0014-4754/94/120979-08$1.50 + 0.20

Multi-author Reviews

Heat shock proteins: the hsp70 family

The editors wish to thank Dr. Ulrich Feige and Dr. Barbara Polla for having coordinated this review.

Hsp70 – a multi-gene, multi-structure, multi-function family with potential clinical applications

Dedicated to our research laboratories, past, present and future, as the most wonderful place for thought and progress.

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Key words. Heat shock protein; hsp70; chaperone.

Introduction

The response of cells or organisms to environmental stress such as heat shock or chemicals is connected to the induction or enhancement of the synthesis of a number of proteins, called heat shock proteins (HSP). Attention was drawn to this by the classic experiments of Ferrucio Ritossa, who exposed salivary glands of *Drosophila busckii* to heat, dinitrophenol or salycilate[61]. Ritossa observed a new chromosomal puffing pattern following these stresses. It is noteworthy that type and severity of stress determine the HSP response and as a consequence the outcome for a cell: survival or death. It is important to note that a mild stress can prepare cells to cope with a subsequent severe stress, which unprepared cells would normally not survive. Following analysis of the heat shock response an array of HSP have been described (for reviews and additional references see 45, 46). On the basis of molecular weight and amino acid sequence homologies, HSP are grouped into families (see 5, 29, 30, 45, 46, 78 and refs therein). Defined functions in different compartments of a cell were found to be assigned to HSP families and to distinct members within the families. This is exemplified by the hsp70 family as shown in the table.

Interestingly, the pattern of the heat shock or stress response is dependent on the type and the extent of stress, and also on the type of stressed cells. Apparently cells or organisms have developed during evolution the ability to cope with stresses in an optimal way. As one example of the fine tuning of a stress response, the stress response of macrophages during phagocytosis of particles depends on the type of particle[56] but also on the phenotype of the phagocytosing cell[38].

HSP are not only induced during stress. Some family members are constitutively expressed (HSC, heat shock cognates), which indicates that HSP fulfil important tasks in cells under normal conditions. One of the functions of HSP is chaperoning proteins during synthesis, folding, assembly and degradation (for review see 2, 5, 12, 25–27, 29, 45, 46, 62, 63, 73, 78). The potential interplay between hsc73 (the constitutive hsp70) and hsp72 (the inducible hsp70) has been discussed in detail elsewhere[5,8].

HSP are abundant proteins in cells. Up to 5% of the cellular protein might be HSP. Considering this and the high conservation of HSP during evolution (see 5, 30), one would expect that the immune system would treat HSP as self-antigens, and that immune reactions to HSP would be rare. However, the opposite is true. Bacterial hsp60 was termed 'common antigen', because in all individuals following bacterial infection(s) antibodies against bacterial hsp60 were found and antisera from infected individuals cross-reacted with hsp60 from other bacteria[81]. Such cross-reactive antibody production to HSP could be expected to lead to autoimmune disease. However, autoimmune disease is nowhere near as common as are bacterial infections. Why? Apparently there are mechanisms to prevent autoimmune disease despite the fact that autoimmunity to HSP is present[3,11,19,28,39,74,77,79]. Furthermore, T or B cell immune responses to HSP may lead to cross-reactions with self-HSP or another self-antigen which is not an

Table 1. Hsp70 family members[a]

Protein/*Gene* name	Other names	Localization/expression
hsp70	72K, hsx70, hsp71, sp71, hsp68, hsp70	Cytosol (nucleus after stress) Major heat-inducible hsp70 Some basal expression in human cells Serum-stimulated Higher expression in dividing cells
hsp72	hsp70B′, hsp70	Cytosol (nucleus after stress) No basal expression Heat-inducible
hsc73	p72, 73K, hscp73, scp73, hsc70, hsc71, hsp70, hsp73	Cytosol (nucleus after stress) High basal expression Slightly heat-inducible
grp78	BiP, p78, grp80	Endoplasmatic reticulum High basal expression in secretory cells and some transformed cells induced by glucose deprivation, glycosylation inhibitors, ionophores, etc.
Stch		'ATPase core' of hsp70; resembles BiP in terms of distribution and induction
Mt-hsp70	grp75, p71	Intramitochondrial hsp70
PBP74		Cytoplasmic vesicles
HSP70RY		?
Mortalin		Cytoplasmic, nuclear membrane
hsp70-hom		Constitutively expressed in spermatogenic cells only
hsp70.2	p70	Constitutively expressed in spermatogenic cells only

[a]Modified from Terlecky et al.[71]. See text and refs 17, 30, 31, 46, 51, 71 for additional information.

HSP. A theoretical analysis of potential cross-reactivities based on protein and DNA sequence data was performed by Jones et al.[37]. Because the role of HSP in immunity, autoimmunity and autoimmune disease has recently been reviewed in detail, this particular field is not covered in this issue and the reader is referred to these reviews[11,19,28,39,74,77,79]. However, it is noteworthy that two examples have been reported in the meantime which show that HSP peptides can interfere with an ongoing autoimmune disease. In the case of adjuvant arthritis in Lewis rats and insulin-dependent diabetes in NOD mice, treatments with mycobacterial hsp65 peptide 180–188[20] and mouse hsp60 peptide 436–460 (peptide p277)[18], respectively, have been shown to stop not only the immunological but also the inflammatory processes. In addition, pretreatment with mycobacterial hsp65 was reported to prevent a number of arthritic diseases in animals (for review see 79).

Research on prokaryotic HSP such as GroEl (hsp60) or DnaK (hsp70) has dominated the field of HSP research in the past (for information on this the reader is referred to a number of recent reviews: 26, 45, 46, 73). However, in the meantime a great deal of knowledge has accumulated on mammalian HSP as well. To us the hsp70 family is attractive because of the protective role of hsp70 in inflammation, ischemic disease, infection, their potential role in antigen processing and their adjuvanticity. The intention of this Multi-author Review is to summarize knowledge on mammalian hsp70 emphasizing aspects of protective functions of hsp70 in cells or organisms. In addition, the issues as to whether there might be a regulatory role of hsp70 in cytokine biosynthesis and the question whether failure of in vivo or in vitro aged cells to cope with environmental stresses might be due to failure of HSP synthesis induction are also considered.

The specific focus of this series is to present a detailed update on the still-growing hsp70 family (see table 1 and the contribution by Günther and Walter[30]) and to provide some possible directions for future clinical applications, an even faster-growing field. There is no definitive general agreement on nomenclature, and matching between gene, protein and function is still difficult to make. In this series, genes will be referred to in italic, specific proteins in lower case and heat shock proteins in general in upper case.

Hsp70, a multi-gene family – structure and function

Members of the hsp70 family were recognized for their peptide-binding functions in protecting nascent protein chains after synthesis, in protein translocation through membranes, protein refolding after denaturation, or during protein degradation[5,7,12,26,29,45,46,70–73] (see below). To give an example: BiP is a specific peptide-binding protein which binds to immunoglobulin heavy chains (IgH) before assembly of the immunoglobulin molecule (Ig)[31].

Genes for hsp70 are widely distributed through the genome, on chromosomes 1, 5, 6 (within the gene loci of

the major histocompatibility complex MHC), 14, and 21. The classification, structure, and chromosome location of the *hsp70* genes is reviewed in detail by Günther and Walter[30]. An interesting upcoming issue in this field is the possibility of association of diseases, and in particular autoimmune diseases, with polymorphism within the *hsp70* genes, not only in the coding regions, but also in the regulatory regions[10,53,59]. Hsp70 family members share high amino acid sequence homology. However, it is important to note that the sequence conservation of BiP of different species is much higher than of BiP and other hsp70 family members within a species[30,31]. This indicates that BiP function is so important that during evolution BiP genes were highly conserved.

HSP synthesis is turned on by heat shock factor (HSF). HSF is synthesized constitutively and thus HSP synthesis is not regulated via the induction of HSF synthesis. A simple model for the regulation of hsp70 synthesis is that hsp70 is complexed to HSF in the cytoplasm. Upon stress some (nascent) proteins are denatured and therefore hsp70 is consumed by binding to these proteins to prevent them from undergoing harmful interactions with other proteins resulting in aggregates of denatured protein in stressed cells. As a consequence, HSF is liberated and migrates to the nucleus where it turns on HSP synthesis[47]. However, as with many simple models reality is much more complicated. In vertebrates several HSF have been described[47]. HSF1 is the homolog of the general HSF found in evolutionary earlier eukaryotes. HSF1 has the characteristics of a transcription factor responsible for the induction of heat shock gene transcription. During heat shock the HSF1 monomer trimerizes in the cytoplasm, acquires DNA binding activity, phosphorylation of HSF1 is increased and HSF1 migrates to the nucleus. The pattern of HSF1 distribution in cells at 37 °C is cytoplasmic, whereas in cells heat shocked at 42 °C a characteristic nuclear distribution pattern of HSF1 is found. This is completely different for HSF2 which is found as a dimer in the cytoplasm of cells at 37 °C and 42 °C. Upon exposure of K562 cells to hemin, HSF2 trimerizes and migrates to the nucleus. In a series of elegant studies Sistonen and colleagues demonstrated that HSF1 and HSF2 upon activation show additive DNA binding activity, but synergistic induction of hsp70 gene transcription[47]. This is interesting in view of the present knowledge of preferred HSF1 and HSF2 binding sites which are composed of inverted adjacent pentamers that contain the primary sequence 5′-nGAAn-3′ (for details see 47). HSF2 is unable to bind to the first pentamer of *hsp70*, whereas HSF1 binds to all five sites. HSF trimer-trimer cooperativity appears to have a greater role in HSF1 as compared to HSF2 binding. Exchanging the oligomerization domains between HSF1 and HSF2 transferred the trimer-trimer cooperativity from HSF1 to HSF2 (see ref. 47 and refs therein, and the contribution on gene regulation of *hsp70*, which will appear in a forthcoming issue of Experientia).

It appears that the transcriptional response of HSP induced after heat shock is proportional to the duration and the severity of the stress. The half life of HSF1 DNA binding activity is much longer in vitro as compared to the in vivo situation. Therefore it has been concluded that an HSP (hsp70) might be involved in the down-regulation of HSF1 DNA binding activity. Taking this model as a starting point and considering the differential response of cells to various stresses, it is evident that the regulatory processes must involve more than hsp70 as the sole regulator of HSF DNA binding activity (for a detailed discussion and references see 48). Recently it has become obvious that posttranscriptional regulation of HSP also contributes to their expression (ref. 48 and Jacquier-Sarlin et al., submitted).

The structure of hsp70 (see fig. 1) consists of two domains: an ATPase and a peptide-binding domain. The ATPase domain of hsp70 is of high structural homology to the ATPase domains of hexokinase[21] and actin[22]. This has been experimentally proven by expression and crystallization of the truncated ATPase domain of hsp70 followed by X-ray crystallographic analysis[21]. Interestingly, a gene called *Stch* has been found that codes for a microsomal stress protein with high homology to the 'ATPase core' of hsp70[51]. On the other hand, the peptide-binding domain was modelled on the structure of MHC class I antigen[23] (see fig. 1). However, experimental proof for this is still missing. The ongoing discussion includes questions of whether the hsp70 peptide-binding domain is more similar to MHC class II than MHC class I, because MHC class I accepts only short peptides for binding whereas MHC class II accepts also longer peptides. The latter is easily understandable since the MHC class II peptide-binding groove is open at both ends. Common sense would predict that it is more likely for the peptide-binding groove of hsp70 to be open at both ends, so that peptide chains can hang out (see also 24).

For BiP (GRP78) the peptide-binding specificity has been studied in detail using a bacteriophage library displaying octapeptides. In these studies it was found that BiP favorably binds to peptides which contain large hydrophobic residues in alternate positions[7]. This fits the idea that chaperones bind to sequences which in the native protein are not accessible. Binding and release of peptides to hsp70 is an ATP-dependent process[24,73]. However, ATP hydrolysis appears not to be necessary for peptide release, because peptide release is found before ATP hydrolysis occurs and is also induced with non-hydrolysable analogs of ATP[52]. On the other hand, using small peptides, it has been shown that peptides as short as heptapeptides fully stimulate ATPase activity of BiP[24]. It is noteworthy that peptide binding stimulates ATPase activity of BiP and inhibits

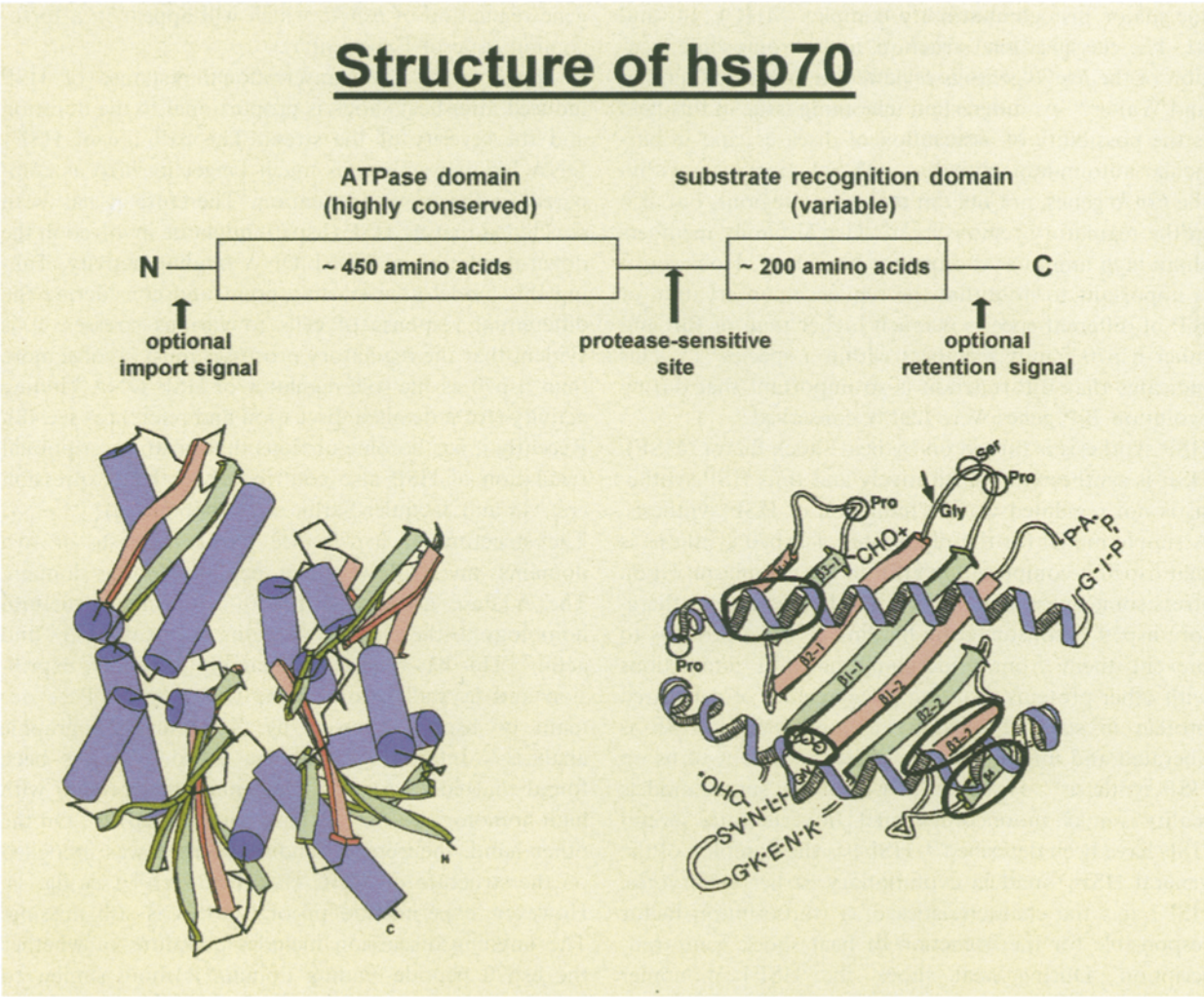

Figure 1. The structure of hsp70 consists of two major domains, a highly conserved N-terminal ATPase domain and a less conserved C-terminal peptide binding domain (top). Some hsp70 contain N-terminal extensions for targeting to the endoplasmatic reticulum or to mitochondria (see ref. 70). BiP contains a C-terminal peptide involved in retention of the protein in the ER (see ref. 31).
The structure of the ATPase domain (left) was derived by x-ray crystallography of the ATPase fragment of bovine hsc70[21,22]. The schematic drawing was produced by Flaherty et al.[21], using a program written by Lesk and Hardman[41].
The peptide binding domain (right) was modelled on the structure of human MHC Class I molecules[6] by Flajnik et al.[23]. Capital letters indicate major insertions. Gaps (deletions) are indicated with bold circles (N-terminus of the helical domain 1; strands 3 and 4 of the α-2 domain; cysteines forming the S-S bond in the α-2 domain). Potential glycosylation sites (CHO), location of prolines, and chymotryptic recognition site of hsc70 (arrow at gly) are indicated.
(Figure adapted with permission from refs 21, 23, 29.)

ATPase activity of mycobacterial hsp70[60]. The function of BiP is discussed in detail in the contribution by Haas[31].

During the life of a cell under normal conditions, nascent proteins have to be protected from improper folding or aggregation after synthesis[12,70]. In addition, for transport through membranes proteins have to be in the unfolded state[33,46,70]. Hsp70 is involved in this transport process through membranes. In this series this area is covered by Stuart et al.[70] who summarizes our understanding of the transport of proteins through the mitochondrial membrane. It is tempting to speculate that during this process hsp70 family members with identical or similar peptide-binding properties have to be present on both sides of the membrane.

HSP also contribute to the degradation of misfolded proteins, for which clearly defined pathways exist. In this series the lysosomal targeting and degradation of such proteins is dealt with in the contribution by Terlecky[72]. There is further evidence for a role of members of the hsp70 family in proteolysis of antigens (Jacquier-Sarlin et al., in preparation).

The chaperoning function of hsp70 might indeed indicate a role in antigen processing and presentation (see refs 13, 16, 65 and refs therein). When foreign particles are taken up by a professional antigen presenting cell (APC), protein antigens are proteolytically degraded. Binding of the resulting peptides to hsp70 might rescue them from total degradation and save them for antigen presentation. It is interesting to note that B cells and

monocytes show enhanced antigen processing and/or presentation after heat shock[13,43].

How the antigenic peptide is handed over to MHC class II (one of the antigen presenting molecules) is the subject of intense research at present[50]. Class II molecules are apparently not stable as heterodimers of an α and β chain. They are stabilized by the invariant chain (I_i). Mixing peptides in vitro with MHC $\alpha\beta$-I_i complexes does not yield peptide binding to MHC class II[9]. Thus I_i may function as a chaperone for MHC class II $\alpha\beta$. MHC class II-I_i complexes migrate as trimers to the compartment where antigen is processed. Experiments with cathepsin B and D suggest a staged release of I_i from MHC class II[9]. A member of the hsp70 family might be involved in the loading of MHC class II with the antigenic peptide because an antiserum against hsp70 can inhibit peptide loading during antigen processing[55]. The potential role of HSP in antigen processing and presentation is discussed in the contribution by Pierce[55].

Potential clinical applications of hsp70

The protective role of HSP in general is viewed at present in enabling cells under stress to cope with denatured proteins, to prevent their aggregation, and to facilitate renaturation of these proteins, or their degradation. As mentioned above, mildly pre-stressed cells are better prepared to cope with a subsequent severe stress. This protective effect was initially termed, as for heat shock, thermotolerance. The concept of thermotolerance has been expanded, on the basis of experimental data, to a more general protection against injury, in particular against oxidative injury, which is involved in the pathogenesis of many diseases, either inflammatory or degenerative.

Production of reactive oxygen species (ROS) viewed as a first line of defense against invading pathogens, might damage host cells and simultaneously induce a protective HSP response in them[36]. During infection other inflammatory mediators are activated and induction of HSP synthesis may also help to prevent the damaging effects of these mediators to the host. This has been demonstrated in the case of tumor necrosis factor α (TNF-α)[35]. In the article by Jacquier-Sarlin and colleagues protective effects of hsp70 in inflammatory processes are discussed[36].

What induces the synthesis of protective hsp70 molecules in cells? The anti-viral prostaglandins of the cyclopentenon-type have been identified as inducers of hsp70 synthesis and were found to have protective roles during early and late phases of viral infection in cells (see contribution by G. Santoro[64]). In studies aiming to unravel the mechanisms of inhibition of cytokine biosynthesis, using drugs such as auranofin, or chemical inducers of hsp70 such as arsenite or iodoacetamide, it appeared that induction of hsp70 synthesis was inversely correlated to inhibition of cytokine biosynthesis[66]. However, hsp70 seems to have at most an indirect role in this regulatory process (see contribution by T. Hall[32]), but it appears from these studies that a better understanding of the mechanisms of hsp70 induction may lead to new possibilities to inhibit cytokine biosynthesis.

HSP are also involved in chaperoning intracellular receptors (such as steroid receptors[57,58]) and possibly immunomodulating drugs. In particular, one such novel immunosuppressive drug, deoxyspergualin (DSG), binds to hsc70 with high affinity. It appears that this binding might be involved in the mode of action of deoxyspergualin because non-immunosuppressive analogs of DSG do not bind to hsc70 (see ref. 49 and refs therein).

Members of the hsp60 and hsp70 family have been recognized as immunodominant antigens. In other words they appear to be preferred targets of the immune system (for reviews see for example refs 39, 74, 81). However, hsp60 and hsp70 are not only targets in immune responses, they are active players in the game: hsp70 constitutes an adjuvant comparable to complete Freund's adjuvant. Adjuvants help to stimulate a strong immune response to an antigen, without necessarily inducing an immune response to the adjuvant itself. Immunity to tumors, for example, can be induced by immunization with an hsp70 preparation purified from tumor cells but not with hsp70 preparations purified from other sources[67,68]. The explanation for this is that hsp70 molecules purified from tumor cells contain tumor-specific peptides as a result of the peptide binding activity of hsp70. In the contribution by Srivastava this is discussed with a more general view to the functioning of the immune system[68]. Further support for the adjuvanticity of hsp70 stems from work with covalently linked conjugates of peptides and hsp70[4]. Mice immunized with these conjugates mount a strong immune response to the peptide but not to the hsp70 part of the conjugate. Interestingly, mice have to be primed with BCG (Bacille Calmette Guerin) if conjugates containing mycobacterial hsp65 are used in order to get an immune response to the peptide. The potential of hsp70 as an adjuvant is discussed in the contribution by G. Del Giudice[15]. Using hsp70 as a carrier and an adjuvant may lead to the replacement of vaccines based on live BCG[69] in the long run.

In the case of a parasite infecting a mammal a dual role of hsp70, both as a target for the immune system of the host and as a protective factor (for the parasite!) is again observed. The heat shock response mounted by the parasite upon entering a mammalian host, secondary to the rise in temperature that it experiences, is on the one hand beneficial for the parasite but on the other hand renders it a better target for the host's immune system (see contribution by Maresca and Kobayashi[42]).

For whole organs or organisms hsp70 also fulfils protective roles, and in this series we have chosen two examples: the role of hsp70 in myocardial ischemia[80] and the heat shock response in the central nervous system[40]. It is obvious from work done in these areas so far, that the ability to specifically induce the protective hsp70 response may have important therapeutic application. A discussion of the protective roles of hsp70 would not be complete without mentioning the reduced capability of in vivo or in vitro aged cells to respond to stress by overexpressing hsp70. It might be speculated that the reduced ability to express hsp70 in response to stress may be a common and ultimately deleterious phenomenon underlying the aging process (see contribution by Heydari et al.[34]).

Conclusions and outlook

HSP fulfil functions essential for the survival of cells and organisms under normal as well as under stressful conditions. In the latter, HSP have protective roles that enhance the chances of survival. Research in this field has clearly established that mild stress can prepare a cell or organism for coping better with a more severe stress. In other words, a mild stress might be desirable to induce this protective system. The protective effect of fever might relate to HSP overexpression[38]. Has the potential to respond with fever evolved for this reason? A number of routinely used drugs have been shown to induce HSP. Under experimental conditions some drugs downregulate cytokine biosynthesis and upregulate hsp70 synthesis in cells. Although hsp70 might not be directly involved in the downregulation of cytokine biosynthesis, it appears promising as a factor in unravelling the underlying molecular mechanisms. Knowledge of these mechanisms raises the possibility of stimulating stress responses by using old or new drugs to induce physiological protective mechanisms for the inhibition of pathological inflammatory processes, e.g. in rheumatoid arthritis or ischemia. Finally, involvement of hsp70 in antigen processing and presentation in addition to the adjuvanticity of hsp70, may lead to improved vaccines and better strategies to enhance immune responses to tumors and infectious agents.

It appears that basic research on hsp70 has accumulated sufficient knowledge to start evaluating the potential of hsp70 and other HSP for diagnostic, prognostic, and therapeutic applications in a number of diseases[54,75].

Acknowledgments. We would like to thank all the authors of this series for their contribution, Tony J. Hall and Eberhard Günther for stimulating discussions and helpful suggestions, and David B. McKay, Martin F. Flajnik, and Mary-Jane Gething for permission to reproduce the structure of hsp70 from refs 21, 23, 29.

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