Heat Shock Proteins

MARINA T. NECHIFOR, I. F. DUMITRU, DIANA DINU

University of Bucharest, Faculty of Biology, Splaiul Independenţei 91-95,

76201 Bucharest, Romania

Received: 19th August 2002; Accepted: 3th September 2002

Introduction

Heat shock proteins (hsp) are a set of proteins with molecular weight ranging between 10 and 110 kDa, whose expression is induced rapidly and massively as a response to the exposure of cells or organisms to high temperatures (Lindquist and Craig, 1988). Numerous other stress factors (heavy metal ions, UV radiation, anoxia, ethanol, viral infections) induce the synthesis of these proteins (Lindquist 1993; Pockley, 2001). Stress factors which trigger the protein unfolding, misfolding or aggregation trigger a stress response that leads to the induction of gene transcription for proteins with the capacity to stabilise and re-fold proteins, therby re-establishing the balance between protein synthesis, assembly and degradation.

A large number of experiments has demonstrated that the induction of these proteins by moderate stress conditions is generally accompanied by the induction of tolerance to more severe stresses (Hahn and Li, 1990). This action may have some remarkable effects. Thus, for instance, if the fibroblasts that have been transformed in order to express constitutively hsp70 are suddenly transferred from their normal growth temperature to a high temperature (45ºC), one can notice that the survival rate of transformed cells is 1000 times higher when compared to that of the original cell line (Li et al., 1991). Given the magnitude of these effects, it is not surprising that cells have evolved mechanisms to ensure that hsp are produced as rapidly as possible on exposure to stress. Indeed, the speed and intensity of the response are truly extraordinary. Such a response may be conducted only by the combined action of more regulatory mechanisms which act at the level of transcription, RNA processing, RNA turnover and translation. A feature of this regulatory mechanism consists in the involvement of hsp, especially hsp70 in its own synthesis path (Morimoto, 1993; Applegate et al., 1997). **Figure 1** presents the self-adjusting mechanism, according to the model proposed by Morimoto (1998). Thus, the regulation of the hsp expression is controlled by nuclear binding factors known as heat shock factors (hsf), by the hsp themselves as well as by unfolded polypeptides generated under stress conditions. Nuclear factors hsf which are activated as a consequence of their exposure to stress are fixed to heat shock elements (hse) which control the expression of the heat shock genes. The main heat shock transcriptional factor of vertebrates is HSF1.

The generation of heat shock proteins must be only transient, even if exposure to stress is over a prolonged period, as a continued presence of hsp would adversely influence protein homeostasis and it would disturb a variety of intracellular functions. One mechanism by which the activity of HSF1 is regulated is via the binding of hsp70 to its transactivation domain, thereby leading to repression of heat shock genes transcription (Shi et al., 1998). A second mechanism that regulates hsp synthesis is the interaction between heat shock protein binding factor 1 (HSBP1) and the active trimeric form of HSF1 and hsp70, thereby inhibiting the capacity of HSF1 to bind to DNA.

Heat shock proteins are among the most phylogenetically conserved proteins. Some types of hsp are expressed at high temperatures in the case of all organisms. To this end homologous genes have been cloned from both eukaryotes and prokaryotes.

The hsp modules the folding and unfolding of proteins, facilitates the assembly of complex proteins made up of subunits and promotes the degradation of wrongly folded proteins and of denatured proteins (Gething and Sambrook, 1992). Since the key element in the functioning of hsp consists in the prevention of wrong association and the expression of certain inappropriate activities, these proteins may be considered members of the chaperone class proteins (Ellis and van der Vries, 1991). Hsp may also function as enzymes, taking part at metabolic pathways affected by the exposure to stress.

Heat shock proteins have been discovered accidentally by Ferrucio Ritossa, within some genetic experiments concerning the synthesis of nucleic acids at the level of polythene chromosomes from the salivary glands of *Drosophila melanogaster* larvae (Ritossa, 1962). Ritossa has noticed that if the larvae are subjected to a heat shock (the temperature of the incubator in which the larvae are maintained has been accidentally modified by a lab colleague) the specific expression of certain genes is triggered. The product of these genes has been identified much later, the term "heat shock protein" being adopted (Tissieres et al., 1974). The heat shock response is distinct from adaptive responses that an organism may undergo when its environment changes gradually.

Classification of heat shock proteins

Table 1 presents the classification of heat shock proteins, the intracellular location and their functional features. The criterion for the classification of heat shock proteins is represented by their approximate molecular weight.

Main families of hsp

Hsp70 is one of the most important types of heat shock proteins, present in most organisms. The genome of *Escherichia coli*encodes only one type of hsp70 called DnaK. The eukaryote cells encode several types of proteins belonging to the hsp70 family, each of them presenting an identity of at least 50% with the protein of *E.coli*. In yeasts there are 13 different types of hsp70 proteins; a part of them are essential for normal growth, and other types are necessary only for conditions of extreme temperatures.

The multitude of hsp70 of eukaryotes is correlated with their presence in major compartments of the cell. Thus, distinct hsp70 proteins are localized to the endoplasmic reticulum, to mitochondria, and, in plants, to chloroplasts. Other types are present in the nucleus and cytoplasm, but their expression and intracellular distribution may vary. Some types of hsp are expressed constitutively, while others shuttle between the nucleolus, the nucleus and the cytoplasm. In multicellular organisms, tissue-specific versions of the protein also exist.

All members of the hsp70 family bind ATP and have a weak ATPase activity that is stimulated by substrate binding. Hsp70 proteins bind to small peptides, to nascent chains on polysomes, to proteins that have been targeted to the wrong cellular compartment, to certain mutant proteins, to some protein subunits that are expressed in the absence of their partners, and to certain oligomeric proteins in the process of assembly or disassembly. In binding to these substrates hsp70 proteins participate in a variety of protein folding, unfolding, assembly, and disassembly processes that emply the energy of ATP. Thus, a certain hsp70 protein, for instance, catalyzes the disassembly of the clathrin cover, another one facilitates the transport of proteins across cell membranes, and yet another hsp70 protein facilitates the assembly of immunoglobulins in the endoplasmic reticulum. The model that explains the role of hsp70 in these processes assumes that their binding to hydrophobic surfaces prevents random associations and stabilizes target protein in a fully or partially unfolded state. At high temperatures, as proteins unfold, hsp70 binds to sequences that would normally be buried in the hydrophobic core of the protein, preventing aggregation and helping to restore the native structures when temperature return to normal values.

Hsp70 proteins contain two structural domains, an NH_2-terminal ATP-ase domain and a COOH-terminal domain involved in binding the peptidic substrate. The secondary and tertiary structure of the ATPase domain are almost identical to that of actin. The hsp70 peptide-binding domain binds a seven-residue peptide in an extended conformation between a beta-sheet subdomain and alpha-helical subdomain. Two or three hydrophobic residues of the peptide are buried in pockets of the beta subdomain, and peptide backbone NH and CO groups form hydrogen bonds with sidechain and backbone groups of the beta subdomain. The ends of the substrate peptide extend out from either side of the peptide-binding domain. The alpha domain clamps down loops of the beta subdomain, pinning the peptide in place (**Figure 2**).

It is thought that ATP binding to the ATPase domain triggers substrate release by causing the alpha domain to bend upwards at a flexible junction near the middle of the long helix that extends over the peptide.

Hsp60 (also known as GroEL or Chaperonin-60) is one of the most abundant proteins from bacteria, which is expressed constitutively even under conditions of normal temperatures. In eukaryotes, hsp60/GroEL proteins are found in mitochondria and chloroplasts. They have not been identified in other compartments of the eukaryotic cell. Hsp60/GroEL do not present a high affinity for most of hsp70 substrates, but have a high affinity for completely denatured proteins and for the individual subunits of certain

proteins destined for oligomeric assembly. The exercising of the biological function by the GroEL is dependent upon another protein called GroES (hsp10). *In vitro*, GroEL binds denatured proteins and prevents their aggregation. When GroES joins the GroEL complex, ATP hydrolysis promotes the folding of unfolded proteins on the surface of GroEL and their release from the complex. *In vivo*, proteins in the hsp60/GroEL class are required for the assembly of oligomeric protein structures, such as ribulose bisphosphate carboxylase and F_1-ATPase.

Hsp60 proteins themselves have an oligomeric structure being made up of 14 subunits arranged in a cylindrical unit. (**Figure 3**). The proteic substrate with an unfolded conformation is fixed inside the central hydrophobic cavity. The fixing of ATP determines the rotation of hsp60 subunits movement which is coordinated by the fixing of hsp10 which closes the cavity, sequestering the inner substrate. Thus, a space is created wherein proteins may fold, the aggregation with other unfolded proteins being excluded.

Hsp110 forms a family of proteins which have been pointed out in bacteria, fungi, plants and animals. The hsp110 from yeasts and *Escherichia coli* are not indispensable for growth at normal temperatures, but play an important role in the protection of cells at extreme temperatures and against other forms of stress (Sanchez et al., 1992) Hsp110 inhibit the aggregation of proteins thermically induced and maintain denatured proteins in an appropriately folded state. Hsp100 proteins contain two regions of very high homology centered around two nucleotide-binding domains.

Hsp90 have been identified in both prokaryotes and eukaryotes organisms. In prokaryotes these proteins are not indispensable for growth at normal temperatures and exercise only a minor effect upon growth at high temperatures. In the case of eukaryote organisms hsp90 proteins play an essential role, and the quantity of protein needed for growth at high temperatures is proportional to the value of the temperature.

Hsp90 proteins interact with many other cellular proteins, including steroid hormone-receptors, oncogenic tyrosine kinases, calmodulin, actin, tubulin, and casein kinase II. The exact nature and function of these interactions are not well-known currently. In the case of receptors for steroid hormones, it was pointed out the fact that hsp90 can exercise both activator as well as repressing action (Pratt, 1990). Hsp90 proteins differ from other types of heat shock proteins in that their *in vivo* association with the target proteins is stable for a long period of time.

Hsp27 are proteins whose expression is correlated with the cellular growth and differentiation (Ciocca et al., 1993). In the different tissues of the human organism (uterus, vagina, oviduct, skin) there has been identified a high level of hsp27. The constitutive expression of hsp27 is a determining factor of cellular resistance to stress. Experimental studies have proved that the hsp27 expression is tightly linked to the differentiation of keratinocytes in the epidermal (Trautinger et al., 1995) as well as that of hematopoietic cells (Arrigo et al., 1995).

Hsp27 act as mediators of thermo-tolerance and as molecular chaperons. As a response to different stress factors from the extracellular environment, hsp27 are rapidly phosphorylated at two or three serine residues under the action of MAPKAP kinase-2/3 (mitogen-activated protein kinase-activated protein kinase-2/3) (Rogalla et al.,1999). The expression and the phosphorylation of hsp27 are regulated by the inflammatory cytokines tumor necrosis factor α (TNFα) and interleukin I (IL-I). This suggests a possible role of hsp27 as a protective factor in inflammatory processes. Although the physiological role of hsp27 is not fully understood, it is common knowledge that the modulation of the expression of this type of stress proteins determines the reorganization of the actin cytoskeleton. (Wieske et al., 2001).

Heme oxygenase (heme, hydrogen-donor: oxygen oxidoreductase, EC 1.14.99.3) is a type of heat shock protein with enzyme function. Heme oxygenase exists under the form of two isoenzymes, HO-1 and HO-2, with molecular weight of approximately 32kDa. HO-2 is the constitutive form which is expressed independently of stress factors, while HO-1 is the inducible form whose expression is amplified under oxidative stress conditions which are generated by various environmental factors (heavy metals, UVA radiation) (Applegate et al., 1995) Both enzymes mediate the resistance to oxidative stress on the path of transforming the heme group with pro-oxidant action into biliverdin, carbon monoxide and ferrous iron (**Figure 4**). The electrons required for catalytic turnover of the enzyme are provided, in mammalian systems, by NADPH-cytochrome P450 reductase. The product of the reaction of degradation of the heme group, biliverdin, exercises an antioxidant action.

Ubiquitin is a small size protein (76 aminoacids), highly conserved from a phylogenetic point of view, that has been found in all eukaryotic cells, but not in prokaryotic cells. Ubiquitin exists both in a monomeric form and in a form that is covalently attached to other proteins or to other ubiquitin molecules. Ubiquitin is a compact globular protein with a COOH-terminus that extends away from the main body of the protein into the aqueous space.

Ubiquitin is involved in a wide variety of cellular processes. One of its best-characterized functions is in the regulation of proteolysis. The conjugation of multiple molecules of ubiquitin to a protein targets it for degradation by the major ATP-dependent proteolytic complex of the cell. Both the expression of the ubiquitin and that of some of the enzymes that conjugate ubiquitin to the other proteins are induced by heat. This mechanism is required for the survival of the cells in the case of a prolonged exposure to temperatures higher than normal growth temperatures. It is assumed that proteins, as they begin to unfold at high temperatures may follow two alternative paths. If they can be repaired by the protective, disaggregating functions of the other heat shock proteins, they will be. If not, they will be targeted by ubiquitinylation for degradation.

If a chain containing multiple copies of ubiquitin binds to a target protein, the protein will be destined to degradation by a multiproteic intracellular complex called 26S proteasome. Recent data suggest that ubiquitinylation may trigger both proteolitic degradation and other “destinies” of target proteins.

The pathway of protein ubiquitinylation

The □-carboxyl group of the terminal Gly residue from the structure of the ubiquitin forms isopeptide bonds with target proteins (which can include another copy of ubiquitin). The isopeptide bond is formed with an □-amino group in the side chain of a Lys residue of the target protein. The mechanism of action is complex and is made up of the following stages:

(a) Activation of ubiquitin. Ubiquitin is activated by the enzyme E1 ("ubiquitin activating enzyme") a protein with a molecular weight of 115-130 kDa, made up of approximately 1000 aminoacids. Plant, yest, human and mouse proteins have 5 conserved Cys residues. In mammalian cells E1 is phosphorylated by the cdc2 kinase, which reflects the involvement of ubiquitin in the control of the cellular cycle. The enzyme E1 hydrolyses the ATP and forms a complex with the AMP-ubiquitin (the adenylated ubiquitin) (equation 1):

$$E1 + ATP + Ub \text{ ---------- } > E1^{\cdot Ub\text{-}AMP} + PPi \qquad \text{(equation 1)}$$

The following stage of the activation process consists in the transfer of ubiquitin at the level of a Cys residue located in the active catalytic site of the enzyme E1, and the forming of a thiol ester link between the COOH-terminus of the ubiquitin and the thiol group of the enzyme E1 (equation 2). This transformation takes place in concert with the adenylation of an additional ubiquitin. These thiol esters are readily cleaved by reducing agents such as mercaptoethanol and dithiothreitol and also by hydroxylamine.

The structure of adenylated ubiquitin is represented in **Figure 5**, and the forming of the thiol ester link is presentend in **Figure 6**.

$$E1^{\cdot Ub\text{-}AMP} + Ub \text{ ---------- } > E1_{\text{-s-co-Ub}}{}^{\cdot AMP\text{-}Ub} \qquad \text{(equation 2)}$$

(b) Ubiquitin conjugation. After the activation stage, the ubiquitin is transferred to the enzyme E2 ("ubiquitin conjugating enzyme") which functions as a ligase. E2 enzymes, in most cases, appear to be the proximal donors of ubiquitin to target proteins. E2 enzymes have a Cys in the active catalytic site, as well as the activating enzyme E1. The ubiquitin is transferred from E1 to E2 forming a thiol ester link in which the Cys from the E2 structure is involved. (equation 3):

$$E1_{\text{-s-co-Ub}}{}^{\cdot AMP\text{-}Ub} + E2\text{-}SH \text{ ----- } > E2_{\text{-s-co-Ub}} + E1^{\cdot AMP\text{-}Ub} \qquad \text{(equation 3)}$$

(c) Formation of the isopeptide bond. The ubiquitin is transferred at the level of an acceptor Lys residue from the structure of the target protein (equation 4). Between the □-amino group of the Lys residue of the target protein and the □-carboxyl group of the Gly terminal residue from the structure of the ubiquitin there is formed an isopeptide bond (**Figure 7**).

$$E2_{\text{-s-co-Ub}} + Protein\text{-}NH_2 \text{ ------- } > E2\text{-}SH + Protein\text{-}NH\text{-}CO\text{-}Ub \quad \text{(equation 4)}$$

More complex structures represented by linear catenations (multi-ubiquitin chains) made up of several molecules of ubiquitin fixed successively by means of isopeptide bonds between the carboxyl group of a Gly residue from the 76 position of a molecule of ubiquitin and the amino group of a Lys residue from the position 48 of the following ubiquitin molecule are set to the target protein. The structures with 4 successive molecules of ubiquitin are recognised by the 26S proteasome (the Ub_4 chains exhibit certain structural characteristics that may be recognised by the proteasome). The Lys substitution from position 48 with Cys results in a mutant ubiquitin which does not support multi-ubiquitin chains and is incapable of targeting proteins for proteolysis.

In the case of certain target proteins it is necessary to have an additional factor for ubiquitination of targets. Thus, some target proteins are recognised and bound by the E3 protein ("recognin") which forms a complex with the target protein and with a ubiquitin-charged E2. E3 does not form covalent links with ubiquitin, it only has the role to identify and sequester suitable target proteins for ubiquitinylation.

The cooperation between the different types of heat shock proteins

Certain types of hsp act together along the processes of folding, assembly and disassembly of proteins. For instance in the mitochondrion hsp70 and hsp60 cooperate along the translocation of the proteins into the organelle, as well as along their assembly together in functional complexes. Thus, hsp70 maintains the protein in an unfolded conformation as it enter into the organelle, maintaining it in a state that is competent for transfer to hsp60/GroEL. In its turn GroEL, promotes its assembly into oligomeric structures. Another example refers to the cooperation between hsp70 and hsp90. Thus, the hsp70 in cytoplasm is associated in a stoichiometric quantities with hsp90-steroid hormone receptor complexes. Antibodies against hsp70 block the association of hsp90 with the receptor *in vitro*, suggesting that hsp70 plays a role in the assembly of complexes of hsp90 and its target proteins.

The role of heat shock proteins in pathological processes

Despite the obvious importance played by hsp in the response to stress, the role of these proteins in the control of pathological processes has been taken under consideration for some time now. The involvement of hsp in the pathological processes in mammals regard the following aspects:

Viral infection induces the expression of hsp. Bacterial viruses use hsp proteins to facilitate the involvement of the replication mechanism of cellular DNA and for the assembly of viral particles. In eukaryotes, heat shock proteins associate with a series of key viral products, such as the of the simian virus 40 (SV40) T antigen, that controls the progression of the cell cycle and cause tissue transformation (cancer).

Hsp are involved in autoimmune diseases. Hsp60 and immune reactivity to members of the hsp60 family have been implicated in autoimmune diseases. Hsp60 is expressed in the synovial fluid of patients with rheumatoid arthritis and juvenile chronic arthritis, and the

T cells from the synovial fluid are activated by hsp60 proteins of the body itself and by hsp65 of bacterial origin (Pope et al., 1992). With children suffering from juvenile chronic arthritis elevated levels of ciculating antibodies to hsp60 has been discovered. A series of experimental data suggests the involvement of hsp60 in the pathogenesis of multiple sclerosis and insulin-dependent diabetes mellitus (type1 diabetes). The constitutive presence of antibodies to hsp70 (hsc) şi has been pointed out in patients with primary biliary cirrhosis and patients with autoimmune hepatitis.

Hsp and the inflammatory process. The alternative between cellular death and survival may be controlled by hsp70 in the case of inflammation. Thus, in cases of acute inflammation, the cells free cytokines, proteases and oxygen species reactive, processes that are accompanied by a massive and rapid decrease of the ATP synthesis, so that cells may die by means of necrosis freeing their toxic content which amplifies the inflammation. In the case of a high rate of synthesis of hsp70 the concentration of ATP is maintained and the cell dies of apoptosis. If the level of hsp70 is balanced, the inflammatory process is limited and it evolves to recovery. Thus, it is considered that the level of hsp70 represents a determining factor in the evolution among necrosis, apoptosis and cure of the inflammation (Cruce et al., 2001).

Emotional, mechanic and oxidative stress induce the expression of hsp. Studies and research on animal models have proved the fact that vascular endothelial cells express a high level of hsp70 whenever blood pressure rises suddenly. In conditions of oxidative stress the hsp expression also amplifies. It was noticed that transgenic mice which express at a high level hsp70 present under conditions of experimental ischaemia minor lesions of the myocardium as compared to the animals in the control lot. This observation suggests that the amplification of the expression of heat shock proteins may determine a protective effect to cardiac failure (Martin et al., 1998).

Neurones are extremely sensitive to stress conditions, sensitivity correlated to the development of certain low intensity stress responses. As different from neurones, glial cells and other non-neurone cells develop a marking response to stress. A specific mechanism of hsp transport from these cells to neurones under conditions of stress has been noticed.

Hsp and the ageing process. Increasing age is associated with a reduced capacity to maintain homeostasis in all physiological systems and it might be that this results, in part at least, from a parallel and progressive decline in the ability to produce heat shock proteins (Bernstein et al., 2000; Gray et al. 2000). An attenuated heat shock protein response could contribute to the increased susceptibility to environmental challenges and the more prelevant morbidity and mortality seen in aged individuals (Rea et al., 2001).

Conclusions

The action of stress factors at cellular level determines the modulation of the expression of specific genes responsible for maintaining cellular homeostasis. Heat shock proteins are extremely versatile and potent molecules, the importance of which to biological processes is highlighted by the high degree to which their structure and function are phylogenetically conserved. Our knowledge of the physiological role of heat shock proteins is currently limited; however, a better understanding of their function and thereby the acquisition of the capacity to harness their power might lead to their use as therapeutic agents and revolutionise clinical practice in a number of areas.

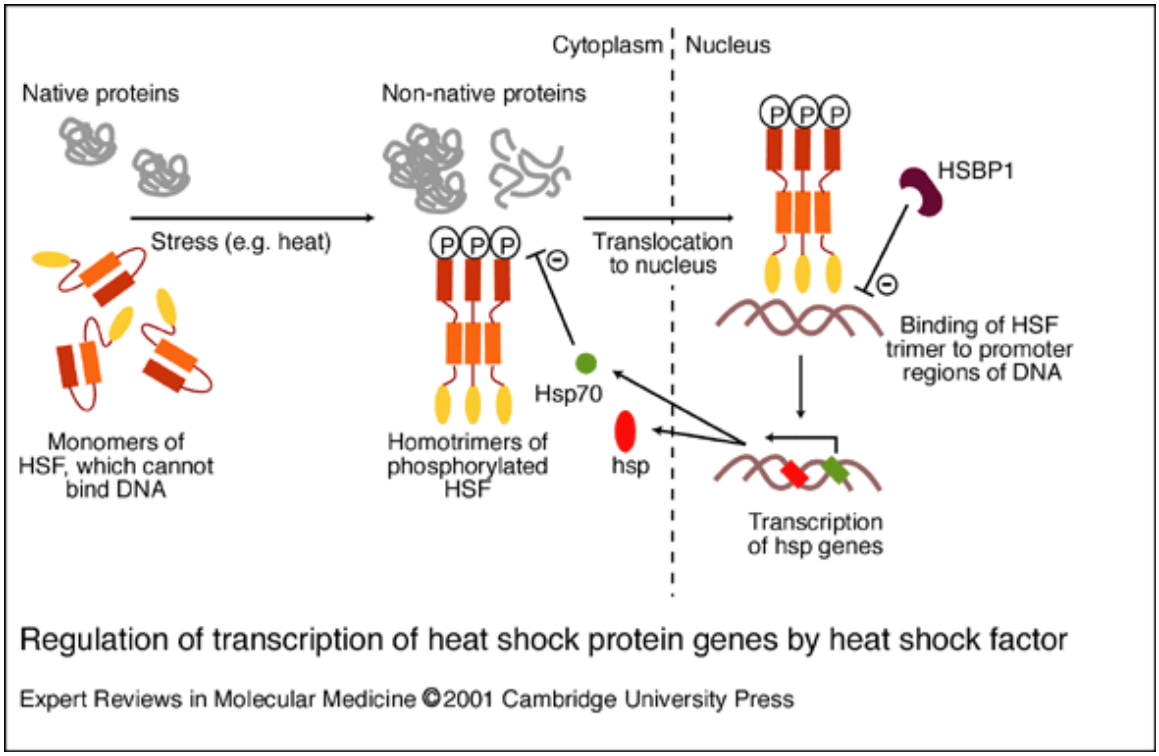

Figure 1. Regulation of transcription of heat shock protein genes by heat shock factor. Heat shock factor (HSF) is present in the cytoplasm as a latent monomeric molecule that is unable to bind to DNA. Under stressful conditions, the flux of non-native proteins (which are non-functional, prone to aggregation, protease-sensitive, and bind to chaperones) leads to phosphorylation (P) and trimerisation of HSF. The trimers translocate to the nucleus, bind the promoter regions of heat shock protein (hsp) genes and mediate hsp gene transcription. The activity of HSF trimers is downregulated by hsps (e.g. Hsp70) and the heat shock binding protein 1 (HSBP1) that is found in the nucleus.

Table 1. Classification of heat shock proteins (Trautinger et al., 1996).

Name	**Molecular weight (kDa, approx.)**	**Cellular location**	**Functional features**
Ubiquitin	8	Cytosol / nucleus	Protein degradation
Hsp10	10	Mitochondria	Cofactor of hsp60
Hsp27	27	Cytosol / nucleus	Growth regulation and differentiation
Heme oxygenase-1	32	Cytosol	UVA-inducible; protection against oxidative damage
Hsp47	47	Endoplasmic reticulum	Collagen chaperone
Hsp56	56	Cytosol	Part of steroid receptor complex
Hsp60	60	Mitochondria	Molecular chaperone
Hsp72	70	Cytosol / nucleus	Molecular chaperone, highly stress inducible
Hsp73	70	Cytosol / nucleus	Constitutively expressed molecular chaperone
Grp75	70	Mitochondria	Constitutively expressed molecular chaperone
Grp78 (Bip)	70	Endoplasmic reticulum	Constitutively expressed molecular chaperone
Hsp90	90	Cytosol / nucleus	Part of steroid receptor complex
Hsp110	110	Cytosol / nucleus	Molecular chaperone, required to survive severe stress

References

- ARRIGO A.P., CHAUFFOUR S., MEHLEN P., JONCA N. Expression of the stress protein hsp27 during differentiation of human leukemic HL-60 cells: possible role in the regulation of cell growth. Canc.Detect.Prev. 19, http://www.cancerprev.org/Journal/Issues/19/1/102/519, (1995).
- APPLEGATE L.A., NOEL A., VILE G., FRENK E., TYRRELL R.M. Two genes contribute to different extents to the heme oxygenase enzyme activity measured in cultured human skin fibroblasts and keratinocytes: implications for protection against oxidant stress. Photochem.Photobiol. 61: 285-291, (1995).
- APPLEGATE L.A., SCALETTA C., FOURTANIER A., MASCOTTO R.E., SEITÉ S., FRENK E. Expression of DNA damage and stress proteins by UVA irradiation of human skin in vivo. Eur.J.Dermatol. 7: 215-219, (1997).
- BERNSTEIN, S.L., LIU A.M., HANSEN B.C. Heat shock cognate-70 gene expression declines during normal aging of the primate retina. Invest.Ophthalmol.Vis.Sci. 41: 2857-2862, (2000).
- CIOCCA D.R., OESTERRICH S., CHAMNESS W.L., MCGUIRE W.L., FUQUA S.A.W. Biological and clinical implications of heat shock protein 27 000 (Hsp27): a review. J.Natl.Cancer Inst. 85: 1558-1570, (1993).
- CRUCE M., PISOSCHI C., STANOIU B., GRIGORESCU M., ARDELEAN A. Factorii de stres si mecanismele moleculare de raspuns la nivel celular. In: Probleme Curente de Biologie Celulara si Moleculara (A.Ardelean, C.Craciun, G.Ardelean, editori), Editura Risoprint, Cluj-Napoca, pp.68-76, (2001).
- ELLIS R.J., VAN DER VIES S.M. Molecular chaperones. Annu.Rev.Biochem. 60: 321-347, (1991).
- GETHING M.-J., SAMBROOK J. Protein folding in the cell. Nature 355: 33-45.
- GRAY, C.C. et al. (2000) Age dependence of heat stress mediated cardioprotection. Ann. Thorac.Surg. 70: 621-626, (1992).
- HAHN G.M., LI G.C. Thermotolerance, thermoresistance, and thermosensitization. In: Stress Proteins in Biology and Medicine (R.I.Morimoto, A.Tissieres, and C.Georgopoulos, eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp.79-100, (1990).
- LI G.C., LI L.G., LIU Y.K. MAK J.Y., CHEN L.L., LEE W.M. Thermal response of rat fibroblasts stably transfected with the human 70-kDa heat shock protein-encoding gene. Proc.Natl.Acad.Sci. USA 88: 1681-1685, (1991).
- LINDQUIST S., CRAIG E.A. The heat shock proteins. Annu.Rev.Genet. 22: 631-637, (1988).
- LINDQUIST S. Autoregulation of the heat-shock response. In: Translational Regulation of Gene Expresion 2 (Joseph Ilan, ed.), Plenum Press, New York, pp.279-320, (1993).
- MORIMOTO R.I. Cells in stress transcriptional activation of heat shock genes. Science 259: 1409-1410, (1993).
- MARTIN J.L., HICKEY E., WEBER L.A., DILLMANN W.H., MESTRIL R. Influence of phosphorylation and oligomerization on the protective role of the small heat shock protein 27 in rat adult cardiomyocytes. Gene Expression 7: 349-355, (1998).

- MORIMOTO R.I. Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev. 12: 3788-3796, (1998).
- POCKLEY A.G. Heat shock proteins in health and disease: therapeutic targets or therapeutic agents ? Exp.Rev.Mol.Med. 21 September, http://www-ermm.cbcu.cam.ac.uk/
  01003556h.htm, (2001).
- POPE R.M., LOVIS R.M. AND GUPTA R.S. Activation of synovial fluid T lymphocytes by 60-kd heat-shock proteins in patients with inflammatory synovitis. Arthritis Rheum. 35: 43-48, (1992).
- PRATT W.B. Interaction of hsp90 with steroid receptors: Organizing some diverse observations and presenting the newest concepts. Mol.Cell.Endocrinol. 74: 69-76, (1990).
- REA I.M., MCNERLAN S., POCKLEY A.G. Serum heat shock proteins and anti-heat shock proteins antibody levels in aging. Exp.Gerontol. 36: 341-352, (2001).
- RITOSSA F.A. A new puffing pattern induced by temperature shock and DNP in Drosophila. Experientia 18: 571-573, (1962).
- ROGALLA T., EHRNSPERGER M., PREVILLE X., KOTLYAROV A., LUTSCH G., DUCASSE C., PAUL C., WIESKE M., ARRIGO A.P., BUCHNER J., GAESTEL M. Regulation of Hsp27 oligomerization, chaperone function, and protective activity against oxidative stress/tumor necrosis factor a by phosphorylation. J.Biol.Chem. 274: 18947-18956, (1999).
- SANCHEZ Y., TAULIEN J., BORKOVICH K.A., LINDQUIST S. Hsp104 is required for tolernce to many forms of stress. EMBO J. 11: 2357-2368, (1992).
- SHI, Y., MOSSER, D.D. AND MORIMOTO, R.I. Molecular chaperones as HSF1-specific transcriptional repressors. Genes Dev. 12: 654-666, (1998).
- SMITH D.F., STENSGARD B.A., WELCH W.J., TOFT D.O. Assembly of progesterone receptor with heat shock proteins and receptor activation are ATP mediated events. J.Biol.Chem. 267: 1350-1356, (1992).
- TISSIERES A., MITCHELL H.K., TRACY, U.M. Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromosome puffs. J.Mol.Biol. 84: 389-398, (1974).
- TRAUTINGER F., KINDÅS-MÜGGE I.,DEKROUT B., KNOBER R.M., METZE D. Expression of the 27-kDa heat shock protein in human epidermis and in epidermal neoplasms: an immunohistological study. Br.J.Dermatol. 133: 194-202, (1995).
- TRAUTINGER F., KINDÅS-MÜGGE I., KNOBER R.M., HÖNIGSMANN H. Stress proteins in the cellular response to ultraviolet radiation. J.Photochem.Photobiol., B: Biol. 35: 141-148, (1996).
- WIESKE M., BENNDORF R., BEHLKE J., DOLLING R., GRELLE G., BIELKA H., LUTSCH, G. Defined sequence segments of the small heat shock proteins HSP25 and aß-crystalin inhibit actin polymerization. Eur.J.Biochem. 268: 2083-2090, (2001).