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highlighted topics

Molecular Biology of Thermoregulation
Invited Review:
Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance

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Kregel, Kevin C. Invited Review: Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *J Appl Physiol* 92: 2177–2186, 2002; 10.1152/japplphysiol.01267.2001.—Cells from virtually all organisms respond to a variety of stresses by the rapid synthesis of a highly conserved set of polypeptides termed heat shock proteins (HSPs). The precise functions of HSPs are unknown, but there is considerable evidence that these stress proteins are essential for survival at both normal and elevated temperatures. HSPs also appear to play a critical role in the development of thermotolerance and protection from cellular damage associated with stresses such as ischemia, cytokines, and energy depletion. These observations suggest that HSPs play an important role in both normal cellular homeostasis and the stress response. This mini-review examines recent evidence and hypotheses suggesting that the HSPs may be important modifying factors in cellular responses to a variety of physiologically relevant conditions such as hyperthermia, exercise, oxidative stress, metabolic challenge, and aging.

stress protein; HSP70; heat stress; exercise; aging; gene expression; molecular chaperone

THERE IS WIDESPREAD INTEREST in the cellular mechanisms utilized by an organism to cope with a disruption in homeostasis. Current research is focused at several levels, ranging from basic molecular biology approaches to therapeutic applications. One reason for this interest, and the complexity associated with the topic, is evidence demonstrating that mammalian species have developed many different ways to deal with stress. Examples at the cellular level include temporary modifications in gene expression to survive changing environments, as well as altering cellular structure and function to deal with more permanent adverse conditions.

One of the "hottest" areas of current research involves a family of highly conserved stress proteins known as heat shock proteins (HSPs). These proteins are ubiquitous, occurring in all organisms from bacteria and yeast to humans. HSPs come in various forms and are categorized into families on the basis of their molecular weights (Table 1). There is substantial evidence that HSPs play important physiological roles in normal conditions and situations involving both systemic and cellular stress. HSPs were first discovered in 1962 (82) and described as a set of proteins whose expression was induced by heat shock and a variety of other stresses. Researchers have subsequently demonstrated that most HSPs have strong cytoprotective effects, are involved in many regulatory pathways, and behave as molecular chaperones for other cellular proteins (41, 63, 73, 106, 107).

Many functional roles for HSPs are known, but the mechanisms for these multiple functions are not entirely understood. It has been postulated that the determination of these mechanisms would permit the design of more precise ways to combat cellular stress in

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Table 1. *Cellular locations and proposed functions of mammalian heat shock protein families*

HSP Family	Cellular Location	Proposed Function
HSP27 (sHSP)	Cytosol, nucleus	Microfilament stabilization, antiapoptotic
HSP60	Mitochondria	Refolds proteins and prevents aggregation of denatured proteins, proapoptotic
HSP70 family:		Antiapoptotic
HSP72 (Hsp70)	Cytosol, nucleus	Protein folding, cytoprotection
HSP73 (Hsc70)	Cytosol, nucleus	Molecular chaperones
HSP75 (mHSP70)	Mitochondria	Molecular chaperones
HSP78 (GRP78)	ER	Cytoprotection, molecular chaperones
HSP90	Cytosol, ER, nucleus	Regulation of steroid hormone receptors, protein translocation
HSP110/104	Cytosol	Protein folding

HSP, heat shock protein; sHSP, small HSP; ER, endoplasmic reticulum.

a variety of clinically relevant settings (e.g., immunologic diseases, cancer, cardiovascular diseases, aging) (6, 29, 37, 47, 72, 96). HSPs also offer the potential to be used as markers of cellular injury and for diagnostic and therapeutic purposes.

The intent of this mini-review is to summarize what is known about the various physiological factors that modulate HSP responses to stressors at cellular and systemic levels as well as to highlight studies suggesting that HSPs play a critical role in the development of thermotolerance and protection from stress-induced cellular damage. Because of space constraints, this mini-review will focus on recent evidence that HSPs may be important modifying factors in an organism's response to a variety of physiologically relevant conditions, such as exercise, hyperthermia, oxidative stress, metabolic challenge, and aging. Although a substantial amount of our understanding regarding the role of HSPs has come from in vitro studies, there is also sufficient evidence that induction of HSPs occurs in vivo in response to a wide variety of stresses.

HEAT SHOCK PROTEIN FAMILIES

Introduction. The HSPs have been extensively studied, especially with regard to their cellular localization, regulation, and functions (6, 41, 63, 71, 106). HSPs are present in both prokaryotic and eukaryotic cells, and their high level of conservation suggests that they play an important role in fundamental cell processes. HSPs were initially discovered in *Drosophila melanogaster* larvae that were exposed to "heat shock" (82), and subsequent studies (97, 98, 105) identified several subsets of these proteins in the 70-kDa range. Over the past 30 years, a large number of additional proteins have been discovered within this family, and these are collectively referred to as "HSPs" (Table 1).

The principal HSPs range in molecular mass from ~15 to 110 kDa and are divided into groups based on both size and function (42, 87, 106). They are present in the cytosol, mitochondria, endoplasmic reticulum, and nucleus, although these locations vary depending on the particular protein. The most well-studied and understood HSPs in mammals are those with molecular masses of ~60, 70, 90, and 110 kDa. These HSPs are expressed at euthermic body temperatures (~37°C) and in conditions of stress (e.g., heat shock) and have distinct locations and functional properties (Table 1). Small-molecular-mass proteins, also termed small HSPs, exhibit tissue-specific expression and include heme oxygenase, Hsp32, Hsp27, αB-crystallin, and Hsp20 chaperone.

The HSP70 family. The primary focus of this mini-review will be on the ubiquitous HSP70 family of proteins, which are the most temperature sensitive and highly conserved of the HSPs. The HSP70s are ATP-binding proteins and demonstrate a 60–80% base identity among eukaryotic cells (5, 18, 62). There are at least four distinct proteins in the HSP70 group (HSP72, HSP73, HSP75, and HSP78), and all of these proteins have several acronyms that can be redundant and confusing.

Proteins in the HSP70 group share common protein sequences but are synthesized in response to different stimuli (Fig. 1). For example, the 73-kDa protein (HSP73 or Hsc70) is constantly produced (hence, the term "constitutive"), whereas the 72-kDa protein (HSP72 or Hsp70) is highly inducible and its synthesis is increased in response to multiple stressors. The molecular structure of the HSP70 group of proteins and descriptions of HSP70 gene regulation will only be briefly covered in this mini-review, as there are several detailed reviews available on these topics (18, 42, 47, 63, 71, 72, 87, 106).

The gene for Hsp70 is a 2,440-base pair gene containing a 212-base pair leader sequence and a 242-base pair downstream or 3′-untranslated region (109). There are at least two regulatory elements in the 5′-region that interact with heat shock transcription factors (HSFs). These HSFs bind to the promoter element during stress and are sufficient to induce Hsp70 transcription. In addition to hyperthermia, a number of stimuli are known to induce Hsp70 transcription, including energy depletion, hypoxia, acidosis, ischemia-reperfusion, reactive oxygen species (ROS), reactive nitrogen species such as nitric oxide, and viral infection (Fig. 1).

An important consideration regarding Hsp70 regulation involves the apparent discordance between transcription of message and Hsp70 translation. There is evidence suggesting that transcriptional activation of the Hsp70 gene is independent of protein synthesis. For instance, in cell culture experiments, Hsp70 mRNA can increase in response to a challenge, although there is little Hsp70 protein produced (11, 40).

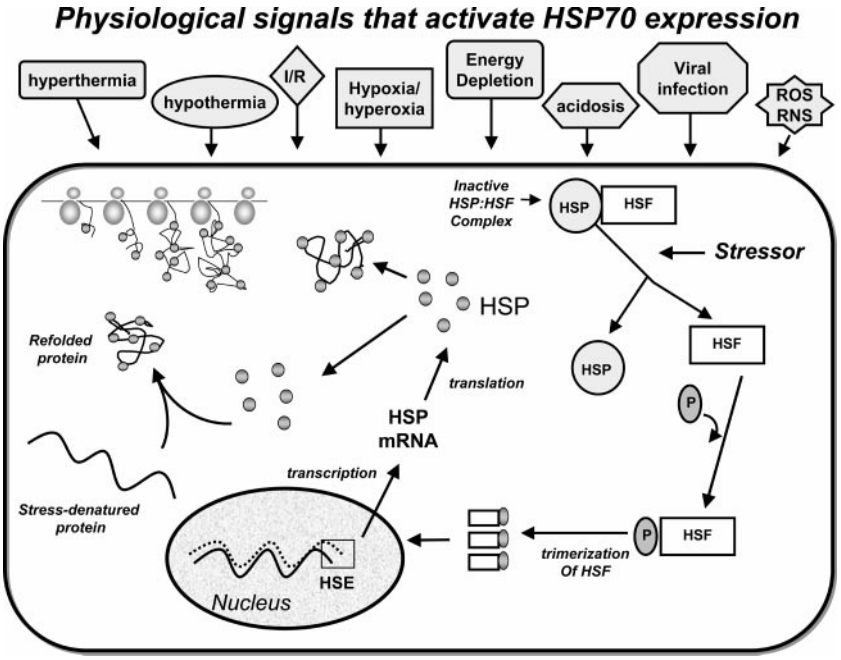

Fig. 1. A summary of some of the major physiological signals that activate the inducible form of the 72-kDa heat shock protein (Hsp70) synthesis (*top*) and a proposed mechanism for increased Hsp70 expression within a cell. Heat shock factors (HSFs), present in the cytosol, are bound by heat shock proteins (HSPs) and maintained in an inactive state. A broad array of physiological stimuli ("stressors") are thought to activate HSFs, causing them to separate from HSPs. HSFs are phosphorylated (P) by protein kinases and form trimers in the cytosol. These HSF trimer complexes enter the nucleus and bind to heat shock elements (HSE) in the promoter region of the Hsp70 gene. Hsp70 mRNA is then transcribed and leaves the nucleus for the cytosol, where new Hsp70 is synthesized. Proposed mechanisms of cellular protection for HSPs include their functioning as molecular chaperones to assist in the assembly and translocation of newly synthesized proteins within the cell and the repair and refolding of damaged (e.g., stress-denatured) proteins. I/R, ischemia-reperfusion; ROS, reactive oxygen species; RNS, reactive nitrogen species.

There are also data demonstrating that both transcriptional and posttranscriptional regulatory steps are required for HSP production (70, 79, 109). Studies of oxidant stress suggest that HSP promoter activity and protein accumulation may be uncoupled (109). Moreover, Bruce et al. (11) demonstrated increased heat shock promoter activation through HSF binding but no Hsp70 message or protein accumulation after exposure of cells to hydrogen peroxide. Mosely et al. (74) investigated the role of posttranscriptional regulation of the human HSP70 gene through its 3′-untranslated region and noted that this region itself is heat responsive. Taken together, these data argue that differences in Hsp70 protein production following cellular changes such as heat or oxidative stress are related to events distal to promoter activation and may include important posttranscriptional regulatory mechanisms that will need to be addressed in future studies.

FUNCTIONAL ROLES OF HSPs

The precise functions of proteins in the HSP70 family have not been completely delineated. However, the high degree of conservation of these proteins across species, coupled with their importance in cell survival in various conditions, suggests that these HSPs are critical for both normal cellular function and survival after a stress. Therefore, one of the primary means to gain insight into HSP70 function in both in vitro and in vivo systems has been to assess their cellular responses after a stress-related induction.

Thermotolerance. One of the first physiological functions associated with the stress-induced accumulation of the inducible Hsp70 was acquired thermotolerance, which is defined as the ability of a cell or organism to become resistant to heat stress after a prior sublethal heat exposure (54, 55, 60, 61, 70, 73). Data from subsequent studies demonstrated that the induction of Hsp70 was associated with the development of tolerance to a variety of stresses, including hypoxia (30, 31), ischemia (69), acidosis (104), energy depletion (88), cytokines such as tumor necrosis factor-α (TNF-α) (45), and ultraviolet radiation (4). The phenomenon of acquired thermotolerance is transient in nature and depends primarily on the severity of the initial heat stress. In general, the greater the initial heat dose, the greater the magnitude and duration of thermotolerance. The expression of thermotolerance following heating will occur within several hours and last 3–5 days in duration. Additional supporting evidence includes observations that have linked the kinetics of thermotolerance induction and decay with parallel changes in HSP70 induction and degradation (54, 59). However, these studies have generally been correlative in nature, with no causal link established between induction of HSP70 and acquired thermotolerance.

The similar kinetics of thermotolerance demonstrated by cells, tissues, and animals suggest that the morbidity and mortality associated with whole body heating is due in part to the dysfunction of some critical target tissues (35, 37, 73, 100, 108). It can be postulated that the development of thermotolerance results from the improved tolerance of the weakest organ and cell systems. Presumably, these tissues are both heat sensitive and vital to the animal. For instance, the small intestine is capable of generating thermotolerance (43) and is also reported to be the tissue most sensitive to heat damage (38). Both the small intestine and whole animal are sensitive to in vivo temperatures ranging from 41°C to 42°C, whereas gastrointestinal disorders are frequently observed after whole body heating (42°C for 120 min) (101) and during heat stroke (90) in humans. In support of this

concept, Flanagan et al. (28) demonstrated that the gut and liver are the first organs to accumulate HSP70 following whole body hyperthermia.

Advances in molecular biology techniques have provided researchers with tools to address the issue of a causal link between HSP induction and thermotolerance more directly. Cellular manipulations that either block HSP70 accumulation or overexpress certain HSPs have been shown to either increase or decrease heat sensitivity (46, 56, 58, 81). For example, transfection of a plasmid containing the *Drosophila* HSP70 gene into a monkey fibroblast cell line produced large increases in HSP70 accumulation in these cells and improved tolerance to a heat shock paradigm (58). Elevations in cellular HSP27 levels via plasmid transfection have also yielded a state of thermotolerance without the need for a conditioning thermal stress (56). Conversely, microinjections of monoclonal antibodies specific for HSP70 inhibited the synthesis of these proteins, resulting in a reduction in thermotolerance (81).

As noted, HSPs appear to play a role in protecting cells from damage generated by a variety of stressors. Their synthesis is associated with protection against light-induced damage to the retina (4) and ischemia-reperfusion injury to the heart (19, 23, 44), liver (7, 12–14), and kidney (102). In addition, studies of cardiac shock followed by resuscitation have revealed that hepatocytes synthesize members of the HSP70 family early in the course of recovery (7, 12–14). The fact that HSP70 message is preferentially translated by a cell under stress to the exclusion of other messages may result in the inability of the cell to produce some proteins or respond to additional signals (70, 79). In this model, the cell may "choose" self-preservation over tissue preservation to the detriment of the organ. This model may be particularly relevant in a situation where HSP70 accumulation could be utilized as a biomarker of cellular injury (35). In this scenario, cells of tissues most at risk would also be the cells most likely to accumulate HSP70 during stress, and this HSP70 accumulation could mark a tissue for potential failure.

Although the precise mechanisms for the improvement in cellular thermotolerance in association with an increase in HSP levels have not been delineated, it is tenable to postulate that proteins in the HSP70 family are involved in preventing protein denaturation and/or processing denatured proteins and protein fragments that are produced by stressors such as hyperthermia. Supporting evidence for this scenario comes from a set of in vitro experiments by Mizzen and Welch (70), who demonstrated that heat stress results in translational arrest within a cell, and this arrest is proportional to both the intensity and duration of the applied heat stress. Subsequent resumption of translation resulted in HSP mRNA being translated into HSPs before the synthesis of other proteins took place within the cell. Interestingly, the period of translational arrest in response to heat stress could be shortened in these experiments if cells were first made thermotolerant.

One interpretation of these results is that a primary function of HSPs during cellular stress is to maintain translation and protein integrity. Cells that were made thermotolerant also produced less HSP during a second challenge compared with previously unheated cells, suggesting there is a regulation of HSP synthesis that is dependent on the levels of these proteins existing within the cell. Although a majority of data in this area has been derived from in vitro methodologies, a unique set of experiments in humans by Moseley and colleagues (84) generated data supportive of this concept. Healthy men performed a challenging exercise protocol in either hot (46°C) or more moderate (30°C) ambient conditions. Leukocytes obtained from subjects after the protocol were then incubated at 41°C. The increase in Hsp70 synthesis in heat-stressed leukocytes was inversely proportional to the length of the initial "conditioning" exercise stress, suggesting that cells regulate the amount of these stress proteins in response to repeated challenges.

An additional issue related to the development of thermotolerance deals with the possibility that HSPs, through their interaction with cellular proteins during translational arrest, play a role in preventing protein denaturation and processing denatured proteins that are generated in response to stressors such as heat. For example, data suggest that the injection of denatured proteins into cells or the generation of abnormal proteins can induce HSP activity (1, 38).

Although these different sets of data clearly demonstrate a broad range of physiological processes that involve the HSPs, the evidence that the HSPs are responsible for cellular thermotolerance is circumstantial rather than conclusive. The variety of stressors used to condition cells will likely induce other important cellular defense proteins in addition to HSPs, such as antioxidant enzymes (34, 35). It should also be noted that thermotolerance can be generated in the absence of HSPs. In these studies, thermotolerance was manifested under conditions of protein synthesis inhibition (i.e., no HSP accumulation) as well as a chronic exposure to a lower temperature than is required for HSP accumulation (57). Other studies have demonstrated that inhibition of transcription during the conditioning heat stress also allows the maintenance of thermotolerance (2). In addition, oxidative stresses, which can confer thermotolerance, may not increase the levels of HSPs (11, 103). In other stresses, such as ischemia, where HSPs are thought to play a role, HSP overexpression has also not been found to confer tolerance (91). Therefore, generating a scenario in which the development of stress tolerance in a cellular system is causally linked to an increase in Hsp70 expression is difficult because organisms and cells respond to stress in a variety of complex ways (49).

The mechanisms contributing to thermal injury vs. thermotolerance are even less clear in the intact organism. One obvious explanation for thermal injury at the cellular level is direct heat damage (53). However, this cellular damage is likely due in part to functional impairment of a tissue or organ (e.g., reductions in

blood flow) and the possible impact of systemic factors such as endotoxin-mediated cytokine production. Moreover, much of the research attempting to gain an understanding of the intact organism's adaptive response to heat has focused on heat acclimatization processes (73). Because the factors involved in heat injury at the whole organism level are complex and the mechanisms contributing to the protective role of HSPs are not well defined, issues such as these remain a central challenge in this field of research.

HSP70 functions associated with stress tolerance. Although the evidence linking stress-induced HSP70 accumulation with tolerance to heat and other stressors is compelling, the mechanisms by which HSPs confer stress tolerance are not well understood. Attention has primarily been focused on the role of HSP70 as a chaperone and its potential ability to contribute to cellular repair processes in response to interventions such as heat, oxidative stress, activation of proteases, release of lysosomal and proteolytic enzymes, and alterations of the cytoskeleton.

Several important cytoprotective functions have been attributed to HSPs and, in particular, the HSP70 family. These include *1*) the folding of proteins in various intracellular compartments, *2*) the maintenance of structural proteins, *3*) the refolding of misfolded proteins, *4*) translocation of proteins across membranes and into various cellular compartments, *5*) the prevention of protein aggregation, and *6*) the degradation of unstable proteins (3, 17, 21, 70, 78). Interestingly, it has also been noted that HSPs can play a role in apoptosis. HSP27, HSP70, and HSP90 proteins are predominantly antiapoptotic, whereas HSP60 is proapoptotic. Moreover, it appears that these HSPs function at multiple points in the apoptotic signaling pathway to elicit this response (29).

Although there are numerous studies available demonstrating the broad range of physiological processes that involve HSPs, including protein translocation, receptor regulation, cytoskeleton stabilization, and management of protein folding and repair, evidence directly demonstrating that the HSPs are responsible for stress tolerance is not conclusive. In addition, the complexity of the integrated response to a physiological challenge in vivo makes it difficult to ascertain what "stressor" is responsible for stimulating an increase in HSP synthesis. In a situation such as an aerobic exercise bout of moderate intensity and duration, additional signals besides an elevation in core temperature (T_c) are present that could potentially activate HSP expression, including acidosis, energy depletion, reductions in blood flow to visceral organs and an associated tissue hypoxia, and generation of ROS (20, 32, 33, 52, 94). Furthermore, in addition to HSPs, cells will express other important stress proteins such as antioxidant enzymes, providing an organism with multiple cytoprotective options.

It is also important to note that there are numerous studies demonstrating that thermotolerance can be generated in the absence of intracellular HSP accumulation. Therefore, it is problematic, especially at the whole organism level, to definitively link an increase in HSP70 expression directly to the acquisition of stress tolerance, partly because mammalian species respond to stress in a multitude of complex, integrated ways.

Immune surveillance and antigen presentation. Although the primary focus of research on HSPs has been directed toward their functions and accumulation inside the cell in response to a physiological stress, there is emerging recognition that HSPs serve as modulating signals for immune and inflammatory responses. This concept was recently detailed in a concise review by Moseley (72). One area of investigation pertinent to the topic of stress tolerance has dealt with the potential role of HSPs in cytokine production. Elevations in intracellular HSP levels have been shown to improve cell tolerance to inflammatory cytokines such as TNF-α and interleukin-1 (45, 75). HSP accumulation within a cell produces both transcriptional inhibition and a decrease in TNF-α and interleukin-1 secretion (24, 93). Kluger et al. (48) demonstrated that heat conditioning and the resultant increase in intracellular Hsp70 levels protected animals from an endotoxin dose that was lethal in unconditioned rats. Moreover, this paradigm was associated with a decrease in serum TNF-α levels after administration of endotoxin in the heat-conditioned animals (48). These results suggest that intracellular HSP accumulation may contribute to a reduction in inflammatory cytokine production with cellular challenge.

Conversely, when HSPs are present on the surface of cells or released into the local extracellular environment during conditions such as necrotic cell death or viral infection, these proteins have an immune-stimulating response. The situation involving cell necrosis is quite relevant to conditions of physiological challenge, such as heat stress, where widespread cellular injury and necrotic cell death have been noted (37).

Hsp70 is also known to facilitate antigen presentation in cells such as macrophages and dendrites (94, 99). When Hsp70 is applied to the environment external to cells, macrophages and lymphocytes produce inflammatory cytokines. Finally, studies have demonstrated the presence of Hsp70 on the surface of tumor cells (76, 83), potentially functioning as recognition molecules for natural killer (NK) cells. Together, these observations demonstrate that HSPs are important modulators of antigen presentation, T-lymphocyte activation, cytokine production, and NK cell killing, placing them in a unique position of contributing to both intracellular and extracellular responses to a physiological stress.

FACTORS AND CONDITIONS THAT MODULATE HSP70 EXPRESSION

Some stimuli and conditions that modulate HSP70 expression. It is well documented that multiple stimuli can induce the in vivo accumulation of HSP70 proteins. As pointed out in Fig. 1, these stimuli include hyperthermia (28, 50, 51, 92), ischemia-reperfusion (15, 69), hypoxia (22, 30, 44), energy depletion (88), acidosis

(104), and ROS formation (103). Because these stimuli are similar to the integrated metabolic changes associated with exercise, it is not surprising that exercise has been demonstrated to induce HSPs.

In one of the initial animal experiments to address the issue of HSP induction with exercise, Locke et al. (67) observed that a single bout of exhaustive treadmill running in rats could increase Hsp70 synthesis in skeletal muscle, lymphocytes, and spleen. Subsequent studies by numerous investigators have confirmed that acute exercise produces increased Hsp70 levels in contracting skeletal muscle as well as critical organs such as the heart, kidney, and liver (50, 66, 68, 85, 86, 92). It is difficult to delineate which of the many stimuli generated during acute exercise are contributing, and to what degree, to the observed increases in cellular Hsp70 accumulation, in part because exercise produces elevations in both T_c and tissue (e.g., contracting skeletal muscle) temperature concurrent with other physiological stimuli.

To investigate whether exercise could increase HSP70 expression independent of changes in T_c, Skidmore et al. (92) designed experiments in which rats were exercised in a cool environment to prevent T_c from increasing above resting levels. HSP70 accumulation was increased in hindlimb locomotor muscles and cardiac tissue after an exercise bout, suggesting that factors other than heat stress may contribute to the accumulation of HSP70 during acute exercise. Although it is likely that the local temperatures of locomotor muscles such as the gastrocnemius and soleus were elevated during treadmill running, data from previous studies indicate that the temperature of contracting skeletal muscles in rats is comparable to T_c at the termination of moderate intensity treadmill exercise (10). Therefore, it is reasonable to assume that stimuli other than increased temperature also contribute to the augmented skeletal and cardiac muscle HSP70 levels that accompany exercise.

Human studies evaluating Hsp70 responses to exercise are more infrequent and less insightful, likely due in part to the difficulties in sample acquisition and the challenging conditions for most experimental designs. These studies have focused primarily on stress protein expression in either skeletal muscle or circulating leukocytes. Two separate studies have demonstrated that a single bout of exercise in untrained subjects can elicit increased Hsp70 mRNA concentrations in vastus lateralis muscle (26, 80). Interestingly, Hsp70 protein levels were assessed 3 h after exercise in one of these studies and remained unchanged from control levels (80). In contrast, Liu et al. (65) found that expression of Hsp70 was increased in human vastus lateralis muscle after 1–4 wk of rowing training.

There are a few additional studies that have evaluated the Hsp70 response to exercise in peripheral blood leukocytes. Ryan et al. (84) found only small increases in Hsp70 expression in blood obtained from young men who performed 2 h of treadmill exercise in warm conditions, whereas Fehrenbach et al. (27) determined that heavy-intensity endurance exercise was associated with an increase in Hsp70 expression in leukocytes. A unique aspect of this study was the observation that the exercise-induced Hsp70 response was blunted in subjects who were aerobically trained compared with untrained controls. In a study with contrasting results (16), exercise and estrogen replacement in young women was found to have no effect on leukocyte Hsp70 levels several hours after a moderate-intensity exercise bout.

Overall, the limited number of studies available in humans does not permit a definitive conclusion to be drawn concerning the impact of exercise on Hsp70 responses. The differences in intensity and type of exercise protocol utilized, along with issues ranging from gender to training status, have likely contributed to the disparate findings in this area. Further research is necessary to address issues related to Hsp70 regulation in humans and to determine whether this regulation is consistent with results obtained in animals and in vitro cell systems.

Aging and altered Hsp70 expression. As noted above, cells, tissues, and whole organisms have the ability to become resistant to stressors such as hyperthermia after a prior sublethal heat exposure (i.e., acquired thermotolerance), and HSPs appear to play a critical role in this process. One important and clinically relevant scenario in which tolerance to thermal stress is reduced is old age. In humans, the aging process is associated with elevated morbidity and mortality rates due to stressors such as heat exposure. For instance, age-related decrements in the stress response are thought to be linked to the increased incidence of death that has been reported for older individuals subjected to prolonged heat waves (39, 89). In our laboratory, we have noted that older animals are less thermotolerant and have higher mortality rates than their younger counterparts when presented with repeated heat challenges (37).

There are several potential mechanisms that likely contribute to reduced stress tolerance with aging, including alterations in HSP70 accumulation and function. Investigators have been examining the stress protein response in aged organisms to determine whether a deficit in HSP70 is a potential explanation for the reduced thermotolerance in older populations (9, 25, 36, 64). In vitro studies have demonstrated that aged human (64) and rat (25) fibroblasts respond to heating with lower HSP70 mRNA and total protein levels. In vivo data, primarily from tissues extracted from intact animals (8, 37, 51, 77), suggest that the expression of HSP70 and its potential protective role in cellular stress may decline with advancing age. Moreover, studies taking advantage of cDNA expression array technology determined that aging results in altered gene expression in response to heat stress that is indicative of decreased stress protein expression (110).

A recent study by Hall et al. (37) addressed this issue in more detail by investigating whether a reduction in cellular ability to mount an appropriate stress protein

response in senescent animals following consecutive heating trials is associated with a decline in thermotolerance. Young and old Fischer 344 rats were heat stressed (T_c of 41°C) twice, separated by 24 h. Liver samples were obtained at several time points in the subsequent 48-h recovery period, and representative samples were then evaluated for Hsp70 expression. Several pieces of evidence from these studies suggested that there is a functional link between age-related decrements in Hsp70 expression and pathophysiological responses to heat stress. First, immunoblot and immunohistochemistry results demonstrated that the magnitude of the Hsp70 response was markedly reduced with age. In young animals, a robust Hsp70 response was present in the vicinity of the central vein, which is functionally associated with reduced blood flow and lower oxygen tensions. In contrast, senescent animals had relatively low Hsp70 expression in this region (Fig. 2). Second, older animals showed extensive liver injury in the central vein region that corresponded to the diminished Hsp70 response in these regions and a reduced ability to survive consecutive heat stresses. Conversely, liver injury was markedly lower in the young cohort. Finally, a comparison of the stress-induced patterns of Hsp70 expression showed distinct differences in the two age groups. Young rats responded to heat stress with a strong pattern of nuclear and cytoplasmic Hsp70 expression that was maintained for 48 h in hepatocytes located in central vein regions (Fig. 2). In aged animals, nuclear and cytoplasmic Hsp70 expression was both delayed and reduced, although there was a transient increase in Hsp70 expression at 2 h of recovery that subsequently diminished at later time points. These results suggest that the blunted stress protein response in older animals may have functional consequences that correlate with the increased cellular injury and reduced stress tolerance that is associated with advancing age.

SUMMARY AND FUTURE DIRECTIONS

In this mini-review, an attempt was made to summarize the physiological factors that modulate HSP responses to stressors at cellular and systemic levels. From the literature presented, it should be evident that the HSP70 family of proteins is essential for cellular survival from heat stress and other types of physiological challenge. It is clear that these proteins are ubiquitously present in cells under both normal and stressful conditions and that their structure is well conserved among species. In addition, there is a large body of evidence to support the role of HSPs for improving cell survival to otherwise lethal challenges.

Despite the significant amount of progress that has been made regarding biochemical and structural features of HSP70, the mechanisms by which these proteins provide protection against cellular stress are still not thoroughly understood. Delineation of these mechanisms will have significant implications both clinically and at a basic science level. Furthermore, technological advances will enhance the ability of researchers to greatly extend experiments addressing HSP functions and mechanisms from cell culture into animals and humans. On the basis of research performed over the past decade, we have learned that the induction of HSP70 is not confined to heat shock paradigms involving extreme conditions in culture systems and lower species. Instead, HSPs are synthesized in animals and humans in response to many relevant physiological (e.g., heat stress, exercise, energy depletion) and pathological (e.g., viral infections, cytokine release) conditions.

Finally, it is still uncertain whether HSPs can be utilized in a therapeutic setting. Although gene therapy programs have made impressive advances in recent years, the overexpression of HSP70 has proven to be problematic. Thus the answers to many of these questions await further study.

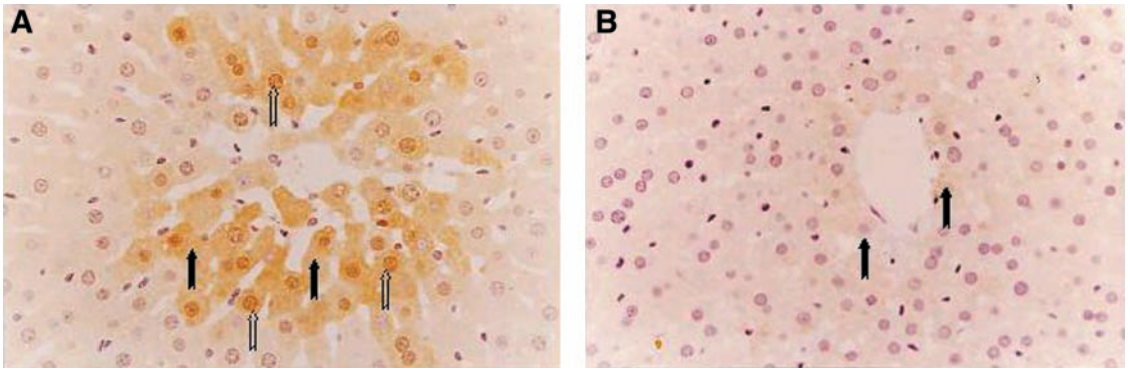

Fig. 2. Cellular localization and zonal distribution of immunoreactive Hsp70 protein in the liver of young and old rats after heat stress (Ref. 37). Liver biopsies were collected from young (*left*) and old (*right*) Fischer 334 rats 12 h after a heat stress protocol, and sections were stained with a monoclonal antibody specific for Hsp70. There was no evidence of immunoreactive Hsp70 in hepatocytes from young or old euthermic control animals (not shown). However, at 12 h of recovery from heat stress, strong cytoplasmic (solid arrows) and nuclear (open arrows) staining for anti-Hsp70 was observed in hepatocytes surrounding the central vein region in young rats (*A*). Conversely, in old rats (*B*), only weak cytoplasmic expression (solid arrows) was observed in hepatocytes in proximity to the central vein. Magnification = ×40.

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