Review

Stress (Heat Shock) Proteins
Molecular Chaperones in Cardiovascular Biology and Disease

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Abstract—How a cell responds to stress is a central problem in cardiovascular biology. Diverse physiological stresses (eg, heat, hemodynamics, mutant proteins, and oxidative injury) produce multiple changes in a cell that ultimately affect protein structures and function. Cells from different phyla initiate a cascade of events that engage essential proteins, the molecular chaperones, in decisions to repair or degrade damaged proteins as a defense strategy to ensure survival. Accumulative evidence indicates that molecular chaperones such as the heat shock family of stress proteins (HSPs) actively participate in an array of cellular processes, including cytoprotection. The versatility of the ubiquitous HSP family is further enhanced by stress-inducible regulatory networks, both at the transcriptional and posttranscriptional levels. In the present review, we discuss the regulation and function of HSP chaperones and their clinical significance in conditions such as cardiac hypertrophy, vascular wall injury, cardiac surgery, ischemic preconditioning, aging, and, conceivably, mutations in genes encoding contractile proteins and ion channels. (Circ Res. 1998;83:117-132.)

Key Words: stress protein • gene expression • molecular chaperone • transcription factor • vascular biology • aging • ischemia

Physiological stresses ranging from myocardial ischemia to genetic mutations produce disease states in which protein damage and misfolded protein structures are a common denominator.1-9 How does the cell respond? Multiple endogenous pathways are engaged in restoring cellular homeostasis, but one well-characterized mechanism that involves protein folding is the heat shock family of stress proteins, or HSPs.10,11 Insight into the mechanisms underlying HSP function is provided by 2 main lines of evidence: (1) the correct folding of many proteins in a cell requires protein-folding machinery, the molecular chaperones,12,13 and (2) HSP chaperones repair denatured proteins or promote their degradation after heat shock.14,15

Genetic studies provide convincing proof in different phyla that overexpression of stress proteins is a powerful means of cytoprotection, even in the intact heart.10,16-18 Similarly, biochemical studies have demonstrated that the Hsc70 chaperone increases the productive folding of the common ΔF508 mutation of CFTR. This suggests a physiological role of an HSP chaperone in human diseases.19,20 Divergent mechanisms that produce abnormal or misfolded cellular proteins converge into a common pathway, leading to an increase in the levels of cytoprotective stress proteins, which decrease or neutralize the deleterious effects of acute or chronic stresses.

In this review, we summarize present knowledge about the regulation and function of individual chaperones (eg, Hsp90, Hsp70, Hsp60, Hsp47, Hsp27, and αB-crystallin) in the cardiovascular system. Besides their well-established roles in cell survival (necrosis and apoptosis), we will emphasize the emerging evidence regarding chaperone functions in physiological adaptation during cardiac hypertrophy, ischemic preconditioning, vessel wall injury, oxidative stress, and aging. Although we presently can only speculate about their roles in specific cardiac diseases, we will discuss many potential opportunities to establish whether HSP chaperones either exert an effect on or play a direct physiological role in the natural history of diseases resulting from mutations of cardiac contractile apparatus (eg, hypertrophic cardiomyopathy) and ion channels (eg, long-QT syndrome) in humans.

Definitions and Nomenclature

What Are Stress “Heat Shock” Proteins?
The term “heat shock” protein is a misnomer but remains as a legacy of Ritossa’s serendipitous discovery1 that heat shock produced chromosomal puffs of salivary gland cells in Drosophila. Heat stress (≈5°C above normal growth temperature) upregulates the rapid synthesis of a multigene family of proteins, originally called heat shock proteins,2 which are the result of a response often referred to as the heat shock response.10,21 Prior sublethal heat stress transiently increases the ability of a cell to withstand an otherwise lethal subsequent heat challenge. This phenomenon, or thermotolerance, played a key role in launching numerous studies in both in vitro and in vivo experimental models in which a similar association was found between the heat shock response and protection against either simulated hypoxia or ischemia.
Stress Proteins in the Cardiovascular System

Selected Abbreviations and Acronyms

CFTR = cystic fibrosis transmembrane conductance regulator
ER = endoplasmic reticulum
ERK = extracellularly responsive kinase
Grp = glucose-related protein
HO = heme oxygenase
Hsc = heat shock cognate
HSP = heat shock transcription factor
Hsp = heat shock protein
JNK = c-Jun N-terminal kinase
MAPK = mitogen-activated protein kinase
MAPKAP = MAPK-activated protein kinase
MW = molecular weight
ROS = reactive oxygen species
SAPK = stress-activated protein kinase
TRC = TCP-1 ring complex
VSMC = vascular smooth muscle cell

Indeed, diverse stresses, including heavy metals, amino acid analogues, inflammation, and oxidative/ischemic stress, induce the expression of HSP genes. Consequently, the terms “stress proteins” or “heat shock family of stress proteins” are preferred, although many of these proteins have essential functions during unstressed conditions.13

Stress proteins belong to multigene families that range in molecular size from 10 to 150 kDa and are found in all major cellular compartments. The convention is to name stress proteins of various molecular sizes as follows: Hsp27, Hsp70, and Hsp90; whereas heat shock protein genes are designated as follows: hsp27, hsp70, and hsp90. The distinction between constitutively expressed (eg, Hsc70 and Hsp90) and cognate members of the HSP family and their inducible isoforms (Hsp70 and Hsp90α, respectively) is arbitrary, since accumulating evidence, in physiologically relevant in vivo systems, now indicates that such relationships depend on cell- and tissue-restricted expression.

Cellular Consequences of Heat and Ischemic Stress Are Similar

Like experimental ischemia/reperfusion, heat shock is a stress that disrupts numerous metabolic processes and cellular structures and that culminates in cell death when a critical threshold is exceeded.10,24,25 Both heat stress and ischemia cause extensive damage to the cytoskeleton, including collapse of the threadlike intermediate filament network into large perinuclear aggregates, reorganization of the cytoplasmic network, relocation of actin-containing fibers around the nucleus, and disruption of microtubules and the mitotic spindle.26,27 Mitochondrial swelling, loss of mitochondria, and uncoupling of oxidative phosphorylation are similarly shared features of heat shock and early reversible ischemic injury.26–30

Characteristically, general protein synthesis is inhibited after extreme heat challenge as a result of phosphorylation of initiation factors such as eIF2α, which disrupts ribosomal assembly and inactivates cap-binding proteins.31–33 In contrast, HSP genes are efficiently expressed after heat challenge, in part, as a result of the absence of introns in several inducible (eg, hsp70) genes. In addition, alterations of mRNA splicing and heat-induced stabilization of mRNA are adaptive mechanisms used to efficiently translate stress proteins, which can reach 15% to 25% of total intracellular protein within minutes after physiological stress under these conditions.10,34 Coincidentally, several cytosolic chaperones translocate into the nucleus,27 where heat-induced inhibition of DNA chromatin assembly exposes a nuclease-sensitive conformation, a pathognomonic feature of both heat- and ischemia-induced apoptosis.36 Less dramatic changes are seen in integral membrane proteins, the lipid bilayer, and cellular surface morphology. Cessation of noxious stimuli is followed by rapid and efficient degradation of Hsp mRNAs.37–39

As mentioned previously, prior sublethal heat stress or “hyperthermic preconditioning” profoundly attenuates all of the heat-induced cellular changes to a subsequent severe heat challenge. Moreover, pretreatment with heat produces “cross tolerance” to varying types of physiological stress. For example, protection of the intact ischemic heart after heat pretreatment may last for hours to days.40,41 Insight gained from the physiological roles of Hsp expression during the heat shock response has contributed to current thoughts about chaperone functions in pathological states likely to result in abnormal protein folding.

Attention has primarily focused on the induction of HSP chaperones and the potential repair mechanisms involved in mitigating ischemia/reperfusion injury. Figure 1 schematically summarizes many of these concepts and illustrates the multiple well-recognized mechanisms implicated in ischemic myocardial injury, including oxidative stress/damage, calcium overload and activated proteases, release of proteolytic and lysosomal enzymes, alterations of the cytoskeleton, and complement activation.

Diverse Physiological Stresses Induce HSP Gene Expression Through a Common Mechanism

Rapid induction of stress protein expression is accomplished through mechanisms of transcriptional activation and preferential translation.10,42 HSFs (HSF1 through HSF4) regulate the inducible synthesis of HSPs during development, growth, and adaptation.43–44 Whereas essential single-copy genes encode HSF in Saccharomyces cerevisiae and Drosophila,45,46 multiple HSFs have been identified in chicks, plants, mice, and humans.47–51 Two HSFs (HSF1 and HSF2, encoding proteins of 75 and 72 kDa, respectively) have been identified in the mouse.49 Neither HSF1 nor HSF2 is heat inducible, but HSF1 is hyperphosphorylated in a ras-dependent manner by members of the MAPK subfamilies (ERK1, JNK/SAPK, and p38 protein kinase) during physiological stress.52,53 During unstressed conditions, both DNA-binding activity and transcriptional activity of vertebrate HSF1 are under tight negative control (reviewed in Reference 44). However, it remains controversial whether repression by chaperone Hsp70, sequestration of constitutive phosphorylation on serine residues, or unknown inhibitory regulators are the principal mechanisms underlying stress-inducible activation and rapid deactivation of HSF1.35,54–56

Previous studies have demonstrated that in response to both heat and simulated ischemia, the mechanism(s) for
HSF1 activation is similar, if not identical, in myogenic cells and that depletion of intracellular ATP plays an important role in triggering the HSF1-DNA binding activity. In disease conditions, inducers of HSF1 activation, such as oxidized LDL and reactive nitrogen intermediates, are thought to increase protein damage, which triggers upregulation of HSP gene expression. However, transcriptional activation of the HSF1 pathway does not require new protein synthesis, since the preexisting transactivator (HSF1) is inactive in the unstressed state. Physiological stresses, such as heat and ischemia, induce HSF1 monomers to oligomerize as homotrimers, which then bind to an upstream sequence-specific motif, heat shock element, in the promoter of all stress-inducible HSP genes (Figure 1). We recently established a gene knockout model of Hsf1 and demonstrated in in vitro studies the essential requirement of this regulatory pathway in cellular defense and thermotolerance. In addition, stress protein expression has been implicated in promoting tumor cell survival and protection of the ischemic heart.

Stress Protein Overexpression Enhances the Speed of Physiological Recovery of the Ischemic Heart

A substantial literature describes the induction of Hsp70 by ischemia, and the potential role of Hsp70 in ischemic preconditioning, and an inverse correlation between expression of Hsp70 induced by ischemic or thermal preconditioning and infarct size in animal models. In physiological terms, potential functions for molecular chaperones in the ischemic heart include the following: protein folding of newly synthesized polyptides essential for maintaining oxidative metabolism after myocyte damage, protection of mitochondria from ROS and cytokines such as TNF and translocation of newly synthesized proteins during organellar repair, repair of critical structural proteins after ischemia-induced cytoskeletal alterations, recycling of membrane vesicles, transport of potential toxic byproducts for degradation by the proteasome, suppression of proinflammatory cytokines such as interleukin-1β, suppression of NADPH oxidase and the oxidative burst by the heat shock response, and prevention of apoptosis either through the mitochondrial chaperone Hsp60 binding of cytochrome c and/or Hsp70 binding of cytosolic targets, repair of ion channel, collagen synthesis by Hsp47 chaperone for reparative fibrosis, and modulation of the immune-mediated ischemic injury.

Figure 1. A summary of the major pathophysiological signals that activate HSP synthesis (left of vertical solid line) and the potential functions of stress proteins (right of vertical solid line). Cellular injury is manifested by increased generation of ROS, availability of redox-active iron, peroxidation of membrane lipids, and protein damage, among others. The extracellular sources of ROS (A) could be endothelial cells, VSMCs, and even myocytes in the surrounding tissue; mitochondrial electron transport of molecular oxygen (B) may be the main intracellular source for ROS generation. Intracellular free calcium (C), which increases 10-fold within 10 minutes of reperfusion, has been implicated in the activation of proteases (D), which, in turn, are thought to contribute to intracellular myocardial injury. Damage to the cytoskeleton (E) is an early event of ischemic injury, which causes ventricular dysfunction or "myocardial stunning" when the injury is reversible. Myocardial injury could also occur from recruitment of polymorphonuclear leukocytes (PMNs) by multiple mechanisms into the ischemic territory and by the release of oxygen-derived free radicals (A), proteolytic enzymes (F), extracellular proteases (G), and complement activation (H) (reviewed in Reference 233). Diverse physiological signals are thought to activate the stress-inducible HSF1 (I), which binds to the sequence-specific heat shock element (HSE) (J), contained in the promoters of all HSPs (K). Although the precise mechanism of Hsp70-mediated ischemic protection is poorly understood, it is widely attributed to the biological property of "molecular chaperones," which are proposed to assist in the assembly or repair of newly synthesized or damaged proteins. In physiological terms, potential functions for molecular chaperones in the ischemic heart include the following: protein folding of newly synthesized polypeptides essential for maintaining oxidative metabolism after myocyte damage, protection of mitochondria from ROS and cytokines such as TNF and translocation of newly synthesized proteins during organellar repair, repair of critical structural proteins after ischemia-induced cytoskeletal alterations, recycling of membrane vesicles, transport of potential toxic byproducts for degradation by the proteasome, suppression of proinflammatory cytokines such as interleukin-1β, suppression of NADPH oxidase and the oxidative burst by the heat shock response, and prevention of apoptosis either through the mitochondrial chaperone Hsp60 binding of cytochrome c and/or Hsp70 binding of cytosolic targets, repair of ion channel, collagen synthesis by Hsp47 chaperone for reparative fibrosis, and modulation of the immune-mediated ischemic injury.
Molecular chaperones, like Hsp70 and αB-crystallin, are proteins that facilitate the folding, assembly, and disassembly of other proteins but are not part of the finished product. Since many proteins require chaperones to fold, these proteins are essential components in the final stage of the central dogma of molecular biology: DNA → RNA → polypeptide → folded protein. In vitro chaperones function to prevent aggregation of other proteins under conditions of stress and to promote restoration of enzymatic activity of denatured protein substrates or enzymes (eg, citrate synthase, β-galactosidase, or luciferase) on removal of the stress.

Figure 2 shows schematically the reaction cycle of chaperone Hsc70 in relation to recently identified co-chaperones and molecular substrates in the cell. For example, chaperone Hsp40 plays a major catalytic function in loading target substrates onto the Hsc70 binding/release cycle. Although the mechanisms of these functions are still emerging, the major functions of molecular chaperones are to (1) transiently bind and delay the folding of nascent polypeptide chains until synthesis is complete, (2) maintain polypeptide chains in an appropriate conformation suitable for translocation across organelle membranes, (3) prevent aggregation from intramolecular or intermolecular interactions, (4) actively disassemble clathrin-coated vesicles, (5) hold steroid aporeceptor complexes in ligand competent states (Hsp90 and co-chaperone), and (6) assist in degrading toxic metabolites by promoting ubiquination and proteasome lysis (Figure 1L through 1P).

**Stress Proteins Can Function as Molecular Chaperones**

The Case for Molecular Chaperones in Cardiac Diseases

Widespread clinical interest into the biological functions of molecular chaperones extends over a range of human pathologies from degenerative conditions such as Alzheimer’s disease, prions, amyloidosis, cataract formation, sickle cell disease, cystic fibrosis, and various cardiac diseases including myocardial ischemia. Early events that reestablish timely reflow of the ischemic myocardium either through thrombolitics, direct angioplasty, or spontaneous clot lysis are essential for enhancing myocardial salvage and reducing morbidity and mortality. Nonetheless, recurrent ischemia either from rupture of an unstable plaque or congestive heart failure can complicate the clinical course of an acute myocardial infarction. In uncomplicated acute myocardial infarction, physiological recovery at the cellular level begins within minutes but could last for weeks to months before myocardial repair is complete. Thus, endogenous protective mechanisms have clinical relevance in mitigating the effects of ischemic heart disease.

Biochemical Activities of HSP Regulatory Pathway and Chaperones During Myocardial Ischemia

In previous work undertaken to define the proximate stimulus to HSF activation, we observed that severe intracellular...
acidosis (pH 6.7) was insufficient to induce the DNA binding of HSF1 in cultured myogenic cells exposed to simulated ischemia, if ATP stores were preserved. In contrast, severe ATP depletion (65%) stimulated DNA binding of HSF1, even if pH was maintained within the normal range. In the intact ischemic heart, 15 minutes of ischemia, which produces reversible injury, is associated with a similar reduction (65%) of high-energy ATP stores, whereas lethal injury is found with prolonged ischemia (>40 minutes) and >90% depletion of high-energy pools. The $K_m$ for the weak ATPase activity of bovine Hsc70 is 1 to 2 μmol/L. 3 orders of magnitude below the millimolar concentrations of intracellular adenine nucleotide pools. Therefore, ATP-dependent activation of the HSF1 regulatory pathway and the biochemical properties of molecular chaperones are unlikely to be adversely affected during periods of transient ischemia or reversible myocardial ischemic injury.

Proof of principle indicating a cardioprotective effect of Hsp70 in transgenic animals subjected to ischemia/reperfusion suggests that pharmacological or genetic methods to increase stress protein expression in the myocardium of patients at risk of acute ischemic events might limit ischemic injury. However, additional basic knowledge is needed regarding (1) their relationships to other endogenous pathways involved in myocardial protection from oxidative stress/damage, (2) functional specificity among chaperone members of the HSP multigene family, and (3) the contribution of this pathway during acute ischemia and other physiological states that trigger the heat shock response, before clinical application.

**Stress Proteins and Antioxidant Pathways for Cardioprotection**

Since the 1970s, the hypothesis that free radical scavengers can ameliorate oxidative damage during ischemia/reperfusion has been pursued by clinicians and researchers. In model systems ranging from transgenic Drosophila to mice, overexpression of catalase, superoxide dismutase, or glutathione peroxidase tends to be protective against oxidative stress. Oxidative stress, from ischemia/reperfusion, also plays a central role in the injury of vital organs such as the brain, kidney, and heart. ROS are thought to contribute to ventricular dysfunction, or “myocardial stunning,” arrhythmias, and progressive cell damage or death after ischemic injury (Figure 1).

Discrepant results have been reported from attempts to deliver antioxidants during and after myocardial ischemia/reperfusion. Exogenous antioxidants, which are restricted to the interstitial spaces, may have limited ability to protect intracellular proteins against ROS. For example, the hydroxyl free radical (-OH), believed to be the main agent of oxidative damage, is so highly reactive with a typical substrate that its half-life at 37°C is $7 \times 10^{-10}$ seconds. Thus, it is difficult to envision that the administration of exogenous antioxidants, at concentrations that are physiologically feasible, can effectively prevent -OH-induced macromolecular damage. A potentially more effective strategy may be to physiologically minimize the production of -OH. Indeed, overexpression of members of the HSP family may provide one such avenue.

Several studies have reported that during protection against myocardial ischemia, upregulation of stress protein levels correlates with increases in the enzymatic activity of catalase, suggesting potential additive or synergistic interactions of these endogenous pathways against oxidative stress. An important unanswered question is whether functions of stress proteins, as molecular chaperones, complement the unique functions of antioxidant enzymes in protecting against oxidative stress/damage.

**Molecular Chaperones of the Cytosol/Nuclear Compartments**

The Table shows the major classes of HSPs, the intracellular compartments, their putative functions, and potential significance in cardiovascular biology.

**Hsp70 Chaperones**

Members of the Hsp70 family are the most widely studied and abundant group in eukaryotic cells. In the cytosol, Hsp70 binds to nascent polypeptides before their release from the ribosome. All members of the Hsp70 chaperone class possess two distinct domains: a highly conserved N-terminal ATPase domain and a more divergent C-terminal domain, which binds short hydrophobic peptides of target substrates (Figure 2A). Hsp70 chaperone function requires the N-terminal ATPase domain, which, interestingly, is similar structurally to rabbit skeletal muscle actin despite little sequence similarity.

These structure/functional relationships of Hsp70 likely confer in vivo chaperone activity in cardioprotection. In this regard, virtually nothing is known about the constitutive Hsc70 chaperone, which shares >80% sequence homology with Hsp70. Conceivably, modest upregulation of the constitutive Hsc70 could promote substantial cardioprotective benefit. However, upregulation of HSPs beyond a critical threshold may have deleterious cellular consequences. Distinct functions between Hsp70 members were reported recently to exist in regions outside the peptide binding domain, suggesting additional levels of complexity to chaperone functions in vivo.

**Hsp90 Chaperones**

Figure 2 shows that the chaperone Hsp90 is a component of the reaction cycle involving the chaperone Hsc70 complex and newly synthesized proteins. In addition, members of the Hsp90 family, Hsp90α and Hsp90β, which constitute 1% to 2% of the total soluble cytoplasmic proteins, have the best characterized in vivo functional relationships with target proteins, the steroid hormone receptors. Hsp90 with chaperone partners, Hsp70 and Hsp56, directly binds, stabilizes, and maintains the aporeceptor complex in an inactive conformation. Ligand binding (eg, estrogen) to the aporeceptor complex triggers ATP hydrolysis by Hsp90, which dissociates from an “activated” receptor that can now bind to the sequence-specific recognition motif and induce the transcription of target genes. In addition, elevated levels of Hsp90 expression destabilize the estrogen receptor/estrogen responsive element complex and downregulate estrogen-responsive
target gene expression, indicating a regulatory feedback loop.

Hsp90 chaperone functions are mediated by signal transduction pathways involving various protein kinases of both tyrosine and serine-threonine types, casein kinase II, the heme-regulated eIF-2α, and a variety of other cellular proteins, such as calmodulin, actin, and tubulin (reviewed in Reference 106). Finally, Hsp90 chaperones of *Saccharomyces cerevisiae* are essential for survival under all conditions, supporting their important physiological roles in lower eukaryotes.

### Cytosolic Chaperones of Special Interest in Cardiac and Vascular Biology

Unlike the ubiquitous Hsp70 and Hsp90 counterparts, specific members of the small MW HSPs (HO-1 or Hsp32, Hsp27, αB-crystallin, and Hsp20 chaperones) exhibit tissue-specific expression and function, which are critical in the cardiovascular system. These chaperones play a pivotal role in maintaining cellular homeostasis, particularly during stress conditions such as ischemia, hypoxia, and inflammation.

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**Major Classes of HSPs in Cardiovascular Biology**

<table>
<thead>
<tr>
<th>Major Stress Proteins and/or Family</th>
<th>Cellular/Organelle (Tissue)</th>
<th>Cochaperones/Protein Targets</th>
<th>Cellular Function (Inducer)</th>
<th>Potential Cardiovascular Significance (Pathophysiological Mechanisms)</th>
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<tbody>
<tr>
<td>Hsp110</td>
<td>Nucleolus/cytoplasm</td>
<td>Hsc70, Hsp40</td>
<td>Thermotolerance (heat stress)</td>
<td>(Ischemic cross tolerance)</td>
</tr>
<tr>
<td>Apo-1 (mouse)</td>
<td>Cytoplasm</td>
<td></td>
<td>Protein refolding (heat stress)</td>
<td>...</td>
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<tr>
<td>Hsp105</td>
<td>Cytoplasm</td>
<td></td>
<td></td>
<td>...</td>
</tr>
<tr>
<td>Osp94</td>
<td>(Renal medulla)</td>
<td></td>
<td>(Osmotic and heat stress)</td>
<td>(Hypersosmolar stress, dehydration)</td>
</tr>
<tr>
<td>Hsp90</td>
<td>Cytoplasm</td>
<td>Hsp70, Hsp56, p23</td>
<td>Aporeceptor function (heat stress)</td>
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</tr>
<tr>
<td>Hsp90β (Hsp84)</td>
<td>Cytoplasm</td>
<td>Immunophils, cyclophilins (steroid receptors)</td>
<td>...</td>
<td>(Immunosuppressive therapy and cardiac transplantation)</td>
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<tr>
<td>Grp94</td>
<td>ER</td>
<td>94-Kinase, Grp78</td>
<td>Calcium binding chaperone (glucose starvation)</td>
<td>(Ischemia)</td>
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<td>Hsp70</td>
<td>Cytoplasm, peroxisome</td>
<td>Hsp40</td>
<td>Protein folding</td>
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<tr>
<td>Hsp70, inductible</td>
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<td>RAD46, platelets, Hsp40</td>
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<td>mHsp75</td>
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<td>Translocation and protein folding</td>
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<tr>
<td>Grp78/Bip</td>
<td>ER</td>
<td>Grp94</td>
<td>Protein folding (unfolded proteins)</td>
<td>(CFTR binding)</td>
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<td>...</td>
<td>(Myocardium)</td>
<td></td>
<td>(Self-antigen)</td>
<td>Chagas' disease (molecular mimicry)</td>
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<tr>
<td>Small Hsp</td>
<td>Cytoplasm</td>
<td></td>
<td>(Hypoxia, HIF-1 transcription factor)</td>
<td>Antioxidant properties</td>
</tr>
<tr>
<td>Heme oxygenase (HO-1, HO-2, Hsp32)</td>
<td>Cytoplasm</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hsp27</td>
<td>Cytoplasm/nucleus</td>
<td>p38 kinase, αβ-crystallin</td>
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<tr>
<td>αB-Crystallin</td>
<td>Cytoplasm</td>
<td>Hsp27</td>
<td>Cytoskeletal stabilization at Z bands</td>
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<td>Cytoplasm</td>
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<td>MKBP</td>
<td>Cytoplasm</td>
<td>Myotonic dystrophy protein</td>
<td>Protection of Z bands and neuromuscular junction</td>
<td>Myotonic dystrophy</td>
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<td>Assorted</td>
<td>ER</td>
<td>Procollagen I and III</td>
<td>Quality control of collagen synthesis</td>
<td>(Reactive and reparative interstitial fibrosis)</td>
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<td>ER</td>
<td>Hsp70, Hsc70</td>
<td>Chaperone functions</td>
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<tr>
<td>Hsp40 (Hdj-1)</td>
<td>Cytoplasm</td>
<td>Hsp70, Hsc70</td>
<td>Chaperone functions</td>
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<td>Mitochondria</td>
<td>Chaperonin 10</td>
<td>Protein import/folding</td>
<td>(Cytochrome c and apoptosis)</td>
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<tr>
<td>TRIC (also called CCT)</td>
<td>Cytoplasm</td>
<td>Actin/tubulin</td>
<td>Protein folding</td>
<td>...</td>
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</tbody>
</table>

mHsp75 indicates mitochondrial Hsp75; HIF-1, hypoxia-inducible transcription factor 1; and MKBP, myotonic dystrophy protein kinase.
restricted expression, suggesting potential specialized properties in the cardiovascular system.

**Inducible HO (Hsp32)**

HO is the rate-limiting enzyme in the degradation of heme to biliverdin (a potent antioxidant), molecular iron, and carbon monoxide. Three related single-copy genes encode HO isoforms: HO-1, HO-2, and HO-3.108–110 HO-1 is a bona fide 32-kDa stress protein (Hsp32) that is induced by diverse physiological stresses, including hypoxia, ischemia/reperfusion, heme, hydrogen peroxide, and several heavy metals (selenium, arsenite, cobalt, cadmium, and stannous ions).108–110 Inducible Hsp32 (HO-1), the most widely expressed isoform, is present in myocardial cells.111

Hsp32, like the inducible form of NO synthase mediates guanylyl cyclase–dependent platelet inhibition and vasodilation of VSMCs.112–114 However, physiologically relevant hemodynamic forces (shear stress and cyclic strain) induce HO-1 mRNA expression but not inducible NO synthase expression, suggesting specificity of this stress response pathway to physiological signals.115 Either endogenously released or exogenous administered NO induces a 3- to 6-fold increase in Hsp32 (HO-1) gene expression and CO production in VSMCs;116; similarly, the inhibitor tin protoporphyrin-IX prevents platelet aggregation through induction of HO-1 gene expression and CO production in aortic VSMCs.117 In rat VSMCs, angiotensin II treatment decreases Hsp32 (HO-1) mRNA expression in a calcium-dependent manner.117 However, angiotensin II–induced hypertension increases Hsp32 (HO-1) mRNA expression in rat aorta, suggesting the overriding influences of hemodynamic factors in vivo.118

Several regulatory pathways are involved in the induction of Hsp32 gene expression, most notably the HSF1 and the hypoxia-inducible transcription factor-1 pathways.119–122 Although direct evidence of a chaperone function of Hsp32 is lacking, its overexpression in several cell types protects against oxidative stress/damage.123–125 Accordingly, studies to define the precise regulatory pathways stimulated by heavy metals, hypoxia, and oxidative stress could provide novel insight into the biological roles of Hsp32 (HO-1) in modulating oxidative stress/damage during ischemia/reperfusion, vascular tone (e.g., hypertension), and inhibition of platelet aggregation and/or VSMC proliferation after balloon angioplasty.

**Hsp25/27 Chaperone**

After its discovery as an inhibitor of actin polymerization,126 chaperone Hsp 25/27 (Hsp25 in mice and Hsp27 in humans) has been demonstrated to play a major role in actin filament dynamics in diverse cell types. Physiological stimuli (oxidative stress, cytokines, and growth factors) dramatically increase the phosphorylation of human Hsp27 at Ser15, Ser78, and Ser83 residues, which is essential for acquired tolerance.127–129 Hsp25/27 phosphorylation is catalyzed by the MAPks (p38-MAPks, JNKs, or SAPKs) and ERKs.130 In the perfused adult heart, both p38-MAPK and JNK/SAPK are activated after ischemia/reperfusion.131 In response to ROS treatment, activation of p38-MAPK increases MAPKAP kinase 2 activity, which phosphorylates Hsp27.132

In human endothelial cells, inhibition of vascular endothelial growth factor–induced p38-MAPK activation abolishes Hsp27 phosphorylation, actin polymerization, and cell migration, suggesting a possible link between Hsp27 and angiogenesis.133 Together, available evidence places the p38-MAPK as an upstream activator of stress-inducible Hsp25/27 phosphorylation, and this pathway underlies the effect of p38-MAPK on the reorganization of filamentous actin, accumulation of stress fibers, and the recruitment of vinculin at focal adhesion sites.134 It will be important next to determine whether Hsp25/27 exerts vasoprotective actions in response to hemodynamic forces or vessel wall injury. However, direct analysis will likely require an Hsp27 gene knockout model.

**αB-Crystallin Chaperone (Hsp22)**

Whereas Hsp27 is detected in endothelial cells, VSMCs, and cardiomyocytes, the αB-crystallin chaperone is expressed in cardiomyocytes only.135 Both Hsp27 and αB-crystallin are structurally related bona fide HSPs with in vitro chaperone activity but, unlike Hsp70, are not ATP-binding proteins.136–139 Heightened interest into the regulation and function of αB-crystallin (Hsp22), a major structural protein of the ocular lens, is related to its tissue-restricted expression in striated myogenic lineages with high oxidative capacity, such as the heart and type I skeletal muscle fibers.140 In nonlenticular tissues, αB-crystallin postnatal expression increases and reaches its highest levels in the adult heart (∼1% to 3% of the total soluble protein), followed by skeletal muscle and the kidney.140 Previous immunohistochemical studies have localized the highest αB-crystallin expression in the cardiac conduction fibers of the adult heart.141 Whether an alteration of αB-crystallin expression could lead to abnormalities of the conduction system is presently unknown but raises an intriguing possibility.

Although αB-crystallin expression has been localized to Z bands of the cytoskeleton, in a pattern similar to desmin and actin,142 recent studies suggest that this interaction is much more transient and dynamic with respect to intracellular targets, depending on the physiological conditions. In unstimulated cardiac myocytes, biochemical studies indicate that αB-crystallin is highly soluble and remains in the cytosolic fraction; heat or ischemia triggers rapid translocation of αB-crystallin into the insoluble cytoskeletal/nuclear fractions, aggregation, and specific interactions at Z bands (Reference 142 and I.J. Benjamin, unpublished data, 1998). The physiological significance for the tendency of both αB-crystallin and Hsp25/27 to form large hetero-oligomeric complexes (500 to 800 kDa) both in vivo and in vitro after physiological stresses remains a mystery.134 Although αB-crystallin chaperone provides cytoprotection to cardiomyocytes,72 the regulatory mechanisms of posttranslation modifications such as phosphorylation, glycation, and deacetylation on αB-crystallin function await direct analysis in the cardiovascular system.

**Stress Proteins and Striated Muscle Development and Differentiation**

Increased small MW HSP chaperone expression has been described during periods associated with increased protein
synthesis, protein degradation, and cellular reorganization, such as myogenic differentiation and embryogenesis. The tissue-restricted expression of αB-crystallin during skeletal muscle myogenesis may require the MyoD family of basic helix-loop-helix transcription factors, which bind the essential E-box enhancer contained in αB-crystallin promoter. We recently reported that expression of αB-crystallin, but not Hsp27, is directly linked to increases in oxidative metabolism in skeletal muscle after chronic nerve stimulation. However, the physiological role of upregulation of Hsp27 expression, which precedes early differentiation of murine embryonic stem cells, remains to be established in myogenic lineages.

Much less is known about the regulatory mechanisms involved in αB-crystallin–restricted expression in cardiac myocytes. αB-Crystallin is abundantly expressed in early cardiac development beginning at embryonic day 8.5, suggesting a role either as a structural protein or as a molecular chaperone in myofiber stabilization. Since Myo-n-like factors are absent in the heart, in vitro binding studies of cardiac nuclear extracts have implicated the transcriptional activities of an upstream stimulating factor at the E-box element and the serum response factor at a reverse CArG box in the αB-crystallin promoter. So far, an in vivo developmental survey reveals that αB-crystallin expression is unaffected in skeletal muscle of myf5 null mice or the heart of d-HAND null mice at embryonic day 9.0 (I.J. Benjamin, unpublished data, 1998).

Other Cytosolic/Nuclear Chaperones

Several other HSPs that exist in the cytosolic/nuclear compartment are of potential interest in cardiovascular biology. For example, the cytosolic 20-kDa protein, p20, is abundantly expressed in heart, skeletal, and smooth muscle and copurifies with the chaperones αB-crystallin and Hsp27. Although p20 expression is induced by neither heat nor chemical stress, it contains the homologous C-terminal “α-crystallin domain” shared by all members of the small MW HSPs. In VSMCs, p20 is a substrate for both cAMP and cGMP protein kinases, suggesting a regulatory role to postulate functions in physiological maintenance of vascular tone and adaptation to vessel wall injury.

A fourth member of the small HSPs (besides Hsp27, αB-crystallin, and p20), a myotonic dystrophy protein kinase–binding protein associates and increases the activity of dystrophy protein kinase and prevents its heat-induced denaturation in vitro. Myotonic dystrophy protein kinase, unlike Hsp27 or αB-crystallin, is upregulated in the skeletal muscle of patients with myotonic dystrophy, suggesting that this novel protein may be involved in the pathogenesis of this disease.

Members of the Hsp110 family exhibit chaperone and cytoprotective functions, although details about their relative expression in myocardial cells and regional distribution in the cardiovascular system await further characterization. Additional Hsp110 family members include Hsp105, Apg-1, and Osp94. There is intense interest in identifying a mammalian homologue of yeast Hsp104, which, instead of preventing protein aggregation, seems to resolubilize insoluble protein aggregates.

Mitochondrial Hsp70 Chaperone System

All organisms possess ATP-dependent mechanisms for protein folding and assembly within organelles. Translocation of proteins across the mitochondrial membrane requires mitochondrial chaperone Hsp70 in the matrix, where folding into the native state is completed (see Figure 1).

Mitochondrial Chaperonin System

Besides Hsp70-like chaperones, mitochondrial chaperonins Hsp60 and Hsp10 constitute a separate system that provides a sequestered environment for folding a subset of proteins in vivo. These 7-membered rings are arranged as cylindrical structures in which ATP-dependent protein folding occurs in their central cavity. Evidence from in vitro studies suggests the Hsp70 chaperones and chaperonin systems function cooperatively in protein folding and assembly in eukaryotes (reviewed in Reference 13).

Cytosolic Chaperonin System

TRiC chaperonin is considered the functional equivalent of chaperonin Hsp60/Hsp10 in the eukaryotic cytosol. The TRiC complex, which consists of either 8- or 9-membered double rings of ~55 to 65 kDa subunits, is required for folding of actin and tubulin in vivo. Chaperonin TRiC requires additional components, such as Hsp40, which stimulates the Hsc70 ATPase, for protein folding in the cytosol (see Figure 2). Available evidence suggests that TRiC chaperonin functions in the final stages of folding during translation of a limited number of polypeptide domains.

Implications

Mitochondrial chaperones and chaperonins are only modestly induced by physiological stress in cardiomyocytes and the heart. However, the location of mitochondrial Hsp70 chaperones and chaperonins at major sites of ROS production could serve to complement both enzymatic and nonenzymatic defense mechanisms to diminish oxidative injury and increase the rate of physiological recovery after ischemic injury. Whether overexpression of either mitochondrial Hsp75 chaperone or chaperonin can provide equivalent or superior protection against ischemic injury is presently unknown. Another important question concerns whether the overlapping functions of Hsp70 chaperones and Hsp60 or TRiC/Hsp40 chaperonin systems are coordinated for de novo protein folding and potential cytoprotection during the pathogenesis of heart disease.

Molecular Chaperones in ER

Glucose-Regulated Proteins

Analysis of ER chaperones may be of particular clinical interest, because the ER functions to degrade or repair proteins damaged after myocardial ischemia or after mutant proteins (eg, CFTR or thyroglobulin) fail to fold properly. Anoxia, glucose starvation, and calcium ionophores induce members of the Ca2+–binding glucose-regulated class of stress proteins, Grp170, Grp94, and Grp78/BiP. Variable changes in the expression of Grp78 protein have been reported after ischemia. Although “guilt by association” with cytosolic chaperone is the presumed role of chaperone
Grp function in protein surveillance and quality control, more studies of their expression are needed in pathophysiological states associated with the expression, posttranslational glycosylation, and secretion of abnormal proteins via the ER-Golgi pathway.

**Hsp47 Chaperones**
The 47-kDa collagen-binding glycoprotein Hsp47 is a member of the serpin (serine protease inhibitor) superfamily and is highly induced by heat stress or pathophysiological states (eg, hepatic fibrosis) associated with increased collagen synthesis. Hsp47 resides in the ER and contains the C-terminal Arg-Asp-Glu-Leu ER retention signal. Hsp47 binds transiently to collagen types I to IV and avidly to denatured collagen substrates; thus, its role in procollagen processing and transport seems firmly established. Future studies must now address the likely biological and clinical relevance of Hsp47 expression at the onset and progression of pathophysiological states, such as myocardial infarction, idiopathic and hypertrophic cardiomyopathies, and hypertension, in which myocardial fibrosis is prominently featured.

**Current Challenges and Future Directions**
Physiological recovery of the ischemic heart begins within minutes, but the rate of cellular ischemic repair, which may last for days to weeks, is critical for the reduction of subsequent cardiovascular morbidity and mortality. The idea that stress proteins can speed the physiological recovery of reversible myocardial injury is based on experimental evidence indicating that multiple proximate “signals” can activate the heat shock response and, thereby, evoke the endogenous protective mechanisms of Hsps. Major clinical events such as unstable angina, recurrent occlusion after thrombolytic therapy, and acute exacerbation of chronic angina are potential physiological inducers of cytoprotective stress proteins. The next logical step would be to consider the possibility that related members of the multigene stress protein family confer similar or additional functional benefits. Because ischemia and other physiological perturbations disrupt the normal structure-function relationships of intracellular proteins, future studies must establish whether molecular chaperones, either alone or in combination, alleviate ischemic damage by accelerating physiological recovery of the myocardial cell in the intact organism.

**Experimental Models for Stress Protein Research**
Transgenic models of Hsp70 overexpression (gain of function) and hsf1-deficient mice (loss of function), and their subsequent characterization, are beginning to illuminate their physiological roles in vivo. One exciting direction of such efforts could lead to integrated approaches into the physiological roles of entire systems or regulatory networks in genetically modified animal models of human diseases. However, the wealth of existing knowledge of physiological studies in animals larger than the mouse should not be disregarded. Studies to define the role of HSP expression in myocardial stunning using conscious animals could justify the development of transgenic rat and rabbit models. Potential limitations of such strategies include substantially greater costs from the longer gestation periods, longer time to reach sexual maturity, and smaller litter sizes of such larger species. However, investigators with expertise in molecular biology and molecular physiology undertaking collaborative projects increase the chances of success, while avoiding the duplication of efforts.

**Does Stress-Inducible Expression of HSP Genes Affect Infarct Size, Arrhythmogenesis, and Myocardial Remodeling After Acute Myocardial Infarction?**
Previous studies have demonstrated that pretreatment (24 hours) with heat stress attenuates free radical release in the isolated rat heart and reduces the amount of arrhythmias, a major functional hallmark of ischemia/reperfusion injury (reviewed in Reference 92). Together, these studies correlate potential interactions between these protective pathways but are inadequate to establish a cause and effect relationship. The development of an Hsf1 gene knockout mouse model provides a powerful experimental approach to determine whether a deficiency in stress-inducible HSP synthesis, during physiological perturbations such as myocardial ischemia, affects the outcome of postinfarction injury and the repair mechanisms in the intact organism. Results of such studies can help establish causality, elucidate potential mechanisms by which HSP synthesis relates to myocardial ischemia, and evaluate the participation of stress proteins during early and late ischemic preconditioning. Timely characterization of several knockout models, currently in development in laboratories worldwide, should continue to foster fruitful collaborations among investigators. Further progress should accelerate the flow of new knowledge into related areas such as posts ischemic reactive and reparative fibrosis, postreperfusion inflammation, and myocardial stunning.

**Stress Protein Expression and Mechanisms of Ischemic Preconditioning**
Ischemic preconditioning is the most powerful experimental maneuver that reproducibly protects the heart against subsequent ischemic challenge. However, controversy exists about the precise roles of stress proteins in this well-characterized phenomenon. Several mechanisms involving protein kinase C, adenosine receptors, and their relationships to signal transduction pathways have been implicated in ischemic preconditioning. Sufficient evidence indicates a lack of correlation between stress-inducible expression (eg, Hsp70) and early preconditioning, which is short-lived and lasts between 1 and 3 hours, depending on the model and species.

**Is the Chaperone Family of Stress Proteins Unlikely to Have Physiological Relevance in Early Preconditioning?**
In our opinion, studies in this area have either prematurely dismissed or paid inadequate attention to the potential importance of the small MW Hsps, such as αB-crystallin and Hsp27. These chaperones seem likely to be candidates for the “first line of defense” against nonlethal stress. Whether oligomerization of small MW Hsps contributes to the me-
Molecular Chaperones and Cell Death Pathways

Stress proteins are ideal candidates to play key regulatory roles in cell survival and death pathways involving DNA damage and protein synthesis, repair, and degradation. Maneuvers that increase Hsp70 expression after heat shock, sodium butyrate exposure, and constitutive or regulated overexpression inhibit apoptosis in a variety of cell types. The proto-oncogene c-myc potentiates heat shock–induced apoptosis, in contrast, Bcl-2 overexpression augments thermotolerance-induced cellular survival. Tissue-specific expression of Hsp70-2 prevents apoptosis in certain mitotic cells through a mechanism involving cell cycle control. Hsp70 chaperone function in regulating apoptosis may be at the level of signal transduction, as it has been implicated in the stress-activated kinase pathway.

Recent studies in our laboratory using hsf1-deficient cultured cells have established the role of stress-inducible Hsps to render cells thermotolerant to heat-induced apoptosis. The present study provides a genetic model to examine potential interdependent relationships between stress-inducible Hsps and the mechanisms involved in cell survival and/or cell death pathways. Sublethal heat stress protects mitochondria against oxidative stress and prevents cell death by apoptosis. Given their strategic locations in all major organelles, it is tempting to speculate that multiple Hsps may combat oxidative stress/damage by refolding damaged repressors of the cell death pathway or preventing their degradation. Alternatively, repressing the release of suicide activators such as cytochrome c could occur through interactions with mitochondrial chaperones and chaperonins.

In addition, results of several recent studies have implicated the small MW Hsp25/27 in cell survival pathways involving cell differentiation and oxidative stress/damage. Previous studies have demonstrated that overexpression of Hsp25/27, like the antiapoptotic protein Bcl-2, increases the levels of the antioxidant glutathione and resistance to Fas/APO-1–mediated apoptosis, although whether this occurs directly remains unclear. Withdrawal of mouse embryonic stem cells from the cell cycle induces upregulation of Hsp25/27 mRNA, which is accompanied by decreases in phosphorylation and increases in oligomerization of Hsp25/27 protein. Antisense reduction of Hsp25/27 reverses these changes through the prolongation of the cell cycle, reduction in glutathione levels, and acceleration toward apoptosis. Together, these provocative findings suggest that during proliferation and differentiation of myocardial cells, the Hsp25/27 chaperone, and others, can reduce oxidative stress/damage and prevent apoptosis through a novel redox-dependent mechanism.

Does Stress-Inducible Expression of HSP Genes Affect the Natural History of Chronic Cardiovascular Diseases, Including Aging?

Numerous studies have correlated the induction of Hsp expression and pressure overload by aortic banding, acute hypertension, exposure to vasoactive agents or left ventricular hypertrophy, and growth factor expression in several cell types. Decreases in the expression of HSP genes and the DNA-binding activity of HSF1 are reported to occur during the aging process in the rodent myocardium. There is increasing evidence suggesting that oxidative stress/damage may be a major causal factor in the aging process. The level of oxidative stress and the susceptibility of tissues to experimentally induced oxidative stress seem to increase during the aging process. Whether the increased amounts of molecular oxidative damage, observed during the aging process, are causally associated with a decreased activity of HSF1 and, by implication, Hsp gene expression is presently unknown. The availability of transgenic and gene knockout models will enable future studies to establish the role of stress-inducible heat shock gene expression during normal aging or physiologic adaptation to disease-associated cardiac risk factor(s).

Molecular Chaperones in Cardiac Disease States Caused by Expression of Mutant Proteins

Elevated levels of misfolded or denatured proteins, as well as microinjection of abnormal proteins, are potent inducers of HSP gene expression. Recent studies indicating that the Hsc70 chaperone interacts indirectly with the CFTR harboring the common ΔF508 folding mutation support this general notion of their biological role in human diseases.

From the perspective of chaperone biology, cardiac diseases that arise from mutations in genes encoding components of the contractile apparatus or ion channel proteins are essentially problems of abnormal proteins. Multiple mutations of sarcomeric proteins have been implicated in the pathogenesis of familial hypertrophic cardiomyopathy, including myosin heavy and light chains, troponin I and T subunits, myosin binding protein C, and tropomyosin (Reference 6 and reviewed in Reference 7). Whereas chaperone Hsp27 plays a role in actin polymerization and chaperonin TRIC is involved in actin and tubulin folding, the physiological role of chaperone and chaperonin systems in folding and assembly of sarcomeric structures, under normal conditions...
or in disease, remains a mystery. Conceivably, chaperones can influence either the repair or degradation of mutant sarcomeric proteins, which, ultimately, affect the structure-function relationships and phenotype of the disease. In vitro analyses are first needed to determine whether chaperone function can affect productive protein folding caused by the relevant mutations, which can be validated in appropriate animal models.

Molecular Chaperones in Vascular Biology

Numerous opportunities exist to dissect chaperone functions during the synthesis and secretion of biological active peptides and proteins, cellular proliferation, intracellular signaling, and cytoskeletal rearrangement. Results of such studies may identify the specific HSP protein targets and the specificity among the various cell types in promoting the vascular protective effects of estrogen. The essential roles of Hsp90 and co-chaperones in steroid receptor biology suggest that HSP chaperones play a clinically significant role in the effects of hormonal replacement therapy in postmenopausal women. Epidemiological studies have found that the protective benefit against coronary heart disease in premenopausal women is abolished in the postmenopausal years. Potential protective actions of estrogen have been attributed to its antioxidative and vasoprotective properties, lowering of blood lipids and lipoproteins, and direct effects on the vessel wall. It will be important to define whether stress-inducible Hsp expression in genetically modified mice plays a role in the vascular response to injury in male and female animals.

Molecular Chaperone and Immunological Diseases

In contrast to their well-established cytoprotective roles, certain stress proteins have been implicated in the pathogenesis of cardiovascular diseases. For example, elevated serum levels of antibodies against the bacterial homologue of mammalian Hsp60 have been demonstrated in patients with cardiomyopathy and diabetes, in asymptomatic individuals with carotid stenosis, and in atherosclerotic lesions in rabbits and humans (reviewed in Reference 222). One hypothesis is that autoimmune disease results from cross-reactivity of immunogenic peptides, which are derived from bacterial and mitochondrial chaperones and Hsp60 (chaperonin) and are recognized by activated γδ T lymphocytes. Humoral immune responses and seropositive markers against the Hsp70 family (especially ER Grp78 and Hsc70) of the protozoan parasite Trypanosoma cruzi have been implicated in the pathogenesis of Chagas’ disease, the most common cause of congestive heart failure in Latin America (see Table). Although these correlative studies have inherent limitations, future research to establish causality could open avenues to develop vaccines or other novel therapies for treatment and prevention.

Potential Therapeutic Applications of Molecular Chaperones

Strategies that could increase the rate of physiological recovery after postinfarction stunning and ventricular dysfunction remain important goals in the management of patients with acute myocardial infarction. Because maneuvers using either tissue or whole body hyperthermia are cumbersome and impractical in conscious humans, pharmacological strategies that increase stress protein expression for isothermal protection have potential merit against ischemic damage to the heart, kidney, and brain. Proteasome inhibitors, which transiently elevate the level of unfolded proteins inside cells, is one potential approach. Alternative approaches may involve the development of small molecules and peptides that mimic the in vivo actions of chaperones with therapeutic benefits.

Perspectives

The role of stress proteins in cardioprotection has been acknowledged as one of the most important future directions of research in ischemic heart disease. Since the number of affected individuals is so large, a therapeutic intervention that contributes to a small change in post–myocardial infarction morbidity and mortality, for example, can have dramatic effects on overall clinical outcomes. Opportunities to address the physiological roles of cytoprotective chaperones in cardiac diseases need to be expanded to include their likely roles during chronic conditions (atherosclerotic, hypertension, diabetes, genetic disorders, and valvular heart disease) that converge through common pathways, resulting in heart failure and sudden death. Strategic alliances among research teams could forge new directions and accelerate progress in this promising area, which, ultimately, could succeed in exploiting endogenous pathways to enhance physiological health and to reduce physiological attrition associated with cardiovascular diseases.

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