Int J Clin Lab Res 25: 59–70, 1995

International Journal of Clinical & Laboratory RESEARCH

Reviews

Heat-shock proteins and molecular chaperones: implications for pathogenesis, diagnostics, and therapeutics

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Received: 10 October 1994

Summary. Cells react to physical (e.g., heat) or chemical (e.g., anoxia, low pH) stressors, mounting a stress (heat-shock) response. Most genes are turned down or off, while a few are activated. The latter encode the stress or heat-shock proteins (Hsps), whose levels increase in stressed cells. Various Hsps are molecular chaperones. These, and other molecular chaperones that are not Hsps, help the other cellular proteins to achieve their native state (correct folding or functional conformation), reach their final destination (e.g., the endoplasmic reticulum or the mitochondria), resist denaturing by stressors, and regain the native state after partial denaturation. Thus the Hsps and molecular chaperones occupy the stage's center whenever and wherever there is cellular and tissue injury caused by local or systemic stressors via protein damage. This feature, their participation in protein folding and transport, and their evolutionary conservation within the three phylogenetic domains, strongly suggest a vital role for Hsps and molecular chaperones. Their importance in pathogenesis, and as diagnostic markers and prognostic indicators, is beginning to be appreciated. The role of Hsps and molecular chaperones in cell recovery from injury by a variety of noxae of clinical and surgical relevance is also being assessed. Consequently, the potential of these molecules (and corresponding genes) as targets for treatment or as therapeutic tools is emerging and is being explored. Stroke, myocardial infarction, inflammatory syndromes, infectious and parasitic diseases, autoimmune disorders, cancer, and aging are but some examples of conditions in which Hsps and molecular chaperones are being scrutinized. The era of Hsp and molecular chaperone pathology has dawned. It is likely that genetic and acquired defects of Hsp and molecular chaperone structure and function will be identified, and will play a primary, or auxiliary but determinant, role in disease.

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Key words: Heat-shock protein – Molecular chaperone – Stressor – Stress response – Heat-shock response

Introduction

All cells found in nature today are thought to have evolved starting billions of years ago and have been classified into three evolutionary lines or phylogenetic domains: Bacteria (eubacteria), Archaea (formerly archaebacteria), and Eucarya (eucaryotes) [1]. The former two domains encompass the procaryotes, while Eucarya comprise all organisms, uni- or multicellular, with nucleated cells. Humans are eucaryotes, and human pathogens are found within the Bacteria and the eucaryotes, but none has yet been reported among the Archaea. Nevertheless, the Archaea are important from the medical and public health standpoints for a variety of reasons. For example, Archaea are phylogenetically closer to eucaryotes than are bacteria. This means that archaeal cells may be used as experimental models to solve biochemical and molecular biological problems pertinent to human biology and pathology that are difficult to investigate with the more complex eucaryotic cells or organisms. Also, a group of archaeal species are key to bioconversion technology for processing organic wastes from cities, industries, and agriculture, and, therefore, could play a central role in environmental and public health if duly utilized [2]. Some archaea have been detected in the intestinal tract of mammals, including humans [3], in the periodontal space of humans and other primates [4], and in the vagina [5]. The study of the role of the archaeal organisms in these human and animal ecosystems is only in its infancy; it promises to be a challenging opportunity for medical microbiology and human ecophysiology.

Today, a well-rounded treatment of heat-shock proteins (Hsps), molecular chaperones, and heat-shock genes pertinent to human medicine must include a parallel discussion of data from humans, eucaryotes other than humans, eubacteria, and archaea. This type of com-

Table 1. Cell stressors that induce stress (heat-shock) genes and cause increase of stress proteins[a]

Heat
Anoxia, hypoxia (ischemia)
Infections, inflammation
Fever
Ultraviolet light
Alcohols (ethanol, butanol, others)
Hypersalinity (hyperosmolarity)
Hyposalinity (hyposmolarity)
High or low pH (alkalosis, acidosis)
H_2O_2
Chemotherapeutic agents
Mutagens, carcinogens, teratogens
Anesthetics
Arsenate, arsenite
Nicotine
Nalidixic acid
Metals (Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, others)
Benzene and derivatives
Phenols and derivatives
Insecticides
Pesticides

Cd^{2+}, Cadmium; Cu^{2+}, copper; Zn^{2+} zinc; Pb^{2+}, lead

[a] Stress genes may also be activated by signals linked to the cell cycle and differentiation-development, and by mitogens, denatured self proteins, and growth factors [6, 10, 12–14]

prehensive approach provides the best standpoint to understand the evolution and contemporary structure and function of the heat-shock genes and Hsps.

A living cell, when exposed to a sudden environmental change, for example a temperature or pH up-shift or a rise in the salinity of the pericellular fluid, suffers a shock or stress [6]. The physical and chemical agents that cause cell stress are called cell stressors (in short, stressors, Table 1). If the cell survives, it mounts a stress response, also known as the heat-shock response, although the latter term ought to be reserved for stress caused by heat. The landmarks of the stress response are visible in the protein pattern of the cell's lysate revealed by one-dimensional gel electrophoresis [usually sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)] [6, 7]. Most normal protein bands fade or disappear after heat-shock, while others (half a dozen or more) become prominent or appear as new bands. The proteins that become prominent or appear in response to a stressor are called stress proteins or Hsps. Here again, the name Hsps should only be used to designate those that characterize the response to heat.

Some of the stress proteins are molecular chaperones [8]. These are molecules that help other proteins to acquire the correct folding while they are being synthesized in the ribosome, or shortly thereafter, or to regain a correct folding (re-folding) after partial denaturation, due to a cell stressor for example, or to reach their final destination in the cell [8–10]. Furthermore, molecular chaperones protect polypeptides from denaturation and aggregation in the face of stress and aid in the formation of multimolecular assemblies, in the disassembly of such complexes, in the stabilization of molecules and molecular complexes, and in the presentation of proteins to proteases for degradation. It may be inferred that molecular chaperones are ubiquitous and promiscuous. They are present in every cell, tissue, and organ, and are capable of interacting with many different kinds of proteins. It must be borne in mind that not all molecular chaperones are stress proteins and, vice versa, not all stress proteins are molecular chaperones.

A bit of history, names, classification

It is generally acknowledged that a seminal contribution to the understanding of the heat-shock response was made at the International Laboratory of Genetics and Biophysics in Naples (Italy) at the beginning of the 1960s [11]. It was observed that the polytene chromosomes of the salivary glands of the fruit fly *Drosophila melanogaster* showed "puffs" after the fly had been exposed for a short time to a temperature slightly higher than that which is optimal for growth and development. Nearly 12 years later it was observed that the heat-induced chromosomal puffing was accompanied by overexpression of a set of genes, the heat-shock genes [7]. The chromosomal puffs are sites where gene transcription is taking place, and puffs caused by heat contain heat-shock genes and their transcripts or mRNAs. These will then direct the synthesis of Hsps in the ribosome.

Since these early observations, many more studies of the stress response have been carried out with all kinds of organisms, from bacteria to higher eucaryotes, including human cells [6, 10, 12–14], and more recently with archaeal cells [15]. Organisms that have been and are commonly used for these investigations, in addition to *D. melanogaster*, are yeasts, particularly *Saccharomyces cerevisiae*, bacteria (of which *Escherichia coli* is the most studied), and rodents such as mice and rats. Research in this area has thus evolved from the observation of chromosomal puffs under the microscope to the biochemical characterization of many Hsps, cloning and characterization of heat-shock genes and operons, manipulation of these genes, and the development of the molecular chaperone concept. The evolution of this concept is not exclusively linked to the progress made in the study of the heat-shock response and Hsps, but it is also connected with a change in the ideas concerning how a protein achieves a final, functional conformation, and stays that way even under stress [8].

It ist known from experiments in the early 1970s that an unfolded polypeptide has in its own amino acid sequence all the information it needs to achieve a native state [16]. While this is beyond argument, other factors are now known to participate in protein folding. Molecular chaperones are part of the process [8, 9, 17, 18].

Proteins traverse unstable, easily self-aggregating stages or conformations during synthesis, transport, assembly into oligomers and secretion. They may even go through these "fragile" conformations during the normal exercise of their functions as mature molecules. Molecular chaperones have evolved to protect proteins when they most need outside assistance, i.e., when they are passing through unstable stages. Molecular chaperones are

thus instruments of the cell for building its protein components with functionally correct conformations. These instruments are also used by the cell to maintain the correct protein conformation during stress and to mediate its recovery after partial loss due to the action of a stressor [9, 10, 19]. Furthermore, molecular chaperones mediate the translocation of proteins from their site of origin to their final destination, namely to the place where they are supposed to function, be it the mitochondria, the endoplasmic reticulum (ER), a preferred locale within the cytoplasm, or the chloroplast (in plant cells). Hence, molecular chaperones must be expected to interact closely with the target proteins, and be able to recognize proteins in need of help. Molecular chaperones must, therefore, be able to recognize sites in proteins that are not exposed in the native state, but are exposed in the non-native ones.

The name Hsp for all stress proteins, not only those typical of the response to heat shock, and the term heat-shock response for any stress response, regardless of the causative stressor, are in general use. Thus stress proteins are named Hsp followed by a number which indicates the apparent molecular mass in kilodaltons (kDa) as determined by SDS-PAGE. The best known in eucaryotes are Hsp8, 28, 58, 72, 73, 90, and 110 [10, 13]. There are also stress proteins that were first identified as typical of the cell's response to glucose starvation [10, 13]. These are called Grp (for glucose-regulated protein) followed by a number that indicates the apparent molecular mass. The best characterized in eucaryotes are Grp78, Grp94, and Grp170.

Hsps and Grps occur in the various compartments of the eucaryotic cell: the nucleus, nucleolus, cytosol, mitochondria, ER, and chloroplast (in plant cells). In the procaryotic cells, which do not have organelles, Hsp and Grp proteins are located in the cytoplasm, the cytoplasmic membrane, and the periplasmic space.

There are Hsps that are synthesized in the absence of stress, i.e., they are constitutively expressed, in contrast to counterparts that are only expressed in response to stress [10, 13]. The latter are the inducible Hsps. If in a cell there are two versions of an Hsp, one inducible and the other constitutive, the latter is called the cognate protein, i.e., heat-shock cognate protein abbreviated Hsc. Examples are the Hsp70 and Hsc70 of eucaryotes.

Hsps are classified in practice into families according to their apparent molecular mass [10, 13]. Thus the $\geq$100-, 90-, 70-, 60-, and 40-kDa families have been delineated, plus the small Hsps family, which includes proteins that are $\leq$30 kDa. Table 2 lists Hsps most relevant to this review, which focuses on clinical and laboratory aspects pertinent to mammals and human medicine. As explained above, examples from eubacteria and archaea are also discussed to provide a more solid basis to build upon as we engage in the analysis of the medical aspects of the stress response and molecular chaperones, and to present an outlook of possible areas of exploration as an extension of clinical practice and research.

The limits between the families are not well defined, but the classification helps to organize newly obtained information and classify novel Hsps. Furthermore, proteins of a family may interact with other proteins, referred to as partners or cohorts, to exercise their functions, and these cohorts may differ substantially in molecular mass [9, 17]. For example, the 70-kDa protein DnaK, which represents the procaryotic Hsp70, is a molecular chaperone that interacts with DnaJ (approximately 43 kDa), also a molecular chaperone, and with GrpE (approximately 24 kDa). The three form a complex which acts as a molecular chaperone machine [9]. Likewise, the bacterial 60-kDa protein GroEL, a molecular chaperone of the group also known as chaperonins (abbreviated Cpn) [8], interacts with GroES, which is only 10 kDa. The above examples also introduce the concept that Hsps interact with one another and with non-Hsps to accomplish their objectives in the cell. A chain of reactions usually occur between an Hsp and auxiliary molecules that lead to the formation of complexes better suited for chaperoning target proteins.

Table 2. Stress (heat-shock) proteins and molecular chaperones of interest in pathology and clinical and laboratory medicines[a]

Mass (kDa)	Name	Location
101–200	Grp170	ER
	Hsp110	Nucleus, nucleolus, cytosol
81–100	Hsp90 (Hsp83, Hsp87)	Cytosol
	Grp94 (ERp99)	ER
65–80	Hsp72 (inducible Hsp70, Hsp70i)	Nucleus, cytosol
	Hsp73 (constitutive Hsp70, Hsc70)	Nucleus, cytosol
	Clathrin-uncoating ATPase (CUATPase)	Cytosol
	Grp78 (BiP)	ER
	Grp75 (mtc70)	Mitochondria
55–64	Hsp60 (TCP-1)	Cytosol
	Hsp58 (Cpn60)	Mitochondria
35–54	HSJ1	Cytosol
	HDJ1	Cytosol
	Hsp40 (human DnaJs)	Nucleus, cytosol
>35 (including small Hsps)	Hsp27 (Hsp28)	Cytosol
	Human GroES	Cytosol
	Ubiquitin	Nucleus, cytosol
Other	Calnexin	ER
	PPIases (immunophilins)	Cytosol, ER, mitochondria
	PDIase	Er

ER, Endoplasmic reticulum; kDa, kilodaltons; Hsp, heat shock protein

[a] Referenes [10, 12–14, 20, 22, 23, 25, 26, 32, 35–42, 47–50, 54]

The Hsps and molecular chaperones

Grp170

Stressors such as glucose starvation, glucosamine, calcium ionophores, chelants, and anoxia affect the functions of the ER and cause synthesis of Grps, chiefly Grp78 and Grp94, and to a lesser extent that of another protein of 150–170 kDa, named Grp170 [10, 20]. The latter resides

in the lumen of the ER, in the pre-Golgi compartment. It co-precipitates with Grp78 (the B-cell immunoglobulin-binding protein) and Grp94, when lysates of stressed Chinese hamster ovary cells are reacted with anti-Grp170 antiserum. It also co-precipitates with immunoglobulin in lysates of B-cell hybridomas expressing surface IgM, or cytoplasmic immunoglobulin light-chain only, or secreted antigen-specific IgG. These and other data suggest that Grp170 is retained in the ER lumen and is constitutively expressed, and that it might play a role in immunoglobulin folding and assembly in co-operation with Grp78 and Grp94.

The Hsp100 family

Hsp110. This protein is constitutively expressed and is present at low levels in the cytoplasm, nucleus, and nucleolus of mammalian cells [10, 13]. It increases after heat shock in the nucleolus near the region where rRNA genes are being transcribed. Its functions have not yet been elucidated.

Hsp104. This molecule is the yeast's homolog of the mammalian Hsp110 [21]. It plays a key role in cell survival under extreme conditions and in acquired thermotolerance [10, 21]. This is a cell state characterized by the capacity to survive a lethal temperature induced by a previous exposure to a sublethal temperature. Hsp104 promotes protein disaggregation as it confers thermotolerance. In procaryotes, the homolog molecules also promote protein disaggregation and participate in the replication of certain plasmids and phages. The *E. coli* representatives of this family are the Clp proteins: ClpA and ClpX which serve as cohorts for the protease ClpP [10, 21].

Hsp90. This protein occurs in the eucaryotic cytosol and is very abundant even in the absence of cell stress [10, 13]. It increases still further after heat shock. It is one of the most abundant proteins in mammalian cells and participates in a number of interactions with several important molecules. For instance, it binds to steroid hormone (glucocorticoids, estrogen, progesterone, testosterone) receptors and stabilizes them [10, 22]. Hsp90 is believed to increase the hormone receptor binding affinity for the hormone and also prevent the binding of the receptor to the corresponding DNA elements while not bound to the hormone. Hsp90 seems to change the configuration of the receptor molecule in such a way that DNA binding is prevented while hormone binding is favored. The cohorts of Hsp90 are Hsp56 (of the immunophilin family of *cis-trans* prolyl isomerases), p50, and p23 (50 and 23 kDa, respectively), and a 63-kDa protein.

While Hsp90 is cytosolic, its counterpart in the ER is Grp94 [10, 13, 23]. The latter resides in the lumen of the ER and its function(s) is not yet fully understood. The information available up to this point suggests that Grp94 is involved in protein secretion pathways. In yeasts, the homologs are Hsp83 and Hsc83, while HtpG is the *E. coli* counterpart [10, 12, 13, 24].

The Hsp70 family

Hsp70. Important components of the Hsp70 family in mammalian cells are Hsp72 and Hsp73 [10, 13]. The former is restricted to stressed cells, whereas Hsp73 is constitutively expressed in all cells and tissues. Thus, Hsp73 is known as the constitutive Hsp70 or as the cognate heat-shock protein 70, or Hsc70. In contrast, Hsp72 is referred to as the inducible mammalian Hsp70. The two proteins are very similar in amino acid sequence and are present in the nucleus and cytoplasm (both are in fact considered to be examples of cytosolic Hsp70). They seem to be involved in the translocation of proteins from the cytosol into either the ER or the mitochondria, and in protein maturation (folding) during and after synthesis in the ribosome.

Hsp70 also plays a role in disassembling multiprotein complexes or protein polymers. This is evident, for example, in bacterial systems developed for studying phage λ and plasmid P1 replication [9]. In eucaryotes, disruption of clathrin cages in endocytic vesicles is mediated by a constitutively expressed Hsp70, the clathrin-uncoating ATPase [25].

Another important member of the Hsp70 family is Grp78, or BiP (for binding protein), also named immunoglobulin heavy chain binding protein, which resides in the ER's lumen [10, 26]. It binds immunoglobulin heavy chains and would thereby participate in the assembly of molecules that are being prepared for secrection out of the cell. Still another important member of the Hsp70 family is the mitochondrial homolog Grp75 of mammalian cells. In yeasts, the proteins SSA1–4, KAR2, and SSC1 are the cytosolic, ER, and mitochondrial homologs, respectively [24, 27].

The procaryotic counterpart is the DnaK protein found in bacteria and some archaea [8, 9, 15]. The bacterial and archaeal DnaKs have the cohorts known as DnaJ and GrpE. DnaK and DnaJ are molecular chaperones, and both interact with one another, and with GrpE, in a variety of situations in which protein assembly, disaggregation, and oligomerization occur [8, 9, 28].

DnaK and the eucaryotic Hsp70 proteins all possess nucleotide binding capacity, particularly for ADP and ATP. These proteins are amongst the most conserved molecules across the three phylogenetic domains. They have been found in bacteria, eucaryotes, and in archaea [15, 29, 30]. Interestingly, however, Hsp70 (DnaK) homologs have not been detected in several archaea, including extreme thermophiles and one species of methanogen [31]. While these are negative results and ought to be considered with caution, the absence of DnaK represents an evolutionary puzzle. It also poses the questions of how cells lacking this Hsp that plays a central role in cell survival and adaptation have managed to thrive, and of what have they evolved to replace the chaperone functions of DnaK. When present, the Hsp70-DnaK proteins are highly conserved in terms of amino acid sequence. Consequently, they have been chosen for phylogenetic analyses [29, 30].

The Hsp60 family or chaperonins

Hsp60. Proteins of these family are called chaperonins [8, 32]. The eucaryotic cytosol contains a ring-shaped molecular chaperone composed of several different subunits coded by separated but related genes. This multimolecular assembly includes the *t*-complex polypeptide 1 (TCP-1), and is called CCT (for chaperonin-containing TCP-1), or TRiC (for TCP-1 ring complex), or TCP-1 complex [32].

The role of the complex in the heat-shock response and in thermotolerance was made evident by studies in archaea [33]. It was found that the archaeon *Sulfolobus shibatae* (extreme thermophile) has a cytoplasmic chaperonin termed TF55 (for thermophilic factor of approximately 55 kDa). The TF55 amino acid sequence is 40% homologous with that of the mammalian TCP-1. TF55 becomes a prominent band in the SDS-PAGE pattern of *S. shibatae* cells that have been heat shocked and made thermotolerant.

The bacterial Hsp60 is GroEL, which resides in the cytoplasm [8, 9]. Equivalents are found in eucaryotes, like the Hsp60 of the mitochondrial matrix and the Rubisco binding protein of the chloroplast inner compartment [8–10, 12, 13, 32].

The TCP-1 complex of eucaryotes includes the 60-kDa subunit and several others that are smaller but equally important for the formation of an efficient chaperone machine [32, 34, 35]. In bacteria, GroEL has as the smaller cohort a 10-kDa molecule named GroES, which is also referred to as co-chaperonin [8, 9, 28, 32]. Recently a human gene encoding a human GroES homolog has been cloned and sequenced [36].

The TCP-1 complex resides in the eucaryotic cytosol, where it mediates folding of non-native proteins to the native state. By comparison, the Hsp70 chaperones maintain proteins in an unfolded and relatively extended conformation to avoid misfolding while they mature to acquire the correct, final, folded state.

The mammalian and yeast mitochondrial homolog is the Hsp58 or Hsp60 [10, 13]. This protein is encoded by nuclear genes, is synthesized in the cytoplasm, and is finally translocated to the mitochondria. In this location, it is assumed that Hsp58 forms a complex similar to that formed by the cytosolic chaperonin TCP-1, to carry out the same type of chaperone activity. Like the Hsp70 proteins, the 60-kDa Hsps are conserved in the three phylogenetic domains. This suggests that they are important for survival and adaptation to changing environments.

The Hsp40 family

Hsp40. The prototype protein of this group is called DnaJ. It plays a role as Hsp and molecular chaperone in protein biogenesis, thermotolerance, DNA replication, and other important cellular activities. DnaJs have been extensively studied in bacteria, particularly in *E. coli* [8, 9, 28]. The Hsp70 protein DnaK plays an important role intracellularly, while associated with DnaJ (and GrpE) [9, 28, 37]. DnaJ homologs found in human cells are the proteins HSJ1, HDJ1, and Hsp40 [10, 13, 38–42]. They are present in the cytosol. Other eucaryotic homologs have been identified in the mitochondria of *S. cerevisiae* (protein SCJ1), cytosol and nucleus (protein SIS1), and cytosol (protein YDJ1). Similar equivalents have been reported for other eucaryotes, bacteria, and an archaeal species [43]. Therefore, DnaJ, like its partner Hsp70-DnaK, is conserved in the three phylogenetic domains. The degree of conservation of the amino acid sequence is lower than that observed for Hsp70-DnaK if the entire molecule is considered. However, DnaJ possesses short sequence stretches of eight amino acids that are highly conserved in all species examined. These highly conserved "motifs" are called signatures, and their functional role has not been elucidated. However, one may assume that the function of the signatures is critical for cell survival and adaptation, considering their conservation with virtually the same sequence in all molecules examined.

The small Hsp family

GrpE. This Hsp is about 23 kDa and has been found in bacteria, archaea, and the mitochondria of *S. cerevisiae* [9, 28, 44, 45]. Thus far it has not been demonstrated in other eucaryotes, or even in the cytosol of *S. cerevisiae*. This is puzzling since GrpE plays an important role in many cellular functions as a partner or cohort of DnaK and DnaJ, in bacteria, in the mitochondria of *S. cerevisiae*, and presumably also in archaea. The questions of what replaces GrpE in the eucaryotic cytosol, or why GrpE cannot be detected in it, have not yet been answered. Like that of DnaJ, the amino acid sequence of GrpE, as a whole, is considerably less conserved than that of Hsp70-DnaK. However, like DnaJ, GrpE possesses signatures (of 6 and 9 amino acids) that are highly conserved [44]. Their biological role has not been elucidated.

Hsp27/28. In the fruit fly *D. melanogaster* and in plants, heat shock induces synthesis of several low molecular mass ($\leq$30 kDa) Hsps [46]. In contrast, mammalian and avian cells show only one (or very few) of these light Hsps, that is within the range 25–30 kDa. The protein is referred to as Hsp28, or Hsp27, or Hsp24 [10, 12–14, 24]. Proteins of this low molecular mass family resemble the α-crystallin of the eye lens, and like it, form large aggregates. These aggregates are located in the cytoplasm near the Golgi apparatus. Hsp27/28 relocates to the nucleus after heat shock. It suppresses protein aggregation and heat inactivation. Hence, it is assumed that Hsp27/28 plays a critical role in the establishment of thermotolerance. In *D. melanogaster*, low molecular mass Hsps are developmentally regulated.

Hsps involved in proteolysis

Several Hsps are molecular chaperones that protect polypeptides in various ways so that they acquire and

maintain (or regain) a functional conformation and assemblage [8–10, 17–19, 24, 28, 32, 47]. In contrast, some Hsps also participate in protein degradation, perhaps when the damage caused by a cell stressor is too advanced to allow reconstitution, even with the help of molecular chaperones [19, 28, 47, 48]. Polypeptides with irreversible damage must be eliminated. To achieve this purpose, the cell is equipped with proteolytic enzymes collectively called proteases. Some Hsps interact with damaged polypeptides and "present" them to the proteases for digestion. The best known example of an Hsp that helps in protein degradation is ubiquitin (approximately 8 kDa) [10, 19, 47–49]. In eucaryotes, this protein "tags" the 26-S proteasome where proteolysis occurs. Procaryotic functional equivalents of ubiquitin are the Lon (90 kDa) and Clp proteins, which are also present in the mitochondria of eucaryotes (e.g., the PIM1 protein, which is a Lon homolog) [9, 10, 13, 19, 28, 47, 48]. The Clp proteins also have dual roles in *E. coli*: molecular chaperoning, protection and proteolysis.

Molecular chaperones involved in protein secretion

A group of proteins are dedicated to chaperone polypeptides destined to be excreted [47, 48]. These "dedicated" chaperones help the target protein to be transported out of the cell, to find its way through the cell membrane. There is not much information concerning the chaperone machines involved in this translocation mechanism in eucaryotes. The best known system operates in *E. coli*. The chaperone molecule is SecB, which is a homopolymer of four subunits of 16 kDa each. SecB interacts with SecA, which in turn interacts with SecY during translocation of the target peptide from the cell's inside to its outside. The PaD protein plays a role similar to that of SecB. PaD resides in the periplasmic space of Gram-negative bacteria and seems to be involved in the assembly of pili structures.

Calnexin

This protein has recently been recognized as a possible member of the molecular chaperone superfamily [48]. It is an integral membrane protein of the ER that protrudes into the ER's lumen. It is required for the oligomeric assembly of class I proteins of the MHC [50]. Calnexin interacts with the target peptide in a manner influenced by the degree of glycosylation of the latter.

Peptidyl-prolyl cis-trans isomerases

These molecules are present in the cytosol, ER, and mitochondria of eucaryotic cells, and some are known under the name immunophilins [18, 47, 48]. They catalyze *cis-trans* isomerization of proline residues, so that the polypeptide acquires its functional conformation. The immunophilins are interesting also because they are inhibited by immunosuppressant drugs like cyclosporine and FK506.

Yeasts have at least eight different immunophilin genes [48, 51]. In *S. cerevisiae*, two peptidyl-prolyl-*cis-trans* isomerases (PPIases), one in the ER and the other in the cytosol, are Hsps necessary for survival under heat stress. Two PPIases have been identified in *E. coli*, one located in the cytoplasm and the other in the periplasmic space [52]. In *Legionella pneumophila*, the *mip* gene encodes a PPIase homolog necessary for virulence [53].

Protein disulfide isomerase

This enzyme catalyzes the isomerization and oxidation of intramolecular disulfide bonds, and thereby enhances correct protein folding [47, 48, 54]. Protein disulfide isomerase (PDIase) is a subunit of several enzyme complexes in the ER. In *S. cerevisiae* it is located in the lumen of the ER and is essential for viability [55]. The periplasmic space of Gram-negative bacteria may be considered an equivalent of the ER's lumen and is the most oxidizing environment of these microbes. The PDIase homolog of *E. coli*, termed DsbA, resides in the periplasmic space. Other similar proteins, DsbB and DsbC, are located in the inner membrane and are in solution in the periplasmic space, respectively [56, 57].

Intramolecular chaperones

Some polypeptides possess a segment of sequence which acts as a chaperone with regard to the rest of the molecule. This segment, prosequence, or intramolecular molecular chaperone occurs in an array of secreted proteases [58]. The prosequence is a cleavable portion of the molecule which enhances proper folding and transport of the proteolytic domain. The cleavable prosequence is a high-affinity inhibitor of the protease activity, presumably to prevent digestion of the very cell that synthesizes the proteolytic enzyme.

The stress (heat-shock) response and molecular chaperones in experimental pathology and clinical and laboratory medicine

The stress response is highly conserved in all living cells of the three phylogenetic domains: Bacteria, Archaea, and Eucarya [6, 12–15]. This observation alone strongly suggests a critical biological role for stress genes and proteins. It is not yet clear, however, what role the stress response, genes, and proteins play in causing and/or preventing cellular injury and disease and in recovery from stress or illness. Consequently, it is not clear at the present time how the stress response, genes, and proteins might help in diagnosis and in assessing prognosis, or how they could be modulated and manipulated for preventing and treating disease. Ongoing research will soon elucidate some of these points. What follows is a brief survey of some of the most promising investigations.

Aging

One aspect of getting old is the accumulation of damage. Cells, tissues, organs, and molecules are damaged by physical and chemical agents or stressors throughout life [59]. Some stressors are endogenously produced by injured cells, thus perpetuating a potentially pathogenetic cycle of events. Modern civilization entails greater and greater exposure to cell stressors present in foods, water, air, soil, medicines, maternal milk, etc., some of which are listed in Table 1. Genetic systems have evolved to deal with cell stressors and their consequences, represented by damaged or foreign molecules. In the face of stress, many genes are down-regulated or turned off. In contrast, other genes are activated and their products increase by a combination of transcriptional and post-transcriptional regulatory mechanisms. These increased gene products, the Hsps, are supposed to enhance cell resistance to stressors, survival, and recovery from injury. It seems that these mechanisms become less and less efficient with advancing age [59].

Experiments have shown that the stress response occurs rapidly (within minutes) after a cell or animal is exposed to a stressor [6, 10, 59]. In the whole animal, the response can be localized at the site of tissue lesion and also elsewhere. For example, in the rat overexpression of *hsp70* occurs in various tissues in the course of ether anesthesia, surgery, and elevation of the body temperature even by only 1.5 °C above the physiological level [59]. Particularly interesting is the demonstration that placing rats in a well-ventilated, comfortable restraining device causes an increase of the *hsp70* gene expression in the adrenal and blood vessels [59]. Thus, a comparatively minimal trauma caused by a restraining maneuver induces the stress response. This response depends on the endocrine hormonal system and seems to be regulated at the transcriptional level. Hypophysectomized rats do not show a stress response upon restraining. Adrenocorticotropic hormone treatment induces *hsp70* gene expression in the adrenals. The promptness and intensity of the stress responses due to restraining and hormones decline considerably as the age of the adult rat increases [59].

Age and diet

It is claimed that the only known means to slow down the aging process is a caloric restriction in the diet [60]. Hepatocytes from old rats responded to heat shock with increased synthesis of Hsp70, and *Hsp70* mRNA, but up to levels that were 40%–50% lower than those observed with hepatocytes of young adult rats. This age-dependent effect was due to a decline in *hsp70* gene transcription in the hepatocytes of the old rats compared with young counterparts. This trend was eliminated by caloric restriction. Hepatocytes from old rats that had been fed only 60% of the ad libitum diet showed *hsp70* gene transcription levels similar to those of hepatocytes from young rats.

Ischemia, Hypoxia

Perhaps the most dramatic effects of vascular occlusion with interruption of the blood flow are felt in the heart and the brain. In these and other organs the central necrotic focus of the infarct is surrounded by cells damaged in various degrees. A gradient of injury forms which declines away from the infarct's center. Cells that have sustained reversible damage mount a stress response, which is most pronounced upon reperfusion [10, 13, 61, 62]. Such response has been observed not only in the heart and brain, but also in the kidney and liver.

Heart

Increased transcription of the *hsp70* gene in the myocardium has been observed in cardiac hypertrophy due to aortic stenosis and other situations with augmented heart workload [61, 63, 64]. The biochemical mechanism of the *hsp70* gene induction upon ischemia of the heart wall has not been elucidated yet, but glucose deprivation, acidosis, and low ATP levels may play a role. In addition, denatured unfolded peptides may contribute to stimulate *hsp70* expression. Important points that remain to be clarified are whether all myocardial cell types, i.e., cardiomyocytes, Purkinje fibers, endothelial cells, and fibroblasts, are equally affected by ischemia in terms of stress response, and whether other heat-shock genes, such as *hsp60*, are also induced in addition to *hsp70*.

Recently, expression of the ubiquitin, *hsp27*, and *hsp60* genes was studied in normal and in briefly ischemic and reperfused (stunned tissue) porcine myocardium [65]. Increased levels of ubiquitin and *Hsp27* mRNAs were detected in the stunned myocardium, but no changes in the *hsp60* transcript levels were observed.

A new *hsp70* gene has been identified in the rat myocardium [66]. The gene is induced by hypoxia and heat-shock through a mechanism that involves the same heat-shock elements for both stressors, although activation by a temperature up-shift reached levels severalfold higher than those induced by hypoxia. This observation lends support to the idea that a slight temperature elevation could be used as a means of enhancing resistance to hypoxia prior to an operation which will decrease circulation in the heart's wall. Indeed, the possibility of protecting the myocardium from the consequences of ischemia and hypoxia by preconditioning via a mild stress is under investigation [48, 61]. For this purpose, drugs that induce expression of *hsp70* (Table 1) may be more convenient and safer than a temperature elevation. One can imagine that such preconditioning might be applied preoperatively, before cardiopulmonary bypass for example. Further along this line of reasoning, preventive gene grafting in cells at risk can be envisaged. For this, means ought to be developed to deliver the gene to the target cell and to insert it in the proper chromosomal site.

Brain

Cerebral ischemia may be localized (stroke) or generalized (as in cardiac arrest or cardiocirculatory shock due to other mechanisms) [62, 67]. Expression of the inducible *hsp72* gene has been investigated in the brain by applying immunohistochemistry with anti-Hsp72 antibodies and dot blotting, and in situ hybridization with nucleic acid probes for *Hsp72* mRNA, in tissue sections [62, 67–69]. After global ischemia, *Hsp72* expression predominated in neurones. Expression was manifest at the level of transcript but was considerably less pronounced at the level of protein (detection of Hsp72 with antibody). These observations indicated that, although *Hsp72* transcription was augmented, translation did not follow to the same extent, or if it did Hsp72 was degraded faster than under normal conditions.

Focal ischemia due to vascular occlusion leads to infarction of the zone irrigated by the occluded vessel. The lesion has a necrotic center surrounded by an area in which a gradient of cellular injury occurs, which declines away from the center towards irrigated regions. Expression of the *hsp70* gene parallels this gradient, and is not restricted to neurones but also affects other brain cell types [10, 62, 67–69]. In general, the data indicate that *hsp72* transcription and high levels of Hsp72 occur preferentially in surviving cells closer to irrigated areas.

Tolerance to ischemia has been observed, reminiscent of thermotolerance, after a mild heat shock [62, 69, 70]. Thus, the phenomenon of cross-tolerance applies also to the pair ischemia and heat as cell stressors of brain cells. As in the case of cardiac surgery, protection of brain cells may then be achieved by a preoperatory treatment with drugs, for example, aimed at increasing the levels of Hsp72 in cells at risk. The difficulties with this approach are the same as those mentioned for the heart. Recently, it has been reported that neurones and glia transfected with an expression vector containing the inducible *hsp70* gene (*hsp70i*) increased cell survival after severe stress [71]. The data suggested that overexpression of the *hsp70i* gene protected the neurones and glia from the denaturing effects of thermal stress.

Little is known beyond *hsp70* gene expression in the brain upon global ischemia. There is scarce information on expression of other stress genes and levels of molecular chaperones other than Hsp70. Apparently, ubiquitin mRNA is augmented as a consequence of global ischemia, but details on this Hsp's role in pathogenesis or cell survival in the brain are lacking.

Hyperthermia

Since a temperature elevation, i.e., fever, is a very common component of illness, and since a temperature upshift induces the stress response, it follows that the latter response must be a frequent manifestation of many clinical syndromes and diseases. It is not yet established what role the stress response plays in pathogenesis or whether the Hsps participate in the organism's reaction to the disease. The possibility exists that Hsps are useful diagnostic markers and prognostic indicators. In this regard, measuring levels of inducible versus constitutive *hsp70* gene transcripts and protein products has been shown to be critical [68]. This is particularly pertinent to measurements in tissues such as brain, in which constitutive expression of *hsp70* is relatively high [13, 68]. To detect cell stress due to hyperthermia (or ischemia) in these tissues with high levels of constitutive *hsp70* expression, measurement of the inducible *hsp70* gene expression is necessary to document that the cells have indeed been stressed and have responded.

The potential of Hsps as therapeutic adjuvants in the management of diseases with fever ought to be investigated. Likewise, the consequences of a chronic stress response due to protracted fever, with elevated Hsp levels in the tissues and blood, could be deleterious [10, 72] and must also be investigated. It may be necessary sometimes to turn down the stress response to avoid disregulation of the genes involved, and to prevent homeostatic imbalance due to an excess of Hsps. These considerations are all the more relevant for the management of infectious diseases.

Infections and parasitic diseases

When a bacterium or parasite invades the tissues of a complex organism (host), it is attacked by the latter, and itself mounts a stress response [72]. The bacterial or parasitic Hsps from destroyed cells enter the host's extracellular fluids and blood circulation and gain access to the host's immune system. This, in turn, mounts an immune response against the foreign Hsps, which are known to be strong immunogens. Anti-microbial Hsp antibodies appear in the serum and constitute a definite sign of infection. The host's cells also mount a stress response because of the usually accompanying fever and also because of other reasons, among which cellular injury by the invading organism and its toxins is a major one. Increased levels of the host's own Hsps may be found in the circulation, which serves as an indicator of cell stress. These self Hsps in increased levels may induce autoimmunity, the consequences of which have not yet been elucidated [72]. Cross-reactivity between the foreign Hsps and host proteins of the stress proteins superfamily, or of any other group, may also lead to autoimmunity.

To fully characterize the role of Hsps in infectious and parasitic diseases more investigation is needed. The participation of Hsps in pathogenesis and in the host's eventual success against the invading organism, if indeed success is achieved, has to be elucidated. Straightforward medical applications can be envisaged in the development of diagnostic tests based on the measurement of Hsp levels in biological fluids, which could also be used for monitoring disease, tissue lesion progression, and the patient's response to treatment.

The mycobacterial antigens that are Hsps have been reviewed [73–75]. The heat-shock response of *Mycobacterium tuberculosis* has been characterized [74]. A temperature up-shift from 37 °C to 42 °C induced increased syn-

thesis of DnaK, GroEL, and GroES. In contrast, Schwann cells from mice and monkeys responded to heat shock and infection with *Mycobacterium leprae* by increasing the synthesis of Hsp70 only [76]. Infection of the J774 macrophage-like cells of mouse origin with *Listeria monocytogenes* induced transcription of the *hsp70* and *hsp90* genes [77].

Similar observations have been reported for parasites and parasitic diseases. For example, a major immunogen of *Schistosoma mansoni* has been found which is homologous to Hsp70 [78]. It has been reported that constitutively expressed Hsp70s in *S. mansoni* and *Schistosoma japonicum* induced a significant antibody response in humans infected with either one of these parasites [79]. The Hsp70 proteins of these two *Schistoma* species are not cross-reactive. Their respective, distinctive antigenic sites are located near their C-terminal ends, where the amino acid sequence similarities are lowest. A detailed comparison of the sequences revealed few amino acid differences between the two schistosomal proteins and between them and the human homologs. The conclusion was drawn that even small structural differences can elicit discriminatory antibody responses. We have made similar observations comparing an archaeal DnaK with the homolog of *E. coli* (Macario et al., unpublished data). Expression of Hsp65 and 67, and of a Hsc70 of *Leishmania donovani* has been observed in macrophages infected with this parasite [80]. The suggestion was made that these proteins play a pathogenetic role, although this remains to be proven.

Trypanosoma cruzi has a cytoplasmic/nuclear Hsp70 and a mitochondrial homolog (mtp70) [81]. Both are potent ATPases that bind ATP avidly, while mtp70 but not Hsp70 possesses autophosphorylation activity. The role of these proteins in the host's reaction to the parasite or in pathogenesis has not yet been elucidated.

Autoimmunity

Immune reactions against self can be beneficial or pathogenic. Antibodies and T cells are involved which recognize self antigens and react with and against them. For example, elimination of senescent or damaged cells and molecules by the immune system seems necessary for maintaining physiological homeostasis. This mechanism could very well apply to the elimination of damaged or abnormal Hsps. It is possible that an alteration of such homeostatic mechanisms could lead to autoimmunity against normal Hsps. Likewise, the presence of foreign Hsps released by bacteria and parasites, with antigenic determinants resembling those of the host's Hsps or those of other host's (non-Hsp) molecules, may induce autoimmunity against the host's structures. Increased levels of host's Hsps have been found in a number of pathological conditions, including inflammatory syndromes and autoimmune diseases [10, 48, 72]. Examples are rheumatoid arthritis, ankylosing spondylitis, chronic gastritis, Crohn's disease, Graves' disease, multiple sclerosis, psoriasis, systemic lupus erythematosus, inflammatory myopathies, polymyositis, and insulin-dependent diabetes mellitus. The role of self Hsps in the development and progression of these pathological conditions is not yet completely understood. However, investigation in this area is intensive because Hsps are potentially convenient targets as diagnostic markers and as prognosis and response-to-treatment indicators. In a recent study on the antigenicities of the human inducible Hsp72 and constitutive Hsc73, it was shown that at least three regions in Hsp72 are potentially strong T-cell-dependent immunogens [82]. In addition, a redistribution of Hsp72 to the cell surface was observed in stressed cells, which would increase the probability of an autoimmune reaction against Hsp72 and the cells coated with this protein.

Hsps as immunogenic carriers

Hsps are considered to be strong immunogens [72, 75]. Consequently, they have been used as carriers for less immunogenic molecules or haptens, to elicit antibodies against these weak immunogens. The same approach has been proposed to prepare vaccines against pathogens. One example is the use of Hsps as carriers for malarial antigens [83].

Cancer

It has been shown that immunization with either one or the other of these three Hsps, Hspgp96, Hsp90, and Hsp70, isolated each from a different tumor, elicited a specific immune response against the tumor of origin [84, 85]. gp96 primed $CD8^+$ T cells and induced anti-tumor immunity in vivo [86]. Heat shock and other stresses induce, in tumor cells, resistance to cytotoxicity mediated by tumor necrosis factors. At least part of this resistance is due to Hsp70; Hsp27 plays no role [87]. Studies with human breast cancer cell lines have shown that Hsp27 plays a role in the establishment of tumor cell resistance to drugs such as doxorubicin [88].

The role of Hsps in oncogenesis was investigated by examining the action of ansamycins on *v-src*-mediated malignant transformation [89]. It was found that geldanamycin binds Hsp90 in a stable and specific manner, inhibits formation of the Hsp90-pp60$^{v\text{-}src}$ heteroprotein complex and thereby inhibits malignant transformation. Mouse Hsc70 binds p53 in cells transformed by mouse mutant p53 and *ras*. The *hsc70* gene efficiently suppressed focus induction (transformation) by mutant p53 plus *ras*, and by *myc* plus *ras* [90]. This anti-oncogenic effect required a complete and functional Hsc70 molecule.

Cystic fibrosis

Deletion of the amino acid 508, phenylalanine or Phe-508, from the transmembrane conductance regulator is the most common variant of cystic fibrosis (mutation ΔF508). The Δ508 cystic fibrosis transmembrane conductance regulator (CFTR) is unstable and possesses a glycosylation pattern specific to the ER. Recently it has been

reported that, while the normal CFTR is translocated to the plasma membrane with the assistance of the chaperone Hsp70, the Δ508 CFTR is retained in the ER [91]. Newly synthesized CFTR complexes with Hsp70. The wild type CFTR dissociates from Hsp70 before it is transported to the Golgi, and then it is degraded in the lysosomes. In contrast, the complex Hsp70-Δ508 CFTR is retained in the ER and then is rapidly degraded in a pre-Golgi, non-lysosomal compartment. The mutation Δ508 in the CFTR affects its translocation to the proper cell's locale because it affects the binding of CFTR with the molecular chaperone that would direct it towards its physiological destination. This is one clear example of pathology in which failure of a molecular chaperoning step leads to serious disease.

Perspectives for the future

Progress is to be expected in the next decade on the following aspects: (1) novel Hsps and molecular chaperones will be discovered in many more eucaryotes and procaryotes; (2) more stress genes will be cloned and sequenced, and their regulation will be elucidated; (3) hence, the structure, function, and evolution of Hsps and molecular chaperones will be better understood as more molecules from a progressively larger number of species will be characterized, particularly those from archaea that live in extreme environments; (4) the physiological role of Hsps and molecular chaperones in humans will be defined and their role in pathogenesis will be assessed; (5) consequently, the potential of Hsps and molecular chaperones as diagnostic markers, prognostic indicators, and therapeutic targets or tools will become clearer, and exploited accordingly; (6) it will be learned when and how to manipulate the stress response, proteins, and genes, to prevent or cure diseases in which these molecules are involved. The era of molecular chaperone pathology has dawned. It is likely that many diseases will be found to be caused or perpetuated by disorders in the chaperoning functions of one or another chaperone, due to genetic or acquired defects.

Acknowledgements. I thank Dr. Everly Conway de Macario for critical discussions and Tracy L. Godfrey for word processing. The author's work was supported by grant No. 706-RIER-BEA-85 from NYSERDA.

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