



**HAL**  
open science

# Integrating the Cell Stress Response: A New View of Molecular Chaperones as Immunological and Physiological Homeostatic Regulators

Brian Henderson

► **To cite this version:**

Brian Henderson. Integrating the Cell Stress Response: A New View of Molecular Chaperones as Immunological and Physiological Homeostatic Regulators. *Cell Biochemistry and Function*, 2009, 28 (1), pp.1. 10.1002/cbf.1609 . hal-00526195

**HAL Id: hal-00526195**

**<https://hal.science/hal-00526195v1>**

Submitted on 14 Oct 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



## Integrating the Cell Stress Response: A New View of Molecular Chaperones as Immunological and Physiological Homeostatic Regulators

Journal:	<i>Cell Biochemistry &amp; Function</i>
Manuscript ID:	CBF-09-0154.R1
Wiley - Manuscript type:	Review Article
Date Submitted by the Author:	27-Jul-2009
Complete List of Authors:	Henderson, Brian; University College London, Eastman Dental Institute
Keywords:	Chaperonin, Cell Stress, Moonlighting Proteins, Immune Function, Heat Shock Proteins
Abstract:	<p>The response of cells to stress was first documented in the 1960s and 1970s and the molecular nature of the families of proteins that subserve this vital response, the molecular chaperones, were identified and subjected to critical study in the period from the late 1980s. This resulted in the rapidly advancing new field of protein folding and its role in cellular function. Emerging at the same time, but initially largely ignored, were reports that molecular chaperones could be released by cells and exist on the outer plasma membrane or in the body fluids. These secreted molecular chaperones were found to have intercellular signalling functions. There is now a growing body of evidence to support the hypothesis that molecular chaperones have properties ascribed to the Roman god Janus, the god of gates, doors, beginnings and endings, whose two faces point in different directions. Molecular chaperones appear to have one set of key functions within the cell and, potentially, a separate set of functions when they exist on the cell surface or in the various fluid phases of the body. Thus it is a likely hypothesis that secreted molecular chaperones act as an additional level of homeostatic control possibly linking cellular stress to physiological systems such as the immune system. This review concentrates on three key molecular chaperones: Hsp10, Hsp60 and the Hsp70 family for which most information is available. An important consideration is the role that these proteins may play in human disease and in the treatment of human disease.</p>

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



For Peer Review

1  
2  
3 **Review for Cell Biochemistry and Function**

4 **Integrating the Cell Stress Response: A New View of Molecular Chaperones as Immunological and**  
5 **Physiological Homeostatic Regulators.**

6  
7 Brian Henderson

8  
9 Division of Microbial Diseases, UCL Eastman Dental Institute, University College London.  
10  
11

12  
13  
14 Address for correspondence: Professor Brian Henderson  
15 Division of Microbial Diseases  
16 UCL Eastman Dental Institute  
17 University College London  
18 256 Gray's Inn Road  
19 London WC1X 8LD  
20 United Kingdom  
21 Tel: (44)+207 915 1190  
22 E-mail:b.henderson@eastman.ucl.ac.uk  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Abstract**

The response of cells to stress was first documented in the 1960s and 1970s and the molecular nature of the families of proteins that subserve this vital response, the molecular chaperones, were identified and subjected to critical study in the period from the late 1980s. This resulted in the rapidly advancing new field of protein folding and its role in cellular function. Emerging at the same time, but initially largely ignored, were reports that molecular chaperones could be released by cells and exist on the outer plasma membrane or in the body fluids. These secreted molecular chaperones were found to have intercellular signalling functions. There is now a growing body of evidence to support the hypothesis that molecular chaperones have properties ascribed to the Roman god Janus, the god of gates, doors, beginnings and endings, whose two faces point in different directions. Molecular chaperones appear to have one set of key functions within the cell and, potentially, a separate set of functions when they exist on the cell surface or in the various fluid phases of the body. Thus it is a likely hypothesis that secreted molecular chaperones act as an additional level of homeostatic control possibly linking cellular stress to physiological systems such as the immune system. This review concentrates on three key molecular chaperones: Hsp10, Hsp60 and the Hsp70 family for which most information is available. An important consideration is the role that these proteins may play in human disease and in the treatment of human disease.

## INTRODUCTION

The founder of Cell Biochemistry and Function, Joe Chayen, was a pioneer systems biologist who attempted to integrate the methodologies of biochemistry and cytology to generate what he termed 'multiphase biochemistry' [1]. Chayen's idea was to integrate the biochemistry of the individual cell within the framework of the whole organ in which the cell was present. Using quantitative cytochemistry to probe the biochemical activity of cells within sections of a tissue, allowed region-specific biochemistry to be practised, and the relationships between individual cells within a cellular complex to be defined. In 2009 integrative/integrated biology is now a key buzzword with the term 'integrated biology' producing 10,400,000 hits on a Google search.

This review focuses on a specific aspect of 'multiphase biology', specifically the multiphase biology of molecular chaperones. These are variously known as heat shock proteins (Hsps) or cell stress proteins, the latter being a more correct term as many stresses can induce the synthesis of these proteins, which are essential proteins involved in maintaining cellular homeostasis. This review will focus on the emerging findings that molecular chaperones, which were initially believed to be purely intracellular proteins with purely intracellular functions, are now seen to be 'multiphase proteins' present on the extracellular membranes of cells and in the extracellular fluids, including the blood. These proteins are emerging with a multitude of extracellular functions from involvement as receptors for key signalling molecules to acting as insect toxins. This review will provide an overview of the emerging evidence of the multiphasic biology of molecular chaperones.

## AN INTRODUCTION TO MOLECULAR CHAPERONES

Ron Laskey, currently the Charles Darwin Professor of Embryology, and a guitar-toting lecturer, was the originator of the term 'molecular chaperone'. Laskey's group at the University of Cambridge was studying the packaging of DNA into nucleosomes. These are oligomeric 'particles' containing 146bp of DNA wrapped around an octamer of the basic nuclear proteins known as histones. Nucleosome formation occurs rapidly in amphibian eggs once they become fertilised, with the basically charged histones binding to the negatively charged DNA. The nucleosomes can be dissociated with buffers containing high salt concentrations and it was therefore expected that DNA and histones should self-assemble into nucleosomes. Experimentally, this is not the case. Removal of the salt resulted in the formation of non-specific aggregates, but no nucleosomes [2]. Laskey's group showed that homogenates of amphibian eggs (they used *Xenopus*) added to the histone/DNA mixture would promote nucleosome formation. They purified what turned out to be an abundant active component and identified it as an acidic nuclear protein that they called nucleoplasmin. This protein alters the interaction between the histones and the DNA such that nucleosome formation is favoured over aggregate formation. Nucleoplasmin has two important properties which shaped the subsequent development of the concept of molecular chaperones and chaperoning. The first is that the final product, the nucleosome, does not

1  
2 contain nucleoplasmin. The second, is that if the conditions are right, nucleosomes can form in the absence of  
3 nucleoplasmin. Gentle dialysis of dissociated nucleosomes, which slowly lowers the salt concentration, allows  
4 them to reform naturally. This means that the nucleoplasmin does not provide steric information for the  
5 generation of the nucleosome, but simply provides a means of enabling the interactions of the DNA and  
6 histones to be modified such that the natural self assembly is favoured over the formation of non-productive  
7 complexes (Fig 1). Now, in prior centuries and still in some parts of the world, the men and women cannot meet  
8 alone and chaperones are required to prevent untoward interactions. Laskey used this analogy to describe  
9 nucleoplasmin as a 'molecular' chaperone which prevented 'unhealthy' interactions between the histones and  
10 the DNA [2].

11  
12 The discovery of the best studied molecular chaperone, chaperonin (Cpn)60 (also known as Hsp60), can be  
13 traced back to two distinct areas of research: (i) the synthesis of the major chloroplast protein, Rubisco and (ii)  
14 the genetics of phage synthesis in *E. coli*. Without going into detail, it was shown that these two proteins were  
15 evolutionarily related and that they were involved in protein folding. This led John Ellis, who has been a tireless  
16 exponent of the science of molecular chaperones [3], to define these proteins as 'a large and diverse group of  
17 proteins that share the property of assisting the non-covalent assembly/disassembly of other macromolecular  
18 structures but which are not permanent components of these structures when these are performing their normal  
19 biological functions' [4] (Fig 1). To aid the reader Table 1 provides details of some of the molecular chaperones  
20 relevant to this review and their cellular locations.

21  
22 Thus by the late 1980s/early 1990s, and with the beginnings of the identification of the 30-odd protein families  
23 that are now defined as molecular chaperones, it was believed that these were essential intracellular proteins  
24 that solved the problem caused by the enormous amount of protein that exists in each cell. It is this so-called  
25 'protein crowding' that is believed to promote protein misfolding and to be the evolutionary pressure for the  
26 development of chaperoning [5,6]. However, at exactly the same time as this was happening, individuals in the  
27 bacteriological and immunological communities were also identifying chaperonin (Cpn)60 as a powerful  
28 immunogen in bacterial and other microbial infections and this protein was entitled 'common antigen' as it was  
29 present in all bacteria examined [7,8]. Other molecular chaperones have also been found to exert powerful  
30 immunological responses. An excellent example are the peptidylprolyl isomerases, a number of which are,  
31 unexpectedly, targets for the major immunosuppressive drugs such as cyclosporine, tacrolimus and rapamycin  
32 [9]. Such drugs are essential for allowing organ transplantation to take place.

33  
34 The finding that molecular chaperones are powerful immunogens may have suggested that these proteins were  
35 secreted or existed on the cell surface. However unbeknown to the scientific community a molecular chaperone  
36 had been found to be present in the blood one year prior to Laskey's coining of the term molecular chaperone.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Early pregnancy factor (EPF) [10] is an immunosuppressive protein [11] that appears in the first trimester. However, it took until 1994 to verify that EPF was the chaperonin, chaperonin (Hsp)10 [12]. Now Hsp10 is more usually known as the heptameric complex which caps the annulus in chaperonin 60 allowing this protein to fold proteins [13]. Thus Hsp10 was actually defined as a homeostatic immunosuppressive factor before being found to be an essential part of the intracellular protein folding process.

### **MOLECULAR CHAPERONES: A PARADIGM REVOLUTION**

With the introduction of the concept of protein chaperoning there was an exponential increase in the numbers of papers dealing with this process and with the identification of new molecular chaperones. Tucked away in the literature were the beginnings of a paradigm revolution in molecular chaperone biology. In the original paradigm molecular chaperones were intracellular proteins, because it was within cells that their actions were required. They had no extracellular functions and therefore were not expected to be found outside of cells. Thus in 1989 when Hightower and Guidon revealed that members of the Hsp70 family of proteins were released by cultured rat embryonic cells [14] it came as a surprise. This work supported earlier studies from Tytell and co-workers who had previously shown similar proteins being transferred from squid glial cells to neurons [15].

Not surprisingly, the concept that molecular chaperones could be released from cells and have additional, perhaps non-folding, functions was largely ignored as it did not fit in with the then powerfully growing paradigm of molecular chaperone biology. An obvious reason for ignoring the non-folding actions of molecular chaperones was the fact that biological science was still working within another potent paradigm.

ONE GENE → ONE PROTEIN → ONE FUNCTION

### **MOONLIGHTING PROTEINS**

Many proteins have enzymic activity and it is sensible to assume that the enzymic active site, which may take up only a fraction of the protein (in terms of volume or surface area) is the only part of the protein with biological activity. The rest of the protein has evolved to generate the circumstances (topology) of the active site and no more. The concept of protein moonlighting was introduced into the scientific literature by Campbell and Scanes in 1995 in an article in which they discussed the immune modulating activity of endocrine peptides [16]. Constance Jeffery has been most active in bringing this concept to the attention of the protein world [17,18]. As an example of the unexpected nature of moonlighting, the enzymes of the glycolytic pathway, beloved of all undergraduate students in the biological sciences, with a few exceptions, have moonlighting functions [19]. For example, phosphoglucose isomerase (PGI), which functions as its name suggests, is also a neuroleukin [20], an autocrine motility factor, important in tumour metastasis [21], a differentiation and maturation mediator for myeloid cells [22] and an implantation factor [23]. To compound this discussion of protein moonlighting – the receptor for autocrine mobility factor/PGI also exhibits ubiquitin E3 ligase activity in the endoplasmic reticulum



1  
2 [24]. Enolase, glyceraldehyde 3 phosphate dehydrogenase, triose phosphate isomerase, pyruvate  
3 dehydrogenase, all have additional activities over and above their enzymic ones [20]. The emergence of this  
4 finding, that individual proteins can have multiple functions, is opening up a whole new view of evolution and its  
5 effects on protein structure and function. Perhaps this is one explanation for the small number of genes that  
6 mammals have. If each gene product has multiple functions then it provides a much richer protein-function  
7 landscape than if each gene only provides one biological activity. It now appears that one of the major groups  
8 of moonlighting proteins are the molecular chaperones, which are increasingly being shown to exhibit  
9 unexpected biological functions. It is these functions that will be discussed in the remainder of this review.  
10 Given the evolved function of molecular chaperones to interact with other proteins it is perhaps not surprising  
11 that they have evolved this capacity to 'molecularly multi-task'.  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21

## 22 **MOLECULAR CHAPERONE SEQUENCE CONSERVATION AND BIOLOGICAL FUNCTION**

23  
24 Before discussing the novel roles that molecular chaperones play, it is important to clarify the relationship  
25 between the homology of molecular chaperones and their extracellular biological actions. Molecular  
26 chaperones have extremely conserved sequences and the chaperonin 60 protein from mammals and from  
27 bacteria will share 50% sequence conservation. This is sufficient for workers on the folding aspects of these  
28 chaperones to assume that these proteins are functionally identical and to talk about Hsp60 proteins as if they  
29 have universal actions no matter how much their sequences may differ. Now, it is well known to biologists that  
30 single residue changes in a protein can dramatically modify its biological activity. The classic example of this is  
31 the substitution of valine for glutamic acid in haemoglobin which results in the precipitation of the deoxygenated  
32 form of the protein thus leading to the sickling of erythrocytes. It is beginning to appear that molecular  
33 chaperones from different sources can have widely different biological actions in spite of an appreciable  
34 sequence conservation. Examples of this include the Hsp70 proteins Hsp70a (now HSPA1) and BiP (now  
35 HSPA5). Thus HSPA1 is well recognised as a proinflammatory ligand causing activation of human and murine  
36 monocytes [25]. In contrast, HSPA5, which has 64% sequence identity to HSPA1, is a potent monocyte  
37 inhibitor and anti-inflammatory protein which is starting trials as a therapy for the chronic inflammatory disease,  
38 rheumatoid arthritis [26,27]. *Mycobacterium tuberculosis* is one of a proportion of bacteria that have more than  
39 one gene coding for chaperonin 60 proteins and in this organism the proteins are termed chaperonin 60.1 and  
40 60.2 (Hsp65). These proteins exhibit around 61% sequence identity. Attempts to inactivate both chaperonin 60  
41 genes reveals that, only the gene encoding the 60.1 protein can be inactivated [28]. Surprisingly, the  
42 chaperonin 60.1 protein of *M. tuberculosis* turns out not to be a molecular chaperone (it does not fold proteins),  
43 but to be a potent virulence factor [28]. Moreover both proteins differ markedly in their ability to modulate the  
44 formation of the major cell population, the osteoclast, which is responsible for homeostatic bone resorption.  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2 Thus the chaperonin 60.1 protein is a potent inhibitor of osteoclast formation while the chaperonin 60.2 protein  
3  
4 neither promotes nor inhibits osteoclastogenesis [29].  
5

## 6 **MOLECULAR CHAPERONES: CELL SIGNALLING PROTEINS AND RECEPTORS FOR CELL SIGNALLING** 7 8 **PROTEINS** 9

10 The thesis being developed in this review is that molecular chaperones (and related proteins such as protein  
11 folding catalysts – proteins such as thioredoxin and protein disulphide isomerase) have moonlighting functions  
12 and the nature of these functions depends on where these proteins exist in the organism. Within the cell it is  
13 assumed that these proteins largely act as molecular chaperones – although this is open for discussion.  
14  
15 Molecular chaperones are also found on the surfaces of bacteria and of eukaryotic cells where they can  
16 functions as receptors for a diverse, and sometimes bizarre, set of client ligands. In other examples the  
17 presence of a molecular chaperone on the surface of a cell has some other, as yet, undefined function. The  
18 best example of this is the role of certain molecular chaperones in sperm capacitation in the mouse [30]. The  
19 third site for molecular chaperones is in the extracellular fluids of the body where, it is assumed, they function as  
20 intercellular signals for a variety of cells. Another complication to our understanding of the general biology of  
21 the non-folding aspects of molecular chaperones is the significant differences in the numbers of selected  
22 molecular chaperones that different species have and in the use to which they put them. For example humans  
23 have only one functional Hsp60 (HSPD1) gene. In contrast, a not inconsiderable proportion of bacteria have  
24 more than one Hsp60 gene [31]. The differences in the activities of the *M. tuberculosis* Cpn60 proteins has  
25 been described [28,29] and an even more distinct difference in folding function [32] and signalling activity [33] is  
26 seen with the three Cpn60 proteins of the soil bacterium *Rhizobium leguminosarum*. *Drosophila melaogaster*  
27 has four Hsp60 proteins and each seems to serve a distinct function [34]. We must bring this information under  
28 the one umbrella to fully appreciate the systems biology of the molecular chaperone/cell stress protein fraternity.  
29 In this review, attention will focus on the non-folding actions of the molecular chaperones and protein folding  
30 catalysts of eukaryotes with only selected examples being taken from the literature on bacterial chaperones.  
31 For more detail on the signalling and other actions of bacterial molecular chaperones the reader is referred to  
32 [35].  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52

### 53 **Extracellular Signalling Actions of Eukaryotic Molecular Chaperones** 54

55 Most of these studies focus on the ability of recombinant molecular chaperones to interact with immune cells  
56 and activate or inhibit them. The literature suggests that molecular chaperones have a major role to play in  
57 immune homeostasis. The review of the literature will be restricted to Hsp10, Hsp60 and Hsp70 as these are  
58 the proteins which have been most closely studied and for which most reliable information is available. This  
59 review will not deal with the functioning of intracellular molecular chaperones.  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

### **Hsp (Chaperonin)10**

The first reported signalling function of a molecular chaperone was not recognised as such. This was the actions of early pregnancy factor (EPF) [10,11] which occurred in the 1970s and suggested that this moiety was immunosuppressive. It was not until 1994 that EPF was identified as chaperonin 10 or Hsp10 [12] and since then it has been shown that recombinant human Hsp10 has immunosuppressive activity *in vitro* and in animal models [36-38]. The Cpn10 protein from *M. tuberculosis* is also able to inhibit the induction of adjuvant arthritis in the rat [39]. These findings have persuaded the Australian Biotech company CBio Ltd to develop human Hsp10 (renamed XToll) for the treatment of rheumatoid arthritis. Preliminary clinical trial data shows some efficacy in patients with rheumatoid arthritis [40,41]. A phase II clinical trial of Hsp10 in a small group of patients with multiple sclerosis has shown that this protein is well tolerated but did not produce statistically significant effects [42]. A larger trial is now called for. Of relevance is the finding that circulating levels of Hsp10 in patients with periodontal disease are lower than in matched, disease-free, controls and blood levels come back to normal only after effective therapy [43]. These findings suggest that circulating Hsp10 levels are controlled by local levels of inflammation and support the hypothesis that this molecular chaperone is a homeostatic controller of inflammation. In a small study of patients with ovarian cancer it was found that HSP10 was present in the sera and ascites of patients, but was not detectable in controls. Ovarian tumour cells in culture released Hsp10. The sera which contained Hsp10 was able to inhibit the expression of a key T lymphocyte signalling protein CD3-zeta and specific removal of this protein resulted in the loss of this T cell suppressive activity [44].

Expanding the biological roles of Hsp10 is the report that erythropoietin stimulated human umbilical vein endothelial cells (HUVECs) to release Hsp10. To determine if this release was biologically relevant, the effect of Hsp10 was examined on erythroid cell differentiation. It was shown that Hsp10 decreased the proliferation of the erythroleukemia cell line K562 and increased the amounts of the erythroid differentiation markers glycophorin A and haemoglobin in TF-1 cells. Such changes were associated with specific alterations in intracellular signalling [45].

In addition to having a role in early pregnancy, potentially by acting as an immunosuppressive factor to help in preventing immune responsiveness to the early embryo, it has recently been reported that Hsp10 is present on the surface of the mouse spermatozoa where it may aid in sperm capacitation [46]. Thus in addition to having biological actions in the body fluids, Hsp10 also may have functions when on the outer cell membrane.

### **Hsp (Chaperonin)60**

The second molecular chaperone to come to the attention of the scientific community because of its alleged intercellular signalling activity was Hsp60. It was initially reported that the chaperonin 60 protein (specifically the

1  
2 Cpn60.2 or Hsp65 protein) of *M. tuberculosis* stimulated human peripheral blood-derived monocytes to release  
3 a variety of pro-inflammatory cytokines [47]. This finding started off a search for the mechanism by which this,  
4 and other, chaperonin 60 proteins stimulated monocyte activation and to discriminate the effects from that of the  
5 key Gram-negative cell wall component, lipopolysaccharide (LPS). While this article focuses on mammalian  
6 (mainly human) Hsp60 proteins, much of our information of the signalling properties of Hsp60 proteins have  
7 come from the study of bacterial proteins; as this may have relevance to the properties of the human protein it  
8 will be briefly discussed. A major area of current research in immunology and infectious diseases research is  
9 the activation status of human or rodent monocytes [48]. Macrophages exposed to interferon gamma (IFN $\gamma$ ) or  
10 to LPS are called classically-activated macrophages and are 'activated' in a way that promotes the presentation  
11 of antigens to T lymphocytes (e.g. upregulation of MHC class II proteins) or kills bacteria (production of reactive  
12 oxygen radicals). However, it has been found in more recent years that other signals can induce a range of  
13 other macrophage activation states that have been termed alternative activation [48]. The finding that *M.*  
14 *tuberculosis* Hsp60.2 protein stimulated the production of pro-inflammatory cytokines suggested that it was  
15 inducing a classically-activated state. However, while the exposure of human monocytes to *M. tuberculosis*  
16 Hsp60.2 induced the production of the same amount of cytokines released by cells exposed to IFN $\gamma$  together  
17 with LPS, cells stimulated with Hsp60.2 did not show the increased expression of Fc $\gamma$ -receptors, MHC class II  
18 proteins or the release of reactive oxygen intermediates. Thus *M. tuberculosis* Hsp60.2 is not inducing a  
19 classically activated state [49]. Further evidence that the Hsp60.2 protein from *M. tuberculosis* produces an  
20 activation state different from that of other cell activators, such as LPS, is the finding that this mycobacterial  
21 protein induces cultured human vascular endothelial cells to synthesise the classic leukocyte adhesion  
22 receptors (ICAM, VCAM, E-selectin) in a cytokine (IL-1, TNF $\alpha$ )-independent manner [50]. This contrasts with the  
23 literature, which suggests that the synthesis of these adhesion molecules requires the prior induction of IL-1 or  
24 TNF $\alpha$  [51].

25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48 Later in the 1990s, attention turned to the human Hsp60 protein with Hubert Kolb and co-workers being the first  
49 to show that this protein could stimulate human and mouse monocytes to synthesise pro-inflammatory cytokines  
50 (including TNF $\alpha$  and the Th1 cytokines IL-12 and IL-15) and mouse cells to produce nitric oxide. Human Hsp60  
51 also synergised with IFN $\gamma$  in inducing cytokine synthesis [52]. Activation of human monocytes and macrophages  
52 was then shown to depend, like LPS, on binding to CD14 with activation of p38 mitogen-activated protein (MAP)  
53 kinase [53] and Kolb's group reported that human Hsp60 failed to activate monocytes from the LPS-insensitive  
54 C3H/HeJ mouse which has a non-functional Toll-like Receptor (TLR)4 LPS-interacting protein. The inference  
55 was that human Hsp60 had to bind to, or somehow interact with, monocyte cell surface TLR4 to induce cell  
56 activation [54]. This has been the start of an unfinished journey into the 'receptorology' of molecular  
57  
58  
59  
60

1  
2 chaperones, which is still a confusing aspect of these proteins and is a major problem with the biology of Hsp60  
3  
4 proteins. Kolb's work pointed to TLR4 as the key receptor for human Hsp60 and Vabulas and colleagues, using  
5  
6 cell transfection methodology, reported that human fibroblasts transfected with TLR2 or TLR4 plus MD-2 (a co-  
7  
8 receptor required for cell activation) gain responsiveness to Hsp60, while TLR2- or TLR4-defective cells  
9  
10 displayed limited response. Moreover, these workers found that the Hsp60 had to be endocytosed to activate  
11  
12 cells. Further analysis of human Hsp60 binding, using fluorescently-labelled recombinant Hsp60, revealed that  
13  
14 it bound to cells and could be competed with unlabelled Hsp60 but not by unlabelled Hsp70, Hsp90 or gp96.  
15  
16 The labelled Hsp60 bound to TLR4 negative cell lines (although it did not activate the cells), showing that the  
17  
18 primary receptor is another cell surface protein. These findings clearly show that Hsp60 has properties distinct  
19  
20 from the Hsp70 and Hsp90 family members [55]. Comparison of Hsp60 proteins from mammals and bacteria  
21  
22 revealed that human, rat and mouse Hsp60 proteins all cross-compete for binding to murine macrophages.  
23  
24 However, it was surprising to find that hamster Hsp60 could not compete the binding of human Hsp60. Neither  
25  
26 did the Hsp60 proteins from *E. coli*, *Chlamydia pneumonia* or *Mycobacterium bovis* compete with the human  
27  
28 Hsp60 protein for binding to mouse macrophages [56]. Using human Hsp60 truncation mutants and  
29  
30 overlapping human Hsp60 synthetic peptides, the site in Hsp60 binding to macrophages was identified as the C-  
31  
32 terminal region – residues 481-500 [57].

33  
34 There is still obviously much to learn about the ligand-receptor systems that drive Hsp60 stimulation of myeloid  
35  
36 cells. More recent studies of human Hsp60 have revealed that this protein has unexpected effects on  
37  
38 macrophages, dendritic cells and on lymphocytes. There are a number of reports that human Hsp60 can  
39  
40 promote dendritic cell maturation and activate mature dendritic cells [58]. Addition of Hsp60 to murine bone  
41  
42 marrow-derived dendritic cells (DC) potently stimulated the secretion of IL-1 $\beta$ , IL-12 and TNF $\alpha$ , but only a small  
43  
44 amount of IL-10 suggesting a Th1 bias. The dendritic cells exposed to Hsp60 were able to induce proliferation  
45  
46 of allogeneic T lymphocytes. These changes in DC behaviour were mirrored by changes in kinase signalling.  
47  
48 Of interest was the finding that the MAP kinase activation induced by Hsp60 was also mediated by LPS but that  
49  
50 the kinetics of the LPS activation was slower than that of Hsp60 [59]. Another study has suggested that unlike  
51  
52 LPS, human Hsp60 stimulates both murine macrophages and dendritic cells to secrete IFN $\alpha$  and neutralisation  
53  
54 of this cytokine blocked Hsp60-induced IFN $\gamma$  production by DCs. This suggests that IFN $\alpha$  production  
55  
56 contributes to Hsp60-specific immune stimulation [60].

57  
58 The interaction of Hsp60 with T lymphocytes opens up a considerable debate, because it was recognised from  
59  
60 about the time of the identification of Hsp60 as a molecular chaperone, that this protein was also a potent  
immunogen and T/B cell modulator. An early finding was that human T cells from healthy individuals, and from  
sites of disease, were able to recognise Hsp60 proteins, including the human protein and that such reactivity

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

contributed to the autoimmune-type phenomena often found in infections [61]. One of the most puzzling aspects of the Hsp60 molecule is its association with T cell modulation and immunity. An early observation was that T cell immunity to a specific epitope in the *M. tuberculosis* Hsp60.2 protein could be responsible both for inducing AND preventing adjuvant arthritis in the rat [62]. Adjuvant arthritis is a curious chronic inflammatory and massively destructive disease of the joints of rats which somewhat resembles human rheumatoid arthritis. Thirty years after this discovery the mechanisms of disease induction and cure by Hsp60 is still somewhat mysterious but is now becoming the basis for specific therapies [63]. In most of this work the Hsp60 has been regarded as an immunogen with limited specific biological activity in its own right. The finding that Hsp60 proteins can modify the actions of myeloid cells has prompted a reappraisal of the direct influence of Hsp60 proteins on lymphocyte functions.

Hill Gaston was among the first to show that human Hsp60 stimulated the proliferation of naive human T lymphocytes [64]. Broeler and co-workers [65] then showed that human Hsp60 promotes antigen-specific production of IFN $\gamma$  in conditions when the antigenic stimulus is not sufficient to activate T lymphocytes. This same group then reported that human Hsp60 could act in the stimulation of CD8 lymphocytes [66]. Another study showed that when added to murine T cells (in the presence of peritoneal exudate cells as antigen-presenting cells (APCs)) human Hsp60 induced the specific secretion of large amounts of IFN $\gamma$ . In contrast, dendritic cells (DCs), which are efficient APCs, released less IFN $\gamma$  when exposed to Hsp60. The ability to induce IFN $\gamma$  is dependent on the co-stimulatory pairing of CD80/86 with CD28. Similar results were seen with recombinant murine Hsp60, although one difference was the finding that the murine protein did not bind to CD14. This is in spite of the fact that the human and murine proteins share >98% sequence identity [67], emphasising the fact that it is not possible to treat the Hsp60 proteins as a single biological entity.

How does human Hsp60 cause the activation of T cells? Irun Cohen's group have examined the interaction of highly purified recombinant Hsp60 with T lymphocytes in the past five years. The first conclusion was that the direct action of human Hsp60 with T lymphocytes required the participation of TLR2 [68]. In this study T cell adhesion to fibronectin was stimulated to the same extent as established activators of this process and at concentrations as low as 0.1ng/ml (around 100pM). Surprisingly, Hsp60 inhibited T cell chemotaxis induced by the chemokines CXCL12 and CCL19 and with long-term exposure down-regulated the expression of the chemokine receptors CXCR4 and CCR7 [68]. This finding that human Hsp60 has inhibitory actions was expanded in later papers from this group. HSP60 is also reported to inhibit synthesis of the transcription factors: T-bet, NF- $\kappa$ B, and NFATp and to increase production of GATA-3, leading to decreased secretion of pro-inflammatory TNF $\alpha$  and IFN- $\gamma$  but increased production of the anti-inflammatory cytokine, IL-10. Administration of Hsp60 to Balb/c mice with ConA-induced hepatitis inhibited clinical, serological and histological markers of



1  
2  
3 disease and increased levels of the transcription factors SOCS3 and GATA-3 while downregulating Tbet  
4 expression in T lymphocytes [69]. It was further shown that Hsp60 stimulates SOCS3 expression in T  
5 lymphocytes via the TLR2 receptor causing signalling via the JAK/STAT pathway which modulates T cell  
6 chemotaxis. A surprising finding was the concentration dependency of these effects. Hsp60 was active at  
7 concentrations of 0.1 to 1.0ng/ml (approximately 0.1-1.0pM) but not active in the range 10-100ng/ml but again  
8 active at concentrations of 1µg/ml. One possible explanation is the involvement of different receptors with  
9 different affinities or of some form of receptor activation complex such as is postulated for LPS [70]. A  
10 fascinating suggestion is that Hsp60 acts as a co-stimulator of human T-regulatory cells (Tregs). Cohen's group  
11 has presented data that Hsp60 co-stimulation of Tregs leads to activation of various intracellular kinases and  
12 the suppression of target T cells both by cell-cell contact and by the secretion of TGFβ and IL-10 [71]. Addition  
13 of Hsp60 (but not *E. coli* GroEL or *M. tuberculosis* Cpn60.2) to naive murine B cells stimulated cell proliferation,  
14 the release of IL-6 and IL-10 and increased surface expression of CD40, CD69 and CD86. This cell modulation  
15 was dependent not on TLR2 binding but on the TLR4/MyD88 signalling system. Thus both mammalian T and B  
16 cells respond to Hsp60 in unexpected ways [72].

17  
18 Two other aspects of the Hsp60 protein will be briefly discussed. The first concerns the insect known as the  
19 Doodlebug. The larval form of this insect lives in sandy soil and hunts prey. It captures its prey by biting them  
20 and injecting a paralysing neurotoxin. It turns out that the neurotoxin is actually produced by a symbiotic  
21 salivary bacterium – *Aerobacter aerogenes*. When this toxin was isolated and sequenced it turned out to be  
22 the Hsp60 protein of the bacterium and a protein that was virtually identical to *E. coli* GroEL. Indeed, single  
23 amino acid mutations can turn GroEL, which has no neurotoxic properties, into a potent neurotoxin [73]. So like  
24 a molecular Meccano® set one can turn GroEL into a new type of molecule with minimal tinkering. The second  
25 use to which Hsp60 has been put has been briefly touched on with regard to Hsp10 and sperm capacitation. It  
26 was reported in 2004 that mouse sperm capacitation required the presence of Hsp60 on the sperm head and  
27 that this protein had to undergo tyrosine phosphorylation to trigger conformational changes facilitating the  
28 formation of a functional zona pellucida receptor complex on sperm surface [74]. Surprisingly, the same role for  
29 Hsp60 **cannot be accorded to human sperm** capacitation [75] revealing major differences in the role played by  
30 molecular chaperones in different species.

### 31 **Hsp70 Family**

32  
33 Humans encode at least 12 Hsp70 proteins [76]. This brings with it some degree of uncertainty in the literature  
34 as it is often not exactly clear what Hsp70 protein individual authors are describing. For readers who want to  
35 know more about these and other molecular chaperones see [77] which describes new guidelines for the  
36 nomenclature for molecular chaperones (also Table 1). This has not been used in this review as most of the  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2 literature that readers would refer to use the older nomenclature. However, it is likely that this will change to the  
3 new nomenclature in the coming years.  
4

5  
6 Alexzander Asea and Stuart Calderwood were the first to describe that commercially available recombinant  
7 Hsp70 (the exact protein was not specified, but was probably Hsp70-1a (HSPA1A) or Hsc70 (HSPA8))  
8 stimulated human monocytes to produce pro-inflammatory cytokines by a mechanism involving rapid  
9 intracellular calcium flux [78]. They termed Hsp70 a **chaperokine**, a term that does not seem to have caught the  
10 scientific imagination. Importantly, this calcium flux was not seen in cells stimulated with LPS [78], which is  
11 always a potential contaminant in *E. coli*-derived recombinant proteins. Other groups repeated and extended  
12 this finding with murine monocytes [79] and showed that purified murine Hsp70 (a mixture of Hsp70 proteins)  
13 stimulated nitric oxide production [80]. Initial studies employing selective inhibitors and appropriately  
14 transfected cell lines came to the conclusion that 'Hsp70' activation of myeloid cells was through a receptor  
15 complex involving CD14, TLR2 and TLR4 [79,81]. Thus the original hypothesis was that 'Hsp70' was a pro-  
16 inflammatory signal interacting with the classic innate immune signalling receptors.  
17  
18

19  
20 A consensus appeared to have been reached by early 2000 concerning the receptors and intracellular signalling  
21 pathways utilized by mammalian 'Hsp70'. These appeared to be the same as were being interacted with by the  
22 majority of the Hsp60 proteins. However, Tom Lehner in London, who has concentrated on the Hsp70 (DnaK)  
23 protein from *M. tuberculosis*, reported that this recombinant protein, but not human 'Hsp70', stimulated human  
24 monocyte chemokine and cytokine synthesis through binding to CD40, a member of the TNF receptor family  
25 [82,83]. Biological activity resided in the C-terminal peptide-binding part of the molecule [83]. Further analysis  
26 using synthetic peptides has identified activating and inhibiting epitopes within this region [84]. In contrast,  
27 Ulrich Hartl and colleagues have reported that human 'Hsp70' binds to CD40 via the amino-terminal nucleotide  
28 binding domain when in the presence of ADP (but not when ATP was present) and activates NF- $\kappa$ B [85]. Hsp70  
29 proteins interact with co-chaperones such as DnaJ (Hsp40), Hip and Bag-1. Hartl has shown that Hip competes  
30 with 'Hsp70' for binding to CD40 [85]. The potential binding specificity of 'Hsp70' is further confused as a range  
31 of other receptors have been claimed to bind to 'Hsp70'. Lehner has recently reported that *M. tuberculosis*  
32 Hsp70 binds to the chemokine receptor/HIV co-receptor, CCR5 [86]. This raises the obvious question of  
33 whether members of the human Hsp70 family bind to this receptor – which could have interesting implications  
34 for the control of HIV infection. Human 'Hsp70' has been reported to bind to a range of other human cellular  
35 receptors including: CD91 [87], the scavenger receptors (SRs) such as LOX-1 [88,89] and C-type lectin  
36 receptors [90]. To further confuse the situation with the 'Hsp70' receptor, Stuart Calderwood, who has done  
37 much of the work on identifying receptors for Hsp70 (including identifying CD14/TLR2/TLR4 as receptors [78]),  
38 now reports that human 'Hsp70' only binds to members of the scavenger receptor family ([90] and personal  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2 communication). One member of the human Hsp70 family (Hsp70-8 also called Hsc70, Hsp73 or in the new  
3 proposed nomenclature HSPA8) has been reported to bind to EW1-2, - an early activation marker of dendritic  
4 cells [91]. Binding of HSPA8 to EW1-2 resulted in enhanced chemokine-dependent migration of activated mature  
5 dendritic cells but their antigen-specific stimulatory actions were attenuated [91]. Hartl has recently shown how  
6 complex are the interactions of Hsp70, and its two separate domains, with the surface of leukocytes [92].  
7  
8  
9

10  
11 As will be described, 'Hsp70' is present in human blood and a number of studies report that circulating  
12 concentrations are negatively correlated with symptoms of cardiovascular disease suggesting that 'Hsp70' is  
13 'cardioprotective' [93-95]. This possibly flies in the face of the evidence that 'Hsp70' is a pro-inflammatory  
14 factor. This was the situation with Hsp60 until more recent studies, described above, revealed that the human  
15 and *M. tuberculosis* Hsp60.1 proteins can inhibit leukocyte activation [see 29,69]. Thus it is possible that Hsp70  
16 proteins may have anti-inflammatory properties at low concentrations.  
17  
18  
19

20  
21 In fact, another Hsp70 family member, BiP, has been shown to be a potent anti-inflammatory protein. Valerie  
22 Corrigan's group identified BiP as an autoantigen in rheumatoid arthritis [26 41]. Assuming BiP would induce  
23 arthritis, the recombinant protein was administered to mice and rats, but without effect [26 41]. However,  
24 administration to rodents with experimental arthritis inhibited the initiation and/or severity of disease [26 41].  
25  
26 The explanation appears to be that BiP (in contrast to 'Hsp70') stimulates leukocytes to produce an anti-  
27 inflammatory cytokine network involving the predominant synthesis of IL-10 and IL-4 [27,96]. To gain more  
28 information about the effects of BiP on monocyte activation, Affymetrix microarrays were used to measure  
29 global transcriptional responses of these cells to BiP. Two normal subjects were used, and a single time point,  
30 24h, was selected for study. The results were remarkable in showing changes in around 900 genes, with 75%  
31 of these genes being downregulated. Of note, was the 3 fold downregulation of HSP70-1a mRNA levels  
32 following BiP stimulation. This suggests the hypothesis that Hsp70 family members regulate each other's  
33 synthesis. To date, the receptor for BiP has not been identified [97] and it is not any of those that binds 'Hsp70'  
34 [97]. Corrigan has shown that BiP is present in synovial fluid and serum of rheumatoid patients [97]. Henderson  
35 has recently shown that patients with periodontitis, have significantly lower circulating levels of BiP than  
36 comparable normal controls [43].  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52

53  
54 The most distant members of the Hsp70 family, the Hsp110/SSE and Grp170 proteins, share similarity to Hsp70  
55 but have additional domains. These proteins act as regulatory factors for Hsp70 [98,99] and, importantly, they  
56 have also been implicated as potent extracellular signalling proteins [100,101].  
57  
58

59  
60 In conclusion, the evidence provided in this review and other studies support the hypothesis that 'Hsp70'  
proteins are potent intercellular signalling molecules with either pro- or anti-inflammatory actions, working  
through a bewildering variety of cell surface receptors to provide homeostatic or pathological network signaling

1  
2  
3 important in immune and other body functions [102]. The confusing nature of the literature is almost certainly  
4 the result of different workers using different proteins (either purified single proteins, 'purified' mixtures of  
5 proteins or recombinant proteins). Post-translational modifications (which will not be present in recombinant  
6 proteins prepared in *E. coli*) could influence activity. A more serious issue hindering the study of the Hsp70  
7 system is a small number of papers which claim that the activity of 'Hsp70' protein is due to either contaminating  
8 *E. coli* components such as LPS [103] or flagellin [104], or to nucleotides left over as part of the isolation  
9 procedure [105]. The flagellin paper [104] focused on Hsp70 stimulation of T cells. However, Henderson has  
10 shown that recombinant *E. coli* flagellin is a very weak activator of human monocytes [33]. and Figueiredo and  
11 coworkers have recently reported that 'Hsp70' expressed in eukaryotic cells is still active as a T lymphocyte  
12 stimulator[106], refuting the work of Gao and Ye [103,104]. In addition, the work of Lehner and co-workers has  
13 comprehensively ruled out LPS as a contributor to the cellular effects produced by *M. tuberculosis* Hsp70 [84].

#### 24 **MOLECULAR CHAPERONES ARE FOUND IN THE EXTRACELLULAR FLUID AND ON THE EXTERNAL** 25 **PLASMA MEMBRANE**

26  
27  
28 Interesting as these studies are, they could be irrelevant if the molecular chaperones involved are not secreted.  
29  
30 There is now increasing evidence for the passage of molecular chaperones both to the cell surface and to the  
31 extracellular fluids of the body. It is also emerging that proteins such as Hsp70 family members and Hsp60 can  
32 act as receptors for a variety of ligands.

33  
34  
35 The Hsp10, Hsp60 and HSPA9 (also known as Hsp70-9, Grp75, mtHsp75, Mortalin) proteins are nuclear-  
36 encoded proteins that target to the mitochondrion where they are considered to have their only functions [107].  
37  
38 The remaining Hsp70 family members are found in the cytosol, nucleus, lysosomes and ER [107]. However, it  
39 has been known for some years now, thanks to the work of Radhey Gupta, that these mitochondrial chaperones  
40 can be found in other parts of the cell including the outer surface of the plasma membrane [108]. Thus Hsp60  
41 has been directly observed on the outer plasma membrane by using surface-labelling techniques linked to  
42 electron microscopy [109]. This Hsp60 protein is also found in other non-mitochondrial locations in cells. For  
43 example, in pancreatic acinar cells and pituitary cells (both secretory cells), Hsp60 is found in zymogen granules  
44 and growth hormone granules respectively [110]. Indeed, Hsp10 has also been immunolocalised in these sites  
45 [111]. The mitochondrial form of Hsp70 (HSPA9) has also been localised in the plasma membrane and in  
46 different cellular granules [reviewed in 108]. Intriguingly, many bacteria utilise cell surface Hsp60 and Hsp70 as  
47 bacterial adhesins which bind with a wide variety of host ligands [35].

48  
49  
50 Other members of the Hsp70 family have also been found in the plasma membrane. Possibly the most  
51 intriguing finding of Hsp70 in plasma membranes is the hypothesis forwarded by Triantafilou, based on  
52 biophysical measurements, that the receptor for the Gram-negative inflammogen, LPS, contains a number of

1  
2 proteins including Hsp70. The other members of this so-called LPS activation complex are Hsp90, CXCR4 and  
3 growth differentiation factor (GDF)5 [112,113]. Other workers have reported that Hsp70 members in plasma  
4 membranes act as 'receptors' for viruses. This was reported for human T-cell lymphotropic virus type 1 [114].  
5  
6 Other viruses proposed to bind to cell surface Hsp70 (type normally unknown) include Dengue [115] and  
7 Japanese encephalitis virus [116].  
8

9  
10 There is still some degree of reluctance to accept the findings that molecular chaperones can escape from cells.  
11 This is a prejudice largely based upon ignorance of the number of mechanisms that eukaryotic cells (and  
12 bacteria) have for secreting proteins. It is now clear that a number of mechanisms in addition to the ER-Golgi-  
13 signal peptide mechanism exist for the secretion of proteins from eukaryotic cells [117]. The first secretion  
14 mechanism identified for Hsp70 release was through the formation of exosomes. These are small membrane  
15 vesicles that are secreted by numerous cell types. It was shown that Hsp70 (non-mitochondrial) was released  
16 by human peripheral blood mononuclear cells, in both unstressed and heat shocked states in the form of  
17 exosomes [118,119]. Exosomal release has also been proposed for the Hsp60 protein [120] although as a  
18 mitochondrial protein the secretion pathway is likely to be more complex than that of Hsp70. One of the most  
19 fascinating stories about protein secretion centres around IL-1. This key early response pro-inflammatory  
20 cytokine does not have a leader sequence and it is only in very recent years that the mechanism of secretion  
21 has begun to yield to investigation. There is evidence that IL-1 secretion is linked to a complex mechanism  
22 involving secretory lysosomes, phospholipase A<sub>2</sub> and the purinoceptor P2X7 [121,122]. The mechanism of  
23 release of Hsp70 has been described as being similar to that of IL-1, at least in respect to the involvement of the  
24 secretory lysosomal compartment [123]. Currently, there is no known mechanism to explain the secretion of  
25 Hsp10.  
26  
27

28 Thus there are appearing mechanisms to account for the presence of molecular chaperones in body fluids. This  
29 review will end with a brief review of the literature on the presence of Hsp10, Hsp60 and Hsp70 in body fluids.  
30

### 31 **Hsp10**

32 As described, this was the first molecular chaperone to be identified in the blood as early pregnancy factor.  
33 Little more is known of this protein in body fluids. In a recent study Henderson & colleagues analysed blood  
34 from patients with periodontitis, a chronic inflammatory disease of the gums and compared them with age and  
35 gender matched controls. Surprisingly, Hsp10 was found in the majority of the bloods from the controls. In  
36 contrast, the levels of Hsp10 in patients with periodontitis were significantly lower. The patients were then  
37 divided into two groups. One had a very rigorous form of treatment which, in essence, cured the disease. The  
38 others were given a lesser form of treatment which meant that disease returned. In the first group, levels of  
39 circulating Hsp10 returned to normal whereas the second group remained lower – similar to pretreatment levels.  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 These findings suggest Hsp10 is a normal component in the plasma and that levels of Hsp10 are able to be  
4 influenced by degrees of inflammation. This seems to be at variance with the concept of Hsp10 only appearing  
5 in the blood during early pregnancy. This may depend on the sensitivity of the assays being used. The other  
6 interesting finding is that levels are controllable. However, while Hsp10 levels showed marked changes in  
7 circulating concentrations there was no changes in Hsp60 levels in these periodontal patients[43]. This finding  
8 is particularly intriguing as the Hsp60 (HSPD1) and Hsp10 (HSPE1) genes are linked head to head with 17 kb of  
9 DNA making up both genes which consist of 12 and 4 exons, respectively. The first exon of the human HSP60  
10 gene is non-coding and the first exon of the human HSP10 gene ends with the start codon. The region in  
11 between is a bidirectional promoter with the transcriptional activity of the promoter fragment in the HSP60  
12 direction being approximately twice that in the HSP10 direction under normal growth conditions [124]. Given  
13 this arrangement it is not clear how the levels of both proteins in blood could differ so markedly with one being  
14 responsive to environmental conditions and the other not [43]. Much more work is required to determine how  
15 levels of these two proteins are controlled in the circulation.  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27

### 28 **Hsp60**

29  
30 Graham Pockley was the first to determine the presence of Hsp60 in the blood of normal individuals (in  
31 transfusion samples) [125]. Given the work of Georg Wick, who had proposed a role for immunity to Hsp60 in  
32 the pathogenesis of atherosclerosis and the development of coronary artery disease [126], it seemed sensible  
33 to see if there was any correlation between Hsp60 levels and heart disease. Wick and co-workers looked at  
34 levels of circulating Hsp60 in participants in the Bruneck Study, an Italian study of a general population to  
35 identify disease risk factors. This revealed that circulating levels of Hsp60 were significantly elevated in subjects  
36 who showed signs of atherosclerosis as determined by measuring the common carotid artery intima/media  
37 thickness. Multiple logistic regression analysis determined that these associations were independent of age,  
38 sex, and other risk factors [127]. Follow up studies provided data confirming an association between high levels  
39 of blood Hsp60 and early carotid atherosclerosis [128]. Pockley studied a cohort of patients with borderline  
40 hypertension, compared to a comparable normotensive group, and reported that those with borderline  
41 hypertension had elevated levels of circulating Hsp60 which was associated with intima-media thicknesses  
42 [129]. However, there was no difference between Hsp60 levels in individuals with established hypertension and  
43 normotensives [130].  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55

56  
57 The Whitehall Study, a large prospective study of 10,000 British civil servants established that psychological  
58 stress is an important risk factor for cardiovascular disease [131]. Measurement of circulating Hsp60 in a small  
59 subset of these subjects revealed a correlation between blood Hsp60 levels and psychological distress in  
60 females [132]. In a larger cohort there was a significant correlation between circulating Hsp60 levels and

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
psychological distress in both males and females [133] and analysis of vascular damage by flow-mediated vasodilation measurements showed that Hsp60 levels correlated with such damage [134]. Measurement of Hsp60 levels in a cohort of healthy teenagers revealed that only 25% had circulating levels of Hsp60 but that these were the individuals who showed vascular damage as determined by flow mediated vasodilatation measurements [135]. This suggested that Hsp60 in the blood may be a predisposing factor for the development of atherosclerosis. Given the fact that Hsp60 has profound effects on leukocytes this may not be so far-fetched. One of the most fascinating findings about Hsp60 in the circulation is that only 50-60% of the population manifest this protein in their blood and that this seems to be a genetically defined event. Measurement of Hsp60 levels in individuals over a period of 30 days revealed complete stability in circulating levels. More strikingly, in blood samples taken 3 years apart, the same individual had virtually the same levels of Hsp60 in their blood. The other very striking finding is the range of concentrations of Hsp60 found in blood. 40-45% of the human race have no measurable Hsp60. These individuals have no circulating factors which interfere with the immunoassays used to measure Hsp60. The remaining 55-60% of the human race can have nanograms/ml, micrograms/ml or, in a small proportion of the population milligrams/ml. This is a million-fold range. No other protein has such a range of circulating levels [133]. We have used peptide fingerprinting and MALDI-TOF MS to identify the circulating protein, which is the intact Hsp60 monomer which has been into the mitochondrion and lacks the mitochondrial import peptide. How this protein exits from the mitochondrion and then from the cell is a key question, as is the source of the Hsp60 and its cellular effects.

### **Hsp70**

38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
Again Graham Pockley was first to report that Hsp70 was present in the blood of normal individuals [136]. The first evidence for changes of Hsp70 levels in disease was a study of patients with peripheral and renal vascular disease [137]. However, measurement of Hsp70 levels in a cohort of individuals who have been assessed for signs of coronary artery disease has revealed a negative correlation between Hsp70 concentrations in the blood and risk of coronary artery disease [138]. In a separate study progression of intimal-medial thickness of the carotids (a standard measurement of atherosclerosis development) was less pronounced in individuals with high levels of circulating Hsp70 [139]. Thus the hypothesis would have to be modified to one which classes Hsp70 as a protective factor for heart disease. A number of other reports have revealed the presence or changes in Hsp70 levels in various conditions or under various treatment regimes. This literature is rather diverse and to maintain the focus of this article the interested reader is advised to do the appropriate literature searches.

### **CONCLUSIONS**

At the time of writing, 22 years have elapsed between John Ellis's seminal paper in Nature [140] on the function of the Hsp60 protein as a protein-folding chaperone and our emerging view that molecular chaperones are

1  
2 serious moonlighting proteins with a growing array of diverse functions which are unrelated to protein folding.  
3  
4 These functions are generated by molecular chaperones present on the cell surface or in the extracellular fluids.  
5  
6 Some of the functions of a selected trio of human and mouse proteins have been described in this article and  
7  
8 the role of bacterial homologues briefly touched upon. While the functions of molecular chaperones inside the  
9  
10 cell are now generally clear and sensible, the growing functions for cell surface and secreted molecular  
11  
12 chaperones tend to create a feeling that 'this can't quite be right'. For example, the finding that around 60% of  
13  
14 the population have Hsp60 in their circulation and that levels of this protein can range from a few nanograms  
15  
16 per ml to mgs/ml raises a plethora of questions, few of which can yet be answered. These results reveal that  
17  
18 we have still a long way to go to understand the extracellular biology of chaperone proteins.

19  
20 The most likely explanation for the evolution of moonlighting activity in the molecular chaperones is the  
21  
22 enormous importance of the cell stress response to the survival of the individual cell and to the organism which  
23  
24 these cells generate. It is a sensible hypothesis to suggest that stress in any particular part of the organism  
25  
26 needs to be integrated into the homeostatic regulation of the whole organism. This is what is believed to be the  
27  
28 evolutionary rationale for the signalling actions of molecular chaperones. They are the archetypal 'danger  
29  
30 signals' as stress is the ubiquitous danger for all organisms. Thus it is likely that in the next decade the real  
31  
32 biology of molecular chaperones will be identified as being a continuum between the protein folding actions  
33  
34 within the cell to selective cell signalling actions on the cell surface and in the wider body milieu. In a previous  
35  
36 publication we have termed this phenomenon – STRESS BROADCASTING [141]. A very simple diagrammatic  
37  
38 view of this hypothesis is presented in Figure 2.

## 41 REFERENCES

- 42  
43 1. Henderson B. Out of one eye: A life integrating cellular biochemistry and function. *Cell Biochem Funct* 2003;  
44  
45 **21**: 201-206.  
46  
47 2.Laskey RA, Honda BM, Mills AD. Finch JT. Nucleosomes are assembled by an acidic protein that binds  
48  
49 histones and transfers them to DNA. *Nature* 1978; **275**: 416-420.  
50  
51 3.Ellis JR. Personal perspective: From chloroplasts to chaperones: how one thing led to another. *Photosynth*  
52  
53 *Res* 2004; **80**: 333-43.  
54  
55 4.Ellis RJ 2005 Chaperone function: The orthodox view. In *Molecular Chaperones and Cell Signalling* (eds  
56  
57 Henderson B, Pockley AG) Cambridge University Press, Cambridge; 3-21.  
58  
59 5.Martin J. Chaperonin function - effects of crowding and confinement. *J Mol Recognit* 2004; **17**: 465-472.  
60  
6. Ellis RJ. (2007) Protein misassembly: macromolecular crowding and molecular chaperones. *Adv Exp Med Biol*  
2007; **594**: 1-13.



- 1  
2  
3 7.Shinnick TM, Vodkin MH, Williams JC. The *Mycobacterium tuberculosis* 65-kilodalton antigen is a heat shock  
4 protein which corresponds to common antigen and to the Escherichia coli GroEL protein. *Infect Immun* 1988;  
5 **56**:446-451.  
6  
7
- 8 8.Young DB. Chaperonins and the immune response. *Semin Cell Biol* 1990; **1**: 27-35  
9
- 10 9.Kang CB, Hong Y, Dhe-Paganon S, Yoon HS. FKBP family proteins: immunophilins with versatile biological  
11 functions. *Neurosignals* 2008;**16**:318-325.  
12  
13
- 14 10.Morton H, Rolfe B, Clunie GJ. An early pregnancy factor detected in human serum by the rosette inhibition  
15 test. *Lancet* 1977; **1**:394-397.  
16  
17
- 18 11.Noonan FP, Halliday WJ, Morton H, Clunie GJ. Early pregnancy factor is immunosuppressive. *Nature* 1979;  
19 **278**:649-651.  
20  
21
- 22 12.Cavanagh AC, Morton H. The purification of early-pregnancy factor to homogeneity from human platelets  
23 and identification as chaperonin 10. *Eur J Biochem* 1994; **222**:551-560.  
24  
25
- 26 13.Horwich AL, Farr GW, Fenton WA. GroEL-GroES-mediated protein folding. *Chem Rev* 2006; **106**:1917-1930.  
27  
28
- 29 14.Hightower LE, Guidon PT. Selective release from cultured mammalian cells of heat-shock (stress) proteins  
30 that resemble glia-axon transfer proteins. *J Cell Physiol* 1989; **138**:257-266.  
31  
32
- 33 15.Tytell M, Greenberg SG, Lasek RJ. Heat shock-like protein is transferred from glia to axon. *Brain Res* 1986;  
34 **363**:161-164.  
35  
36
- 37 16.Campbell RM, Scanes CG. Endocrine peptides 'moonlighting' as immune modulators: roles for somatostatin  
38 and GH-releasing factor. *J Endocrinol* 1995; **147**:383-396.  
39  
40
- 41 17.Jeffery CJ. Moonlighting proteins. *Trends Biochem Sci* 1999; **24**:8-11.  
42  
43
- 44 18.Jeffery CJ. Molecular mechanisms for multitasking: recent crystal structures of moonlighting proteins. *Curr*  
45 *Opin Struct Biol* 2004; **14**:663-668.  
46  
47
- 48 19.Henderson B. Moonlighting in protein hyperspace: Shared moonlighting proteins and bacteria-host cross-  
49 talk. In *The Influence of Cooperative Bacteria in Animal Host Biology* (eds McFall-Ngai M, Henderson B, Ruby  
50 EG) pp347-374. Cambridge University Press 2005.  
51  
52
- 53 20.Faik P, Walker JI, Redmill AA, Morgan MJ. Mouse glucose-6-phosphate isomerase and neuroleukin have  
54 identical 3' sequences. *Nature* 1988; **332**:455-457.  
55  
56
- 57 21.Funasaka T, Raz A. The role of autocrine motility factor in tumor and tumor microenvironment. *Cancer*  
58 *Metastasis Rev* 2007; **26**:725-735  
59  
60
- 22.Haga A, Komazaki S, Funasaka T, Hashimoto K, Yokoyama Y, Watanabe H, Raz A, Nagase H. AMF/G6PI  
induces differentiation of leukemic cells via an unknown receptor that differs from gp78. *Leuk Lymphoma* 2006;  
**47**:2234-2243.

- 1  
2  
3 23. Schulz LC, Bahr JM. Glucose-6-phosphate isomerase is necessary for embryo implantation in the domestic  
4 ferret. *Proc Natl Acad Sci U S A* 2003; **100**:8561-8566.  
5  
6 24. Chiu CG, St-Pierre P, Nabi IR, Wiseman SM. Autocrine motility factor receptor: a clinical review. *Expert Rev*  
7  
8 *Anticancer Ther* 2008; **8**:207-217.  
9  
10 25. Asea A, Kraeft S-K, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK. Hsp70  
11 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone  
12 and cytokine. *Nature Med* 2000; **6**: 435-442.  
13  
14 26. Corrigan VM, Bodman-Smith MD, Fife MS, Canas B, Myers LK, Wooley PH, Soh C, Staines NA, Pappin  
15 DJC, Berlo SE, van Eden W, van der Zee R, Lanchbury JS, Panayi GS. The human endoplasmic reticulum  
16 molecular chaperone BiP is an autoantigen for rheumatoid arthritis and prevents the induction of experimental  
17 arthritis. *J Immunol* 2001; **166**: 1492-1498.  
18  
19 27. Corrigan VM, Bodman-Smith MD, Brunst M, Cornell H, Panayi GS. Inhibition of antigen-presenting cell  
20 function and stimulation of human peripheral blood mononuclear cells to express and anti-inflammatory cytokine  
21 profile by the stress protein BiP. *Arthr Rheum* 2004; **50**: 1164-1171.  
22  
23 28. Hu Y, Henderson B, Lund PA, Tormay P, Liu HL, Gurcha SS, Besra GS, Coates ARM. A *Mycobacterium*  
24 *tuberculosis* mutant lacking the *groEL* homologue *cpn60.1* is viable but fails to induce an inflammatory response  
25 in animal models of infection. *Infect Immun* 2008; **76**:1535-1546.  
26  
27 29. Winrow VR, Mesher J, Meghji S, Morris CJ, Fox S, Coates ARM, Tormay P, Blake D, Henderson B. The two  
28 homologous chaperonin 60 proteins of *Mycobacterium tuberculosis* have distinct effects on monocyte  
29 differentiation into osteoclasts. *Cell Microbiol* 2008; **10**:2091-2104.  
30  
31 30. Mitchell LA, Nixon B, Aitken RJ. Analysis of chaperone proteins associated with human spermatozoa during  
32 capacitation. *Mol Hum Reprod* 2007; **13**:605-613.  
33  
34 31. Lund PA. Microbial molecular chaperones. *Adv Microb Physiol* 2001; **44**:93-140.  
35  
36 32. George R, Kelly SM, Price NC, Erbse A, Fisher M, Lund PA. Three GroEL homologues from *Rhizobium*  
37 *leguminosarum* have distinct in vitro properties. *Biochem Biophys Res Commun* 2004; **324**:822-888.  
38  
39 33. Lewthwaite J, George R, Lund PA, Poole S, Tormay P, Sharp L, Coates AR, Henderson B. *Rhizobium*  
40 *leguminosarum* chaperonin 60.3, but not chaperonin 60.1, induces cytokine production by human monocytes:  
41 activity is dependent on interaction with cell surface CD14. *Cell Stress Chaperones* 2002; **7**:130-136.  
42  
43 34. Sarkar S, Lakhota SC. The Hsp60C gene in the 25F cytogenetic region in *Drosophila melanogaster* is  
44 essential for tracheal development and fertility. *J Genet* 2005; **84**:265-281.  
45  
46 35. Henderson B, Allan E, Coates ARM. Stress wars: The direct role of host and bacterial molecular chaperones  
47 in bacterial infection. *Infect Immun* 2006; **74**:3693-3706.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



- 1  
2  
3 36. Athanasas-Platsis S, Zhang B, Hillyard NC, Cavanagh AC, Csurhes PA, Morton H, McCombe PA. Early  
4 pregnancy factor suppresses the infiltration of lymphocytes and macrophages in the spinal cord of rats during  
5 experimental autoimmune encephalomyelitis but has no effect on apoptosis. *J Neurol Sci* 2003;**214**:27-36.  
6  
7  
8 37. Zhang B, Walsh MD, Nguyen KB, Hillyard NC, Cavanagh AC, McCombe PA, Morton H. Early pregnancy  
9 factor treatment suppresses the inflammatory response and adhesion molecule expression in the spinal cord of  
10 SJL/J mice with experimental autoimmune encephalomyelitis and the delayed-type hypersensitivity reaction to  
11 trinitrochlorobenzene in normal BALB/c mice. *J Neurol Sci* 2003;**212**:37-46.  
12  
13  
14 38. Johnson BJ, Le TT, Dobbin CA, Banovic T, Howard CB, Flores Fde M, Vanags D, Naylor DJ, Hill GR,  
15 Suhrbier A. Heat shock protein 10 inhibits lipopolysaccharide-induced inflammatory mediator production. *J Biol*  
16 *Chem* 2005;**280**:4037-4047.  
17  
18 39. Agnello D, Scanziani E, Di GM, Leoni F, Modena D, Mascagni P, Introna M, Ghezzi P, Villa P. Preventive  
19 administration of *Mycobacterium tuberculosis* 10-kDa heat shock protein (hsp10) suppresses adjuvant arthritis  
20 in Lewis rats. *Int Immunopharmacol* 2002;**2**:463-474.  
21  
22 40. Vanags D, Williams B, Johnson B, Hall S, Nash P, Taylor A, Weiss J, Feeney D. Therapeutic efficacy and  
23 safety of chaperonin 10 in patients with rheumatoid arthritis: a double-blind randomised trial. *Lancet*  
24 2006;**368**:855-863.  
25  
26 41. van Eden W. XToll, a recombinant chaperonin 10 as an anti-inflammatory immunomodulator. *Curr Opin*  
27 *Investig Drugs* 2008;**9**:523-533.  
28  
29 42. Broadley S, Vanags D, Williams B, Johnson B, Feeney D, Griffiths L, Shakib S, Brown G, Coulthard A,  
30 Mullins P, Kneebone C. Results of a phase IIa clinical trial of an anti-inflammatory molecule, chaperonin 10, in  
31 multiple sclerosis. *Mult Scler* 2009; **15**:329-336.  
32  
33 43. Shamaei-Tousi A, D'Aiuto F, Nibali L, Steptoe A, Coates AR, Parkar M, Donos N, Henderson B. Differential  
34 regulation of circulating levels of molecular chaperones in patients undergoing treatment for periodontal  
35 disease. *PLoS ONE* 2007;**2**:e1198.  
36  
37 44. Akyol S, Gercel-Taylor C, Reynolds LC, Taylor DD. HSP-10 in ovarian cancer: expression and suppression  
38 of T-cell signaling. *Gynecol Oncol* 2006;**101**:481-486.  
39  
40 45. Dobocan MC, Sadvakassova G, Congote LF. Chaperonin 10 as an endothelial-derived differentiation factor:  
41 Role of glycogen synthase kinase-3. *J Cell Physiol* 2009; **219**:470-476.  
42  
43 46. Walsh A, Whelan D, Bielanowicz A, Skinner B, Aitken RJ, O'Bryan MK, Nixon B. Identification of the  
44 molecular chaperone, heat shock protein 1 (chaperonin 10), in the reproductive tract and in capacitating  
45 spermatozoa in the male mouse. *Biol Reprod* 2008;**78**:983-993.  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 47.Friedland JS, Shattock R, Remick DG, Griffin GE. Mycobacterial 65-kDa heat shock protein induces release  
4 of proinflammatory cytokines from human monocytic cells. *Clin Exp Immunol* 1993;**91**: 58-62.  
5  
6 48.Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: An immunologic functional  
7 perspective. *Annu Rev Immunol* 2009; **27**:451-83.  
8  
9 49.Peetermans WE, Raats CJ, Langermans JA, van Furth R. Mycobacterial heat-shock protein 65 induces  
10 proinflammatory cytokines but does not activate human mononuclear phagocytes. *Scand J Immunol*  
11 1994;**39**:613-617.  
12  
13 50.Verdegaal ME, Zegveld ST, van Furth R. Heat shock protein 65 induces CD62e, CD106, and CD54 on  
14 cultured human endothelial cells and increases their adhesiveness for monocytes and granulocytes. *J Immunol*  
15 1996;**151**:369-376.  
16  
17 51. Thornhill MH, Haskard DO. IL-4 regulates endothelial cell activation by IL-1, tumor necrosis factor, or IFN-  
18 gamma. *J Immunol* 1990; **145**:865-872.  
19  
20 52.Chen W, Syldath U, Bellmann K, Burkart V, Kolb H. Human 60-kDa heat-shock protein: a danger signal to  
21 the innate immune system. *J Immunol* 1999;**162**:3212-3219.  
22  
23 53.Kol A, Lichtman AH, Finberg RW, Libby P, Kurt-Jones EA. Cutting edge: heat shock protein (HSP) 60  
24 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear  
25 cells. *J Immunol* 2000;**164**:13-17.  
26  
27 54.Ohashi K, Burkart V, Flohé S, Kolb H. Cutting edge: heat shock protein 60 is a putative endogenous ligand of  
28 the toll-like receptor-4 complex. *J Immunol* 2000;**164**:558-561.  
29  
30 55.Habich C, Baumgart K, Kolb H, Burkart V. The receptor for heat shock protein 60 on macrophages is  
31 saturable, specific, and distinct from receptors for other heat shock proteins. *J Immunol* 2002;**168**:569-576.  
32  
33 56.Habich C, Kempe K, van der Zee R, Burkart V, Kolb H. Different heat shock protein 60 species share pro-  
34 inflammatory activity but not binding sites on macrophages. *FEBS Lett* 2003; **533**:105-109.  
35  
36 57.Habich C, Kempe K, Burkart V, Van Der Zee R, Lillicrap M, Gaston H, Kolb H. Identification of the heat shock  
37 protein 60 epitope involved in receptor binding on macrophages. *FEBS Lett* 2004; **568**: 65-69.  
38  
39 58.Bethke K, Staib F, Distler M, Schmitt U, Jonuleit H, Enk AH, Galle PR, Heike M. Different efficiency of heat  
40 shock proteins (HSP) to activate human monocytes and dendritic cells: superiority of HSP60. *J Immunol*  
41 2002;**169**:6141-6148.  
42  
43 59.Flohé SB, Brüggemann J, Lendemans S, Nikulina M, Meierhoff G, Flohé S, Kolb H. Human heat shock  
44 protein 60 induces maturation of dendritic cells versus a Th1-promoting phenotype. *J Immunol*. 2003;**170**:2340-  
45 2348.  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 60. Osterloh A, Kalinke U, Weiss S, Fleischer B, Breloer M. Synergistic and differential modulation of immune  
4 responses by Hsp60 and lipopolysaccharide. *J Biol Chem* 2007; **282**:4669-4680.  
5  
6 61. Lamb JR, Bal V, Rothbard JB, Mehlert A, Mendez-Samperio P, Young DB. The mycobacterial GroEL stress  
7 protein: a common target of T-cell recognition in infection and autoimmunity. *J Autoimmun* 1989; **2** Suppl:93-  
8 100.  
9  
10 62. van Eden W. Heat-shock proteins as immunogenic bacterial antigens with the potential to induce  
11 and regulate autoimmune arthritis. *Immunol Rev* 1991; **121**:5-28.  
12  
13 63. Wieten L, Broere F, van der Zee R, Koerkamp EK, Wagenaar J, van Eden W. Cell stress induced HSP are  
14 targets of regulatory T cells: a role for HSP inducing compounds as anti-inflammatory immuno-modulators?  
15 *FEBS Lett* 2007; **581**:3716-3722.  
16  
17 64. Ramage JM, Young JL, Goodall JC, Gaston JS. T cell responses to heat-shock protein 60: differential  
18 responses by CD4+ T cell subsets according to their expression of CD45 isotypes. *J Immunol* 1999; **162**:704-  
19 710.  
20  
21 65. Breloer M, Dorner B, Moré SH, Roderian T, Fleischer B, von Bonin A. Heat shock proteins as "danger  
22 signals": eukaryotic Hsp60 enhances and accelerates antigen-specific IFN-gamma production in T cells. *Eur J*  
23 *Immunol.* 2001; **31**:2051-2059.  
24  
25 66. Moré SH, Breloer M, von Bonin A. Eukaryotic heat shock proteins as molecular links in innate and adaptive  
26 immune responses: Hsp60-mediated activation of cytotoxic T cells. *Int Immunol* 2001; **13**:1121-1127.  
27  
28 67. Breloer M, Moré SH, Osterloh A, Stelter F, Jack RS, Bonin Av A. Macrophages as main inducers of IFN-  
29 gamma in T cells following administration of human and mouse heat shock protein 60. *Int Immunol*  
30 2002; **14**:1247-1253.  
31  
32 68. Zanin-Zhorov A, Nussbaum G, Franitza S, Cohen IR, Lider O. T cells respond to heat shock protein 60 via  
33 TLR2: activation of adhesion and inhibition of chemokine receptors. *FASEB J* 2003; **17**:1567-1569.  
34  
35 69. Zanin-Zhorov A, Bruck R, Tal G, Oren S, Aeed H, Hershkoviz R, Cohen IR, Lider O Heat shock protein 60  
36 inhibits Th1-mediated hepatitis model via innate regulation of Th1/Th2 transcription factors and cytokines. *J*  
37 *Immunol* 2005; **174**:3227-3236.  
38  
39 70. Triantafilou K, Triantafilou M, Dedrick RL A CD14-dependent LPS receptor cluster. *Nat Immunol* 2001;  
40 **2**:338-345.  
41  
42 71. Zanin-Zhorov A, Cahalon L, Tal G, Margalit R, Lider O, Cohen IR Heat shock protein 60 enhances CD4+  
43 CD25+ regulatory T cell function via innate TLR2 signaling. *J Clin Invest* 2006; **116**:2022-2032.  
44  
45 72. Cohen-Sfady M, Nussbaum G, Pevsner-Fischer M, Mor F, Carmi P, Zanin-Zhorov A, Lider O, Cohen IR.  
46 Heat shock protein 60 activates B cells via the TLR4-MyD88 pathway. *J Immunol* 2005; **175**:3594-3602.  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 73.Yoshida N, Oeda K, Watanabe E, Mikami T, Fukita Y, Nishimura K, Komai K, Matsuda K. Protein function.  
4 Chaperonin turned insect toxin. *Nature* 2001; **411**:44.  
5  
6 74.Asquith KL, Baleato RM, McLaughlin EA, Nixon B, Aitken RJ. Tyrosine phosphorylation activates surface  
7 chaperones facilitating sperm-zona recognition. *J Cell Sci* 2004;**117**:3645-3657.  
8  
9 75.Mitchell LA, Nixon B, Aitken RJ. Analysis of chaperone proteins associated with human spermatozoa during  
10 capacitation. *Mol Hum Reprod* 2007;**13**:605-613.  
11  
12 76.Hageman J, Kampinga HH. Computational analysis of the human HSPH/HSPA/DNAJ family and cloning of a  
13 human HSPH/HSPA/DNAJ expression library. *Cell Stress Chaperones* 2009;**14**:1-21.  
14  
15 77.Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, Cheetham ME, Chen B, Hightower  
16 LE. Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones* 2009;**14**:105-  
17 111.  
18  
19 78.Asea A, Kraeft S-K, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK. Hsp70  
20 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone  
21 and cytokine. *Nature Med* 2000; **6**:435-442.  
22  
23 79.Vabulas RM, Ahmad-Nejad P, Ghose S, Kirschning CJ, Issels RD Wagner H. Hsp70 as endogenous  
24 stimulus of the Toll/interleukin-1 receptor signal pathway. *J Biol Chem* 2002; **277**:15107-15112.  
25  
26 80.Panjwani NN, Popova L, Srivastava PK. Heat shock proteins gp96 and Hsp70 activate the release of nitric  
27 oxide by APCs. *J Immunol* 2002; **168**:2997-3003.  
28  
29 81.Asea A, Rehli M, Kabingu E, Boch JA, Bare O, Auron PE, Stevenson MA, Calderwood SK. Novel signal  
30 transduction pathway utilizing extracellular HSP70. *J Biol Chem* 2002; **277**:15028-15034.  
31  
32 82.Wang Y, Kelly CG, Karttunen JT, Whittall T, Lehner PJ, Duncan L, MacAry P, Younson JS, Singh M,  
33 Oehlmann W, Cheng G, Bergmeier L, Lehner T. CD40 is a cellular receptor mediating mycobacterial heat shock  
34 protein 70 stimulation of CC-chemokines. *Immunity* 2001; **15**:971-983.  
35  
36 83.Wang Y, Kelly CG, Singh M, McGowan EG, Carrara A-S, Bergmeier LA, Lehner T. Stimulation of Th1-  
37 polarizing cytokines, C- chemokines, maturation of dendritic cells, and adjuvant function by the peptide binding  
38 fragment of heat shock protein 70. *J Immunol* 2002; **169**:2422-2429.  
39  
40 84.Yang Y, Whittall T, McGowan E, Younson J, Kelly C, Bergmeier LA, Singh M, Lehner T. Identification of  
41 stimulating and inhibitory epitopes within the heat shock protein 70 molecule that modulate cytokine production  
42 and maturation of dendritic cells. *J Immunol* 2005; **174**:3306-3316.  
43  
44 85.Becker T, Hartl F-U, Wieland F. CD40, an extracellular receptor for binding and uptake of Hsp70-peptide  
45 complexes. *J Cell Biol* 2002; **158**:1277-1285.  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 86.Whittall T, Wang Y, Younson J, Kelly C, Bergmeier LA, Peters B, Singh M, Lehner T. Interaction between  
4 CCR5 chemokine receptors and microbial Hsp70. *Eur J Immunol* 2006; **36**:2304-2314.  
5  
6 87.Basu S, Binder RJ, Ramalingam T, Srivastava PK. CD91, a common receptor for heat shock proteins gp96,  
7 Hsp90, hsp70 and calreticulin. *Immunity* 2001; **14**:303-313.  
8  
9 88.Delneste Y, Magistrelli G, Gauchat J, Haeuw J, Aubry J, Nakamura K, Kawakami-Honda L, Goetsch L,  
10 Sawamura T, Bonnefoy J, Jeannin P. Involvement of L0x-1 in dendritic cell-mediated antigen cross-presentation  
11 *Immunity* 2002; **17**:353-362.  
12  
13 89.Theriault JR, Mambula SS, Sawaura T, Stevenson MA, Calderwood SK. Extracellular HSP70 binding to cell  
14 surface receptors present on antigen-presenting cells and endothelial/epithelial cells. *FEBS Lett* 2005;  
15 **579**:1951-1960.  
16  
17 90.Theriault JR, Adachi H, Calderwood SK. Role of scavenger receptors in the binding and internalization of  
18 heat shock protein 70. *J Immunol* 2006; **177**:8604-8611.  
19  
20 91.Kettner S, Kalthoff F, Graf P, Priller E, Kricek F, Lindley I, Schweighoffer T. EWI-2/CD316 is an inducible  
21 receptor of HSPA8 on human dendritic cells. *Mol Cell Biol* 2007;**27**:7718-7726.  
22  
23 92.Zitzler S, Hellwig A, Hartl FU, Wieland F, Diestelkötter-Bachert P. Distinct binding sites for the ATPase and  
24 substrate binding domain of human Hsp70 on the cell surface of antigen-presenting cells. *Mol Immunol* 2008;  
25 **45**:3974-3983.  
26  
27 93.Pockley AG, de Faire U, Kiessling R, Lemne C, Thulin T, Frostegard J. Circulating heat shock protein and  
28 heat shock protein antibody levels in established hypertension. *J Hypertension* 2002; **20**:1815-1820.  
29  
30 94.Pockley AG, Georgiades A, Thulin T, de Faire U, Frostegard J. Serum heat shock protein 70 levels predict  
31 the development of atherosclerosis in subjects with established hypertension. *Hypertension* 2003; **42**:235-238.  
32  
33 95.Zhu J, Quyyumi AA, Wu H, Csako G, Rott D, Zalles-Ganley A, Ogunmakinwa J, Halcox J, Epstein SE.  
34 Increased serum levels of heat shock protein 70 are associated with low risk of coronary artery disease.  
35 *Arterioscler Thromb Vasc Biol* 2003; **23**:1055-1059.  
36  
37 96.Brownlie RJ, Myers LK, Wooley PH, Corrigan VM, Bodman-Smith MD, Panayi GS, Thompson SJ. Treatment  
38 of murine collagen-induced arthritis by the stress protein BiP via interleukin-4-producing regulatory T cells: A  
39 novel function for an ancient protein. *Arthr Rheum* 2006;**54**: 854-863.  
40  
41 97. Corrigan VM, Panayi GS (2005) BiP, a negative regulator involved in rheumatoid arthritis. In *Molecular*  
42 *Chaperones and Cell Signalling* (eds Henderson B, Pockley AG) pp234-248. Cambridge University Press.  
43  
44 98.Dragovic Z, Broadley SA, Shomura Y, Bracher A, Hartl FU. Molecular chaperones of the Hsp110 family act  
45 as nucleotide exchange factors of Hsp70s. *EMBO J* 2006; **25**:2519-2528.  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 99.Raviol H, Sadlish H, Rodriguez F, Mayer MP, Bukau B. Chaperone network in the yeast cytosol: Hsp110 is  
4 revealed as an Hsp70 nucleotide exchange factor. *EMBO J* 2006; **25**:2510-2518.
- 5  
6 100.Colgan SP, Pitman RS, Nagaishi T, Mizoguchi A, Mizoguchi E, Mayer LF, Shao L, Sartor RB, Subject JR,  
7  
8 Blumberg RS. Intestinal heat shock protein 110 regulates expression of CD1d on intestinal epithelial cells. *J Clin*  
9  
10 *Invest* 2003; **112**:745-754.
- 11  
12 101.Manjili MH, Park JE, Facciponte JG, Wang XY, Subject JR. Immunoadjuvant chaperone, GRP170, induces  
13  
14 'danger signals' upon interaction with dendritic cells. *Immunol Cell Biol* 2006; **84**:203-208.
- 15  
16 102.Csermely P, Korcsmaros T, Kovas IA, Szalay M, Sou C (2008) Systems biology of molecular chaperone  
17  
18 networks In Novartis Foundation Symposium 291 *The Biology of Extracellular Molecular Chaperones*. pp45-  
19  
20 58.Wiley.
- 21  
22 103.Gao B, Tsan MF. Endotoxin contamination in recombinant human heat shock protein 70 (Hsp70)  
23  
24 preparation is responsible for the induction of tumour necrosis factor- $\alpha$  release by murine macrophages. *J Biol*  
25  
26 *Chem* 2003; **278**:174-179.
- 27  
28 104.Ye Z, Gan YH. Flagellin contamination of recombinant heat shock protein 70 is responsible for its activity on  
29  
30 T cells. *J Biol Chem* 2007; **282**:4479-4484.
- 31  
32 105.Bendz H, Marincek BC, Momburg F, Ellwart JW, Issels RD, Nelson PJ, Noessner E. Calcium signaling in  
33  
34 dendritic cells by human or mycobacterial Hsp70 is caused by contamination and is not required for Hsp70-  
35  
36 mediated enhancement of cross-presentation. *J Biol Chem* 2008; **283**:26477-26483.
- 37  
38 106.Figueiredo C, Wittmann M, Wang D, Dressel R, Seltsam A, Blasczyk R, Eiz-Vesper B. Heat shock protein  
39  
40 70 (Hsp70) induces cytotoxicity of T-helper cells. *Blood* 2009;**113**:3008-3016.
- 41  
42 107.Daugaard M, Rohde M, Jäättelä M. The heat shock protein 70 family: Highly homologous proteins with  
43  
44 overlapping and distinct functions. *FEBS Lett* 2007; **581**:3702-3710.
- 45  
46 108.Gupta RS, Ramachandra NB, Bowes T, Singh B. Unusual cellular disposition of the mitochondrial  
47  
48 molecular chaperones Hsp60, Hsp70 and Hsp10. *Novartis Found Symp* 2008;**291**:59-68.
- 49  
50 109.Soltys BJ, Gupta RS. Cell surface localization of the 60 kDa heat shock chaperonin protein (hsp60) in  
51  
52 mammalian cells. *Cell Biol Int* 1997; **21**:315-320.
- 53  
54 110.Cechetto JD, Soltys BJ, Gupta RS. Localization of mitochondrial 60-kD heat shock chaperonin protein  
55  
56 (Hsp60) in pituitary growth hormone secretory granules and pancreatic zymogen granules. *J Histochem*  
57  
58 *Cytochem* 2000; **48**:45-56.
- 59  
60 111.Sadacharan SK, Cavanagh AC, Gupta RS. Immunoelectron microscopy provides evidence for the presence  
of mitochondrial heat shock 10-kDa protein (chaperonin 10) in red blood cells and a variety of secretory  
granules. *Histochem Cell Biol* 2001; **116**:507-517.



- 1  
2 112.Triantafilou K, Triantafilou M, Dedrick RL. A CD14-independent LPS receptor cluster. *Nat Immunol* 2001;  
3 2:338-345.  
4  
5  
6 113.Triantafilou K, Triantafilou M, Ladha S, Mackie A, Dedrick RL, Fernandez N, Cherry R. Fluorescence  
7 recovery after photobleaching reveals that LPS rapidly transfers from CD14 to hsp70 and hsp90 on the cell  
8 membrane. *J Cell Sci* 2001; **114**:2535-2545.  
9  
10  
11 114.Sagara Y, Ishida C, Inoue Y, Shiraki H, Maeda Y. 71-kilodalton heat shock cognate protein acts as a  
12 cellular receptor for syncytium formation induced by human T-cell lymphotropic virus type 1. *J Virol* 1998;  
13 72:535-541.  
14  
15  
16 115.Reyes-Del Valle J, Chávez-Salinas S, Medina F, Del Angel RM. Heat shock protein 90 and heat shock  
17 protein 70 are components of dengue virus receptor complex in human cells. *J Virol* 2005; **79**:4557-4567.  
18  
19  
20 116.Das S, Laxminarayana SV, Chandra N, Ravi V, Desai A. Heat shock protein 70 on Neuro2a cells is a  
21 putative receptor for Japanese encephalitis virus. *Virology* 2009; **385**:47-57.  
22  
23  
24 117.Nickel W, Rabouille C. Mechanisms of regulated unconventional protein secretion. *Nat Rev Mol Cell Biol*  
25 2009;**10**:148-155.  
26  
27  
28 118.Lancaster GI, Febbraio MA. Exosome-dependent trafficking of HSP70: a novel secretory pathway for  
29 cellular stress proteins. *J Biol Chem* 2005; **280**:23349-23355.  
30  
31  
32 119.Asea A. Mechanisms of HSP72 release. *J Biosci* 2007; **32**:579-584.  
33  
34  
35 120.Gupta S, Knowlton AA. HSP60 trafficking in adult cardiac myocytes: role of the exosomal pathway. *Am J*  
36 *Physiol Heart Circ Physiol* 2007; **292**:H3052-3056.  
37  
38  
39 121.Andrei C, Margiocco P, Poggi A, Lotti LV, Torrisi MR, Rubartelli A. Phospholipases C and A2 control  
40 lysosome-mediated IL-1 beta secretion: Implications for inflammatory processes. *Proc Natl Acad Sci U S A*  
41 2004; **101**:9745-9750.  
42  
43  
44 122.Piccini A, Carta S, Tassi S, Lasiglié D, Fossati G, Rubartelli A. ATP is released by monocytes stimulated  
45 with pathogen-sensing receptor ligands and induces IL-1beta and IL-18 secretion in an autocrine way. *Proc Natl*  
46 *Acad Sci U S A* 2008; **105**:8067-8072.  
47  
48  
49 123.Mambula SS, Calderwood SK. Heat shock protein 70 is secreted from tumor cells by a nonclassical  
50 pathway involving lysosomal endosomes. *J Immunol* 2006; **177**:7849-7857.  
51  
52  
53 124.Hansen JJ, Bross P, Westergaard M, Nielsen MN, Eiberg H, Børglum AD, Mogensen J, Kristiansen K,  
54 Bolund L, Gregersen N. Genomic structure of the human mitochondrial chaperonin genes: HSP60 and HSP10  
55 are localised head to head on chromosome 2 separated by a bidirectional promoter. *Hum Genet* 2003; **112**:71-  
56 77.  
57  
58  
59  
60

- 1  
2  
3 125.Pockley AG, Bulmer J, Hanks BM, Wright BH. Identification of human heat shock protein 60 (Hsp60) and  
4 anti-Hsp60 antibodies in the peripheral circulation of normal individuals. *Cell Stress Chaperones* 1999; **4**:29-35.  
5  
6 126.Wick G, Knoflach M, Xu Q. Autoimmune and inflammatory mechanisms in atherosclerosis. *Annu Rev*  
7  
8 *Immunol* 2004; **22**:361-403.  
9  
10 127.Xu Q, Schett G, Perschinka H, Mayr M, Egger G, Oberhollenzer F, Willeit J, Kiechl S, Wick G. Serum  
11 soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. *Circulation*  
12  
13 2000; **102**:14-20.  
14  
15 128.Xiao Q, Mandal K, Schett G, Mayr M, Wick G, Oberhollenzer F, Willeit J, Kiechl S, Xu Q. Association of  
16 serum-soluble heat shock protein 60 with carotid atherosclerosis: clinical significance determined in a follow-up  
17 study. *Stroke* 2005; **36**:2571-2576.  
18  
19 129.Pockley AG, Wu R, Lemne C, Kiessling R, de Faire U, Frostegård J. Circulating heat shock protein 60 is  
20 associated with early cardiovascular disease. *Hypertension* 2000; **36**:303-307.  
21  
22 130.Pockley AG, De Faire U, Kiessling R, Lemne C, Thulin T, Frostegård J. Circulating heat shock protein and  
23 heat shock protein antibody levels in established hypertension. *J Hypertens* 2002; **20**:1815-1820.  
24  
25 131.Marmot MG. Understanding social inequalities in health. *Perspect Biol Med* 2003; **46**(3 Suppl):S9-23.  
26  
27 132.Lewthwaite J, Owen N, Coates A, Henderson B, Steptoe A. Circulating human heat shock protein 60 in the  
28 plasma of British civil servants: relationship to physiological and psychosocial stress. *Circulation* 2002;**106**:196-  
29 201.  
30  
31 133.Shamaei-Tousi A, Steptoe A, O'Donnell K, Palmen J, Stephens JW, Hurel SJ, Marmot M, Homer K, D'Aiuto  
32 F, Coates AR, Humphries SE, Henderson B. Plasma heat shock protein 60 and cardiovascular disease risk: the  
33 role of psychosocial, genetic, and biological factors. *Cell Stress Chaperones* 2007;**12**:384-392.  
34  
35 134.Ellins E, Shamaei-Tousi A, Steptoe A, Donald A, O'Meagher S, Halcox J, Henderson B. The relationship  
36 between carotid stiffness and circulating levels of heat shock protein 60 in middle-aged men and women. *J*  
37 *Hypertens* 2008; **26**:2389-2392.  
38  
39 135.Halcox JP, Deanfield J, Shamaei-Tousi A, Henderson B, Steptoe A, Coates AR, Singhal A, Lucas A.  
40 Circulating human heat shock protein 60 in the blood of healthy teenagers: a novel determinant of endothelial  
41 dysfunction and early vascular injury? *Arterioscler Thromb Vasc Biol* 2005; **25**:e141-142.  
42  
43 136.Pockley AG, Shepherd J, Corton JM. Detection of heat shock protein 70 (Hsp70) and anti-Hsp70 antibodies  
44 in the serum of normal individuals. *Immunol Invest* 1998; **27**:367-377.  
45  
46 137.Wright BH, Corton JM, El-Nahas AM, Wood RF, Pockley AG. Elevated levels of circulating heat shock  
47 protein 70 (Hsp70) in peripheral and renal vascular disease. *Heart Vessels* 2000; **15**:18-22.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



- 1  
2  
3 138.Pockley AG, De Faire U, Kiessling R, Lemne C, Thulin T, Frostegård J. Circulating heat shock protein and  
4 heat shock protein antibody levels in established hypertension. *J Hypertens* 200; **20**:1815-1820.  
5  
6 139.Pockley AG, Georgiades A, Thulin T, de Faire U, Frostegård J. Serum heat shock protein 70 levels predict  
7 the development of atherosclerosis in subjects with established hypertension. *Hypertension* 2003; **42**:235-238.  
8  
9 140.Ellis RJ. Proteins as molecular chaperones. *Nature* 1987; **328**:378-379.  
10  
11 141.Maguire M, Coates ARM, Henderson B. Chaperonin 60 unfolds its secretes of cellular communication. *Cell*  
12 *Stress Chaperones* 2002; **7**:317-329.  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

**Table 1. Molecular Chaperones, Alternative Names and Cellular Dispositions**

<b>Molecular Chaperone</b>	<b>Alternative Names</b>	<b>Cellular Disposition</b>	<b>Secreted</b>
Heat shock protein (Hsp) 10	chaperonin (Cpn)10, GroES in <i>E. coli</i>	mitochondrion	yes
Cyclophilins	peptidylprolyl isomerases	cytoplasm	yes
Hsp27	Hsp25, Hsp28	cytoplasm	yes
Hsp60	chaperonin (Cpn)60 GroEL in <i>E. coli</i>	mitochondrion	yes
Hsp70 (12 human genes)	DnaK in bacteria HSPA 1 onwards (new nomenclature)	all cell sites	some
BiP (Hsp70 family member)	Grp78, HSPA5	endoplasmic reticulum	yes
Hsp70-9	HSPA9 Grp75, mtHsp75, mortalin	mitochondrion	not established
Hsp90		cytoplasm	unclear

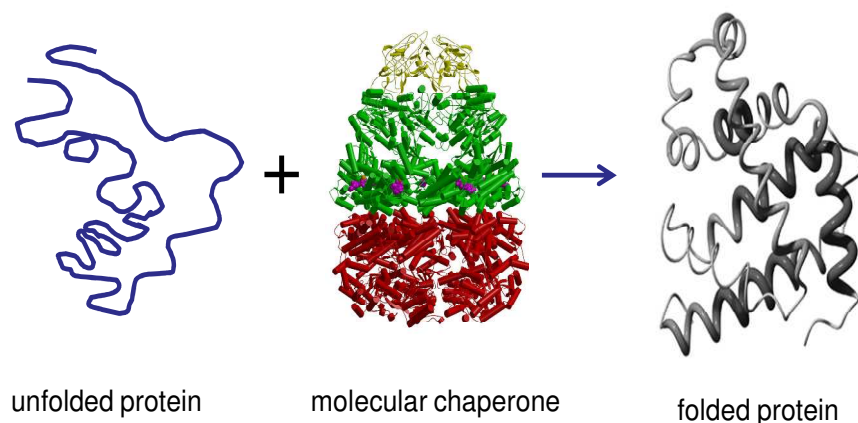


Figure 1. This schematic diagram encapsulates the basic biology of intracellular molecular chaperones (in this case Hsp60 or chaperonin 60). The function of molecular chaperones is to prevent unfolded proteins going to one of the potentially huge number of folding states (configurations) that they are capable of attaining. The molecular chaperone assists the unfolded protein to achieve its single correct three-dimensional configuration (by whatever mechanism it has evolved to generate this folded state) without becoming a constituent of the final folded protein. Molecular chaperones have evolved, it is believed, to aid protein folding inside the cell because in this milieu the protein concentration is enormously high leading to proteins inappropriately interacting with each other and failing to fold properly.

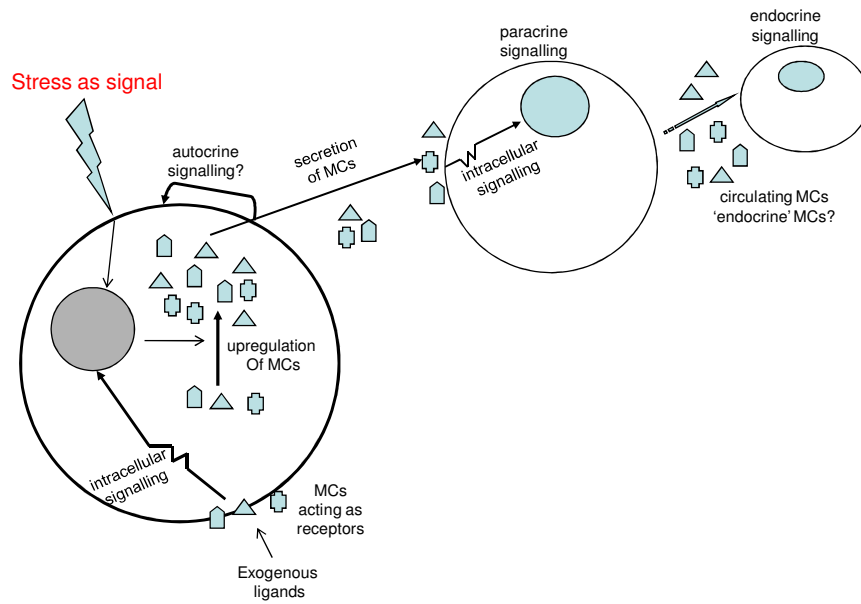


Figure 2. A simplified schematic diagram suggesting how stress received by a cell could lead to the upregulation of levels of intracellular molecular chaperones (MCs) which could lead either to these proteins exiting the cell to exist on the cell membrane (where they could act as receptors for host or microbial ligands) or to exit the cell and diffuse into the extracellular milieu to act as signals for other cells. If this process is amplified and sufficient cells are activated it could result in sufficient MC entering the blood for it to be measured. Thus it is envisaged that. Like cytokines, the MCs could act as autocrine, paracrine or endocrine signals. It is not clear if the very high levels of Hsp60 in some individuals could be explained in this way and possible alternative pathways may exist for the release of Hsp60.