Pharmacol. Ther. Vol. 80, No. 2, pp. 183–201, 1998

ISSN 0163-7258/98 $19.00
PII S0163-7258(98)00028-X

Associate Editor: P. K. Chiang

Heat Shock Protein 70 kDa: Molecular Biology, Biochemistry, and Physiology

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ABSTRACT. Heat shock proteins (HSPs) are detected in all cells, prokaryotic and eukaryotic. *In vivo* and *in vitro* studies have shown that various stressors transiently increase production of HSPs as protection against harmful insults. Increased levels of HSPs occur after environmental stresses, infection, normal physiological processes, and gene transfer. Although the mechanisms by which HSPs protect cells are not clearly understood, their expression can be modulated by cell signal transducers, such as changes in intracellular pH, cyclic AMP, Ca^{2+}, Na^{+}, inositol trisphosphate, protein kinase C, and protein phosphatases. Most of the HSPs interact with other proteins in cells and alter their function. These and other protein–protein interactions may mediate the little understood effects of HSPs on various cell functions. In this review, we focus on the structure of the HSP-70 family (HSP-70s), regulation of HSP-70 gene expression, their cytoprotective effects, and the possibility of regulating HSP-70 expression through modulation of signal transduction pathways. The clinical importance and therapeutic potential of HSPs are discussed. PHARMACOL. THER. 80(2):183–201, 1998. **© 1998 Elsevier Science Inc.**

KEY WORDS. Heat shock proteins, Ca^{2+}, cAMP, inositol trisphosphate, gene regulation, heat shock factors.

CONTENTS

ABBREVIATIONS. BiP, bacterial GRP-78; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; cAMP, cyclic AMP; GroEL, *E. coli* chaperonin 60 kDa protein; GRP, glucose-regulated protein; HSC-70, heat shock cognate 70 kDa; HSE, heat shock element; HSF, heat shock transcription factor; HSP, heat shock protein; HSP-70s, HSP 70 kDa family; $InsP_3$, inositol 1,4,5-trisphosphate; MDJ, mitochondrial DnaJ homolog; MHC, major histocompatibility complex; mt-HSP-70, mitochondrial GRP-75; pH_i, intracellular pH; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; Scj, *S. cerevisiae* DnaJ; Sec, *S. cerevisiae* protein; Sis, yeast DnaK homolog; TNF, tumor necrosis factor; Ydj, yeast DnaJ; YGE, mitochondrial GrpE-homolog Yge1P.

1. INTRODUCTION

Heat shock proteins (HSPs) are present in both prokaryotic and eukaryotic cells. Their highly conserved structure suggests that they play a role in fundamental cellular processes.

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This article was written by the authors in their private capacity. No official support or endorsement by the U.S. Department of the Army is intended or should be inferred.

As the name suggests, HSPs are induced in cells exposed to sublethal heat shock. The first report on HSPs appeared in 1962; after *Drosophila* salivary gland cells were exposed to 37°C for 30 min and then returned to their normal temperature of 25°C for recovery, a "puffing" of genes was found to have occurred in the chromosome in the recovering cells (Ritossa, 1962), accompanied by an increase in the expression of proteins with molecular masses of 70 and 26 kDa (Tissieres *et al.*, 1974). These proteins were named "heat shock proteins." Since then, a large number of proteins in addition to these, also collectively referred to as HSPs, have been discovered.

Heat shock is not the only stimulus that can induce and increase synthesis of HSPs. Exposure of cells to amino acid analogs (Kelley and Schlesinger, 1978; Li and Laszlo, 1985), glucose analogs (Pouyssegur *et al.*, 1977), heavy metals (Levinson *et al.*, 1980), protein kinase C (PKC) stimulators (Ding *et al.*, 1996), Ca^{2+}-increasing agents (Ding *et al.*, 1996), ischemia, sodium arsenite (Johnson *et al.*, 1980), microbial infections, nitric oxide, hormones, and antibiotics also induce the expression of HSPs. The nomenclature for HSPs in prokaryotic and eukaryotic cells differs (Table 1).

HSPs are present in the cytosol, mitochondria, endoplasmic reticulum, and nucleus. They typically have a relatively long half-life (48 hr in human epidermoid cells). HSP-78, -75, -60, and -10 are present mainly in the organelles, whereas HSP-110, -90, -73, -72, and -20 are detected in the cytosol and the nucleus (Table 1). Increased expression of HSPs has been shown to be protective in many cultured cells and animal tissues. The protection probably is mediated by the HSPs' capacity to function as molecular chaperones to prevent inappropriate protein aggregation and to mediate transport of immature proteins to the target organelles for final packaging, degradation, or repair. In this review, we mainly discuss what is known about the induction and regulation of the HSP 70 kDa family (HSP-70s). We also describe the relationship of HSP-70s to signal transduction and other cytosolic or nuclear proteins, the protective roles of HSP-70s, and the clinical implications of HSP-70s.

2. HEAT SHOCK PROTEINS

The best understood HSPs are those with molecular masses of 60, 70, 90, and 110 kDa. These major HSPs are expressed at 37°C in the absence of heat shock. HSP-70 and -90 are observed in all organisms, whereas HSP-110 is present mainly in mammalian cells. A second group of HSPs (sometime referred as minor HSPs) are induced under conditions of glucose deprivation and include glucose-regulated proteins (GRP) 34, 47, 56, 75, 78, 94, and 174 kDa (Sciandra and Subjeck, 1983). A third group of HSPs is the low-molecular-mass HSPs; these have molecular masses of about 20 kDa; these are found at elevated levels in heated *Drosophila* cells (Tissieres *et al.*, 1974) and ischemic cardiomyocytes (Mestril *et al.*, 1994). A small portion of their amino acid sequence is similar to mammalian α-crystallin (Ingolia and Craig, 1982). In our laboratory, heating fails to increase these low-molecular weight HSPs in human epidermoid A-431 cells, human mammary cancer cells, Jurkat cells, rat pituitary, and rat thyroid cells.

2.1. *Molecular Structure of the Heat Shock Protein 70 kDa Family*

HSP-70s are highly conserved and demonstrate a 60–78% base identity among eukaryotic cells and a 40–60% identity between eukaryotic HSP-70 and *Escherichia coli* DnaK (similar to the HSP-70) (Bardwell and Craig, 1984; Craig, 1985; Lindquist, 1986; Caplan *et al.*, 1993).

All HSP-70s bind ATP (Chappell *et al.*, 1987; Milarski and Morimoto, 1989). Figure 1A shows the molecular structure of human heat shock cognate 70 kDa (HSC-70),

TABLE 1. HSP Nomenclature and Intracellular Locations

HSP		Cohorts		
Eukaryotic	**Prokaryotic**	**Eukaryotic**	**Prokaryotic**	**Location of HSP**
HSP-110/104	C1P family	?	?	Cytosol/nucleus
HSP-90	HtpG	HSP-56	?	Cytosol/nucleus
GRP-78 (Bip)	DnaK	Sec63, Scj1 Ydj1, Sis1	DnaJ	Endoplasmic reticulum
HSP-73	DnaK	Sec63, Scj1 Ydj1, Sis1	DnaJ	Cytosol/nucleus
HSP-72	DnaK	Sec63, Scj1 Ydj1, Sis1	DnaJ	Cytosol/nucleus
GRP-75	DnaK	Sec63, Scj1 Ydj1, Sis1	DnaJ	Mitochondria/chloroplast
HSP-60	GroEL	HSP-10	GroES	Mitochondria/chloroplast
HSP-56	?	?	?	Cytosol
HSP-47	?	?	?	Endoplasmic reticulum
HSP-20	?	?	?	Cytosol/nucleus
HSP-10	GroES	GroEL	?	Mitochondria/chloroplast
Ubiquitin	?	?	?	Cytosol/nucleus

C1P, HSP-100 homolog; GroES, *E. coli* chaperonin 10 kDa protein; HtpG, HSP-90 homolog.

the constitutive form of HSP-70 present at low levels in unstressed cells. The 44-kDa fragment (amino acid residues 1–386) from the N-terminus has been characterized by X-ray crystallography, which indicates that there are four domains forming two lobes with a deep cleft between (Bork *et al.*, 1992; Flaherty *et al.*, 1990). The 18-kDa peptide-binding domain (amino acid residues 384–543) consists of two four-stranded antiparallel β-sheets and a single α-helix, as determined by multidimensional nuclear magnetic resonance (Morshauser *et al.*, 1995). The 10-kDa C-terminus (residues 542–646) has been predicted using the Garnier algorithm to be primarily α-helix, followed by a glycine/proline-rich aperiodic segment next to the highly conserved EEVD terminal sequence (Hightower *et al.*, 1994). These four terminal amino acids are present in all eukaryotic HSP-70 and -90 and affect the amount of mRNA translated during heat shock (Denisenko and Yarchuk, 1990). Figure 1A also shows the molecular structure of human HSP-70. The 44- and 18-kDa fragments are the same as those composing HSC-70. The 10 kDa C-terminal of HSP-70 differs by 26 amino acid residues relative to HSC-70 and is 6 amino acids shorter (Leung and Hightower, 1997).

Recent biochemical studies of HSC-70 fragments generated using recombinant DNA technology have led to a mapping and characterization of the domains (Fig. 1B). The 44-kDa fragment contains the ATPase domain. The 18-kDa fragment contains the peptide-binding domain that binds unfolded and folded peptides (Wang *et al.*, 1993). Gragerov *et al.* (1994) used DnaK (prokaryotic HSP-70) to screen a phage-display peptide library and found that peptides such as NRLLLTG, containing internal aliphatic residues, are preferred substrates for HSPs and that basic residues favor binding whereas acidic residues disfavor it. Using bacterial GRP-78 (BiP), Flynn *et al.* (1989, 1991) also identified preferred peptide sequence. In eukaryotic cells, HSC-70 binds two different peptide motifs, based on results obtained by screening phage-display peptide libraries. One motif, containing large hydrophobic and aromatic residues such as FYQLALT, is a relatively good stimulator of ATPase activity and probably represents sites used by HSC-70 to distinguish the native form, unfolded protein conformation (Fourie *et al.*, 1994). The second motif, containing a combination of hydrophobic and basic residues such as NIVRKKK, is a poor stimulator of ATPase activity and may be involved in chaperoning proteins to organelles or binding certain unfolded proteins (Takenaka *et al.*, 1995). The binding of NIVRKKK in HSC-70 to phage-display peptides, which occurs primarily by electrostatic interactions, has a K_i of 2.5 μM (Takenaka *et al.*, 1995). Wang *et al.* (1993) indicate that the HSC-70 peptide binding domain appears to be very stable and relatively independent of the rest of the molecule. The binding site may be located

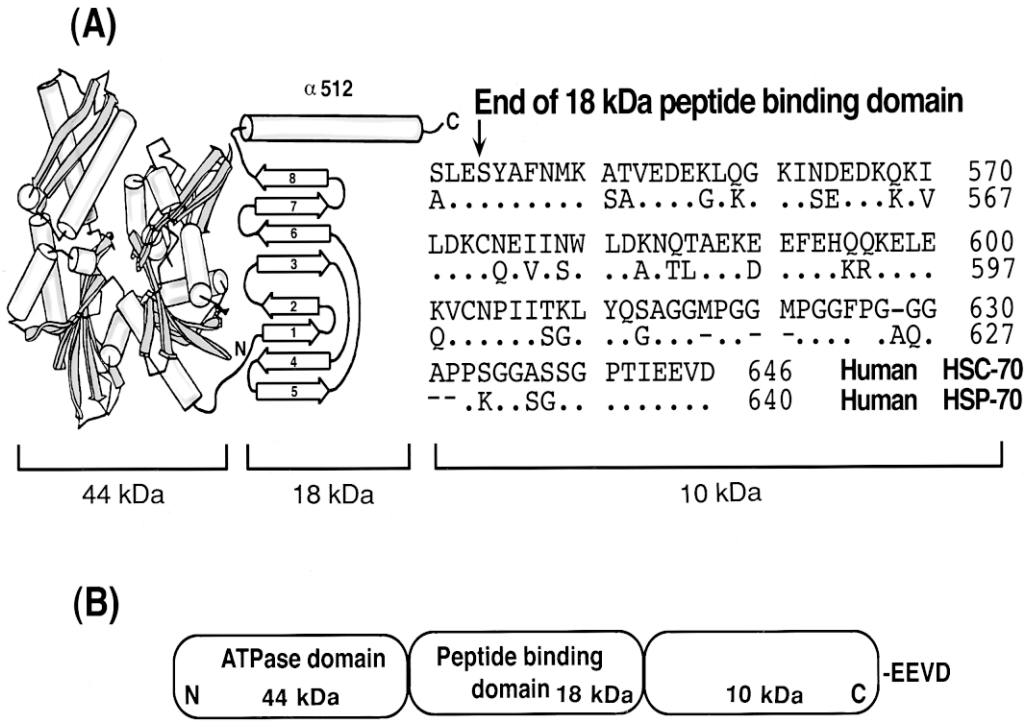

FIGURE 1. Molecular structure of human HSC-70 and human HSP-70. (A) 44-kDa fragment (amino acid residues 1–386) at N-terminus contains 4 domains forming 2 lobes with a deep cleft between. 18-kDa fragment (amino acid residues 384–543) contains two 4-stranded antiparallel β-sheets and single α-helix. 10-kDa fragment (amino acid residues 542–646 for HSC-70 and 542–640 for HSP-70) at C-terminus conserves EEVD terminal sequence. (B) N-terminal 44-kDa domain is ATPase domain; 18-kDa domain is peptide-binding domain; C-terminal 10-kDa fragment carries highly conserved EEVD terminal sequence, which is present in all eukaryotic HSP-70 and HSP-90. Reproduced with permission of L. E. Hightower.

on the α-helix. The potent immunosuppressor 15-deoxyspergualin, a synthetic antitumor agent known to bind HSC-70, could be carried by such a site.

2.2. *Heat Shock Protein 70 kDa Gene Regulation*

2.2.1. Heat shock elements. Most HSP genes, including HSP-27, -60, -70, -78, and -90, have been cloned from several organisms. Genomic footprinting of the human HSP-70 promoter has revealed that heat shock induces a rapid binding of heat shock transcription factors (HSFs) to a region encompassing five nGAAn sequences named heat shock elements (HSEs), three perfect and two imperfect matches to the consensus sequence (Fig. 2). In mammalian and *Drosophila* cells, no HSFs are bound to these HSEs before or after recovery from heat shock (Abravaya *et al.*, 1991; Zimarino *et al.*, 1990). The sequence of HSEs at Site 3 and Site 4 is dyad symmetrical, and HSFs bind preferably to HSEs at Sites 3 and 4 (Kroeger *et al.*, 1993; see Section 2.2.2 for details).

Figure 3 is a schematic representation of the interactions between HSPs and HSF. HSPs are bound to HSFs that reside in the cytosol of mammalian cells under unstressed conditions (Schlesinger, 1990). Under stress conditions such as heat shock and ischemia, HSFs are separated from the HSPs. When HSFs are then phosphorylated by PKC or other serine/threonine kinases, they form a homotrimeric structure (Kroeger *et al.*, 1993). The trimers enter the nucleus, bind to HSEs located on the promoter region of HSP genes, and become further phosphorylated by HSF kinases (Price and Calderwood, 1991). Transcription is then initiated, followed by translation. The newly synthesized HSPs bind to HSFs to prevent further synthesis of HSPs. Li *et al.* (1995) have presented evidence that Ku protein is a constitutive HSE-binding factor. Under unstressed conditions, Ku protein binds to HSE to prevent HSF binding.

Unlike mammalian cells, the HSFs in *Sacchromyces cerevisiae* are constitutively bound to the HSE (Jakobsen and Pelham, 1988; McDaniel *et al.*, 1989; Sorger *et al.*, 1987). It has been proposed that a protein named extragenic suppressor inactivates HSF (Halladay and Craig, 1995). However, Høj and Jakobsen (1994) have suggested that phosphorylation of serine-460 of yeast HSFs causes its inactivation.

2.2.2. Heat shock factors. Four different HSFs have been identified in vertebrates: HSF1, HSF2, HSF3, and HSF4 (Morimoto *et al.*, 1996; Nakai *et al.*, 1997). HSF1 responds to the kinds of stimuli listed in Table 2, whereas HSF2 responds only to hemin (Sistonen *et al.*, 1992). It is known that HSFs have a binding domain, a helical trimerization surface, and a short conserved element (Conserved element-2). The binding domain is responsible for the binding to both protein and HSE. The trimerization surface has leucine zipper coiled-coil motifs involved in trimer formation. In yeast, HSF is encoded by a unique, single copy gene. Although *Drosophila* has been found to have only one gene, other eukaryotic cells have multiple genes encoding HSFs. For example, there are two genes in mice and humans and three genes in chicken and tomatoes. The molecular masses of the HSF monomer in *S. cerevisiae*, *Drosophila*, and humans are 93, 77, and 57 kDa, respectively (Fernandes *et al.*,

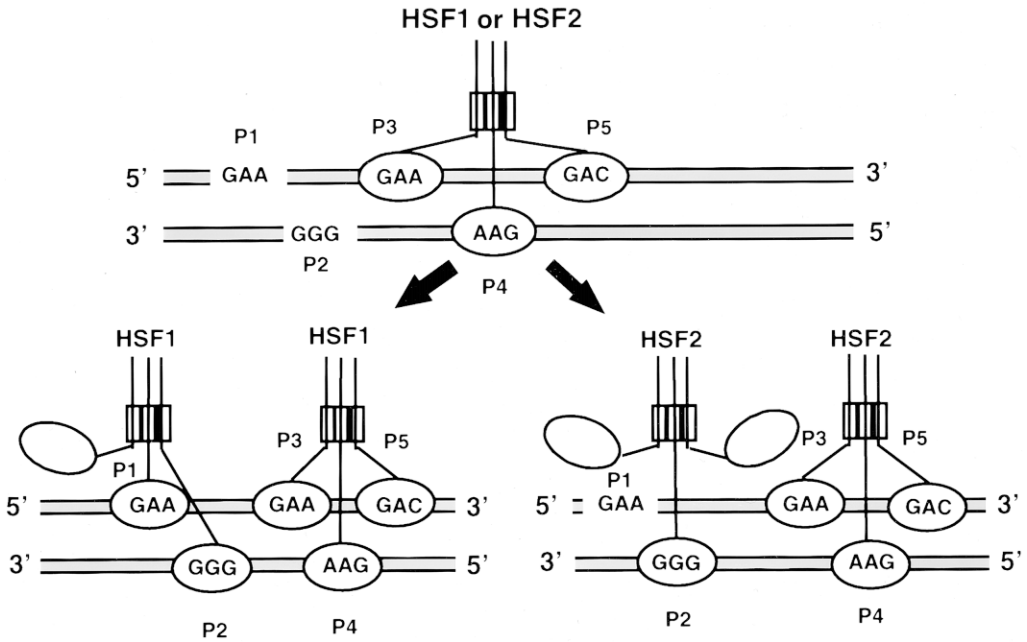

FIGURE 2. Binding of HSFs to HSEs. Three perfect matches of nGAAn and two imperfect matches, nGACn and nGGGn, located on the promoter of HSP-70 gene are present. HSEs at positions 3 (P3) and P4 are symmetrical and favor binding of trimers of HSF1 and HSF2. The second molecule of HSF1 then binds to HSEs at P1 and P2, whereas HSF2 binds to HSE only at P2.

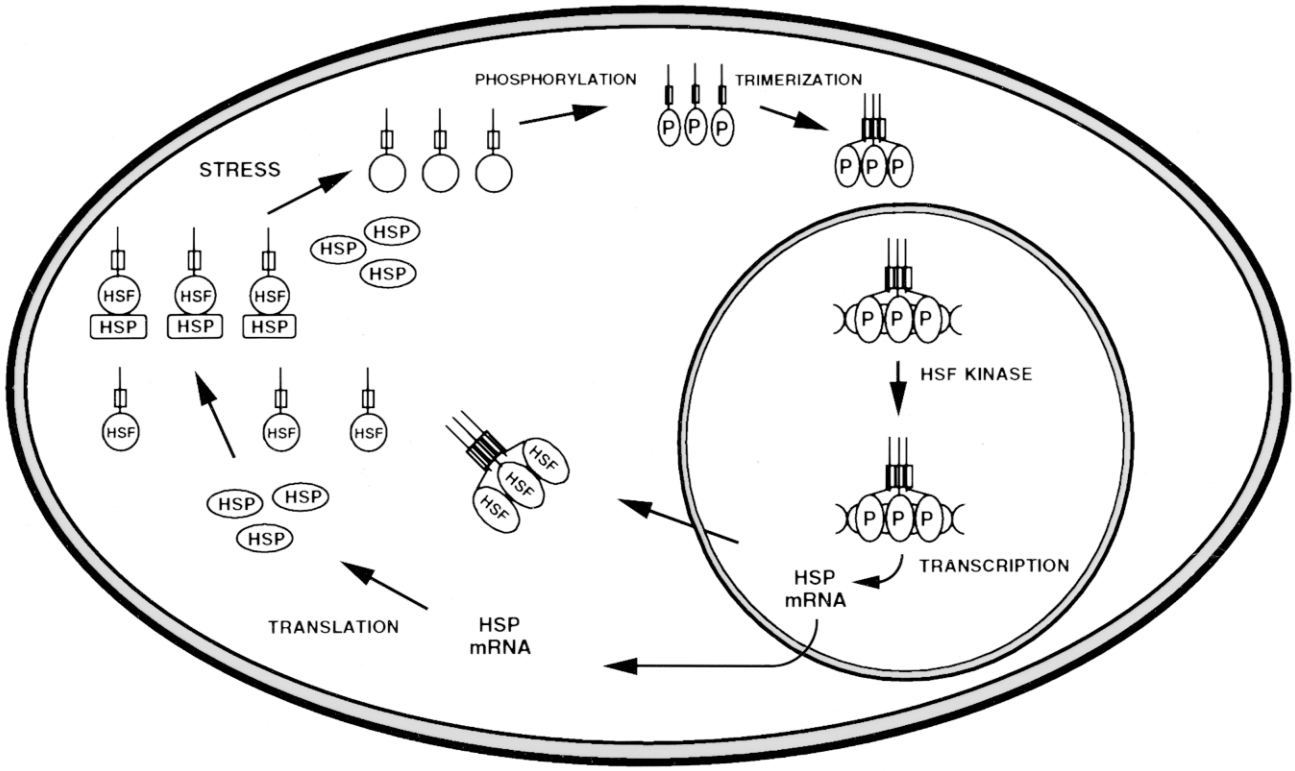

FIGURE 3. Proposed mechanism of stress-induced increase in HSPs in human and *Drosophila* cells. HSFs residing in the cytosol are normally bound by HSP and are inactive. Under stress, such as heat shock, HSFs separate from HSP, are phosphorylated by protein kinases such as PKC, and form trimers in cytosol that enter the nucleus to bind HSEs in the promoter region of HSP gene. HSF is phosphorylated further, and HSP mRNA is transcribed and leaves the nucleus for cytosol. In cytosol, new HSP is synthesized. HSF returns to the cytosol and is bound once again by HSF.

1994; Morimoto *et al.*, 1996). Kroeger *et al.* (1993) reported that mouse HSF1 binds all 5 HSEs, whereas mouse HSF2 does not interact with Site 1 of HSE, but binds to Sites 2–5. Missing-nucleoside analysis demonstrated that the third and fourth nGAAn sites are essential for HSF1 and HSF2 binding. HSF1 and HSF2 trimers bind initially to Sites 3, 4, and 5, the second HSF1 trimer binds to Sites 1 and 2, but the second HSF2 trimer binds to Site 2 only (Fig. 2). The nature of the differences between the specificity of HSF1 and HSF2 binding to Site 1 is not clear.

TABLE 2. Conditions that Induce Expression of HSPs

Physiological	Pathological	Environmental
Cycle of cell division	Viral infection	Heat shock
Growth factors	Bacterial infection	Heavy metals
Cell differentiation	Parasitic infection	Metabolic inhibitors
Tissue development	Fever	Amino acid analogs
Hormonal stimulation	Inflammation	Ethanol
	Ischemia	Antibiotics
	Hypertrophy	Radiation
	Oxidant injury	
	Malignancy	
	Autoimmunity	

HSF3 is constitutively expressed in the avian erythroblast cell line HD6, the lymphoblast cell line MSB, and embryo fibroblasts. It is a homolog of mouse and human HSF1 and HSF2 and is negatively regulated in cells. Its DNA-binding activity is acquired by heat shock (Nakai *et al.*, 1995).

HSF4 is preferentially expressed in the human heart, brain, skeletal muscle, and pancreas. It lacks the carboxyl-terminal hydrophobic repeat that is shared among all vertebrate HSFs. In HeLa cells, HSF4 represses expression of the endogenous HSP-27, -70 , and -90 (Nakai *et al.*, 1997).

In human epidermoid cells, translocation of HSF1 from the cytosol to the nucleus is regulated by cytosolic free Ca^{2+}, PKC (Kiang *et al.*, 1996a; Ding *et al.*, 1996), and the levels of cytosolic HSP-70 (Ding *et al.*, 1998). Furthermore, the level of HSF1 phosphorylation is modulated by cytosolic HSP-70 levels (Ding *et al.*, 1998). Other laboratories have reported similar observations in NIH 3T3 cells (Price and Calderwood, 1991) and HeLa cells (Mosser *et al.*, 1990).

The HSP-70 gene regulation responsible for synthesis of constitutive HSP-70 is not clearly understood, but HSFs seem not to be involved. In quiescent cells, the rat constitutive HSP-70 gene expresses fairly low levels of mRNA that are not heat inducible. In growing cells, this gene expresses high levels of mRNA that are also not inducible by

heat. Its promoter region contains two double HSEs, several SP1-binding sites, and two CCATT boxes within 250 bp of the TATA box (Bienz and Pelham, 1987). The role of HSEs in promoters that do not respond strongly to heat shock is yet to be determined. The rat GRP-78 promoter does not have HSEs, a finding that indicates that other transcription factors are involved in the constitutive expression of this gene (Lin *et al.*, 1986; Ramakrishnan *et al.*, 1995; Zhou and Lee, 1998). Unlike constitutive HSP-70, the HSP-90 gene normally is expressed at a high level and responds to heat shock. In *Drosophila*, there is a single heat-inducible HSP-90 gene. Its promoter contains three HSEs with three good matches to the heat shock consensus sequences adjacent to the TATA-box. These HSEs are not accessible to restriction enzymes or exonucleases under either stressed or unstressed conditions (Wu, 1985), a possible indication that HSFs bind to HSEs of the HSP-90 gene more tightly than to HSEs of the HSP-70 gene.

The rate of degradation of HSFs may determine the level of expression of HSP-70. It has been found that rapid degradation of HSF1 is the cause of decreases in HSP-70 expression in lymphoma cells; these decreases are reversed by treatment with prolactin (Blake *et al.*, 1995). Evidence has been presented suggesting that phosphorylated, trimerized HSF1 translocated to the nucleus is not sufficient in itself to cause increased transcription of HSP-70 (Davidson *et al.*, 1995). Other factors that have to be delineated are apparently involved in the expression of the HSP-70 gene. The regulation of the expression of GRP-78 and -94, and other molecular chaperones, seems also to involve multiple mechanisms (Ramakrishnan *et al.*, 1995; Zhou and Lee, 1998).

2.3. *Stimulation of Heat Shock Protein 70 kDa Induction*

HSPs have been observed in every cell type and tissue, under both unstressed and stressed conditions. An accumulation of evidence has shown that environmental and pathological stresses (Table 2) induce HSPs, especially the inducible form of HSP-70, HSP-72. The degree of induction depends on the level and duration of exposure to stress. The increase is transient, but how long it persists is different in various cell types. Using [^{35}S]methionine incorporation, we have measured newly synthesized HSP-70 and -90 in human epidermoid A-431 cells, rat thyroid cells, human Jurkat cells, rat GH_3 cells, and human breast cancer cells. The maximal rate of HSP synthesis occurs 3–5 hr after heat shock and ceases after 8 hr. The increase in HSP-70 is much greater than in HSP-90 (Kiang *et al.*, 1994). This difference may be due to the fact that the basal levels of HSP-90 are already high or that HSP-90 is not specifically involved in cell-protection mechanisms. The HSP levels after heat shock remain above basal levels for days to weeks, depending on cell types.

Under normal conditions, levels of constitutive HSPs increase during the cell cycle and during development. Two kinds of increases in constitutive HSPs are observed, one transient and the other sustained. The transient increase is exemplified by the ecdysone induction of *Drosophila* HSP-26 and -27 expression during the late larval and early pupal stages (Sirotkin and Davidson, 1982; Mason *et al.*, 1984; see review by Bienz and Pelham, 1987). In mammalian cells, the constitutive HSP-70 gene responds to serum stimulation, which reflects a transient activation of this gene during the S phase of the cell cycle (Milarski and Morimoto, 1986). It is also known that a germ cell-specific HSP-70 is expressed during spermatogenesis (Krawczyk *et al.*, 1987; Zakeri and Wolgemuth, 1987). In human breast cancer cells (Kiang *et al.*, 1997) and other types of cells (Hsieh *et al.*, 1996), GRP-75, -78, and -94 do not respond to heat shock because HSEs are absent on these genes (Lin *et al.*, 1986). Sustained increases are observed with the *Drosophila* HSP-83 and mammalian HSP-90 genes because their HSEs, located on the promoter of the genes, are not accessible to attack by restriction enzymes or exonucleases. Furthermore, the HSPs have a high binding affinity of their HSFs. Also, unlike the constitutive HSP-70s, heat shock can induce increases in HSP-83 and -90.

Production of HSPs can be regulated by hormones (Table 2). HSPs have been shown to be present in human endometrium and their levels correlate with levels of estrogen and progesterone during the menstrual cycle (Tang *et al.*, 1995). In the rat ventromedial hypothalamus, basal HSP-70 levels are lower in females than in males, whereas HSP-90 levels are similar in both sexes. Subcutaneous injection of estrogen causes significant elevation of HSP-70 and -90 in female, but not male, rats (Olazabal *et al.*, 1992). Cultured breast cancer MCF-7 and MDA-MB-231 cells, which express high levels of HSP-70 and -27, exhibit resistance to treatment with doxorubicin, an antitumor agent (Ciocca *et al.*, 1982; Shen *et al.*, 1987). In our laboratory, we found that 17β-estradiol at 1 nM (a physiological concentration) induces GRP-78 and -94 in human breast cancer T47D cells (Kiang *et al.*, 1997). Treatment with catecholamine in the Wistar rat results in an induction of HSP-70 (Udelsman *et al.*, 1994). On the other hand, glucocorticoid agonists are reported to inhibit HSP-70 expression (Heufelder *et al.*, 1993).

2.4. *Heat Shock Protein 70 kDa Functions*

Extensive research indicates that a major function of some HSP-70s is that of a molecular chaperone. The constitutive members of the HSP-70 family, including nuclear/cytoplasmic HSC-70, mitochondrial GRP-75 (mt-HSP-70), and GRP-78 in the endoplasmic reticulum are involved in two distinct chaperone functions in normal unstressed cells. In the first, HSP-70 family chaperones pass the newly synthesized, unfolded proteins to members of the HSP-60 family of chaperonins eventually leading to folded proteins. In the second, these HSP-70 chaperones carry proteins for translocation into different cellular compartments. Both functions involve the interaction of different binding motifs instead of nonspecific hydrophobic interactions. For example, the nuclear localization sequence PKKKRKV of the SV40 large

T antigen is a short, basic peptide located on the surface of the proteins. HSP-70 and HSC-70 bind to the PKKKRKV amino acid stretch and chaperone SV40 large T antigen into the nucleus (Shi and Thomas, 1992). The signal is KFERQ for translocation to lysosomes. If HSC-70 is involved in a similar fashion in carrying peptides during antigen processing by antigen-processing cells, then it may play a role in modifying antigen presentation, either by blocking the presentation of endogenous antigens or by adding new antigens for presentation. Finally, HSC-70 and HSP-70 may serve as cohort (i.e., assistant) proteins to other proteins such as glial-axon transfer proteins. It is known that both HSC-70 and HSP-70 can interact directly with fatty acids and that this interaction may be part of their mode of binding to cell membranes (Guidon and Hightower, 1986). If these proteins indeed do have special membrane-penetrating capability, either intrinsically or in association with transporter complexes, this feature would be very useful in assisting in the design of drug-delivery vehicles. The two β-sheet structures confirmed by nuclear magnetic resonance are the most hydrophobic parts of HSC-70 and thus are likely to be involved in the binding of hydrophobic peptides (Leung and Hightower, 1997).

Cohort proteins play an important role in HSP function. Table 1 lists selected cohort proteins. In *E. coli*, it is evident that the cohort protein DnaJ stimulates the ATPase activity of Dnak. In this way, protein substrates bound by DnaK can be released from DnaK (Caplan *et al.*, 1993; Silver and Way, 1993). Eukaryotic homologs of DnaJ such as HSP-40 (a stress-inducible basic protein isolated from HeLa cells) have been discovered recently (Hattori *et al.*, 1993), but it is not known how HSP-40 assists HSP-70 in performing its function. Other cohort proteins for HSP-70 in eukaryotic cells include *S. cerevisiae* protein (Sec) 63, *S. cerevisiae* DnaJ (Scj) 1, yeast DnaJ (Ydj) 1, and yeast DnaK homolog (Sis) 1 (DnaJ homologs). Unlike cohort proteins of HSP-70, the cohort proteins for HSP-90 and -60 are HSP-56 and -10, respectively, which are themselves classed as HSPs.

HSPs help cells to resist noxious stimuli both *in vitro* and *in vivo* (see Section 4 for details), and are thought to be mediated by an augmentation of functions performed by the inducible members and up-regulated constitutive members of HSP-70, such as solubilization of denatured protein aggregates, facilitation of the restoration of the function of the renatured proteins, and transportation of irreversibly damaged proteins to degradative organelles and proteasomes. Table 3 lists a brief description of each HSP function.

2.4.1. Protein translocation across mitochondrial membranes. The outer membrane of mitochondria has a receptor complex consisting of at least six different proteins that mediate binding of incoming proteins and insertion into the translocation pore (Kiebler *et al.*, 1993). Neupert and Pfanner (1993) proposed that spontaneous "breathing" of the proteins on the outside is sufficient to allow the partial entry and passage of a segment of the unfolded protein through the translocation pore and into the outer and inner membranes. Once that segment has entered, it binds mitochondrial HSP-70, which requires hydrolysis of ATP that exists in the micromolar range in the mitochondrial matrix. The signal peptide of the unfolded protein is then cleaved by matrix processing peptidase. Mitochondrial DnaJ homolog (MDJ) and mitochondrial GrpE-homolog Yge1P (YGE) mediate the folding of the unfolded protein-mitochondrial HSP-70 complex (ATP hydrolysis is required). Alternatively, this complex is caught by HSP-60 for folding (see review by Langer and Neupert, 1994). HSP-60 is a homo-oligomeric protein composed of 14 subunits that are arranged in two-stacked heptameric rings, thereby forming the characteristic barrel-like structure (Hutchison *et al.*, 1989). A schematic representation of the transport of unfolded polypeptides across mitochondrial membranes is presented in Fig. 4.

2.4.2. Protein translocation across membranes of endoplasmic reticulum. BiP has been shown to be associated with the transport of nascent polypeptides across the endoplasmic reticulum, a process requiring ATP binding and hydrolysis (see review by Brodsky and Schekman, 1994). A schematic representation of this process is presented in Fig. 5. A nascent polypeptide first engages an acceptor protein complex at the endoplasmic reticulum membrane, which contains an acceptor protein, a Sec63, and a BiP. This step does not require ATP. The nascent polypeptide then contacts Sec61 located on the membrane. This interaction, requiring ATP, is poorly understood. The third step involves the association of the polypeptide-Sec61 complex with the acceptor protein complex. Finally, once inside the lumen of the endoplasmic reticulum, the translocated polypeptide interacts with BiP before being released from the translocation complex and assuming its native conformation.

TABLE 3. HSP Functions

HSP	Functions
HSP-110/104	Required to survive severe stress for yeasts
HSP-90	Binds steroid receptors to stabilize the receptors in the cytosol and silence their function
GRP-78	Acts as molecular chaperones and provides cytoprotection
GRP-75	Acts as molecular chaperones
HSP-73	Acts as molecular chaperones
HSP-72	Provides cytoprotection
HSP-60	Acts as molecular chaperones
HSP-56	Binds steroid receptors and FK506
HSP-47	Acts as collagen chaperones
HSP-20	Regulates actin cytoskeleton; acts as molecular chaperones; provides cytoprotection
HSP-10	Acts as a cohort of HSP-60
Ubiquitin	Involves a nonlysosomal ATP-dependent protein degradation pathway

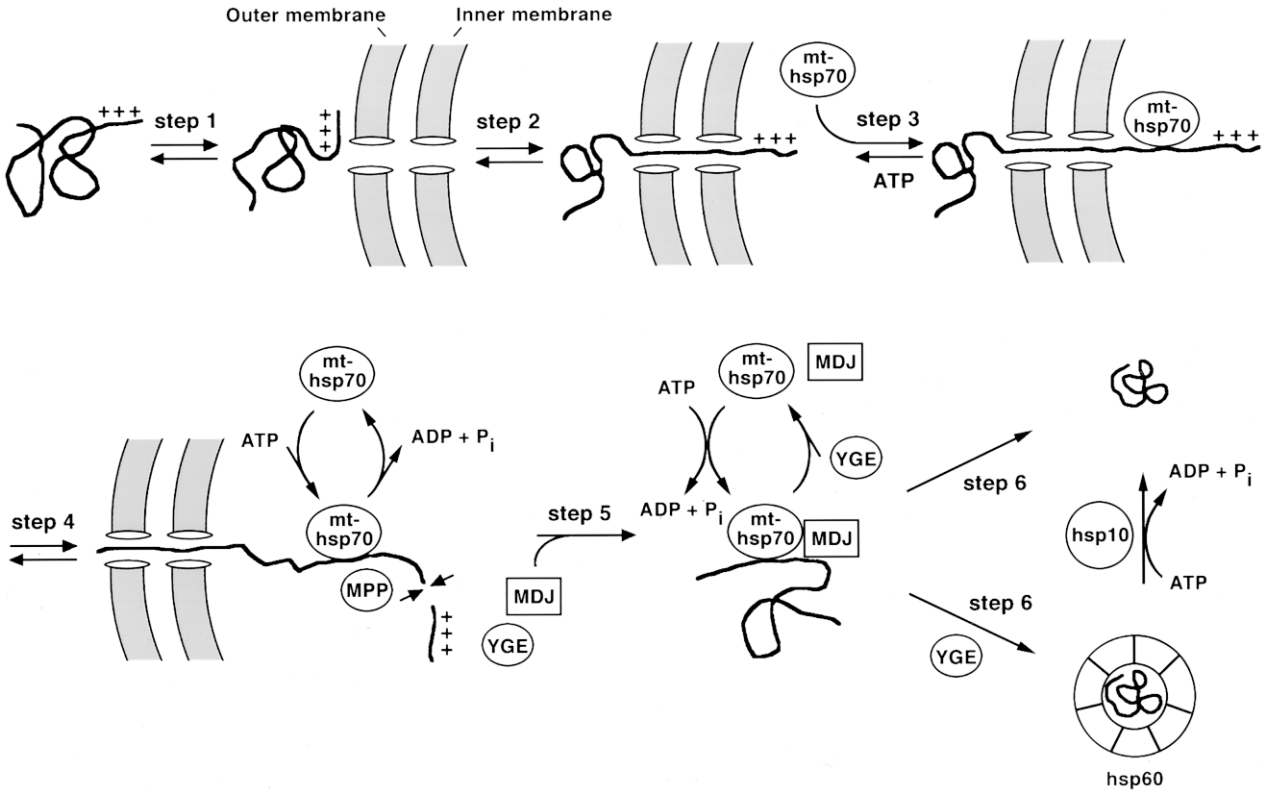

FIGURE 4. Schematic representation of the process by which HSP-70 participates in transit of unfolded protein across the mitochondrial membrane. Protein binds to receptor complex at the outer membrane of the mitochondria (step 1), then enters the translocation pore (step 2). Once it emerges from the translocator, protein binds mitochondrial HSP-70, a step requiring ATP (step 3). The signal peptide of the protein is cleaved by matrix processing peptidase (MPP) (step 4). In a process involving MDJ, YGE, and ATP hydrolysis (step 5), the protein either folds spontaneously as HSP-70 is removed or is folded actively by a process mediated by HSP-60 (step 6).

3. FACTORS THAT MODULATE HEAT SHOCK PROTEIN 70 kDa EXPRESSION

3.1. *Intracellular pH*

The resting basal intracellular pH (pH_i) in most cells ranges between 7.3 and 7.5. A variety of basic cellular functions is known to be triggered by alterations in pH_i beyond this range. Changes in pH_i trigger DNA replication and cell proliferation in some cell systems (Grinstein *et al.*, 1989). Inhibition of intracellular alkalization induced by growth factors leads to blockage of cell growth (Pouyssegur *et al.*, 1984). In 3T3 and Vero cells, an increase in pH_i by 0.2 U is sufficient to induce tumorigenicity and growth (Perona and Serrano, 1988). Additionally, changes in pH_i can alter second messenger levels. In avian heart fibroblasts (Dickens *et al.*, 1990), rat hepatocytes (Yajima and Ui, 1975), and human epidermoid A-431 cells (Kiang, 1991), intracellular acidification decreases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and cellular cyclic AMP (cAMP). Conversely, both $[Ca^{2+}]_i$ and cAMP are increased by intracellular alkalinization.

In extracts of HeLa cells, activation of HSF occurs in cell extracts when the pH is adjusted from 5.8 to 6.4, with maximal activation occurring at pH 6.0 (Mosser *et al.*, 1990). These studies did not address whether changes in pH_i influence HSP-70 expression. Studies performed in our laboratory have shown that changes in resting pH_i neither affect the baseline levels of HSP-70 nor alter the ability of heat shock to induce HSP-70 in human A-431 cells (Kiang *et al.*, 1994). Heat shock is known to acidify cells (Aickin and Thomas, 1977; Yi *et al.*, 1983; Drummond *et al.*, 1988; Kiang *et al.*, 1990; Weitzei *et al.*, 1985; Liu *et al.*, 1996). In cells overexpressing HSP-70 as a result of previous exposure to heat (Drummond *et al.*, 1986; Kiang *et al.*, 1996a) or HSP-70 gene transfection (Kiang *et al.*, 1998), heat shock still acidifies cells; this result suggests that there is no association between HSP-70 and pH_i. Preservation of the ability of cells to reduce pH_i after heat shock probably is functionally important because the return to normal pH_i inhibits various deleterious biochemical processes and thereby promotes cell survival. The heat shock-induced reduction of pH_i is considered to represent a defensive mechanism of cell survival.

3.2. *Cyclic AMP*

cAMP serves as the second messenger in the cell-signaling process of various hormones and cytokines. Heat shock increases intracellular cAMP levels in rabbit epididymis (Kampa and Frascella, 1977), human thymocytes (Lin *et al.*, 1978), and human A-431 cells (Kiang *et al.*, 1991). It has been reported that the activation of many HSP genes in yeast is

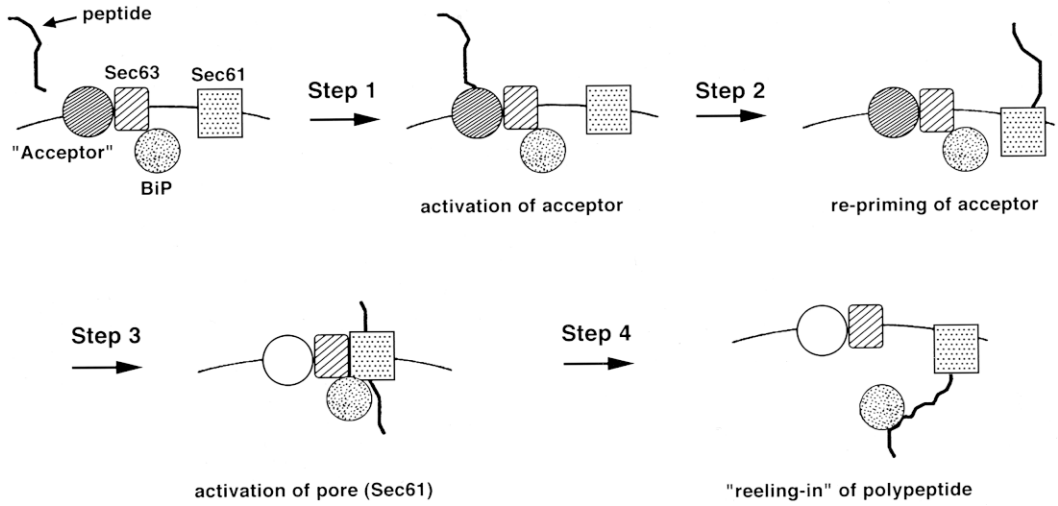

FIGURE 5. Schematic representation of the process by which BiP (HSP-78) participates in nascent polypeptide transport across the endoplasmic reticulum. Nascent polypeptide engages acceptor protein complex at the endoplasmic reticulum membrane, which contains acceptor protein, Sec63, and BiP (Step 1). This step does not require ATP. Nascent polypeptide then contacts Sec61 located on the membrane, an interaction requiring ATP (Step 2). The complex of nascent polypeptide and Sec61 then associates with the acceptor protein complex (Step 3). Finally, in the lumen of the endoplasmic reticulum, the translocated polypeptide interacts with BiP (Step 4) before being released from the translocation complex and assuming its native conformation.

caused by reduced intracellular cAMP levels (Fuhr *et al.*, 1976, 1977). In female C57BL/6J Jcl mice injected with 50 mg/kg dibutyryl cAMP, HSP-70 increases in livers 3 and 8 hr after the injection (Takano *et al.*, 1998). However, in human A-431 cells, treatment with cAMP-stimulating agents does not induce HSP-70 production (Kiang *et al.*, 1994). The different results observed in liver cells and A-431 cells may be attributed to the length of exposure of cells to the cAMP-stimulating agent because in female C57BL/6J Jcl mice, dibutyryl cAMP was injected and it remained at measurable levels for up to 8 hr, whereas A-431 cells were exposed to cAMP-stimulating agents for only 20 min.

In human epidermoid A-431 cells that overexpress HSP-70 following transfection with the HSP-70 gene, basal cellular cAMP remains unchanged. Exposure of these cells to heat shock causes greater increases in cAMP than those found in cells that do not overexpress HSP-70. This enhancement in cAMP production induced by heat shock is a result of increases in the enzymatic activity of adenylate cyclase and phosphodiesterase (J. G. Kiang, unpublished data). Because convincing evidence supports the view that HSP-70 protects many cells from otherwise lethal exposures, the enhanced cAMP production stimulated by high levels of HSP-70 may play a part in the cytoprotective effect of HSP-70.

3.3. *Intracellular Free Calcium*

It has been shown that the binding of HSF to HSE can be activated by Ca^{2+} (Mosser *et al.*, 1990; Price and Calderwood, 1991; Kiang *et al.*, 1994), Mn^{2+}, and La^{2+} (Mosser *et al.*, 1990). Increases in $[Ca^{2+}]_i$ induced by ionomycin (a Ca^{2+} ionophore) promote HSP-70 production in human epidermoid A-431 cells (Ding *et al.*, 1996), MDCK cells (Yamamoto *et al.*, 1994), and rat luteal cells (Khanna *et al.*, 1995). In human epidermoid A-431 cells, we found that ionomycin also increases HSF1 gene expression (Ding *et al.*, 1996). Increases in $[Ca^{2+}]_i$ are involved in promoting HSP-70 mRNA and protein synthesis induced by heat shock, because inhibition of increases in $[Ca^{2+}]_i$ by removal of external Ca^{2+}, addition of the chelator EGTA in the medium, or treatment with bis-(O-aminophenoxy)-N,N,N′,N′-tetraacetic acid, an intracellular Ca^{2+} chelator, greatly attenuates HSF translocation from the cytosol to the nucleus, HSF binding to HSE, HSP-70 gene expression, and protein synthesis (Kiang *et al.*, 1994).

In cells that overexpress HSP-70 as a result of heat shock or HSP-70 gene transfection, increases in $[Ca^{2+}]_i$ induced by heat shock, air hypoxia, or chemical hypoxia are attenuated (Kiang and Koenig, 1996; Kiang *et al.*, 1992, 1996a,b,c, 1998). The attenuation may be due to a desensitization of Na^+/Ca^{2+} exchange systems and other C^{2+}-related mechanisms (Kiang *et al.*, 1996a, 1998). Intracellular Ca^{2+} pools in human A-431 cells are also desensitized. It is not clear whether the size of the pools or their sensitivities to Ca^{2+} mobilizers are modified by HSP-70. Our data show that in human epidermoid A-431 cells, NaCN increases $[Ca^{2+}]_i$ by reducing the K_m and increasing the V_{max} of the Na^+/Ca^{2+} exchangers. In cells overexpressing HSP-70, attenuation of the NaCN-induced $[Ca^{2+}]_i$ increase is a result of a reduction of V_{max} (Kiang *et al.*, 1998). It is possible that HSP-70 acts directly on the Na^+/Ca^{2+} exchanger to reduce its V_{max}, because others have shown that HSPs stabilize protein molecules such as steroid receptors (Tsai and O'Malley, 1994). HSP-70 may modulate the Na^+/Ca^{2+} exchanger by altering the activity of protein kinase A, PKC (Ding *et al.*, 1998), or

phospholipase A_2 (Pastorino *et al.*, 1993), or by affecting the function of Ras, Raf, and pp60v-src kinase (Stancato *et al.*, 1993; Xu and Lindquist, 1993). These possibilities have not been investigated yet. Because sustained elevation of $[Ca^{2+}]_i$ to micromolar levels leads to cell death, this attenuation of the $[Ca^{2+}]_i$ response to heat shock or hypoxia in cells overexpressing HSP-70 is one of the cellular defense mechanisms that promotes cell survival.

3.4. *Intracellular Inositol 1,4,5-Trisphosphate*

Inositol 1,4,5-trisphosphate ($InsP_3$) is important in the regulation of the expression of HSPs because treatment with pertussis toxin, cholera toxin, or forskolin increases the production of $InsP_3$ (Kiang and McClain, 1993). This increased production leads to the increased levels of HSP-70 mRNA and protein in human epidermoid A-431 cells. In contrast, an inhibitor of $InsP_3$ production, U-73122, diminishes the heat-induced increase in the expression of HSP-70 (Kiang *et al.*, 1994). These results suggest that $InsP_3$ is involved in HSP-70 production. It is still unclear how an inhibition of $InsP_3$ production leads to a decrease in HSP-70 production. It has been found that the binding of $InsP_3$ to its receptor alters RNA splicing (Danoff *et al.*, 1991; Mignery *et al.*, 1990; Nakagawa *et al.*, 1991) and regulates the expression of multiple gene products (Berridge and Irvine, 1989; Berridge, 1993; Sudhof *et al.*, 1991). Induction of HSP-70 may be a result of $InsP_3$ binding to its receptors.

In cells overexpressing HSP-70, the basal level of $InsP_3$ is not altered. The heat-induced increase in $InsP_3$ is attenuated as a result of a diminished capacity of heat shock to induce increases in $[Ca^{2+}]_i$ (Kiang and Koenig, 1996). We have not found any changes in the level of $InsP_3$ receptor expression and tyrosine phosphorylation in human epidermoid A-431 cells overexpressing HSP-70 (J. G. Kiang, R. J. H. Wojcikiewicz, and G. C. Tsokos, unpublished data).

3.5. *Protein Kinase C and Protein Phosphatases*

It has been reported that activation of PKC induced HSP-70s (Yamamoto *et al.*, 1994; Khanna *et al.*, 1995; Ding *et al.*, 1996). PKC phosphorylates proteins at serine and threonine residues. We have found that treatment of human epidermoid A-431 cells with phorbol 12-myristate 13-acetate (PMA), a potent PKC stimulator, increases the levels of HSP-70 mRNA and protein, as well as the levels of HSF1 mRNA. Furthermore, treatment of A-431 cells with PMA increases the translocation of HSF1 from the cytosol to the nucleus and the binding of HSF1 to HSE. The level of HSF1 phosphorylation is also increased (Ding *et al.*, 1996). In MDCK cells (Yamamoto *et al.*, 1994) and rat luteal cells (Khanna *et al.*, 1995), treatment with PMA also induces new HSP-70 synthesis.

When human epidermoid A-431 cells overexpress HSP-70, induced either by heat shock or gene transfection, the enzymatic activity of PKC is significantly reduced, whereas that of protein phosphatases 1 and 2A is augmented (Ding *et al.*, 1998). Similar changes have been observed in Jurkat cells transfected with the HSP-70 gene (Liossis *et al.*, 1997).

It is not clear whether reduction of enzymatic activity of PKC and elevation of protein phosphatases favor cell survival. It has been shown that an imbalance of phosphorylation and dephosphorylation leads to apoptotic cell death (Walker *et al.*, 1993; Baxter and Lavin, 1992; Song and Lavin, 1993). Although thermotolerance provided by HSP-70 overexpression is a well-established event, whether transient or long-lasting increases in HSP-70s are a benefit or a detriment to overall cell function and cell survival remains to be determined.

4. PROTECTIVE ROLE OF HEAT SHOCK PROTEIN 70 kDa

4.1. In Vivo *Studies*

Substantial evidence indicates that heat shock is capable of protecting cells, tissues, organs, and animals from a subsequent, normally lethal heating, as well as from other types of noxious conditions, including hypoxia and ischemia/reperfusion. For example, it has been reported that heat shock protects human gastric cells from sepsis-induced injury (Villar *et al.*, 1994), transplanted pig kidneys from warm ischemic injury (Perdrizet *et al.*, 1990), rabbit heart from ischemic-reperfusion injury (Currie *et al.*, 1993), rat small intestine from ischemic-reperfusion injury (Stojadinovic *et al.*, 1995) or ricin toxicity (Stojadinovic *et al.*, 1997), and rat retina from light injury (Barbe *et al.*, 1988). Rats that overexpress HSP-70 induced by methods other than heat shock display protection of the lungs from sepsis-induced injury (Ribeiro *et al.*, 1994) and a reduction in hepatocyte apoptosis induced by tumor necrosis factor (TNF)-α (Takano *et al.*, 1998). Transgenic mice that overexpress HSP-70 demonstrate resistance to adverse effects of lethal heat or ischemia (Marber *et al.*, 1995; Plumier *et al.*, 1995).

4.2. In Vitro *Studies*

Similar evidence has been derived from the study of cultured cells after heat shock or HSP-70 gene transfection to promote overexpression of HSP-70. Workers have reported protection of human monocytes from hydrogen peroxide-induced toxicity (Polla *et al.*, 1987) and apoptosis (Samali and Cotter, 1996) and of guinea pig gastric mucosal cells from ethanol damage (Nakamura *et al.*, 1991). Protection of human A-431 cells from NaCN toxicity (Kiang *et al.*, 1997), of rat FRTL-5 thyroid cells from hypoxia/reoxygenation injury (Kiang *et al.*, 1996c), and of human breast cancer T47D cells and MCF-7 cells from lethal temperature (Kiang *et al.*, 1998) has also been reported. The protective effect of heat shock is likely mediated by overexpressed HSP-70 because there is a lag between heat shock and the development of protection correlated with the production of HSP-70 2 hr after heat shock, and protection is affected when HSP-70 production is inhibited by treatment with inhibitors (Kiang *et al.*, 1996a). Furthermore, HSP-70 also

TABLE 4. Proteins that Complex HSPs

HSP-60	HSP-70	HSP-90
λ-Phage collars	Clathrin-coated vesicles	Steroid receptors
Ribulose-P_2 carboxylase/oxygenase heavy chain	Prepro-α factor	Tyrosine kinases
Cytochrome C	Nucleolar proteins	Eukaryotic initiation factor-2α kinase
F1-ATPase	Immunoglobulin G heavy chain	Yeast PKC
	P53 tumor antigen	Tubulin
	DNA replication-initiation complex	Actin
	Calmodulin	
	SV40 T-antigen	
	Microtubules	

Data from Schlesinger (1990).

provides protection when induced by methods other than heat shock, such as treatment of human breast cancer T47-D cells with estrogen (Kiang *et al.*, 1997), rat vascular smooth muscle cells with nitric oxide-generating agents (Xu *et al.*, 1997), and rat lung cells with arsenite or glutamine (Wischmeyer *et al.*, 1997). Cells transfected with the HSP-70 gene are readily protected from many harmful agents (Li *et al.*, 1991; Kiang *et al.*, 1998; Mestril *et al.*, 1994; Samali and Cotter, 1996; Uney *et al.*, 1993). It should be noted that induction of HSP-70 enhances the hydrogen peroxide cytotoxicity in *Drosophila* (Love *et al.*, 1986) and the TCR/CD3- and Fas/APO-1/CD95-mediated apoptotic cell death in Jurkat cells (Liossis *et al.*, 1997).

Inhibition of HSP-70 expression diminishes cell survival. For example, microinjection of anti-HSP-70 antibody into fibroblasts to neutralize HSP-70 increases vulnerability of the cells to sublethal temperatures (Riabowol *et al.*, 1988). Inhibition of HSP-70 expression in mouse J774A.1 macrophages by the *S. choleraesuis* RF-1 strain increases TNF-α-induced cell death (Nishimura *et al.*, 1997). These results further support the view that HSP-70 in fact is important for the cell survival.

Evidence presented above suggests that harnessing endogenous protective systems such as HSPs can possibly provide therapeutic benefit. Harmless manipulation that increases the synthesis of cytoprotective HSP-70 may prove to be of clinical use in organ transplantation and functional recovery of organs that require reperfusion as a result of lost blood supply.

5. HEAT SHOCK PROTEIN 70 kDa INTERACTIONS WITH OTHER PROTEINS

HSP-70 can distinguish between folded and unfolded forms of the same protein. Clathrin light chains and p53 tumor antigen have been used as models in the study of the binding of HSP-70 to proteins. Table 4 lists proteins that can complex with HSPs. Both p53 (Lam and Calderwood, 1992) and clathrin light chain (DeLuca-Flaherty *et al.*, 1990) are enriched in glycine and proline residues, which allows the protein to assume an accessible, aperiodic structure. Three sequences of amino acids have been reported to interact with HSP-70. They are Pc 81-104, IFAGIKKKSERVDLIAYLKDATSK, a fragment of cytochrome c (Domanico *et al.*, 1993; Vanbuskirk *et al.*, 1989), and KRQIYTDLEMNRLGK and KLIGVLSSLFRPK, both of which are fragments of proteins derived from the vesicular stomatitis virus. Heat shock causes transient phosphorylation of vimentin, which leads to its association with HSP-70 (Cheng and Lai, 1994). This result indicates that HSP-70 may alter cell function by associating and dissociating with vital cellular proteins. On the other hand, HSP-70 may bind to and attenuate the function of proteins that are involved in the killing of tumor cells and may render tumor cells more resistant to drug treatment (Fuqua *et al.*, 1994). It has been hypothesized that HSP-70 interferes with apoptotic mechanisms involved in the elimination of tumor cells (Gabai *et al.*, 1995; Samali and Cotter, 1996). Because proteins that are involved in apoptotic cell signaling are being characterized, the association of HSP-70 with any of these proteins needs to be investigated.

Interaction between HSP-70 and receptors is also evident. Hutchison *et al.* (1994) reported that the reconstitution activity of reticulocyte lysates to a receptor is abrogated by depletion of ATP-binding proteins and restored by addition of purified HSP-70. This reconstitution requires K^+ and ATP, but not Na^+ (Bohen and Yamamoto, 1994; Hutchison *et al.*, 1992; Palleros *et al.*, 1993). It is thought that HSP-70 may be vital to maintaining receptors in ligand-responsive complexes. Steroid receptors (Table 5) are present in the cytosol and contain protein-binding domains (Zalliacus *et al.*, 1995). Two molecules of HSP-90, one HSP-70, one HSP-60, and one HSP-56 can stabilize the steroid receptor (Edwards *et al.*, 1992). The stabilization of steroid receptors deprives them of their capacity to enter the nucleus and initiate their nuclear function.

6. CLINICAL IMPORTANCE OF HEAT SHOCK PROTEIN 70 kDa

6.1. *Immunology and Infectious Diseases*

Several studies have raised the possibility that HSP-70 may be involved in various aspects of the immune system (see review by Kaufmann and Schoel, 1994). Genes encoding

TABLE 5. Receptor-Associated HSPs

HSP	Receptors	Comments
HSP-90	GR, PR, ER, AR, MR, AhR	Conserved, ubiquitous, abundant, phosphorylated
HSP-70	PR, GR	Conserved, ubiquitous, abundant, binds ATP
HSP-60	cPR	Conserved, ubiquitous, abundant, phosphorylated
HSP-56	GR, PR, ER, AR	Conserved, ubiquitous, abundant, binds FK506

AhR, aryl hydrocarbon receptor; AR, androgen receptor; cPR, chicken prolactin receptor; ER, estrogen receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PR, prolactin receptor.
Data from Smith and Toft (1993).

two members of the HSP-70 family are found to reside in the major histocompatibility complex (MHC), and a protein binding motif of HSP-70 is very similar to the peptide-binding cleft of the MHC Class I proteins. A peptide-binding protein named PBP 74 and related to the HSP-70 family is involved in peptide loading of MHC Class II molecules. Deoxyspergulain, an immunosuppressant, is found to bind to HSP-73 specifically (Nadler *et al.*, 1992). Another immunosuppressant, FK506, appears to bind to HSP-56 (Yem *et al.*, 1992). Two of 11 self-peptides isolated from purified Class I HLA-B27 have been shown to be derived from HSP-90. The physiological and pathological relevance of these observations is not clear. It is likely that these two self-peptides are involved in immune processes, such as antigen presentation and cytotoxic cell killing of immune targets, which may lead to autoimmunity (see Section 6.2 for details).

In many bacterial infections in animals, the immunodominant antigen is *E. coli* chaperonin 60 kDa protein (GroEL), an analog of HSP-65. Antibodies raised against GroEL from one bacterial species tend to recognize the protein from all other species of the same genus, but not those of a different genus. In parasitic infections, the parasitic forms of HSP-70 and -90 represent a major target of the immune response. Table 6 lists the infectious diseases in which HSP-70s are the immunodominant antigens of the infecting organisms (Kaufmann and Schoel, 1994). Does the immune response to bacterial and parasitic HSPs protect the host from infection? Several studies have shown that immunization with HSPs purified from pathogens protects against diseases such as blinding trachoma (Zhang and Brunham, 1992), Legionnaires' disease (Blander and Horwitz, 1993), and malaria (Dubois *et al.*, 1984). In some cases, the immunity against pathogenic HSPs tends to exacerbate diseases such as Lyme disease (Morrison *et al.*, 1989; Shanafelt *et al.*, 1991).

TABLE 6. Infectious Diseases in which HSP-70s Are the Immunodominant Antigens

Infectious agent	Disease
Borrelia burgdorferi	Lyme disease
Brugia malayi	Lymphatic filariasis
Chlamydia trachomatis	Trachoma
Leishmania donovani	Visceral leishmaniasis
Leishmania major	Leishmaniasis
Mycobacterium tuberculosis	Tuberculosis
Mycobacterium leprae	Leprosy
Onchocerca volvulus	Onchocercosis
Plasmodium falciparum	Malaria
Schistosoma mansoni	Schistosomiasis
Trypanosoma cruzi	Chagas' disease
Trypanosoma brucci brucci	Trypanosomiasis of cattle

Data from Kaufmann and Schoel (1994).

Generally, HSPs are present only intracellularly. How do T-cells in the host recognize the bacterial or parasitic HSPs? It is suggested that bacterial GroEL is secreted or present on the surface membrane and thus allows T-cells access to the GroEL antigen. The other possibility is that cytolysis of infected cells releases pathogenic HSPs to the extracellular space, where they are detected by host immune cells. Vaccination and immunotherapy against bacterial and parasitic HSPs might prove useful.

The host response to infection provides an example of how HSP metabolism in host immune system cells can harm the immune response. Macrophages in the host respond to infection by releasing cytokines, oxygen free radicals, and nitric oxide, which are involved in killing the infecting cells (Snyder *et al.*, 1992). Cytokines, oxygen free radicals, and nitric oxide have been shown to increase HSP's synthesis in macrophages (Manthey and Vogel, 1992; Vogel and Hogan, 1990). However, increases in HSPs in macrophages in turn act to inhibit the release of cytokines, oxygen free radicals, and nitric oxide (Langermans *et al.*, 1990; Van Dissel *et al.*, 1987; Peetermans *et al.*, 1993; Shigetada *et al.*, 1996; Fincato *et al.*, 1991; Polla and Cossarizza, 1996). In this way, the HSP increase in macrophages may not benefit the host.

6.2. *Autoimmune Diseases*

The idea that HSPs play a role in autoimmune diseases is not well supported (see review by Kaufmann and Schoel, 1994). It has been reported that T-cell receptor αβ-positive T-cells recognize an epitope of HSP-65, and this recognition causes autoimmune diseases, such as adjuvant arthritis (Van den Broek *et al.*, 1989) and nonadjuvant arthritis in mice (Ito *et al.*, 1991) and rats (Anderton *et al.*, 1994; Van Eden *et al.*, 1988). However, T-cell receptor αβ-positive T-cells also recognize a different epitope of HSP-65, and

this recognition enables the modulation of autoimmune diseases in rats (Anderton *et al.*, 1995). HSP-70 may also serve as an antigen that is recognized by a subset of T lymphocytes, which express γ and δ chains in place of the α and β chains (Tamura *et al.*, 1993; Hisaeda *et al.*, 1997). This T-cell subset is disproportionally increased in patients with the systemic autoimmune disease lupus erythematosus, a finding that suggests that HSP-70 plays a role in this disease (Rajagopalan *et al.*, 1990).

Numerous reports have claimed the presence of anti-HSP (e.g., 60 or 70 kDa) antibodies in sera from patients with rheumatic diseases, Graves' disease, and Hashimoto's thyroiditis. Until evidence is produced that unequivocally proves a definitive role of HSPs in the origin and pathogenesis of human rheumatic diseases, we should consider that the anti-HSP antibodies probably represent an effort by the body to protect itself from the HSP-like proteins released by stressed cells and infectious pathogens (Schultz and Arnold, 1993).

6.3. *Cancer Treatments*

It has been found that cancer cells exposed simultaneously to heat and chemotherapeutic agents die at a higher rate than do cells treated with chemotherapeutic agents alone. However, cells exposed to heat before treatment with the chemotherapeutic agents exhibit resistance to drug treatment. For example, cultured breast cancer MCF-7 and MDA-MB-231 cells that express high levels of HSP-70 and -27 exhibit resistance to treatment with doxorubicin, actinomycin D, and amphotericin (Ciocca *et al.*, 1982; Shen *et al.*, 1987; Rice and Hahn, 1987; Hahn and Li, 1990). It is clear that the increased expression of the multidrug resistance protein mediates the development of resistance to many cancer chemotherapeutic agents (Gottesman and Pastan, 1988). Interestingly, the gene that encodes multidrug resistance protein contains an appropriate HSE. Therefore, modulation of the HSP-70 content may enhance the therapeutic efficacy of chemotherapy and perhaps allow the use of lower doses of chemotherapeutic agents.

Two studies have claimed that certain tumor cell lines express HSP-70 on the cell surfaces (Chant *et al.*, 1995; Multhoff *et al.*, 1995a,b). However, these studies have not shown in a definitive manner that HSP-70 proteins are indeed membrane-anchored proteins and not just loosely associated with the outer surface. On the basis of reports that natural killer cells are involved in eliminating cellular targets that express HSP-70 (Blachere *et al.*, 1993; Botzler *et al.*, 1996), it is tempting to speculate that HSP-70 vaccination may have powerful antineoplastic activity (Nakagawa *et al.*, 1991; Rajagopalan *et al.*, 1990; Udono and Srivastava, 1993).

7. TOXICOLOGY

It is known that both environmental and pathological stresses cause an increase in HSPs of host cells. It is believed, but not yet proven, that levels of HSP-70s might be used as a measure of stress, resulting from air/land pollution, elevated temperatures, or mycobacterial and parasitic invasions (Sanders, 1993). With the development of new molecular biology technology, it is possible to develop transgenic organisms in which the promoter region of the HSP-70 gene is hooked to genes that transcribe colors. These organisms can be used to detect the environmental stressors (Welch, 1993). It is likely that approaches using molecular biology techniques will revolutionize toxocological applications that are cheaper and do not require the use of animals to detect environmental stressors.

8. PERSPECTIVE

HSPs were observed accidentally more than 30 years ago. Recent studies of HSPs have advanced our understanding of their physiological and biochemical features (see reviews by Leung and Hightower, 1997; Schlesinger, 1990; Sorger, 1991; Subjeck and Shyy, 1986; Minowada and Welch, 1995; Feige and Van Eden, 1996). Structural and functional studies of HSPs have defined their ability to function as molecular chaperones in processes such as protein maturation and degradation. Their gene expression is triggered by physiological stimuli, pathological intruders, and environmental stressors. Overexpression of HSP-70s is capable of down-regulating signal transduction, altering enzymatic activities, and protecting cells from lethal assault.

HSP-70s appear to play crucial roles in the survival of organisms because they are ubiquitously present in cells under both normal and pathological conditions and their structure is evolutionarily conserved. Despite the impressive progress that has been made toward understanding the structure of HSPs, questions with crucial biological significance remain unanswered. It is often thought that HSPs primarily protect cells by preventing the harmful aggregation of proteins. However, it is obvious that not all of the beneficial functions of HSPs can be explained by this mechanism. It was discussed in Section 5 that HSPs bind to certain proteins through distinct motifs. Little is known about how HSP binding controls the function of, for example, protein kinases and phosphatases. We recently showed that cells overexpressing HSP-70 as a result of transfection with the HSP-70 gene demonstrated diminished protein kinase activity, but enhanced protein phosphatase activity (Liossis *et al.*, 1997; Ding *et al.*, 1998). Unless this effect is due to events directly associated with the process of gene-transfection—which ostensibly was excluded by the appropriate experimental controls—it suggests that HSPs have previously unrecognized functions. Other recent studies have corroborated this idea by showing that HSPs bind to and alter the function of Jun N-terminal kinase and p38 kinase, both of which are stress kinases (Gabai *et al.*, 1997).

It has been shown that HSPs protect cells from noxious stimuli that cause either necrosis or apoptosis. Yet, no studies have addressed the differential effect of HSPs on these two distinct processes. Furthermore, except for one example (Liossis *et al.*, 1997), there are no studies of the effect of

HSPs on surface membrane-initiated cell death or activation. The need for these studies is obvious because cells routinely are engaged in cell-specific, membrane-initiated events.

Advances in molecular biology techniques undoubtedly will provide answers to many of these questions. For example, the production of synthetic HSP binding domains allows for the detailed, direct study of HSP-protein interactions without the complications inherent in studying such interactions *in vivo*.

Is there any realistic hope of using HSPs therapeutically? The answer awaits further study. The *in vivo* overexpression of HSPs may protect certain cells from some kinds of noxious stimuli. We recently have learned that Jurkat cells overexpressing HSP-70 are resistant to heat shock, but are sensitized to T-cell antigen receptor-initiated cell death (Liossis *et al.*, 1997). If this observation also holds for normal lymphocytes, it suggests that heated lymphoid cells might be more susceptible to cell death when they encounter an antigen. The greater susceptibility to cell death would mean a compromise of the immune system.

Similarly, paradoxical results about the role of HSPs have been reported relative to cytokine production. For example, HSPs inhibit the production of TNF-α in lung endothelial cells and thus offer protection against the development of lung injury. However, as mentioned in Section 6.1, HSPs inhibit the production of lymphokines by macrophages that are necessary for the defense against infectious agents. These examples suggest the need for a much better understanding of the function of HSPs.

Acknowledgements–The authors thank Dr. David E. McClain for his critical review of and editorial assistance with this article.

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