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Original Article

Heat Shock Proteins: A Review

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Abstract

Heat shock proteins (Hsps) were first known as proteins whose synthesis was increased by stresses like a rise in temperature. Recently, many of the key Hsps are shown to be intimately involved in protein biogenesis through a direct interaction with a large type of proteins. As a reflection of this role, these Hsps are named as molecular chaperones. Hsp70s interact with incompletely folded proteins, like nascent chains on ribosomes and proteins within the process of translocation from the cytoplasm into mitochondria and the endoplasmic reticulum. Hsp60 additionally binds to unpleated proteins, preventing aggregation and facilitating protein folding. Though less well defined, other Hsps like Hsp90 additionally play vital roles in modulating the activity of variety of proteins. The operate of the proteolytic system is intertwined therewith of molecular chaperones. Many parts of this technique, encoded by heat-inducible genes, are responsible for the degradation of abnormal or misfolded proteins. The budding yeast *saccharomyces cerevisiae* has proven terribly useful within the analysis of the role of molecular chaperones in protein maturation, translocation and degradation.

Key words: Heat shock protein (Hsps)

INTRODUCTION

Heat shock proteins are omnipresent proteins found in the cells of all studied organisms. Many types of stress, including heat, induce expression of a family of genes known as the heat shock protein genes. Heat shock proteins originally were discovered when it was observed that heat shock produced chromosomal puffs in the salivary glands of fruit flies (*Drosophilia*). The DNA sequence that makes up this family of genes is highly conserved across species. This family of genes originally was named because of their expression after exposure to heat. However, the genes are now known to be induced by a wide variety of environmental or metabolic stresses that include the following: anoxia, ischemia, heavy metal ions, ethanol, nicotine, surgical stress, and viral agents. Thus, the term “heat shock protein”

is a misnomer because many agents other than heat induce the expression of the heat shock protein gene. Consequently, "stress protein" is the preferred term (Whitley, 1999). Stress proteins are critically important because they appear to be necessary in the critical step of three-dimensional folding of some newly formed proteins within the cell. In fact, they ensure that newly formed polypeptides proceed correctly through folding and unfolding to eventually achieve a functional shape.

DISCOVERY

It is known that rapid heat hardening can be elicited by a brief exposure of cells to sub-lethal high temperature, which in turn provides protection from subsequent and more severe temperature. In 1962, Italian geneticist Ferruccio Ritossa reported that heat and the metabolic uncoupler2,4-dinitrophenol induced a characteristic pattern of puffing in the chromosomes of Drosophila (Ritossa , 1962).This discovery eventually led to the identification of the heat-shock proteins (HSP) or stress proteins whose expression these puffs represented. Increased synthesis of selected proteins in Drosophila cells following stresses such as heat shock was first reported in 1974 (Schlesinger, 1990).

CLASSIFICATION

Heat-shock proteins are named according to their molecular weight. For example, HSP60, HSP70 and HSP90 (the most widely-studied HSPs) refer to families of heat shock proteins on the order of 60, 70, and 90 kilodaltons in size, respectively (Li and Srivastava 2004). The small 8-kilodalton protein ubiquitin, which marks proteins for degradation, also has features of a heat shock protein (Raboy et al., 1991)

The HSPs have been extensively studied, especially with regard to their cellular localization, regulation, and functions (Benjamin and McMillan 1998). 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" (Ritossa, 1962) and since last 3 decades a large number of additional proteins have been discovered within this family, and these are collectively referred to as "HSPs" (Table 1).

Stress proteins belong to a multigene family and range in molecular size from 8 to 150 kd. The principal heat-shock proteins that have chaperone activity belong to five conserved classes: HSP33, HSP60, HSP70, HSP90, HSP100, and the small heat-shock proteins (sHSPs) (Schlesinger, 1990).

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

Approximate molecular weight (KDa)	**Prokaryotic proteins**	**Eukaryotic proteins**	**Function**
10 kDa	GroES	HSP10	
20-30 kDa	GrpE	The HSPB group of HSP. Eleven members in	

		mammals including HSP27, HSPB6 or HSPB1 (Kampinga, 2009)	
40 kDa	DnaJ	HSP40	Co-factor of HSP70
60 kDa	GroEL, 60kDa antigen	HSP60	Involved in protein folding after its post-translational import to the mitochondrion/chloroplast
70 kDa	DnaK	The HSPA group of HSP including HSP71, HSP70, HSP72, Grp78 (BiP), Hsx70 found only in primates	Protein folding and unfolding provides thermotolerance to cell on exposure to heat stress. Also prevents protein folding during post-translational import into the mitochondria / chloroplast.
90 kDa	HtpG, C62.5	The HSPC group of HSP including HSP90, Grp94	Maintenance of steroid receptors and transcription factors
100 kDa	ClpB, ClpA, ClpX	HSP104, HSP110	Tolerance of extreme temperature

HSP70: Members of the Hsp70 family are the most extensively studied group of stress proteins to date. The HSP70s are ATP-binding proteins and demonstrate a 60–80% base identity among eukaryotic cells (Bardwell and Craig, 1984). Some members of the Hsp70 family are expressed constitutively, and others are strictly stress inducible. The constitutively expressed protein shares about 95% sequence homology (identity of the DNA sequence) with the inducible form of Hsp70. However, little is known about its function within the cell. Upregulation of the inducible form of Hsp70 has been most closely associated with the development of thermotolerance.

HSP90: The 90-kd (HSP90) family of proteins represents one of the most abundant proteins in mammalian cells, yet its synthesis still increases after stress. It appears that HSP90, in conjunction with HSP70 and HSP56, binds, stabilizes, and maintains the estrogen receptor complex in an active confirmation. HSP90 appears to interact with multiple intracellular proteins and signal transduction pathways. HSP90 serves a regulatory role by binding to and either inhibiting or stimulating the activity of its target protein. Thus, HSP70 and HSP90 are ubiquitous in all tissues, but some of the smaller stress proteins may have a more specialized role in the vascular system.

HSP32: HSP32 Hemeoxygenase a rate-limiting enzyme in the degradation of Heme, is stress induced and is abundant in myocardial cells. HSP32 is induced by sheer stress and may mediate nitric oxide–dependent platelet inhibition and vasodilatation. There is no direct evidence that HSP32 functions as a chaperone, but its overexpression during stress events indicates that it may function in this fashion.

HSP25/27: HSP25/27influences the cell cytoskeleton (actin polymerization) and may be involved in cell migration. Physiologic stress increases the phosphorylation of HSP27. Phosphorylation of HSP25 occurs via the mitogen-activated protein kinases. Mitogen-activated protein kinases are involved in the intracellular signaling cascade and are activated during ischemia-reperfusion.

Ubiquitin is a small 8-kd stress protein that may facilitate targeting and removal of other proteins denatured during the stress event.

REGULATION OF HEAT SHOCK PROTEINS

Heat shock proteins are present in cells under normal conditions, but are expressed at high levels when exposed to a sudden temperature jump or other stress. Heat shock proteins stabilize proteins and are involved in the folding of denatured proteins. High temperatures and other stresses, such as altered pH and oxygen deprivation, make it more difficult for proteins to form their proper structures and cause some already structured proteins to unfold. Left uncorrected, mis-folded proteins form aggregates that may eventually kill the cell. Heat Shock Proteins are induced rapidly at high levels to deal with this problem. Increased expression of HSPs is mediated at multiple levels: mRNA synthesis, mRNA stability, and translation efficiency.

Stress proteins also assist in the repair of denatured proteins or promote their degradation after stress or injury. They have been referred to as “molecular chaperones” because of this function.

It is thought that stress proteins are produced in response to nonlethal stress to protect organisms from subsequent severe stress that would otherwise be lethal. In the case of exposure to heat, this phenomenon has been called “thermotolerance” and has launched many experiments in which an association has been found between the heat shock response and protection against other stresses, such as hypoxia or ischemia. The addition of one type of stress may provide protection against other types of insults, which results in cross-tolerance. As examples, stress protein induction by hyperthermia may provide protection during a subsequent arterial injury or exposure to a heavy metal may provide subsequent protection against heat or ischemic injury. This thermotolerance treatment strategy has proved successful in experimental models of cardiac ischemia, arterial injury, endotoxic shock, renal and hepatic ischemia, ethanol-induced gastric ulcerations, and skeletal muscle ischemia-reperfusion.

Many of the stress proteins are present continuously (constitutive expression), and expression of other proteins is increased by stress (stress inducible). Stress proteins are rapidly induced through transcription (messenger RNA production from DNA occurs in minutes) and translation (protein production from messenger RNA) mechanisms. Gene transcription is controlled by heat shock transcription factors. Different members of the heat shock transcription factor family may be activated

by specific stresses. Inactive heat shock factors exist as monomers. However, once activated, they trimerize into an active form that is capable of binding to the promotor site of the stress protein gene and initiating transcription and translation.

The HSP70 family:

HSP70 family of proteins is 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 (Craig, 1985).

Proteins in the HSP70 group share common protein sequences but are synthesized in response to different stimuli 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 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 (Wu, 1995). 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.

An important consideration regarding HSP70 regulation involves the apparent discordance between transcription of mRNA and HSP70 translation. There is evidence suggesting that transcriptional activation of the HSP70 gene is independent of protein synthesis.

FUNCTIONAL ROLES OF HSPs

The function of a protein is determined by its three-dimensional structure. When excessive heat is applied to proteins, chains of amino acids which are folded into spirals, loops and sheets begin to lose their shapes. When the interior of these proteins gets exposed, proteins can adhere and form globs. This can make them dysfunctional. Protein conformational defects are responsible for a number of pathologies, ranging from Alzheimer's disease and oncogenic transformation in humans to heat and drought susceptibility in plants. Chaperones protect against denaturization. Heat Shock Proteins bind to denatured proteins to prevent aggregation. Some Heat Shock Proteins, like HSP104, have the ability to rescue already aggregated proteins.

Stress Tolerance

Production of high levels of heat shock proteins can also be triggered by exposure to different kinds of environmental stress conditions, such as infection, inflammation, exercise, exposure of the cell to toxins (ethanol, arsenic, trace metals, and ultraviolet light, among many others), starvation, hypoxia (oxygen deprivation), nitrogen deficiency (in plants), or water deprivation. As a consequence, the heat shock proteins are also referred to as stress proteins and their upregulation is sometimes described more generally as part of the stress response (Santoro, 2000).

The mechanism by which heat-shock (or other environmental stressors) activates the heat shock factor has been determined in bacteria. During heat stress outer membrane proteins (OMPs) do not fold and cannot insert correctly into the outer membrane. They accumulate in the periplasmic space. These OMP's are detected by DegS, an inner membrane protease, that passes the signal through the membrane to the sigmaE transcription factor (Walsh et al., 2003). However, some studies suggest that an increase in damaged or abnormal proteins brings HSPs into action.

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 the folding of proteins in various intracellular compartments, the maintenance of structural proteins, the refolding of misfolded proteins, translocation of proteins across membranes and into various cellular compartments, the prevention of protein aggregation and the degradation of unstable proteins.

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 (Landry et al., 1982). Data from subsequent studies demonstrated that the induction of HSP70 was associated with the development of tolerance to a variety of stresses, including hypoxia (Guttmanet al.,1980), ischemia (Marber et al., 1995), acidosis (Weitzelet al.,1985), energy depletion(Sciandra and Subjeck, 1983), cytokines such as tumor necrosis factor-α (TNF-α) (Jäättelä and Wissing, 1993) and ultraviolet radiation (Barbe et al., 1988).

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 (Lambert et al., 2002). 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 (Moseley, 1997). 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(Hume and Marigold, 1980)and is also reported to be the tissue most sensitive to heat damage (Henle and Leeper1982). 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) (Vander Zee et al., 1983) and during heat stroke (Shibolet et al., 1976) in humans.

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 (Barbe et al., 1988) and ischemia-reperfusion injury to the heart (Currie et al., 1988), Liver (Bernelli- Zazzera et al., 1992) and kidney (Van Why et al., 1992). 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 (Mizzen and Welch, 1988).

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. 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 (Henle and Leeper,1982). 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 (Hall et al., 2001).

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. Other studies have demonstrated that inhibition of transcription during the conditioning heat stress also allows the maintenance of thermotolerance. In addition, oxidative stresses, which can confer thermotolerance, may not increase the levels of HSPs. In other stresses, such as ischemia, where HSPs are thought to play a role, HSP overexpression has also not been found to confer tolerance. 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 (Krebs and Bettencourt, 1999).

Immune surveillance and antigen presentation

Extracellular and membrane bound heat-shock proteins, especially HSP70 are involved in binding antigens and presenting them to the immune system (Nishikawa et al., 2008).

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

(Moseley, 2000).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 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.

Elevations in intracellular HSP levels have been shown to improve cell tolerance to inflammatory cytokines such as TNF-α and interleukin-1 (Muller, 1993), (Jäättelä and Wissing1993). HSP accumulation within a cell produces both transcriptional inhibition and a decrease in TNF-α and interleukin-1 secretion (Synder et al. , 1992), (Emerson et al., 1992) 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 (Kluger et al., (1997). 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.

HSP70 is also known to facilitate antigen presentation in cells such as macrophages and dendrites. 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, 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.

Role as chaperone

Several heat shock proteins function as intra-cellular chaperones for other proteins. They play an important role in protein-protein interactions such as folding and assisting in the establishment of proper protein conformation (shape) and prevention of unwanted protein aggregation. By helping to stabilize partially unfolded proteins, HSPs aid in transporting proteins across membranes within the cell (Walter and Buchner, 2002).

Cardiovascular

Heat shock proteins appear to serve a significant cardiovascular role. HSP90, HSP84, HSP70, HSP27, HSP20 and alpha B crystallin all have been reported as having roles in the cardiovasculature (Benjamin and McMillan 1998).

HSP90 binds both endothelial nitric oxide synthase and soluble guanylatecyclase, which in turn are involved in vascular relaxation. A chocolate kinase of the nitric oxide cell signaling pathway, protein kinase G, phosphorylates a small heat shock protein, HSP20. HSP20 phosphorylation correlates well with smooth muscle relaxation (Antonova et al., 2007) and is one significant phosphoprotein involved in the process (McLemor et al., 2005). HSP20 appears significant in development of the smooth muscle

phenotype during development. HSP20 also serves a significant role in preventing platelet aggregation, cardiac myocyte function and prevention of apoptosis after ischemic injury, and skeletal muscle function and muscle insulin response (Fan et al., 2005).

HSP27 is a major phosphoprotein during a woman's contractions. HSP27 functions in small muscle migrations and appears to serve an integral role (Salinthone et al., 2008).

CLINICA SIGNIFICANCE

Heat Shock Factor 1 (HSF1) is a transcription factor that is involved in the upregulation of HSP70 protein expression (Xu et al., 2008). Recently it was discovered that HSF1 is a powerful multifaceted modifier of carcinogenesis. HSF1 knockout mice show significantly decreased incidence of skin tumor after topical application of DMBA (7, 12-dimethylbenzanthracene), a mutagen (Dai et al., 2007).

Abnormal levels of stress proteins have been found in a number of disorders, including atherosclerosis, congestive heart failure, fever, infection, aging, Alzheimer's disease, malignant diseases, and autoimmune disorders. There is a growing body of evidence that some stress proteins may be associated with atherosclerosis. Experimentally, arteriosclerotic lesions can be induced by immunization with HSP60/65. HSP60/65 is found in high concentrations in human arteriosclerotic lesions, and there is a correlation between anti-HSP60/65 antibodies and atherosclerosis. In addition to being involved in specific disease processes, the stress proteins may play a key regulatory role in cell death pathways (apoptosis) that involve DNA and protein synthesis. These proteins now are being implicated in the aging process. It appears that there is decreased expression of stress protein genes and decreased activity of HSF-1 during aging. These factors may make aging tissues more susceptible to oxidative stress injury (Whitley et al., 1999).

APPLICATION

Cancer vaccine adjuvant

Given their role in antigen presentation, (Nishikawa et al., 2008) HSPs are useful as immunologic adjuvants in boosting the response to a vaccine (Bendz et al., 2007). Furthermore, some researchers speculate that HSPs may be involved in binding protein fragments from dead malignant cells and presenting them to the immune system. Therefore HSPs may be useful for increasing the effectiveness of cancer vaccines (Binder, 2008).

Anticancer therapeutics

Intracellular heat shock proteins are highly expressed in cancerous cells and are essential to the survival of these cell types. Hence small molecule inhibitors of HSPs, especially HSP90 show promise as anticancer agents (Didelot et al. 2007). The potent HSP90 inhibitor 17-AAG is currently in clinical trials

for the treatment of several types of cancer (Solit and Rosen, 2006). HSP96 also shows promise as an anticancer treatment and is currently in clinical trials against non-small cell lung cancer.

CONCLUSIONS

Cells from nearly all organisms answer a variety of stresses by the rapid synthesis of a extremely conserved set of polypeptides termed heat shock proteins (HSPs). The precise functions of HSPs are unknown, however there's appreciable evidence that these stress proteins are essential for survival at both normal and elevated temperatures. HSPs additionally seem to play an important role in the development of thermotolerance and protection from cellular injury related to stresses like ischemia, cytokines, and energy depletion. These observations recommend that HSPs play a vital role in each normal cellular homeostasis and therefore the stress response. This review prove that the HSPs may be necessary modifying factors in cellular responses to hyperthermia of physiologically relevant conditions like physiological state, exercise, oxidative stress, metabolic challenge and aging.

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