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Is ATF6 the Triage Nurse?

Ivor J. Benjamin

How cardiac cells sense, respond, and adapt to acute and chronic changes of their metabolic and environmental milieu remain among the most enigmatic and fundamental questions in contemporary cardiovascular biology and medicine. With more than 700 000 deaths and 6 million hospital discharges annually in the U.S., ischemic heart disease remains a major public health problem. If prompt therapy is delayed, myocardial injury from depletion of high-energy phosphates from inhibition of oxidative phosphorylation is inevitable. Ischemia/reperfusion (I/R) also unleashes a cascade of cellular and molecular events whose sequalae may sustain organ function at diminished capacity but, beyond a critical balance, threatens the organism's survival. Reactive oxygen and nitrogen species (ROS; RNS), released from the mitochondria and other sources, alter the tertiary and quaternary structures of proteins, exposing their hydrophobic residues to allow conformational tendencies toward protein misfolding and aggregation.[1] Nevertheless, highly sophisticated schemes equipped with molecular sensors, rapid response pathways, and adaptor mechanisms have evolved to mitigate the accumulation of unfolded proteins in a compartment specific manner, principally the cytoplasm and endoplasmic reticulum (ER). Although the cardiovascular field has paid considerably more attention to the cytoprotective mechanisms of heat shock proteins in response to I/R, termed the "classical" heat shock response, a parallel series of events has been unfolding at a breathtaking pace in the ER, with arguably considerable excitement to warrant a sneak preview of the article published in this issue of *Circulation Research*.

To place into perspective the current buzz being garnered by the ER requires a brief discussion of more familiar themes in the field, which were developed thanks to the pioneering efforts of cardiovascular scientists, and others, working on the complementary stress response network, best exemplified by heat shock "stress" proteins and their transcriptional regulators. First discovered by Ritossa in *Drosophila*, the capacity for cells to augment synthesis of distinct classes of heat shock proteins (HSPs) in response to heat and other stressors (eg, hypoxia and ischemia/reperfusion), termed the heat shock response, has emerged as a paradigm for inducible gene expression.[2] Reminiscent of ischemia preconditioning (IPC) in which prior sublethal ischemia confers transient protection to a subsequent prolonged bout of ischemia, increased synthesis of HSPs by different maneuvers was similarly shown to produce cross-tolerance for ischemic cardioprotection. Whereas most HSPs are constitutively expressed in unstressed cells, stress-inducible HSP expression is controlled at the transcriptional level by heat shock transcription factor 1 (HSF1) by mechanisms involving unfolded proteins and redox-dependent stress signals. Transgenic overexpression of Hsp25, Hsp70i, and αB-crystallin can individually confer ischemic cardioprotection in vivo, possibly through their roles as molecular chaperones and antiapoptotic agents, legitimizing their therapeutic potential in the armamentarium for ischemic cardioprotection.[3,4] While the aforementioned Hsps are localized in the cytosol and nucleus, the recent intriguing elucidation of the complementary transcriptional networks that govern ER-resident chaperones has heightened interest in their direct relevance to cardiac biology and (patho)physiology (Figure).

It is generally not widely appreciated that the ER contains the highest concentration of intracellular Ca^{2+}, an essential requirement used by Ca^{2+}-ATPases for intraluminal calcium transport. In contrast to the reducing environment of the cytoplasm, the ER requires an oxidizing state to facilitate disulfide bonding formation during maturation along the protein folding pathway of secretory and membrane-bound proteins, a process termed oxidative protein folding.[5,6] However, most secretory and noncytosolic nascent proteins with exposed hydrophobic residues will readily aggregate if not prevented by the enzymatic activities (eg, peptidyl prolyl isomerase) and specialized properties of molecular chaperones. Accumulation of misfolded proteins, beyond some critical threshold, is sensed in the ER and activates the evolutionarily conserved unfolded stress response (UPR), also termed the ER stress response.[7] This response is mediated by at least three regulatory pathways, two involved in transcription regulation (ATF6, XBP-1) and the third controlling protein translation (eIF2α, kinase). First identified in a yeast one-hybrid screen, ATF6 is a basic leucine zipper (bZIP) protein that binds to the consensus ER stress response element (ERSE) and enhances transcription of genes encoding glucose regulated proteins (Grp) Grp78, Grp94, and calreticulum (Figure).[7] In response to ER stress, endogenous ATF6 is a 90-kDA protein of 670 amino acids, which translocates from the ER to the Golgi coincident with proteolytic cleavage to the transcriptionally competent 50-kDA protein by a process termed regulated intramembrane
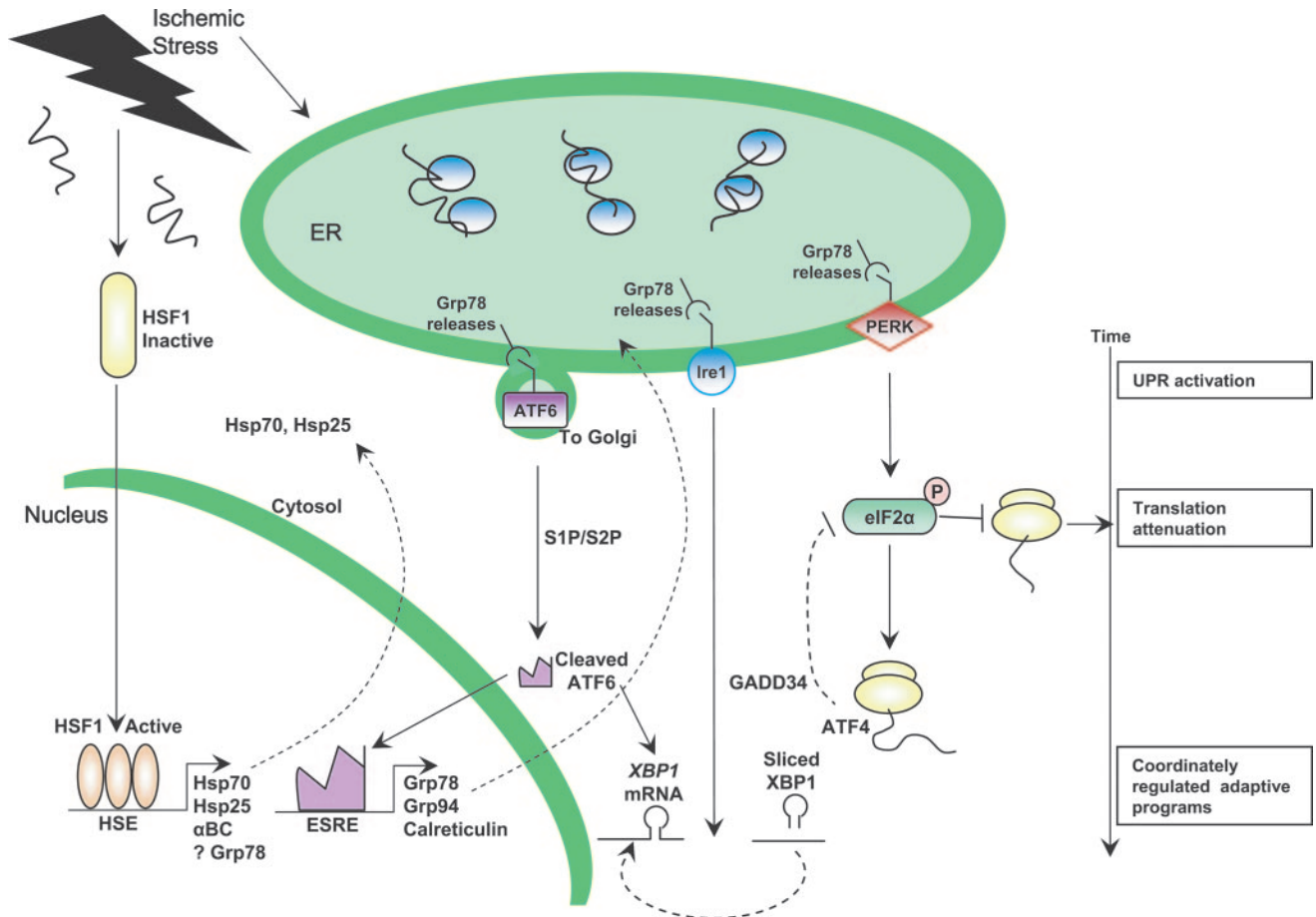
Sensing pathways for the activation of stress response genes encoding molecular chaperones in selective compartments, primarily the cytosol and the lumen of the ER. Ischemia/reperfusion elicits pleiotropic responses that promote protein misfolding and aggregation, the proximate stress signals for two potentially complementary adaptive schemes, termed the heat shock response (HSR) and unfolded protein response (UPR) pathways. HSF1-dependent transcription requires conversion of HSF1 monomers to homotrimers for stress-inducible synthesis of Hsp70i, Hsp25, and αB-crystallin. In the lumen of the ER, proteolytic cleavage of ATF6 in response to ER stress leads to upregulation of ER-resident chaperones such as Grp78 and Grp94 and enhanced synthesis of XBP1 mRNA. Thus, prioritization of ATF6-dependent gene products for synthesis proceeds before XBP1, indicating triage processing of stress signals for handling ER stress.

proteolysis (Rip). Thus, Ca^{2+}-dependent molecular chaperones such as GRP78, Grp94, and calreticulum are strategically poised within the ER to orchestrate the stabilization of protein intermediates, prevent protein misfolding, and whose upregulation function downstream in quality control mechanisms and compartment-specific cellular adaptation.[8]

In the current issue of the Journal, Martindale and workers have examined the functional properties of activation of transcription factor 6 (ATF6), a membrane-bound transcription factor, which activates the ER-stress response in animal cells.[9] The experimental approach uses the cleaved transcriptionally competent form of ATF6 engineered with the Flag epitope and the mutated estradiol receptor (MER) fused to either the N or C termini, respectively. Using a tamoxifen-inducible system, ATF6-MER activation induced the ER-resident molecular chaperones Grp78 and Grp94 in transgenic hearts, which exhibited greater functional recovery, decreased myocardial injury (ie, LDH release and infarct-size), and reduced necrosis and apoptosis compared with control hearts subjected to ex vivo I/R. The authors conclude that activation of the ATF6 limb of the UPR may exert

salutary and beneficial effects against ischemic injury. Although considerable progress has been made on several potential cardioprotective pathways, this current work not only spotlights a coordinately adaptive pathway but exploits a novel approach that bypasses an important step for stress-inducible gene expression originating from the lumen of the ER.

How does a membrane-bound protein such as ATF6 achieve transcriptional competency in the nucleus? Recent investigations have elucidated this novel molecular mechanism of transcriptional regulation, which is elegantly illustrated by two seemingly disparate yet fundamental areas in cardiovascular biology (ie, the ER stress response and lipid biosynthesis). *Rip* is a two-step process by which transmembrane proteins are cleaved to release cytoplasmic domains that move to the nucleus.[10] In the first step, proteolytic processing begins with site-specific cleavage by the Site-1 protease (S1P) of the transmembrane portion of ATF6 directed toward the ER lumen (ie, extracytoplasmic domain) followed by the second attack of the juxtamembrane portion by the Site-2 protease (S2P) to release the transcriptionally active cytoplasmic domain.

Like ATF6, the type-I transmembrane protein kinases PERK and IRE1 reside in the endoplasmic reticulum (ER) where they transmit stress signals after disturbances that exacerbate protein unfolding. Indeed, these luminal domains of PERK and Ire1 are functionally interchangeable, and Grp78 release causes their high molecular weight oligomerization and transcriptional activation. Increased amounts of misfolded proteins in the ER drives the release of Grp78 from Ire1, PERK, and ATF6, uncovering the activation of massive transcriptional programs ($\approx$5% of the genome) that span host defenses, phospholipid biosynthesis, ER-associated protein degradation, and apoptosis.[1,8,11]

Once released from Grp78, oligomerization of Ire1 now binds to the signaling kinase, TRAF2, and activates JNK, p38, and NF-κB. An intrinsic ribonuclease activity of Ire1, containing Ser/Thr kinase domain and endoribonuclease domains, also triggers X box protein-1 (XBP-1), mRNA splicing, and the production of XBP-1 protein, a 41-kDA bZIP-family transcription factor. XBP-1 activation functions in the retrograde transport of misfolded proteins and ER-induced protein degradation.[8] After release from Grp78, PERK, also a Ser/Thr protein kinase, oligomerizes in the ER membrane enabling its autophosphorylation and activation of its kinase domain, targeting inhibition of eukaryotic initiation factor 2α (eIF2α). Phosphorylation of eIF2α dampens global protein synthesis while restricting translation to 'translationally privileged' mRNAs essential for ER stress adaptation and rapid return of cellular homeostasis.[12]

In the present study, evidence that Grp78 and Grp94 are increased in mouse hearts subjected to ex vivo I/R is consistent with I/R-mediated UPR activation but does provide a causal mechanism for Grps in cardioprotective properties.[9] Furthermore, Grp78 and Grp94 are increased at the end of the ischemia–reperfusion protocol, presumably in the myocytes that have survived, and obscuring potential beneficial or detrimental consequences. As demonstrated previously for several cytosolic/nuclear HSPs, future studies are needed to directly test the hypothesis that ER-resident chaperones such as Grp78, Grp94, and calreticulum are effective cardioprotective agents in the physiologically relevant intact organism. As previous studies by this group have implicated other molecular targets of ATF6 (eg, SERCA and ANF), the precise mechanisms for ATF6-dependent activation is far from resolved, and an interesting follow-up for the present studies should include a comprehensive gene expression profiling. A potential limitation of the present work is the sole reliance of ex vivo I/R instead of the more physiologically relevant on vivo I/R for assessment of ischemic tolerance and functional recovery. In this regard, the experimental approaches used by Martindale and coworkers merit attention as the state-of-art for circumventing potential postnatal unanticipated influences associated with unregulated transgene expression.

As cellular adaptation involving UPR proceeds as a classical feedback loop, future work must explore the intriguing possibility of the temporal and spatial relation to the heat shock response. In turn, the capacity of resident chaperones to adequately handle the protein load and, therefore, the recruitment of a transcriptionally coupled regulatory network becomes essential for restoring cellular homeostasis. The potential for coordinate regulation and recruitment of two highly conserved biological schemes, which have evolved to increase the synthesis of resident chaperones, warrants formal experimental analysis. Multiple features of the ER stress response such as Ca^{2+} and oxidative stress, which have potential relevance to ischemic preconditioning, are presently unknown. Lastly, further work on the targeting of UPR using small molecules directed at positive effect and not negative effect of ER response, as controlled by CHOP, has the potential for inducing selective members of the chaperones in a compartment-specific manner in the treatment of cardiac diseases.

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