

Heat shock factor 1 and heat shock proteins: Critical partners in protection against acute cell injury

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Objective: Life-threatening conditions cause severe changes in the organization and conformation of macromolecules, creating urgent requirements for protein repair to ensure survival. As molecular chaperones, heat shock proteins (HSP) that have specialized functions in protein folding are now well established to restore homeostasis in cells and organisms. Augmentation of HSP synthesis is tightly regulated by stress-inducible heat shock factors (HSF), which are part of a transcriptional signaling cascade with both positive (e.g., HSP) and negative (e.g., proinflammatory cytokines) properties. In this review, we discuss the biological roles and mechanisms of HSP-mediated protection in pathophysiologic conditions (ischemia, sepsis, and preeclampsia) and the regulation for stress-dependent HSP synthesis and speculate about future applications for harnessing HSF and HSP partners as cytoprotective agents.

Data Sources: Reactive oxygen species are major pathogenic factors in cell death pathways (e.g., necrosis, apoptosis), in part, because of proteotoxic effects. In intact organisms, forced overexpression of HSP *per se* affords effective counterbalance against

ischemia challenges (e.g., heart and brain) and systemic conditions (e.g., sepsis). Besides stressful conditions, gene-targeting studies have uncovered new functions for heat shock transcription factors (e.g., maintenance of intrauterine pregnancy) in mammals. In parallel, pharmacologic studies using small molecules are paving the way for future prospects to exploit the beneficial properties of HSP, albeit an important but presently elusive goal.

Conclusions: Together, HSF and HSP partners are attractive targets in therapeutic strategies designed to stimulate endogenous protective mechanisms against deleterious consequences of oxidative stress. With further technological advances, it is anticipated that the spotlight on HSP, alone or in combination with other stress response pathways, could, ultimately, reduce injury and accelerate functional recovery of susceptible organs in living organisms including humans. (Crit Care Med 2002; 30[Suppl.]:S43–S50)

KEY WORDS: heat shock proteins; heat shock factor; tumor necrosis factor- α ; ischemia; sepsis; drug targets; and preeclampsia

Although significant strides in health outcomes have been made, acute infarction (e.g., heart and brain attacks) and infections (e.g., sepsis) are still major causes of morbidity and mortality, especially in an aging population in the Western world (1). Even before birth, conditions such as preeclampsia, which affects 3% to 5% of pregnancies, have potential devastating consequences to both mother and fetus (2). Of interest, these conditions evoke endogenous adaptive cellular mechanisms, which, at times, are insufficient to reverse the underlying pathology. Therefore, the delicate balance between life and death requires clinical intervention and intensive life support, during which beneficial cell responses

can be mounted to limit adverse reactions.

Cells respond to stressful conditions by activating genetic programs whose evolutionarily conserved mechanisms have common ancestral origins, from the simplest bacteria to complex organisms including humans (3). In recent years, one such genetic program that has gained increased attention involves the families of heat shock proteins (HSP) and their regulatory partners, the heat shock transcription factors (HSF). Heat shock proteins are classified according to their molecular weight: HSP25, HSP47, HSP60, HSP70/72, HSP90, HSP110 (4). Although many HSP have multiple roles, the most important to date appears to be the capacity to act as protein chaperones, “molecular guardians” that protect vital protein structures and functions. Viewed from this perspective, HSP chaperones play significant roles to mitigate acute challenges from internal or external environmental sources that pose a lethal threat to survival of the

organism. Because HSP are under the transcriptional control of HSF, this regulatory pathway is worthy of our attention for investigation as a novel molecular target for therapeutic intervention (5, 6).

In addition to the biology of HSP, we will review the emerging roles that the regulatory pathways, especially HSF, play in mammalian health and disease. Illustrated by the clinical scenarios pertaining to heart and brain attacks, sepsis, and pregnancy-specific syndrome (preeclampsia), this review tackles three major objectives: (1) to discuss the cellular mechanisms leading to protein damage and defense schemes involving heat shock proteins; (2) to describe the physiologic roles of the heat shock transcription factor 1 (HSF1) regulatory pathway revealed by gene inactivation in mammals; and (3) to provide a conceptual framework for targeting HSF1 as a possible diagnostic and/or therapeutic strategy to reduce proteotoxic damage and to improve clinical outcome.

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National Institutes of Health grant R01 HL (5-R01-HL60667–03), March of Dimes grant 1FY00739, and the Established Investigator Award from the American Heart Association provided support for this work.

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ACUTE CELL INJURY CAUSED BY OXIDATIVE STRESS

Eukaryotic cells have evolved complex schemes to combat and survive over wide ranges of temperatures, pHs, oxygen deficits, and energy supplies. Alterations in metabolic requirements and disorders, such as heart or brain attacks, sepsis, and preeclampsia, can acutely cause severe disruption of the mitochondrial function and the circulatory system, two key homeostatic mechanisms for cell survival.

For example, abrupt discontinuation of blood flow (ischemia) and reperfusion of the myocardium induce an oxidative burst from reactive oxygen species (ROS) that are normal byproducts of the respiratory chain in mitochondria (1). Elevations in ROS levels can have damaging effects on macromolecules, including lipids, DNA, and proteins. In the ischemic heart, acute oxidative stress causes loss of intracellular K^+ , reduction in Ca^{2+} -ATPase activity, and higher levels of intracellular Ca^{2+} , resulting in the severe perturbation of cellular metabolism, deterioration of cardiac function, and the potential genesis of lethal arrhythmias. In the ischemic brain, additional factors (e.g., vascular endothelial growth factor) (7) are thought to disrupt the blood-brain barrier, a factor that extends proteotoxic damage within the ischemic zone.

Sepsis is a clinical syndrome primarily caused by bacterial endotoxin and secondary to complex inflammatory responses, including tumor necrosis factor (TNF)- α production. Multiple events during septic shock have been attributed to TNF- α , including hypertension, metabolic acidosis, hemoconcentration, cardiodepression, and, eventually, death (8). In turn, TNF- α induces the release of ROS, reinforcing the level of oxidative stress and protein damage. Although TNF- α has beneficial roles (9, 10), negative effects caused by the activation of TNF- α cell death pathway imply that the production of this cytokine has to be tightly controlled.

Likewise, preeclampsia, a pregnancy-specific syndrome, is characterized by a low perfusion of the placenta secondary to abnormal placentation. Ischemia of the fetoplacental unit triggers signals to increase its own blood supply, which paradoxically causes vasoconstriction in vital organs of the mother (11). Activated neutrophils and endothelial cells increase the production of ROS, which in the hypoxic placenta releases cytokines and generates

additional oxidative stress. Indeed, on the basis of the hypothesis that oxidative stress plays an important role in the pathogenesis of preeclampsia, clinical trials that have tested antioxidant administration to women in early pregnancy have shown the benefits of such treatment: decreased oxidative stress, endothelial activation, and lower prevalence of preeclampsia (2)

Although important differences exist in clinical onset, the foregoing conditions share a common denominator in protein toxic damage (12–14). In addition, our present pharmacopoeias do not directly target protein misfolding and damage of intracellular molecules whose fate ultimately determines cell death and survival. Hence, the conceptual focus on molecular chaperones as endogenous tools for protein protection and repair has biological foundations deeply rooted in the cell's evolutionary past and therapeutic value in new clinical settings (5, 6).

HSP AND CELLULAR MECHANISMS OF DEFENSE

HSP Are Molecular Chaperones

In unstressed cells, HSP function as molecular chaperones to facilitate the correct folding and assembly of other nascent polypeptides but are not themselves components of the final structure (15). Because ROS induced oxidative damage to macromolecules such as lipids, DNA, and proteins that ultimately affect cellular functions, defense schemes become paramount for cell survival. Of interest, HSP are strategically positioned in the cytoplasm, mitochondria, peroxisomes, and endoplasmic reticulum, providing an organized network for quality control of protein folding in major subcellular compartments (16–18).

Misfolded proteins are thought to be a proximal signal for HSF activation and HSP expression (19, 20). In parallel, small HSP (HSP25) have been proposed to protect against oxidative stress by preventing the inactivation of glucose-6-phosphate dehydrogenase, a key mediator that maintains intracellular GSH pool and redox potential (21).

HSP Limit Cell-Death Mechanisms (Apoptosis)

Apoptosis is an adaptive mechanism that controls cell death without sacrificing the organism's survival (Fig. 1). At

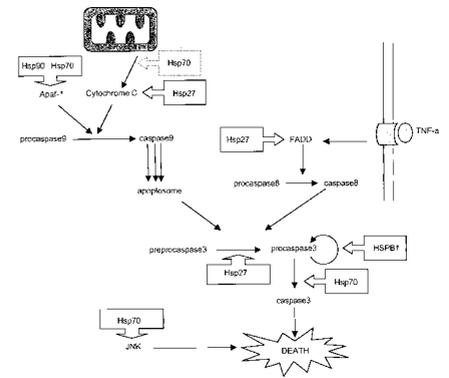


Figure 1. A proposed scheme for integrating the key roles that several heat shock proteins (HSP) play as mediators and regulators in death pathways. Activation of death receptors, such as tumor necrosis factor (TNF) receptor-1, by TNF- α /FasL triggers Fas-associated protein with death domain (FADD) to cleave and activate procaspase-8. Activated caspase-8 is an essential protease whose multifunctional roles include transcriptional, endonuclease, and cell surface activation and reorganization of the cytoskeleton. The small molecular weight HSP25/27 has been proposed to modulate FADD, as does the inhibitor of apoptosis proteins, IAP1-2. Ischemia/reperfusion causes organellar changes in mitochondrial hyperpolarization, membrane swelling, and alterations in permeability transition pore, all of which may release proapoptotic proteins, such as cytochrome *c* and apoptosis-inducing factor. HSP 70 might reduce the cytochrome *c* release, whereas HSP27 prevents caspase activation by cytochrome *c*. Both HSP90 and HSP70 chaperone sequester Apaf-1 upstream of caspase-9 activation, thereby preventing apoptosome formation and procaspase-3 activation downstream. HSP27 and HSPB1 (α B-crystallin) inhibit procaspase-3 activation at two different steps. Protection against cell death is also operated by HSP70 downstream of caspase-3 activation and by negative regulation of JNK.

the same time, stressed cells induce the expression of HSP as key determinants in the regulation of apoptosis (22). The manner in which HSP, including HSP27, HSP70, and HSP90, inhibit apoptosis is beginning to be elucidated (see first report of the positive effect of HSP27,70, Samali and Cotter [23]). HSP interfere with extrinsic (receptor-mediated) and intrinsic (mitochondria-mediated) signaling pathways that lead to proteolytic activation of caspases, the main effectors of apoptosis. HSP27 is a small HSP that can act as a molecular chaperone, regulate actin cytoskeleton organization, and modulate redox parameters. This small HSP seems to exert protection against apoptosis at multiple levels. It has been reported recently that HSP27 can regu-

late the death receptor-mediated pathway, but the exact mechanism remains unknown (24). An increasing number of studies have provided a better description of HSP27 roles in the intrinsic pathway. HSP27 does not inhibit the release of cytochrome *c* from mitochondria but binds the released cytochrome *c* and, thus, prevents the activation of procaspase-9. In addition, HSP27 can act further downstream by blocking the procaspase-3 autoactivation (25). Activation of procaspase-3 involves a multiple-step process, and the other small HSP, α B-crystallin (HSPB1), inhibits this cascade one step downstream of HSP27 (26).

HSP70, the main inducible HSP, seems to be able to inhibit cell death by several mechanisms upstream and downstream of caspase inactivation. In the human acute lymphoblastic leukemia T-cell PEER line, Mosser et al. (27) reported that HSP70 inhibits apoptosis by preventing mitochondrial cytochrome *c* release and activation of procaspases into caspases. In addition, the same study and others show that HSP70 also acts downstream of cytochrome *c* release and upstream of the activation of caspase-3 (see references in Reference 22). In addition, HSP70 might be involved in preventing a proposed caspase-independent cell death by suppressing c-Jun N-terminal kinase activity (28). Both HSP70 and/or HSP90 can bind Apaf-1, a component of apoptosome, thereby inhibiting its formation and the activation of caspase-9 (22).

Molecular Chaperones Confer Organ and Cell Protection

Protection by HSP in Heart and Brain Ischemia. The well-characterized biochemical and metabolic events that accompany ischemic injury are likely to coincide with alterations in the three-dimensional structure and tertiary conformation of numerous proteins (e.g., actin) (14). Because molecular chaperones facilitate repair of misfolded proteins caused by errant either unfolding or misfolding, the hypothesis that ischemia-induced protein denaturation and, ultimately, organ dysfunction could be ameliorated by chaperones has been tested recently. Forced overexpression of the major stress protein, HSP70, in transgenic mice directly protects against myocardial ischemic damage, improves metabolic (i.e., high-energy stores) recovery, enhances functional recovery, and reduces infarct size in the ischemic heart

(30–33). Other members of the HSP multigene family, such as HSPB1, impart similar cytoprotective effects when expressed in cultured cells and when the intact heart is exposed to simulated and ischemic conditions, respectively (34, 34a).

As with the ischemic heart, intensive efforts were focused on the potential neuroprotective benefits that HSP expression might exert during cerebrovascular accidents and strokes (35, 36). Both excitatory (e.g., glutamate) and neurotoxic stimuli (ischemia, epilepsy, trauma, hyperthermia) are well-characterized inducers of HSP expression, such as HSP70 in CA3 pyramidal neurons and dentate granule cells (37). Given the anatomical distribution and diversity of cell types, it is not surprising that more complex interactions and relationships exist in neuronal cells and regions during ischemia and reperfusion. In the rat model of ischemic stroke, for example, maneuvers using adenoviral-mediated gene transfer for major HSP70 overexpression reduces cerebral infarction (38). In isolated astrocytes from HSP70 transgenic mice, the neuroprotection exerted by HSP70 against oxidative stress induced by hydrogen peroxide and, in lesser amounts, hypoglycemic exposure appears to be independent of glutathione levels (39). Hippocampal neurons with high-level expression by transgene HSP70 exhibit increased resistance, but cortical cells do not; however, infarction size was not significantly reduced by transgene HSP70 overexpression 24 hrs after permanent occlusion (39). Likewise, Plumier et al. (40) reported that HSP70 transgenic mice exhibit the same size of infarct area after occlusion of middle cerebral injury, whereas cellular morphology in hippocampus seemed to be protected, indicating a cell-specific beneficial effect of HSP70 expression. Consistent with this notion, Kelly and co-workers (41) demonstrated recently that human HSP70I under the control of the Lmo-1 promoter, which drives high-level expression in postnatal hippocampal neurons but not in glial cells, reduced cerebral infarction by ~50% in the lateral caudate nucleus and posterior thalamus of transgenic ($21 \pm 9.3\%$) compared with nontransgenic mice ($12.5 \pm 9\%$) after bilateral carotid artery occlusion.

Proof of the concept that elevated levels of specific HSP could influence molecular pathways that limit proteotoxic damage and, perhaps, accelerate protein

degradation has been an important milestone for the field of cardio- and neuroprotection, especially as functional recovery could be demonstrated *in vivo*. The mechanisms by which HSP70 and HSPB1, both of which reside in cytosolic compartments, exert their observed beneficial effects remain incompletely understood. Could either tissue-selective or compartment-specific properties of other chaperones and chaperonins (beyond the aforementioned ones) harness more robust cytoprotective properties alone or in combination during pathologic conditions simulating heart and stroke attacks in humans? Answers to such questions must await current and future investigations, but they hold substantial promise for novel strategies to selectively increase cytoprotective molecules, which mitigate ischemic injury, in either susceptible or discrete cellular subpopulations.

Role of the Heat Shock Regulatory Pathway in Septic Shock. Sepsis is a complex syndrome caused by overwhelming release of bacterial endotoxin of Gram-negative organisms in susceptible hosts. In infants and immunocompromised populations, septicemia carries an almost 50% mortality, for which effective therapies have remained elusive until recently. Toxicity from lipopolysaccharide (LPS), the outer coat protein in endotoxin, requires the presence of specific cell-surface receptors that determine susceptibility of the host to the offending reagent. LPS can bind various receptors expressed by monocytes/macrophages and neutrophils activating the release of proinflammatory cytokines, such as TNF- α , a major hallmark of septic shock, which mediates cellular destruction and depression of cardiac function. Animal models that mimic endotoxin shock have proven useful for studies into the mechanisms of sepsis (42).

Prior studies have demonstrated that pretreatments with heat stress and the induction of multiple HSP correlate with improved survival in several rodent models exposed to endotoxin challenge (43–45). Not surprisingly, endotoxemia has been proposed to stimulate the stress response under the control of HSF as a major protective mechanisms (46). The question remains whether the HSF1-dependent up-regulation of HSP can still have a protective effect amenable to therapeutic intervention.

To test the hypothesis that *Hsf1* inactivation, which abrogates the heat shock response *in vivo*, reduces stress tolerance

during inflammatory systemic challenge, we administered LPS intraperitoneally and monitored survival rates in wild-type and mutant mice for 96 hrs. Whereas the survival of wild-type controls and heterozygotes was 63.2% and 60%, respectively, the survival rate of C,129-*Hsf1*^{-/-} mice was significantly lower (35%) after 4 days, indicating that HSF1 expression plays a key survival role during pathologic challenges, mimicking a lethal inflammatory stimuli in mammals, including humans. This reduced survival of *Hsf1*^{-/-} animals, compared with survival of heterozygous controls, correlated mainly with increased production of the proinflammatory cytokine TNF- α but not with stress-inducible HSP expression (47). It was previously shown that HSF1 activity can suppress prointerleukin-1 β gene production in human monocytes in response to *in vitro* LPS stimulation (48). Such evidence for significantly greater TNF- α production in *Hsf1*^{-/-} animals was the first to directly support the role of HSF1 *in vivo* as a negative regulator. This was confirmed by additional investigation of transcriptional regulation of TNF- α by HSF1 (49).

Downstream steps in the sepsis signaling pathway include the activation of the proinflammatory transcription factor, nuclear factor- κ B, by degradation of I κ B. Because heat shock prevents such an activation by inhibiting I κ B degradation, Shanley et al. hypothesized that HSF1 can be involved in this negative control of the inflammatory pathway. By using HSF1-deficient fibroblasts exposed to TNF- α , however, they did not find any difference in I κ B degradation, indicating that HSF1 is acting mainly upstream and downstream of the TNF- α signaling pathway (50). Notwithstanding, suppression of proinflammatory cytokines, such as TNF- α , by HSF1 establishes a *bona fide* compensatory endogenous mechanism for protection during pathologic challenges in mammals, albeit the nature of the mechanisms remains poorly defined.

Role of the Heat Shock Regulatory Pathway in Mammalian Pregnancy. During mammalian development, formation of the placenta is essential for proper nutrient exchange, and any severe defect in this transient organ has potential life-threatening consequences to fetus and mother alike. Placenta growth is partly dependent on inflammatory and hypoxic signals and requires the heat shock regulatory pathway. That stress response genes and, in particular, HSP that are

expressed in human and rodent placental cells (47, 50a) might play a direct physiologic role has been revealed elegantly by gene-targeting studies of the DnaJ-related chaperone, Mrj, and the major cytosolic/nuclear stress protein, HSP90 β , in mice (50b, 51). Regarding clinical investigation, the relationship between pregnancy outcome and expression of the HSP or HSP-antibody complexes in placental tissue was evaluated in normal and preterm birth pregnancies. HSP60- and HSP70-antibody complexes have been identified significantly more often in cases of preterm births; thus, women sensitized for these antibodies might be at increased risk for pathologic pregnancies (52).

BIOLOGY OF HSF FAMILY

The transcription factor HSF1, which was first identified in cellular extracts from *Saccharomyces cerevisiae* and *Drosophila melanogaster*, oligomerizes on activation and binds to heat shock element (HSE) sequences to control stress-inducible expression of HSP genes. Whereas a single HSF exists in yeast and fly, to date three HSF (HSF1, HSF2, and HSF4) have been identified in mammals. HSF3, the fourth member of the HSF family, has been described in chicks but not mammals (53). Differences in splicing variants, posttranslational modifications, and oligomerization enable additional levels of regulation and potential specialized functions for HSF in higher eukaryotes. Pirkkala and co-workers provided a detailed description of the structural properties and presented the species-specific relationship within the HSF family (reviewed in Reference 54).

In mice, HSF1 is ubiquitously expressed but is selectively abundant in ovary, placenta, heart, and fetal brain (55). Unlike HSF1, HSF2 is much less abundant in postnatal tissues, where it can be found mainly in brain and testes (56, 57). In mouse embryos, Eriksson et al. (58) showed early and robust HSF2 expression in heart at 11.5–12.5 days. Separately, Rallu and co-workers (59) demonstrated HSF2 expression until 15.5 days postcoitus; thereafter, HSF2 declines in the perinatal period, becoming more restricted to some regions of the nervous system by birth (59, 60). Taken together, these descriptive studies are important to support the hypothesis of key developmental roles for HSF2, albeit such propositions await further clarifica-

tion from gene-targeting studies currently in progress. The third mammalian paralog, HSF4, is detected in many organs at the messenger RNA level but only in brain and lung at the protein level (61), suggesting that HSF4 expression is regulated by a yet unknown posttranscriptional mechanisms. As with HSF2, the physiologic roles of HSF4 await further characterization in the mammalian genome. Recent studies in transfected lung cells suggest that HSF4 might be a negative regulator for HSF1 (63), although these intriguing findings await confirmation in the intact organism.

HSF1 FUNCTIONS IN MAMMALIAN ORGANISMS

Because most mammalian cells express three HSF, we hypothesize that they should serve unique and complementary physiologic roles. This review focuses on HSF1 because, unlike HSF2 and HSF4, it has substantial information available from recent gene-targeting studies in intact animals. As a positive regulator for stress-induced transcription of heat shock genes, HSF1 is well established among the factors involved in cell protection. This does not preclude other potential targets and other critical functions that HSF1 might exert in the intact organism. Two lines of investigation have provided conclusive and sometimes unexpected answers to this question.

In mice, induced genetic mutations have provided unprecedented challenges and opportunities for biologists to characterize the physiologic roles of specific genes. Such has been the case of *Hsf1* knockout mice, as described originally by our laboratory, that supports hitherto unknown and major new functions for the HSF1 regulator, especially under nonstressed and physiologic conditions (47, 65).

Disruption of HSF1 expectedly eliminates the “classic” heat shock response, abolishes acquired thermotolerance *in vitro*, and increases the susceptibility of HSF1-deficient cells to heat-induced apoptosis (66). Surprisingly, *Hsf1*^{-/-} mice exhibit a complex phenotype, including a partial fetal lethality attributable to placental insufficiency. This is manifested, in part, by reduction of the spongiotrophoblast layer and, thereafter, the disruption of the labyrinth, the major functional layer of the placenta. In addition, survival and fetal growth of *Hsf1*^{-/-} concepti show a variable penetrance and ex-

pressivity depending on the genetic background (47, 66), suggesting that genetic modifiers influence the HSF1 pathway and that other genes or pathways should be interacting with HSF1 itself or with HSF1-like functions. Because *Hsf1*^{-/-} fetuses are grossly normal, HSF1 requirements appear to be restricted to functional extra-embryonic tissues and support the notion that growth retardation develops secondarily to the placental defect.

In adults, we observed that *Hsf1*^{-/-} males are fertile, whereas *Hsf1* null females are sterile. Detailed characterization of reproductive failure in *Hsf1*^{-/-} females has implicated an essential requirement for HSF1 expression for the embryo to develop beyond the first cell cycle after fertilization. It appears that the abundant stores of HSF1, located in the nucleus of immature oocytes, function as an important maternal factor that has an essential role during the first cleavages and, thus, specifies the progression of embryonic development. Female infertility of *Hsf1* knockout mice is actually one of the rare maternal effect mutations described in mammals (65). These data unambiguously establish that HSF1 exerts critical functions that correlate with *in vivo* expression patterns at normal physiologic temperatures and that, importantly, are distinct from the classic heat shock response in mammals.

Beyond the major development requirements, the *Hsf1* knockout model is an invaluable experimental tool to dissect potential pathophysiologic functions of HSF1 activity in mammals. Although *Hsf1*^{-/-} animals have a normal life span, we previously discussed the key requirement for HSF1 activity during endotoxemic challenge and in modulating production of the proinflammatory cytokine TNF- α (47). Using the *Hsf1* mutant should clarify the suspected roles that inducible members of the HSP family play in preconditioning and the early and late events after acute myocardial infarction. By taking advantage of this model, we also anticipate that studies in progress will bring new insight in inheritable forms of cardiomyopathy caused by genetic mutations, most commonly found in sarcomeric structural genes but which on rare occasions occur in either intermediate filament proteins (e.g., desmin) (68) or the small MWHSP, HSPB1 (69).

Others have approached the functions of HSF1 using a gain-of-function strategy to overexpress a constitutively activated

HSF1 isoform *in vivo*. Under the control of the ubiquitously expressed human β -actin promoter, transgenic mice harboring a truncated form of human HSF1 exhibited phenotypical changes only in the heart and testis. Dramatic effects on testicular function appear secondarily to blocking spermatogenesis at the pachytene stage, leading to male infertility. On the other hand, overexpression of activated HSF1 appears to induce cardiac hypertrophy by poorly understood mechanisms. Therefore, the unregulated increase of HSF1 activity is deleterious for specific cellular populations, in part, because of imbalances between proapoptotic and apoptotic mechanisms involving HSP and other genes (70).

Now, compelling evidence has been obtained for HSF1 activity in the regulation of TNF- α both *in vitro* and *in vivo*. Other candidate genes are potential targets, including prointerleukin-1 β , the copper/zinc superoxide dismutase (SOD1), and the multidrug resistance 1 (MDR1)/P-glycoprotein, all of which contain HSE in their promoters for HSE-DNA binding activity (72, 74). The complete compendium of the genes controlled by HSF1 should soon be possible using existing and more refined strategies in genomics, which is predicted to identify non-*hsp* genes as transcriptional targets.

HSF1 MECHANISMS OF REGULATION

A deeper understanding about the regulatory mechanisms of HSF1 activity is widely believed to herald novel possibilities for therapeutic benefits (Fig. 2). Stressful conditions induce the transformation of HSF1 monomers into homotrimers, which migrate into nucleus and bind to specific HSE (i.e., inverted repeats of nGAAn) located within promoter regions of target genes. A formidable task has been to decipher the precise molecular signals that trigger HSF1 activation and regulation of *hsp* genes, under both stress and nonstress conditions. Stress-induced protein damage and abnormal structures have been proposed as a proximal signal triggering the heat shock response in cells (75). Microinjection of abnormal proteins activates the heat shock response, strongly supporting the hypothesis that conformational abnormalities in proteins are stress signals and that chaperone functions serve to protect the

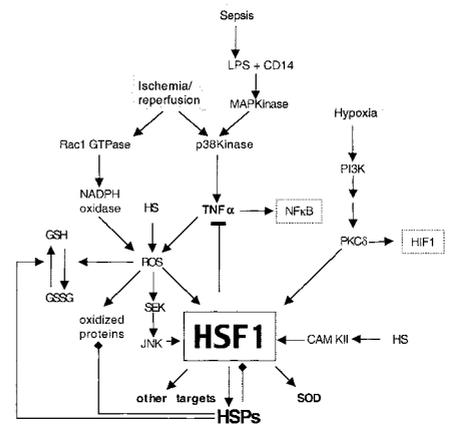


Figure 2. Scheme of heat shock factors (*HSF*) regulatory pathways. HSF1 activity intersects multiple pathways through both cellular- and stress-specific signals. Ischemia/reperfusion stimulates Rac1 GTPase, the proximate stress signal in reduced nicotinamide adenine dinucleotide phosphate (*NADPH*) oxidase and reactive oxygen species (*ROS*) production. Alternatively, lipopolysaccharide (*LPS*)-induced activation of CD14 promotes MAPK-dependent stimulation of the p38 kinase pathway. Up-regulation of tumor necrosis factor (*TNF*)- α by p38 KINASE activation, ischemia-induced nuclear factor (*NF*)- κ B stimulation, and activation of *NADPH* oxidase are additional pathways for ROS production. Emerging evidence suggests that increased ROS generation reduces GSH pool, causes oxidative stress/damage, and serves as a key stress signal, which modulates multiple intracellular processes, including posttranslational modification, and transcriptional activity of HSF1. Stress-induced transactivation and DNA-HSE binding activity of HSF1 requires monomer-to-homotrimer conversion, a property associated with phosphorylation by CAMKII and JNK signaling pathways. Heat shock protein (*HSP*) is the best-known target of HSF1 transcriptional activity, but several studies show that HSF1 controls other genes in a positive or negative way (SOD, TNF- α). HSP (e.g., HSP25/27) are proposed to modulate glutathione levels, thus enabling a testable hypothesis to determine whether redox signaling plays a role in HSF1 activity *in vivo*. In brief, cellular integration of stressful signals depends on co-activation of several transcriptional factors, such as HSF1 and the TNF- α -dependent activation of NF- κ B or the PKC α -dependent activation of hypoxia-inhibitory factor HIF1 α/β .

polypeptides against irreversible or potentially toxic effects to the cell (15, 19).

Evidence for physiologic roles for HSF1 under nonstressed conditions has increased the stakes to identify a physiologic stress signal capable of HSF1 activation, which is likely to be distinct from the classic heat shock response. One hypothesis is that the ROS, the byproducts of normal mitochondrial oxidative phos-

It is conceivable, in regard to the development of small molecules inducing heat shock protein expression, that we are at the threshold in a promising field that spearheads the revolution in chemical biology for widespread applications in reducing morbidity and improving human health.

phorylation, serve as a molecular switch in transcriptional regulation of *hsp* gene expression. ROS are best known for their deleterious effects on macromolecules, including lipids, DNA, and proteins. For example, oxidized proteins usually exhibit an increased carbonylation (a biomarker of protein damage), a decreased enzymatic activity, and changes in thermal stability (77). In addition, many HSF1 inducers have been shown to increase ROS production and/or reduce glutathione levels (e.g., see References 78–80). ROS are common mediators in the pathogenesis of acute myocardial infarction (81), sepsis, and preeclampsia, all of which might engage HSF1 activity.

Because HSF1 activity can restore cellular homeostasis under stressful conditions, a fundamental question remains about the nature of the signals and mechanisms that govern HSF1 activity under normal temperatures and physiologic conditions. Specifically, does oxidation of thiol-containing molecules, such as glutathione or thioredoxin, regulate HSF1 activity in either a direct or indirect way? Treatment with oxidants like hydrogen peroxide induces HSF1 activity (79, 82), in part, through mechanisms that directly modify the structure of HSF1 protein (83, 84). A more complex process is envisioned, however, for signal transduction pathways (e.g., GSK3) that mediate phosphorylation of HSF1 and intermolecular interactions (e.g., HSP90) that maintain HSF1 in a monomeric state to prevent HSE-DNA binding activity.

CURRENT IMPLICATIONS FOR HSF1 AS A THERAPEUTIC TARGET

The potential for therapeutic targeting of HSF1 activity using small molecules remains a promising yet elusive goal. Numerous studies have now explored different pharmacologic agents to assess their effects on HSP expression and/or HSF regulation. Among such agents that stimulate HSF activity is the glucocorticoid agonist and anti-inflammatory agent dexamethasone. At therapeutic concentrations, dexamethasone induces HSF1 activation and HSP expression in isolated adult rat cardiomyocytes and correspondingly increases their resistance to hypoxia (85). Paradoxically, activation of the glucocorticoid receptor can suppress HSF1 activity by an unknown mechanism, suggesting that dexamethasone can modulate the heat shock response as part of its anti-inflammatory actions.

Of interest, other anti-inflammatory drugs, such as salicylate, induce the HSE-DNA binding activity of HSF1 but not the transcriptional up-regulation of *hsp* genes (86). Furthermore, low doses of arachidonic acid administered extracellularly in cells reduce the temperature threshold of HSF1-dependent gene transcription, suggesting that the limited potency of salicylate effects can be augmented during inflammation (87). The molecular targets of salicylate actions are presently unknown, and to date, the *in vitro* effects on stimulating HSF1 activity have not been confirmed *in vivo* in the case of the ischemic heart (88). Because the molecular targets of salicylate actions are presently unknown, for example, on platelets and myocardial cells *per se*, the case of ischemic isolated heart is presently.

Geranylgeranylacetone, a widely used antinuclear drug in Japan, induces HSP70 expression in gastric mucosa cells. In addition, several studies have shown that geranylgeranylacetone administration protects against liver ischemia, suggesting its therapeutic value for pathologic conditions affecting susceptible organs, such as the heart and brain (89, 90).

More recently, Bimoclochol, a hydroxylamine derivative, has been shown to induce the production of HSP70 in HeLa cells as well as myogenic cells and to exert potent cytoprotective effects during tissue injury (91). The correlation between the positive effects of Bimoclochol

and HSP induction activity has been demonstrated in several recent studies (see references in Jednakovits et al. [92]). However, the direct targets of Bimoclochol remain unclear, and Jednakovits et al. suggest that compartment translocation of preformed HSP might be involved in the acute cardioprotective actions. Future efforts should clarify whether the cytoprotective mechanisms of this drug and similar small molecules require the HSF1 regulatory network.

Last, Emiliusen et al. (93) developed a novel delivery system using modules of the HSF signaling pathway to target the transcription of cytotoxic genes. Thanks to the well-characterized HSE and a weak but highly tissue-specific element (Tyr300) in a targeting vector, low-level HSF activity in combination with the Tyr300 element was sufficient to promote the cytotoxic genes for drug delivery. Furthermore, induction of HSP70 expression serves as a negative feedback mechanism that modulates the exogenous vector, suggesting this system will require repeated dosing.

CONCLUSIONS AND PERSPECTIVES

In the ensuing years, the widespread interest in HSP as stress-response mediators, which are induced in many acute pathologic conditions, is likely to be fortified by insights regarding their roles in prevention, diagnosis, and therapeutic applications. Current formidable barriers, which confront clinicians and cell biologists alike, will likely dissipate with greater knowledge of biological pathways and with insights into underlying mechanisms. Considerable enthusiasm exists to integrate cytoprotective molecular pathways, such as HSP/HSF partners, particularly in the field mediating cell death and survival in complex biological organisms. It is conceivable, in regard to the development of small molecules inducing HSP expression, that we are at the threshold in a promising field that spearheads the revolution in chemical biology for widespread applications in reducing morbidity and improving human health.

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