This initial response of protein synthesis suppression and upregulation of ER resident chaperones is designed to resolve the ER stress, restore homeostasis, and enhance survival. If ER stress is severe or prolonged, the UPR may stimulate apoptosis, a significant feature of hypoxia and ischemia/reperfusion injury.[9] Recent reports (reviewed elsewhere[5]) have demonstrated an integral role for ER stress in the heart during prolonged insult, such as pressure overload, or during acute stress, such as ischemia/reperfusion.

Quality Control in the Secretory Pathway

The protein-folding apparatus in the ER prevents the deployment of aberrant protein conformations, retains nascent proteins in an environment suitable for their maturation, favors correct multiprotein assembly by elevating subunit concen-

tration, reduces the risks of protein toxicity by inhibiting aggregation and degrading terminally misfolded proteins, maintains transport homeostasis in the early secretory pathway, ensures posttranslational control of protein expression, guarantees developmental regulation of protein secretion, and stores proteins for regulated secretion. All of these functions translate into the association of nascent proteins with molecular chaperones and enzymes, including BiP/glucose-regulated protein (GRP)78, calreticulin, calnexin, GRP94, and the thiol oxidoreductases protein disulphide isomerase (PDI) and ER protein (ERp)57, which are all involved in generating conformationally competent and functional proteins. BiP/GRP78 and GRP94 have the ability to recognize exposed hydrophobic regions, a common feature of nascent misfolded proteins, thus assisting in protein folding and assembly and preventing premature export. BiP/GRP78 also performs numerous functions including Ca^{2+} buffering, acting as a permeability barrier on the ER luminal side by blocking the Sec61 translocon pore, binding hydrophobic domains, and using ATP to fold nascent proteins, and sensing and binding misfolded proteins, as well as regulating the UPR and ER-associated degradation (ERAD).[10] Calreticulin and calnexin interact with glycoproteins via their lectin binding ability, allowing folding and interactions with enzymes to occur in a regulated manner.[11] Calnexin and calreticulin present polypeptides for disulfide bond formation to a member of the PDI family, ERp57, which directly binds to these molecular chaperones.[12] ERp57, a thiol oxidoreductase, uses the oxidizing environment of the ER to generate disulfide linkages.[13] These enzymes are directly affected by deficiencies in molecular oxygen, because oxygen is the primary donor of the energy for disulfide bond formation. Correctly folded proteins are recognized by folding sensors, followed by transit through the secretory pathway. If the quality control cycle is unable to fold the protein, the misfolded proteins accumulate and trigger a variety of signaling pathways to control resultant ER stress.[4] Strict regulation of ER luminal Ca^{2+} concentration using ER Ca^{2+} buffering proteins is of importance in the maintenance of cellular integrity. Disruption of the Ca^{2+} homeostasis in the ER is a potent trigger of ER stress and apoptosis.

ER Stress and The UPR

UPR activation involves 3 distinct steps: (1) translational attenuation to avoid further accumulation of misfolded proteins in the ER; (2) transcriptional activation of chaperone and protein folding genes; and (3) activation of ER-associated degradation in an attempt to rectify the accumulation of misfolded protein.[4] Three ER integral membrane proteins (PERK [dsRNA-activated protein kinase–like ER kinase], inositol-requiring kinase [IRE]1, and activating transcription factor [ATF]6), in combination with the ER molecular chaperone BiP/GRP78,[15] are primarily responsible for the response to ER stress. ATF6,[16] in conjunction with the transcription factor X-box binding protein (XBP)1, a product of the IRE1-dependent splicing of the XBP1-specific mRNA target genes regulated by promoters containing ER stress response elements, termed ER stress element (ERSE)-I and -II, UPRE (UPR element), and cAMP-response element (CRE) (Table 1).[4] ERSE-I is composed of CCAAT-N9-CCACG (where N is any nucleotide)[17]; ERSE-II, ATTGG-N-CCACG[18]; UPRE, TGACGTGGA[19]; and CRE, TGACGT(C/A)(G/A).[20] A combination of these 3 ER resident transmembrane proteins, IRE1, ATF6 and PERK, in conjunction with BiP/GRP78, results in the downregulation of protein synthesis, consequently leading to a reduced influx of nascent protein into the ER, upregulation of expression of ER chaperones, preparing the ER to handle the elevated level of misfolded proteins, as well as upregulating proteins involved in ERAD.[21]

Table 1. List of Selected Genes Regulated by ATF6 and XBP1 Transcription Factors

Protein Name	Symbol	NCBI Accession No.	Reference
Calreticulin	Calr	NM_007591	58, 108, 109
Calnexin	Canx	NM_001110499	58, 109
GRP94	Tra1	NM_011631	109, 110
GRP78	Hspa5	NM_022310	109–111
XBP1	Xbp1	NM_013842	109, 111
ERp57	Pdia3	NM_007952	109, 110
Derlin-3	Derl3	NM_024440	58, 112
ERO1L	Ero1L	NM_026184	58, 111
ERp72	Pdia4	NM_009787	110
ERp59	Ph4b	NM_011032	108, 109
ERGIC-53	Lman1	NM_027400	113
ATF4	Atf4	NM_009716	111
ATF6	Atf6	NM_001081304	109
P58IPK	Dnajc3	NM_008929	58
PDIa6	Pdia6	NM_027959	58, 109
DNAjb11		NM_026400	108
ERdj4	DNAjb9	NM_013760	108, 111
EDEM1	Edem1	NM_138677	109
MCP1	Ccl2	NM_011333	114
CHOP	Ddit3	NM_007837	109–111
c-Myc	Myc	NM_010849	108
Puma	Bbc3	NM_133234	115
Oasis	Creb3l1	NM_011957	116
Eif2a	Eif2a	NM_001005509	58
Stat3	Stat3	NM_011486	58

ERO1L indicates endoplasmic oxidoreductin-1–like protein; ERGIC-53, endoplasmic reticulum–Golgi intermediate compartment 53; P58IPK, protein kinase inhibitor of 58 kDa; PDIa6, PDI family A, member 6; ERdj3, endoplasmic reticulum DnaJ homolog 3; ERdj4, endoplasmic reticulum DnaJ homolog 4; HSP40, heat shock protein 40; EDEM1, endoplasmic reticulum degradation enhancer, mannosidase α–like 1; MCP1, monocyte chemotactic protein 1; c-Myc, myelocytomatosis viral oncogene homolog; Puma, p53 upregulated modulator of apoptosis; Oasis, old astrocyte specifically induced substance; Stat3, signal transducer and activator of transcription 3.

BiP/GRP78: ER Stress Sensor

BiP/GRP78 is a centrally located modulator/sensor of ER stress. The protein is involved in sensing misfolded protein accumulation in the ER and in conjunction with 3 other ER transmembrane proteins (ATF6, IRE1, and PERK) is responsible for the UPR. BiP/GRP78 was initially identified to bind immunoglobulin heavy chain binding protein (IgH) in B cells.[22] Glucose starvation, one of the downstream effects of hypoxia and

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ischemia, regulates BiP/GRP78.[23] BiP/GRP78 is a monomeric, globular protein with 2 functional domains, an ATP binding domain and a peptide binding domain and functionally sorts and releases terminally misfolded substrates to the ERAD pathway.[10] *O*-Mannosylation may play a role in retaining misfolded ERAD substrates in BiP/GRP78-independent manner.[24] Consequently, impaired *O*-mannosylation induces the UPR, suggesting that this modification reduces the amount of misfolded proteins that need to be recognized by BiP/GRP78.[24]

Similar to other ER resident chaperone and folding enzymes, BiP/GRP78 plays an important role as a Ca^{2+} buffer in the lumen of ER.[25] Association between BiP/GRP78 and nascent polypeptides is stabilized by high Ca^{2+} concentrations.[26] BiP/GRP78 has also been shown to be involved in Ca^{2+} transport from the ER to the mitochondria through transient associations with the ER membrane σ-1 receptor (Sig-1R).[27] Recent studies suggest that BiP/GRP78 plays critical cytoprotective roles in oncogenesis.[28,29] Interestingly, BiP/GRP78 expression is upregulated after an ischemic event, likely because of the activation of the ER stress responses.[30–32]

PERK Pathway

BiP/GRP78 interacts with the ATPase binding domain of PERK (Figure 2A; see also Figure S1 at http://www.cidms.org/pathways/er_stress/s1.html). The transmembrane protein PERK, on activation, triggers the phosphorylation of the translation initiation factor eIF2α at Ser,[51] maintaining it in the GDP bound state, which inhibits protein synthesis by sequestering the $tRNA_{met}$ responsible for initiating the translation of nascent protein.[15] During this period, only select mRNAs, such as ATF4, that contain a structural feature such as cap-independent translation initiation site, a secondary open-reading frame in the 5′ untranslated region, or leaky scanning mechanisms, are translated (Figure 2A).[33,34] ATF4 transcriptional activity induces both prosurvival (early) and proapoptotic (late) transcriptional programs.[35] Prolonged or extreme ER stress uses ATF4 to induce expression of the proapoptotic protein C/EBP homologous protein (CHOP).[35] The PERK-deficient mice develop diabetes resulting from death of pancreatic β-cells.[36] Under nonstressed conditions, endogenous PERK function is inhibited by the activity of prolyl hydroxylase, which uses molecular oxygen to enzymatically modify proline within the consensus LxxLAP motifs. This targets the molecule for ubiquitination and degradation, preventing PERK activity during periods of normoxic conditions.[37] Hypoxia stimulates inhibition of prolyl hydroxylase, promoting activation of PERK, and it reduces postischemic infarct size.[38] Prolyl hydroxylases also modify another oxidative response protein, the Hif1 (hypoxia-inducible factor 1), preventing premature activation of oxidative mechanisms until a hypoxic event occurs.[39] PERK is also responsible for phosphorylating another protein, NRF2, resulting in its translocation to the nucleus where it activates expression of oxidative genes and coordinate upregulation of proteins such as glutathione as a buffer for reactive oxygen species in response to oxidative stress induced by ischemia.[40]

ATF6 Pathway

ATF6α is a transmembrane ER protein that, after directed cleavage, becomes a potent transcription factor (Figure 2B; see also Figure S2 at http://www.cidms.org/pathways/er_stress/s2.html). Under nonstress conditions, BiP/GRP78 remains associated with ATF6α, retaining it in the ER. After an accumulation of misfolded proteins, BiP/GRP78 is sequestered away from ATF6α, resulting in translocation of ATF6α to the Golgi, where it undergoes cleavage by site-1 and site-2 (S1P and S2P) proteases.[16] This yields a soluble basic leucine zipper (bZIP) transcription factor, N-ATF6α, that translocates to the nucleus and induces target genes by binding directly to ERSE or ATF/CRE (Figure 2B).[17] ATF6 can induce expression of more than 30 different genes including BiP/GRP78, proteins involved in ERAD, and XBP1.[41] Interestingly, ATF6α is a glycoprotein that interacts with calreticulin. ER stress triggered by Ca^{2+} depletion induces the formation of a nascent, partially glycosylated form of ATF6α with reduced interaction with calreticulin and faster rate of traverse to the Golgi, resulting in higher transactivation of N-ATF6α gene targets.[42] Because an accumulation of underglycosylated proteins in the ER has the ability to induce the UPR, the glycosylation status of ATF6α may serve as a novel sensor of glycoprotein homeostasis, leading to activation of the UPR. There are 2 isoforms of ATF6 (ATF6α and ATF6β) that are characterized by their divergent transcriptional activity domains.[43] ATF6α is an efficient transcriptional activator and ATF6β, a rather poor transcriptional activator. There appears to be "fine tuning" of the ATF6 branch of UPR with the different isoforms, α and β, regulating the strength and duration of the response.[44] Surprisingly, coexpression of both ATF6α and ATF6β show ATF6β-mediated inhibition of ATF6α transcriptional activity of BiP/GRP78 on stimulation of ER stress, with ATF6β potentially a transcriptional repressor.[43] ATF6α- or ATF6β-deficient mice are viable, whereas a double knockout is embryonic lethal.[45] A dominant-negative mutant of ATF6α leads to increased apoptosis, with the transgenic mice demonstrating an increase in the left ventricle diameter and reduced fractional shortening followed by heart failure and death.[46] ATF6α performs an important role during the ER stress response but also maintains cardiac function under physiological conditions.

IRE1 Pathway

IRE1 is a type I ER transmembrane, bifunctional protein that contains a serine–threonine kinase module and a COOH-terminal endoribonuclease domain in its cytoplasmic region. There are 2 mammalian homologs of IRE1, -α and -β, with IRE1α being ubiquitously expressed and IRE1β being restrictively expressed in the gut.[20,47] As BiP/GRP78 is sequestered away from the ATPase domain of IRE1 due to accumulation of misfolded protein in the ER, IRE1 homodimerizes, causing a conformational modification that is transmitted across the membrane, leading to activation of kinase activity, *trans*-autophosphorylation, and activation of its endoribonuclease activity (Figure 2C; see also Figure S3 at http://www.cidms.org/pathways/er_stress/s3.html).[4] Recent studies determined that the PERK and IRE1 ATPase domains are conserved, and switching the luminal portions of IRE1

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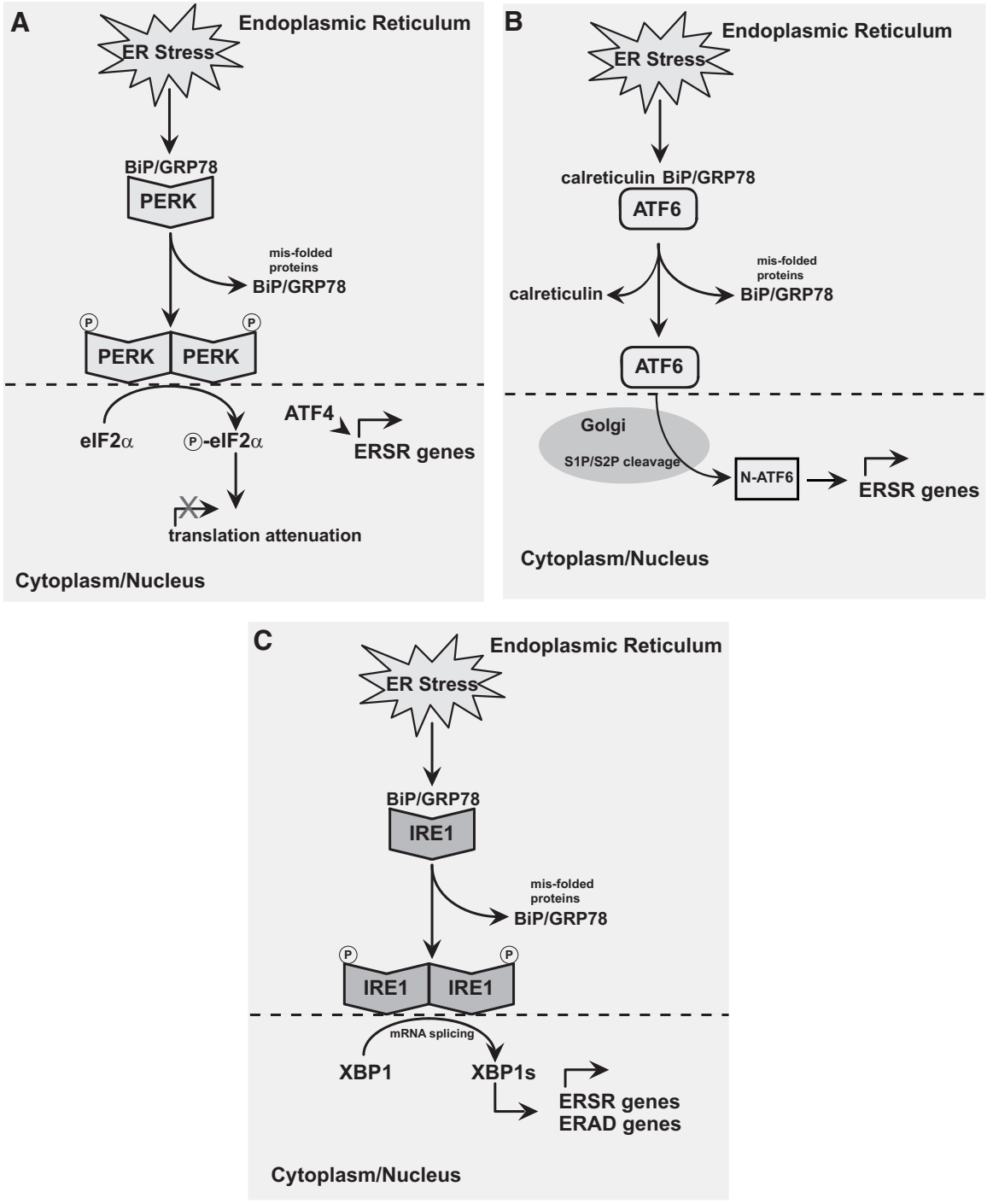

Figure 2. ER stress signaling pathways. A, PERK-dependent pathway activated by ER stress. PERK, a transmembrane kinase and endoribonuclease, interacts with BiP/GRP78 under nonstressed conditions. On activation of ER stress, BiP/GRP78 dissociates from PERK, resulting in dimerization of PERK and activation of its kinase domain, autophosphorylation, and subsequent phosphorylation of eIF2α. Phosphorylation of eIF2α results in attenuation of protein synthesis. However, expression of ATF4 is not inhibited, and the transcription factor induces expression of ERSR-containing genes. **B,** ATF6 pathway. Under nonstress conditions, ATF6, a transmembrane protein localized to the ER, interacts with BiP/GRP78 and calreticulin. After ER stress, BiP/GRP78 and calreticulin dissociate from ATF6, and the protein translocates to the Golgi, where it undergoes cleavage by S1P and S2P proteases. This cleavage yields a cytoplasmic transcription factor (N-ATF6) that translocates to the nucleus and induces ERSR-containing genes. **C,** IRE1 pathway. IRE1 is an ER transmembrane protein containing a serine–threonine kinase domain and a carboxyl-terminal endoribonuclease domain in its cytoplasmic region and binds to BiP/GRP78. Under ER stress conditions, BiP/GRP78 is released from IRE1 followed by IRE1 homodimerization and autophosphorylation. Phosphorylation is essential for IRE1 endoribonuclease activity that is responsible for splicing of XBP1 mRNA, yielding spliced XBP1s mRNA encoding a potent transcription factor. The XBP1s splice variant binds to ERSE-containing promoters and activates ERSE genes. XBP1s also binds to a second *cis*-acting motif, termed the UPRE, resulting in upregulation of genes involved in ERAD.

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and PERK does not affect downstream signaling.[48] IRE1 endoribonuclease activity cleaves 28S rRNA and inhibits protein synthesis, as well as splices and activates XBP1 mRNA (Figure 2C).[49] A 26-nucleotide intron is excised from constitutively synthesized XBP1 (XBP1u) mRNA in a spliceosome-independent manner, and an undefined mechanism then relegates the 5′ and 3′ fragments, yielding a spliced XBP1s mRNA with an altered reading frame.[49] XBP1s splice variant binds to the promoters containing ERSE elements.[19,49] XBP1u C-terminal domain has 2 characteristic domains that are absent from XBP1s. First, it has a nuclear exclusion signal that allows XBP1u to shuttle between the nucleus and the cytoplasm, and, secondly, it has a degradation domain that can only be degraded when interacting with XBP1u and ATF6α or ATF6β. The degradation domain allows for rapid shutdown of the UPR in the absence of ER stress. XBP1s also binds to a second *cis*-acting promoter motif, the UPRE,[19] upregulating genes involved in ERAD. Hypoxia and ischemia triggers UPR activation in cardiomyocytes and in mouse myocardium in vivo.[35] A dominant-negative form of XBP1 inhibits the XBP1-dependent arm of the UPR, leading to an increase in cardiomyocyte apoptosis during hypoxia.[50] Targeted disruption of the XBP1 gene results in embryonic lethality resulting from a developmental disorder during cardiogenesis.[51] In addition to XBP1 mRNA splicing phosphorylated IRE1 recruits TRAF2 (tumor necrosis factor receptor–associated factor-2), ASK1, and the IκB kinase IKK, resulting in stimulation of the stress-activated JNK (c-Jun amino-terminal kinase) pathway.[52] TRAF2 and caspase 12 may form complexes[53] potentially connecting extenuating ER stress and apoptosis.

ER-Associated Degradation

One part of the cellular recovery system that is turned on by the UPR is ERAD.[21] ERAD is triggered when these misfolded proteins are targeted by the ER α1,2-mannosidase I, which cleaves one or more mannose residues from the oligosaccharide. Mannose trimmed oligosaccharides are specifically recognized by EDEM (ER degradation-enhancing 1,2-mannosidase-like protein),[54] which accepts the misfolded proteins from calnexin[55] and selectively trims mannose glycans on the misfolded proteins for recognition by the ERAD lectin, OS-9, which targets the misfolded proteins for retrotranslocation and degradation. The misfolded proteins are retrotranslocated into the cytoplasm where they are deglycosylated by *N*-glycanase, polyubiquitinated, and subsequently targeted to the 26S proteasome for degradation. In nonmuscle cells, the translocation channel was identified as Derlin, distinct from the Sec61 translocon, and forms a complex with cytoplasmic VCP (valosin-containing protein; also termed AAA ATPase p97).[56,57] Many components of the ERAD pathway are induced by the UPR, specifically the ATF6α branch. For example, UPR transcription induces upregulation of the Derlin retrotranslocation channel.[58] Derlin3 may be one of the earliest targets of the UPR as a means for reducing ER protein load and may prove a therapeutic target for treatment of ischemic injury.[58]

Autophagy and ER Stress

Autophagy, a highly conserved process first identified in yeast, is commonly seen in cardiac disease such as hypertrophy and ischemia/reperfusion.[59] Autophagy is used by the cell during both nonstress and stress conditions to maintain homeostasis by sequestering membranes, organelles, and cytoplasmic components into double-membrane vesicles followed by delivery to the lysosome for degradation and recycling. Many types of stress, including nutrient and energy starvation, developmental remodeling, oxidative stress, mitochondrial dysfunction, ER stress, and infections are all observed to activate autophagy as a prosurvival pathway.[60] Excessive insult or injury can lead to severe upregulation in autophagy, resulting in cardiomyopathy and cellular death.[61] Autophagy is directly activated by ER stress, via the IRE1/JNK/p38 pathway and ATF4-dependent activation.[62] Inhibitors of ER stress, as well as siRNA (small interfering RNA) targeted toward IRE1 triggers reduction in autophagy and cell death in cardiomyocytes.[63] Recently, p38 was identified as an upstream protein during autophagy by stabilizing a critical autophagy-related protein (ATG).[64] ATG-deficient mouse embryonic fibroblasts[65] and several deficient strains of yeast[66] exhibit increased sensitivity to ER stress, demonstrating the importance of autophagy for survival of ER stress.

ER Stress in the Heart

A concept of the ER stress response was first described more than 2 decades ago,[67] but only recently has it received significant attention by the cardiovascular community. In nonmuscle cells, ER stress results from a disruption in any of the homeostatic functions of the ER, including protein folding and intracellular Ca^{2+} stores.[4] Other physiological or pharmacological effects such as heat, hypoxia, ischemia, disease, glucose and metabolic starvation, and changes in lipid metabolism are also potent inducers of the ER stress pathway.[35] ER stress has been implicated in a number of diseases including ischemia/reperfusion of the brain and heart,[35,68] heart failure,[35] and diabetes,[69] with the UPR activated in cardiac tissues by decreases in ATP, ER Ca^{2+}, or UDP-glucose.[70] In the heart, hypoxia, ischemia/reperfusion, hypertrophy, pressure overload, and drug-induced insults can result in activation of ER stress.[35] For example, within 24 hours of myocardial infarction,[30] as well as oxygen, serum, and glucose deprivation,[31] there is upregulation of UPR components such as ATF6.[35] Cardiac remodeling in hypertensive heart disease has recently been associated with expression of prostatic androgen repressed message-1 PARM-1.[71] PARM-1 expression is induced by ER stress, which plays a protective role in cardiac myocytes through regulating PERK, ATF6, and CHOP expression.[71] It appears that UPR elements are important for cardiovascular survival, but are they present in the myocardial ER? The UPR is relatively well understood in nonmuscle ER, but has not been fully elucidated in the cardiovascular system. ER stress appears to be intimately involved in the maintenance of cardiovascular homeostasis and as such, demonstrates an important therapeutic target for the treatment of cardiovascular diseases and insults. Figure 3 shows a schematic representation of combined ER stress pathways with special emphasis on their role in cardiac

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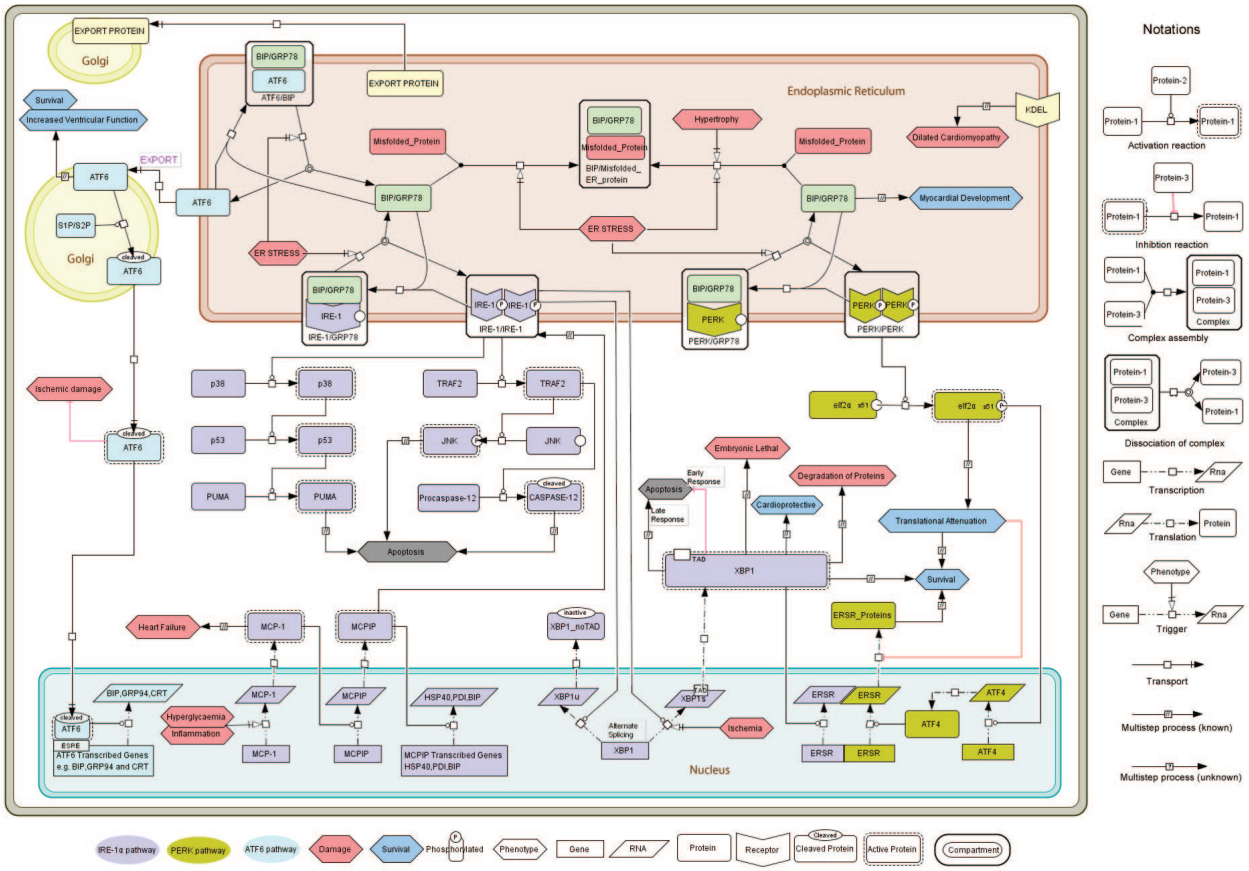

Figure 3. ER stress–induced pathways. Schematic representation of 3 major signaling pathways activated by ER stress: ATF6, IRE1, and PERK. BiP/GRP78, a molecular chaperone, senses the accumulation in misfolded protein, transferring away from UPR mechanisms. The instant cessation in protein synthesis mediated by the ER kinase PERK reduces the translocation of nascent proteins into the ER lumen. The endoribonuclease IRE1 and transcription factor ATF6 mediate the transcriptional activation of genes encoding components that increase protein folding, export, and degradation. Because this activity requires chaperone transcription and translation, it follows the translational attenuation with a slight delay to allow recovery in protein translation. The primary aim of the UPR is to limit damage to the cell by adapting the cell to the situation causing the ER stress. Increased UPR caused by prolonged stress may induce apoptosis of the cell. For additional details, see the text. An extended and interactive version of this figure is available online at http://www.cidms.org/pathways/er_stress/s3.html.

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physiology and pathology (extended and interactive version of the Figure 3 is available online as Figure S4 at http://www.cidms.org/pathways/er_stress/s4.html).

How Do Cardiac Diseases and Insults Trigger ER Stress?

During ischemia, there is significant nutrient and oxygen starvation which leads to the induction of ER stress response genes, including BiP/GRP78, ATF6, XBP1, and IRE1. Glucose starvation leads to activation of specific proteins, the glucose-regulated proteins, or GRPs. These proteins include BiP/GPR78 and GRP94, 2 molecular chaperones that are essential for protein folding in the ER. Upregulation of these proteins is necessary to deal with ER stress, as BiP/GRP78 is involved in activation of the UPR. Oxygen starvation during ischemia results in particular activation/deactivation of proteins, leading to upregulation of the UPR. For example, the activity of PDI is dependent on molecular oxygen and during hypoxia, disulfide bonds are not formed properly because of the lack of O_2, leading to accumulation of misfolded proteins and ensuing ER stress.[72] Cardiac ischemia induces all 3 arms of the UPR: PERK, ATF6, IRE1, and their downstream targets.[31] The transcription factor ATF6 is activated by ischemia but inactivated on reperfusion, suggesting it may play a role in the induction of the ER stress response genes during ischemia that could have a preconditioning effect leading to cell survival during reperfusion.[30]

Hypoxia is one major insult that is a result of a number of cardiac related conditions that trigger ER stress. Oxygen availability becomes limited during hypoxia, either by an ischemic event caused by an occluded artery or by inadequate blood flow caused by other factors. A decrease in the availability of molecular oxygen is a severe stress to the cardiovascular system, and mechanisms to address this deficiency are immediately triggered. Numerous functions within the cell are dependent on normoxic conditions. The oxidative status of the ER dictates formation of disulfide bonds, activity of specific enzymes that regulate other factors, and metabolism. The lumen of the ER is a highly oxidative environment with disulfide bond formation occurring in nascent proteins via oxidoreductases. These enzymes use molecular oxygen as the electron donor.[72] In addition, modification of proline residues via prolyl hydroxylase is dependent on molecular oxygen, with hypoxia inhibiting this enzyme, allowing acti-

vation of downstream targets.[37] During ischemia, resulting from the reduced availability of molecular oxygen, significant changes occur in cardiac energy metabolism.[73] Under conditions of mild hypoxia, the metabolic changes attempt to help the heart adapt to the ischemic insult; however, under severe hypoxia, metabolism has to occur anaerobically, with a massive increase in reactive oxygen species leading to hibernating myocardium, cell death, and, ultimately, cardiovascular dysfunction. In addition, reperfusion after ischemia generates increased oxidative stress as the heart converts back to aerobic respiration, thereby generating lethal levels of reactive oxygen species. Ischemia/reperfusion injury in the heart results in a number of cellular and molecular events that lead to loss of cardiovascular function, such as disruption in ER oxidative state or Ca^{2+} homeostasis, triggering cellular damage and eventually apoptosis.[74] This damage directly impairs ER functions such as protein folding and induces the UPR. The oxidative state of the heart directly influences several proteins, including PDI and prolyl hydroxylase.[37,75] The electron donor used by PDI is ERO1, an ER oxidase. ERO1 uses molecular oxygen for generation of the energy necessary for the formation of the disulfide bond. There are 2 isoforms of ERO1, ERO1α and ERO1β, with both being involved in the transfer of oxidative equivalents to PDI, which in turn oxidizes nascent proteins.[76] As part of the apoptotic response to acute ER stress, CHOP is upregulated by PERK activity, with CHOP in turn activating ERO1α expression.[77] ERO1α then stimulates the release of Ca^{2+} by $InsP_3R$ during the onset of apoptosis.[78] In mice undergoing chronic hypoxia, PDI is increased in myocardial endothelial cells and may play a role during neoangiogenesis to address the infarct areas.[75]

Another part of an ischemic event is a reduction in blood flow, limiting the cell exposure to an energy source. This leads to a disruption in metabolism attributable to D-glucose starvation. The 2 transcription factors that are activated by ER stress, ATF4 and ATF6, trigger downstream activation of gluconeogenesis genes[34] and repression of cholesterogenesis to preserve carbon units.[79] Because glucose units are a necessary part of *N*-linked glycosylation, potentially, this may be a sensing mechanism that triggers a change in glucose metabolism. Furthermore, GRP94 and BiP/GRP78 are up regulated on glucose starvation in an attempt to prevent cellular damage. One significant feature of heart disease is a change in the energy source used by the heart. Fatty acids, glucose, and lactate normally contribute the most to cardiac energy metabolism and this might be sensitive to ER stress responses.[80] The precise role of ER stress in control of cardiac energy metabolism needs further investigation.

Does the Heart Use Adaptive ER Stress As a Means to Limit Disease and Damage?

Adaptive ER stress is a hallmark of nonmuscle tissues and cells that undergo low level extended insult. This has also been termed cytoprotection, functional remodeling, tolerable ER stress, adaptive stress response, ER stress acclimatization, attenuating ER stress and preconditioning ER stress. Many of these terms have been observed in cardiovascular research with pre- and postconditioning as a potential clinically relevant treatment for an ischemic episode.[81,82] Recent evidence demonstrates that hypoxic preconditioning treatment of neonatal cardiomyocytes stimulates low level ER stress, leading to the reduction of ER stress related apoptosis that occurs after sustained hypoxia/reoxygenation.[83–85] This is very similar to heat tolerance, where tissues undergo numerous rounds of brief heat insult, resulting in resistance to further injury, whether it is heat, hypoxia or other. Ischemia/reperfusion is an interesting phenomenon, in that significant damage is generated on hypoxia, but with reperfusion, there is also considerable damage produced with dramatic changes in cardiac metabolic and contractile function as a result of the production of oxygen free radicals, disruption of cation homeostasis, depletion of energy stores (both ATP and glucose), and changes in intracellular signaling. Ischemia induces ATF6 activity, but on reperfusion where a significant amount of damage is generated, ATF6 is inhibited, suggesting that it may instigate ER stress response genes during ischemia that have a preconditioning effect on cell survival during reperfusion.[30] In the reverse, tissues that have undergone acute ischemia, when subjected to numerous episodes of mild ischemia during reperfusion, are able to lessen the total ischemia-reperfusion injury,[86] an ischemic postconditioning. The cardioprotective mechanisms of ischemia postconditioning involve upregulation of ER stress protein synthesis and prevent an increase in cytoplasmic Ca^{2+}.[87] Heat acclimation is also successfully used to generate protection to conditions with ischemia or hypoxia, via reprogramming of gene expression and translational processes that occur during ER stress.[88] It appears that the heart uses ER stress as a means to protect itself from damage as a result of hypoxia or an ischemic event.

Does Cardiac ER Stress Trigger the Fetal Gene Program (Hypertrophic Gene Response) in Failing Heart As a Means for Survival?

One major event observed during cardiac disease is activation of the fetal gene program, also termed the hypertrophic gene program.[89] A return to the fetal gene program protects the stressed heart. This pathway appears to be activated as a means to address remodeling of the cardiac tissue, both metabolically but also physically, to generate an increase in cardiac efficiency. Activation of the fetal gene program leads to protection for the stressed heart but unfortunately also frequently results in cardiovascular hypertrophy, with enlargement of the heart. A number of conditions activate the fetal gene program, including hypoxia, ischemia, hypertrophy and atrophy.[89] Could ER stress via ATF6 or XBP1 turn on the fetal gene program (hypertrophic gene program)? Potentially, this fetal gene program may be modulated by ER stress resulting from the pathological insult via ATF6 and XBP1 transcriptional activation, with resultant activation of the specific cardiac developmental transcriptions factors such as MEF2c (myocyte enhancer factor-2), NFAT (nuclear factor of the activated T cells), and GATA that are involved in cardiac remodeling. Interestingly, MEF2c, an early embryogenic cardiac specific transcription factor, has a putative UPRE DNA interaction motif, specific for the transcription factor, ATF6, located upstream of the translation initiation codon in the promoter as well as a putative ERSE where the

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Table 2. Selected Transcription Factors Relevant to the Cardiovascular System Containing ATF6 or XBP1 Binding Sites in the Promoter

Transcription Factor	ERSE/UPRE
MEF2c (cardiac)	ATF6 site (1)
Nkx2.5 (cardiac)	XBP-1 site downstream of the promoter
Gata-4 (cardiac)	None
Gata-5 (cardiac)	ATF6 site (1) (XBP1 site downstream of the promoter)
Gata-6 (cardiac)	ATF6 site (1)
NFAT-C4 (cardiac)	ATF6 site (1)
Brachyury (mesoderm)	None
Oct4 (pluripotency)	None
Nanog (pluripotency)	None
BMP4 (pluripotency)	None
Sox2 (pluripotency)	ATF6 site and XBP1 site downstream of the promoter
Coup-TF1 (antagonize Nkx2.5)	ATF6 site (1) [XBP1 site downstream of the promoter)
Coup-TF2 (cardiac)	ATF6 sites (2)
Stat3 (growth)	XBP1 site (1) and ATF6 sites (2)
Creb1	ATF6 site (1)

Search was performed using SABiosciences ChIP primer search engine (http://www.sabiosciences.com/chipqpcrsearch.php?app=TFBS). No. of sites identified is indicated in the brackets. BMP indicates bone morphogenetic protein; COUP-TF, chicken ovalbumin upstream promoter transcription factor; MEF2c, myocyte enhancer factor-2; NFAT, nuclear factor of the activated T cells; Nkx2.5, homeobox protein Nkx-2.5; Oct3, octamer-4; Sox2, sex-determining region Y-box2; Stat3, signal transducers and activators of transcription 3.

XBP1 transcription factor binds, situated very close to the translation initiation codon (Table 2). The GATA-6 gene, recognized to be important during cardiac differentiation,[90] has a putative ATF6 transcription factor binding site just upstream of the transcription start codon. The Nkx2–5 transcription factor functions in heart formation and development with the gene containing a putative ERSE where XBP1 may bind. COUP-TFII is upregulated during early cardiomyogenesis[91] and has a number of putative ATF6 binding sites upstream of the initiation codon (Table 2). NFAT-C4 plays an important role during myotube differentiation as well as cardiac development and hypertrophy[92] and contains a putative UPRE ATF6 transcription factor binding site upstream of the translation initiation codon.

ER Stress: Pathological Versus Physiological?

The response to ER stress appears to occur in 2 phases, initially, the pathway is turned on in an attempt to address one consequence of the insult, the accumulation of protein in the ER. The fetal gene program is also activated in an attempt to remodel the diseased tissue. Unfortunately, if the heart continues to be stressed, the UPR triggers autophagy and apoptosis as a last measure to deal with the problem. This may ultimately result in heart failure and death. Therefore, the response to insult or injury can be classified into a pathologically relevant ER stress response and physiologically relevant ER stress response. The 2 main UPR transcription factors, XBP1 and ATF6, mediate induction of ER chaperones, protecting the heart from ischemia/reperfusion injury, whereas activation of the PERK/ATF4/CHOP branch of the UPR triggers the proapoptotic signals. ER stress is demonstrated to be pathologically involved in disease and damage under numerous conditions, including myocardial infarction, ischemia/reperfusion, and pressure induced overload (reviewed elsewhere[5]). A direct disruption in ER function by overexpression of mutant KDEL receptor in transgenic mice leads to activated ER stress and heart failure.[93] Pressure overload induces extended ER stress during hypertrophy with resultant heart failure.[94] In cardiomyocytes, AMP-activated protein kinase (AMPK) is involved in energy homeostasis during hypoxia by inhibiting protein synthesis by phosphorylating and inactivating an elongation factor, eEF2 (eukaryotic elongation factor 2).[95] The AMPK protects cardiomyocytes from damage by down regulation of ER stress.[95] Furthermore, inhibition of ER stress in the heart blocks ischemia/reperfusion injury in mice or hypoxic injury in cultured cardiac myocytes.[35] ER stress can also be physiologically protective and has been demonstrated to enhance survival of mouse myocardium after hypoxia by targeted upregulation of ER stress-associated proteins.[32] A decline in ER Ca^{2+} triggers an increase in BiP/GRP78 expression in H9c2 cardiac myocytes and activates ATF6.[50,96] Overexpression of several UPR elements, including ATF6, BiP/GRP78, and Derlin3, protects cardiomyocytes from ischemia/reperfusion damage.[32,58,97] A prolonged mild ischemic stimulus may lead to the cell having a tolerable amount of ER stress, thereby dictating that further insult will not significantly harm the cell by attenuation of the ER stress response after an acute ischemic episode.[98] Interestingly, overexpression of the cardiac sodium/hydrogen exchanger (NHE1) resulted in upregulation of ER stress.[99] It had been previously determined that NHE1 expression is maladaptive during cardiac function but the unexpected increase in the UPR (during NHE1 overexpression), led to enhanced survival of the mouse heart under ischemic conditions.[99]

In other systems, such as brain ischemia/reperfusion and tumor growth, there are significant changes in ER stress during the insult. There is enhanced expression of BiP/GRP78, CHOP, ATF4, and caspase 12, as well as processing of XBP1 occurring under conditions of brain ischemic insult.[100] Tumors use activated UPR, including hypoxia-mediated activation of XBP1 and induction of GRP94 and BiP/GRP78 as a means for survival, in response to internal hypoxia resulting from exponential tumor growth. These results potentially can be used as a "jumping point" to observe what role ER stress may play in protection of the heart but also a therapeutic target to limit damage caused by autophagy or apoptosis. Therefore, the pathway comprising ER stress, including UPR, ERAD, and apoptosis may present specific therapeutic targets in the treatment of cardiovascular diseases and conditions.

Challenges and Perspectives

Systemic ER stress manifests as chronic metabolic disorders that are often accompanied by hyperglycemia and hyperlipidemia.[101] It is now well accepted that changes in cellular

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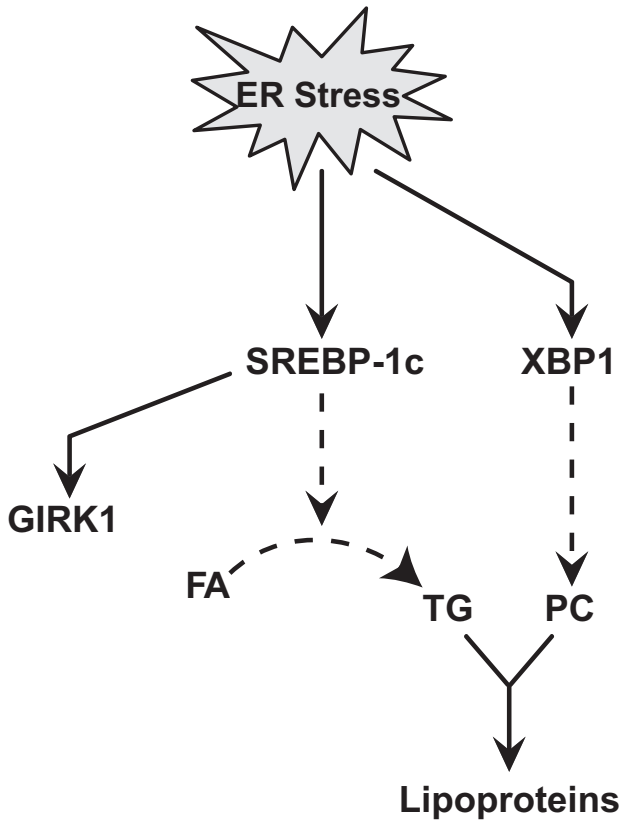

Figure 4. ER stress and energy metabolism. Schematic representation of a potential prosurvival metabolic adaptation to mitigate ER stress in cardiac cells. The stimulation of SREBP1c by ER stress promotes the expression of GIRK1/4 (G protein–coupled inward rectifying K^+ channel 1/4), as well as the synthesis of triglyceride (TG) from fatty acids (FA), resulting in a decreased overuse of fatty acids as fuel. ER stress also activates XBP1, which can induce phosphatidylcholine (PC) synthesis in ER-stressed noncardiac cells. Newly synthesized PC may be used for lipoprotein biogenesis as a means for exporting accumulated triglycerides from stressed cardiac cells.

glucose homeostasis are potent inducers of the UPR. Unlike other cells, the cells of the adult heart preferentially use fatty acids to meet its high energy requirements. However, further increase in the use of fatty acids resulting from certain stress conditions decreases cardiac efficiency and compromises heart function. Intracellular triglyceride accumulation has been observed in stressed hearts and this has been regarded as a hallmark of cardiac lipotoxicity that eventually ends in heart failure.[102,103] An alternate view of lipid accumulation in stressed hearts is that of an intermediate marker of an adaptive response to the elevated intracellular levels of free fatty acids. Specific cardiac conditions such as pressure overload or ischemia are known to cause ER stress[94,104] and have been demonstrated to activate the SREBP1c (sterol response element binding protein 1c) (Figure 4). The activation of SREBP1c and subsequent stimulation of triglyceride synthesis which results in the accumulation of triglyceride may in fact represent a prosurvival adaptation to cope with the detrimental consequences like those associated with chronic metabolic disorders. It is intriguing to note that cardiac cells have been shown to express the genes for apolipoprotein B and microsomal triglyceride transfer protein.[102] Both of these proteins are involved in the biogenesis of lipoproteins in hepatic and intestinal cells. The activation of XBP1 during the UPR response has also been shown to stimulate phosphatidylcholine synthesis.[105,106] Thus, lipoprotein secretion can potentially serve as a mechanism for the export of accumulated triglyceride within cardiac cells. Additionally, SREBP appears to be involved in the stimulation of G protein-coupled inward rectifying K^+ channel GIRK1/4 (G_i-activated potassium channel 1/4), a channel that is activated by parasympathetic signals.[107] The functional consequences of ER stress on glucose and fatty acid metabolism in noncardiac cells have been well studied whereas comparatively little is known about the importance of ER stress in the coordination of the selection and utilization of nutrients for energy production during ER stress in cardiac myocytes. It will be of interest to uncover, in the heart, the mechanisms that coordinate protein synthesis and quality control with energy maintenance and metabolism of other cellular constituents, such as membrane phospholipids.

In conclusion, ER stress in the heart has received significant attention in recent years from basic and clinical scientists because of its association with several cardiac pathologies. ER stress responses, ER Ca^{2+} buffering, and protein and lipid turnover impact many cardiac functions including energy metabolism, cardiogenesis, ischemic/reperfusion, cardiomyopathies, and heart failure. However, many challenges remain, and key questions need to be addressed by both cell biologists and cardiovascular scientists. Is there a functional ER membrane in the heart? How is ER stress activated in cardiac physiology and pathology? Can ER stress be controlled in the context of cardiac pathology? Why and how are some ER stress pathways detrimental to the heart, whereas others promote survival? Can cardiac energy metabolism be influenced by ER stress? Can ER stress be considered a potential target for new drug discovery? In cancer cells and in cardiomyocytes, upregulation of BiP/GRP78 attenuates ER stress–induced apoptosis.[97] Chemical ER chaperones can modulate ER stress, suggesting that small molecules may become future drugs regulating ER stress pathways in disease states. Understanding the contribution of ER proteins and ER-associated pathways to cardiac pathology will need to be addressed by clinical studies with the anticipation of developing novel UPR-targeted therapies for cardiovascular diseases.

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Disclosures

None.

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