

Hsp60 and human aging: *Les liaisons dangereuses*

Francesco Cappello^{1,2}, Everly Conway De Macario³, Antonella Marino Gammazza^{1,2}, Giuseppe Bonaventura¹, Francesco Carini¹, Anna M. Czarnecka⁴, Felicia Farina¹, Giovanni Zummo¹, Alberto J. L. Macario^{2,3}

¹Department of Experimental Biomedicine and Clinical Neurosciences, Human Anatomy Section Emerico Luna, University of Palermo, Palermo, Italy, ²IEMEST, Istituto Euro-Mediterraneo di Scienza e Tecnologia, Palermo, Italy, ³Department of Microbiology and Immunology, School of Medicine, University of Maryland at Baltimore, and IMET, Baltimore (MD), USA, ⁴Department of Laboratory of Molecular Oncology, Department of Oncology, Military Institute of Medicine, ul. Szaserów 128, 01-141 Warsaw, Poland

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Essential background on chaperonology
4. Hsp60 molecular anatomy
5. Extracellular Hsp60 and immune system activation
6. Hsp60 in cell aging
7. Hsp60 in aging-related diseases
 - 7.1. Atherosclerosis and heart failure
 - 7.2. Neurodegenerative disorders
 - 7.3. Degenerative joint diseases
 - 7.4. Other pathologies
8. Summary and perspective
9. Acknowledgements
10. References

1. ABSTRACT

Stressors can cause abnormal intracellular accumulation of Hsp60 and its localization in extramitochondrial sites, secretion, and circulation, with immune system activation. Dysfunction of chaperones associated with their quantitative and qualitative decline with aging (chaperonopathies of aging) characterizes senescence and is a potential causal factor in the physiological deterioration that occurs with it. The role of Hsp60 in aging is not easy to elucidate, because aging is accompanied by pathologies (e.g., cardiovascular and neurodegenerative disorders, osteoporosis, diabetes, cancer, etc.) in which Hsp60 has been implicated but, although those disorders are more frequent in the elderly, they are not unique to them. Therefore, it is difficult to determine what is due to aging and what to an associated disease that can occur regardless of age. Does Hsp60 contribute to the pathogenesis? How and when does Hsp60 interact with the immune system and, thus, contributes to the initiation-progression of the generalized chronic inflammation characteristic of aging? These and related issues are discussed here in the light of reports showing the participation of Hsp60 in aging-associated disorders.

2. INTRODUCTION

Genes for molecular chaperones, and their protein products were identified in the early 1960's and 1970's, respectively (1, 2). After that, the study of chaperones, many of which are heat-shock proteins (Hsps), was very active in prokaryotic and eukaryotic systems, including the bacterial prokaryotes *Escherichia coli* and *Bacillus subtilis*, and the eukaryotes *Drosophila*, and a variety of mammals, plants and aquatic organisms (3-8). In the early 1990's, a molecular chaperone gene was identified for the first time by cloning and sequencing in a prokaryote of the phylogenetic Domain Archaea (9). In later times, a number of studies have examined the role of molecular chaperones in protein folding inside the cell, and chaperones were considered intracellular proteins (10-12). However, in the last several years evidence for extracellular chaperones has progressively accumulated and, nowadays, there is little doubt that various types of molecular chaperones can reside inside and outside cells with defined functions in both locations (12-15). In most recent times, significant advances have occurred in the understanding of detailed structure-function relationships in chaperones from the prokaryotes Archaea (16-18).

This article has been written for a Special Issue on *Frontiers in Molecular Medicine* and, consequently, deals with a theme currently at the outer edge of science: the active participation of defective molecular chaperones in pathogenesis. Diseases in which the primary cause, or one of the most important secondary causes, is a defective chaperone have been called chaperonopathies (19, 20). This unifying concept, encompassing a wide range of pathological conditions with diverse signs and symptoms but sharing important features, is the foundation for the outlining of a new area of Medicine. The scientific, medical, and practical advantages of such unifying approach are multiple and have been discussed elsewhere (21).

The main objective of this article is to present some of the chaperonopathies, focusing on the chaperone Hsp60, that affect aging individuals to illustrate how chaperone defects play a role in senescence and aging-related diseases by failing to interact correctly with physiological partners or by interacting with the wrong partner, namely by getting into “dangereuse liaisons.”

The literature on chaperones is abundant, so as to satisfy the bibliographic appetite of interested readers who are not specialists in this article's topic we have cited, whenever possible, review articles that summarize key issues and provide a rich list of references to original work. In addition, we have discussed some original data from various laboratories, including ours that we considered to be illustrative of important aspects of chaperone failure, and its consequences, during aging. We have also highlighted some of the critical points that merit further investigation with priority.

3. ESSENTIAL BACKGROUND ON CHAPERONOLY

As it may be realized from the preceding paragraphs, chaperonology is an emerging area of science encompassing the study of molecular chaperones in all their aspects, normal and abnormal, as a unit of related topics pertaining to chaperones in physiology and pathology examined from various angles (22). Many chaperones are heat shock proteins (Hsps) and, in this article, we will use the terms chaperone and Hsp interchangeably. This field is important because defective chaperones can contribute to the pathogenesis of a number of diseases, now referred to as chaperonopathies (19, 20, 23). Chaperonopathies can be genetic or acquired, the latter being quite common (20, 24).

The role of chaperones in human cell physiology changes from embryo/foetal life through adulthood to old age, and they are involved in cell senescence (25-27). A number of studies have shown that the stress-induced levels of chaperones tend to decrease with age (28). Pathological post-translational modifications causing malfunction of chaperones seem to be implicated in the aging process by affecting other molecules and supramolecular structures, cells, and tissues (20). There is some evidence indicating that the age-associated appearance of defective chaperones (chaperonopathies of aging) contributes to the

accumulation of defective non-chaperone proteins (proteinopathies of aging) (27, 29-36). This accumulation, in turn, causes a quantitative deficiency of chaperones, which thus become insufficient to deal with the increased demand from proteins in need of assistance for folding, refolding, translocation, etc. (30-32). Alternatively, chaperonopathies and proteinopathies of aging may start independently of one another – perhaps simultaneously – and progress in parallel, in which case the chaperonopathies would have a negative impact on protein homeostasis and, thus, contribute to the aggravation of the proteinopathies (27, 35).

In this article, we will concentrate on Hsp60, the eukaryotic mitochondrial chaperonin that is also found outside mitochondria, and discuss its role in cell senescence and organismal aging.

4. HSP60 MOLECULAR ANATOMY

Chaperonins are a subset of chaperones highly conserved during evolution and with essential roles in cell physiology. They are classified in two groups: Group I, present in bacteria (e.g., GroEL) and eukaryotic organelles (e.g., mitochondrial Hsp60 also called Cpn60), and Group II, found in archaea (e.g., thermosome subunits) and eukaryotic cytoplasm (e.g., CCT or TriC subunits) (37-40).

Hsp60 works in mitochondria together with its co-chaperonin Hsp10 (or Cpn10). In mammalian cells, Hsp60 and Hsp10 are important mitochondrial molecules, playing key roles in both unstressed and stressed cells (41, 42). The human genes for Hsp60 and Hsp10 are localised head-to-head on chromosome 2 and share a bidirectional promoter (43). This probably means that the DNA sequences encoding these chaperonins moved together to the nucleus from a bacterium, according to the endosymbiotic theory (44). Most of what we know about the eukaryotic Hsp60 and Hsp10 structures and functions derives from studies on their prokaryotic homologues, the bacterial GroEL and GroES, respectively, to which they are evolutionarily related.

The typical chaperonin machine in bacteria is formed by 14 GroEL molecules arranged in two stacked heptameric rings delimiting a barrel-like container with an inner cavity large enough to accommodate client polypeptides of up to nearly 60 kDa (40, 45, 46) (Figure 1A). GroES also forms a heptameric ring, which associates with the GroEL barrel at one of its ends, serving as a sort of lid to the GroEL-complex cavity (47). In addition, some information suggests that eukaryotic mitochondrial Hsp60 can form not only the typical two-ringed barrel, but it can also function as a single heptameric ring (48-50) (Figure 1B). Moreover, the majority of the client proteins are affected by the inactivation of Hsp60, but only a small subset of them are affected by the lack of Hsp10, suggesting that in eukaryotic cells Hsp60 and Hsp10 do not always act together as a functional unit (Figure 1C) (51). Unfortunately, the full range of Hsp60-dependent protein substrates in humans has not yet been fully delineated and

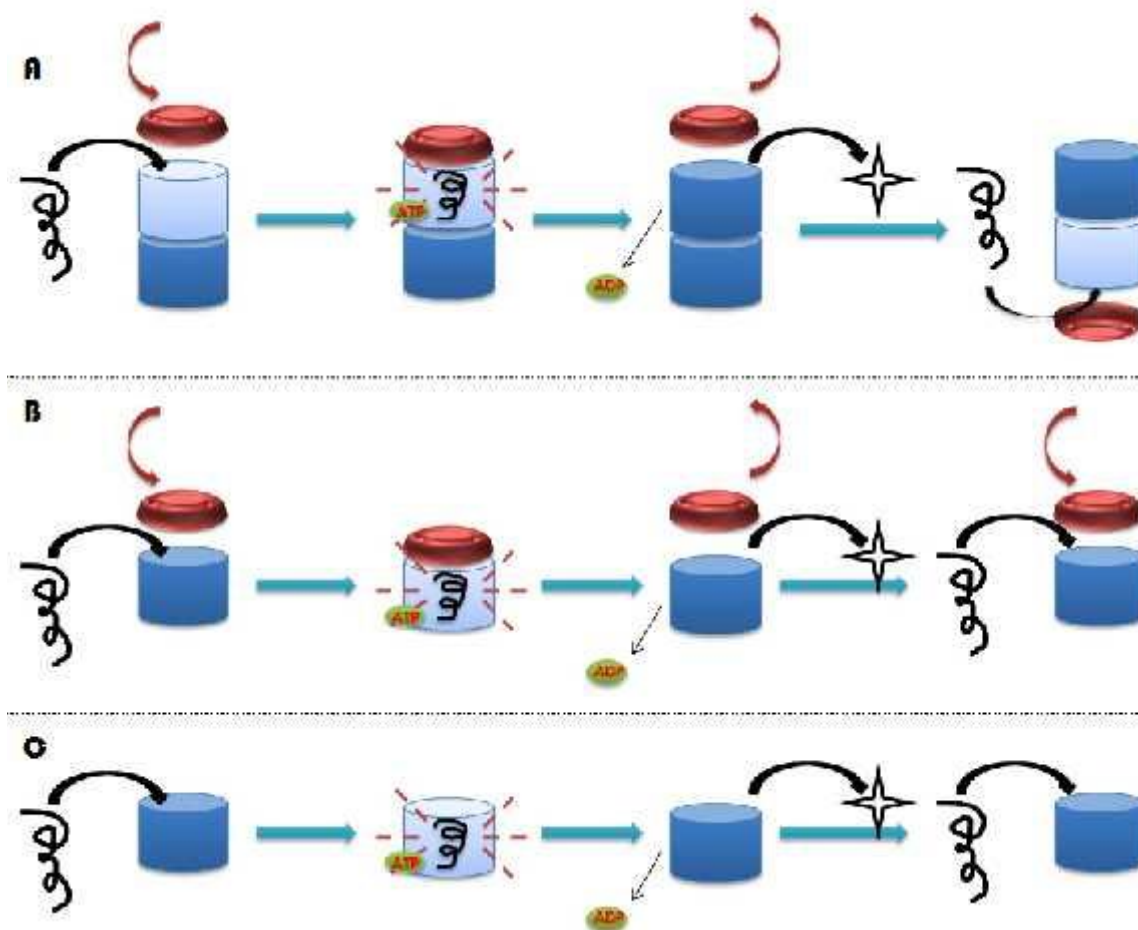


Figure 1. Hsp60 functioning models in human cells. (A) Mammalian mitochondrial Hsp60 forms a double heptameric-ring barrel-like structure with an inner cavity -- the folding chamber -- (blue cylinder), analogously to the prokaryotic homolog GroEL (not shown). A client polypeptide, i.e., a polypeptide in need of assistance for folding (black filament), enters the folding chamber of one of the rings (the light blue cylinder in this figure) in which it will fold to gain the native conformation after the chamber is occluded by the binding of Hsp10 (the homolog of the prokaryotic GroES; represented as a red cap). The process requires energy from ATP hydrolysis. Another chaperoning round begins with the dissociation of Hsp10, the release of the folded protein (four-pointed star) and ADP, and the binding of ATP to the other ring. In addition, there is information suggesting that a similar folding process is carried out by a single Hsp60 heptameric ring with Hsp10 (B), or even by a single ring without participation of Hsp10 (C), but these two possibilities, particularly the last shown in (C) are still controversial.

this lack of information curtails research on the Hsp60 chaperonopathies.

Apart from mitochondria, Hsp60 can be found in other subcellular compartments (e.g., zymogenic granules) as well as in the cytosol and on the cell membrane (52-54). In this context, one of the most interesting issues for investigation is the structure and function of this chaperonin in sites different from the canonical intra-mitochondrial location. For example, some data indicate that cytosolic Hsp60, that is involved in apoptosis activation (55), is sometimes in a monomeric form (56), while other results suggest equilibrium between monomeric and heptameric forms (57). It is likely that both situations are not mutually exclusive. Furthermore, it is also possible that various oligomeric forms of different multiplicities,

depending mainly on the Hsp60 concentration in the cytosol, can occur and function.

In what concerns membrane-associated Hsp60, it is now clear that this association is a pre-requisite for its secretion into the extracellular environment by secretory mechanisms involving the lipid rafts/exosomes pathway, in both stressed and tumor cells (54, 58). In the extracellular environment, Hsp60 encounters leukocytes and participates in immune system regulation, as discussed in the following section.

5. EXTRACELLULAR Hsp60 AND IMMUNE SYSTEM ACTIVATION

Hsp60 has been for long time considered an intracellular protein but the reality is that it can also be found in the extracellular milieu as well as in the

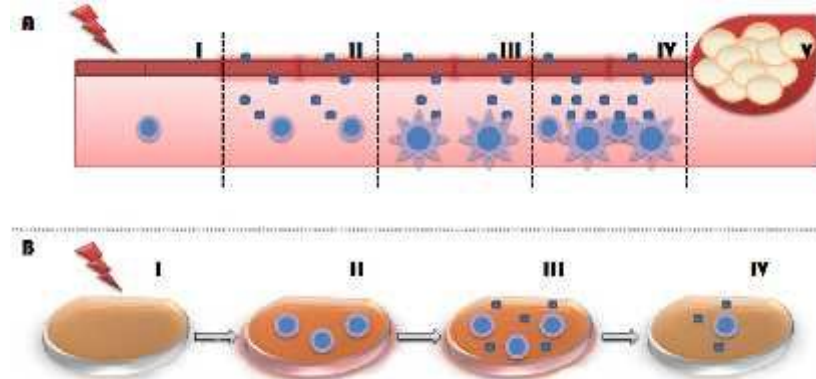


Figure 2. Hsp60 can have both pro- and anti-inflammatory effects. (A) Stress (e.g., hypertension) on vascular endothelial cells (top red thin rectangles) can determine Hsp60 (small blue squares) overexpression, cell membrane localization and secretion (I-II). In turn, Hsp60 can have pro-inflammatory effects on immune cells (lymphocytes, round blue circles with a nucleus; and macrophages, stars with a nucleus; both shown here inside a blood vessel) infiltrating the vessel wall (II-III), which induces, as a feedback effect, a perpetuation of inflammation (III-IV); finally, atherosclerotic lesions develop (IV-V). An incipient atherosclerosis plaque is shown in V (see Ref. 67). (B) In an injured joint (e.g., in rheumatoid arthritis) with inflammation of synovial tissue (I-II) (Hsp60 and lymphocytes are represented as in A, above), Hsp60 peptide administration (III) can exert an anti-inflammatory effect (IV) and contribute to joint lesion improvement (see Ref. 68).

bloodstream and its plasmatic levels seem to be genetically controlled (59). A work on 60 subjects aged between 20 and 96 years of age showed that the serum levels of Hsp60, but not those of anti-Hsp60 auto-antibodies, declined with aging (60). The reason why the antibodies do not decline, and may even increase with age, could be that the immune system reacts not only to the autologous Hsp60 but also to its homolog from microbial pathogens usually present as persistent infections in the elderly (61). Furthermore, it is possible that post-translation modifications occurring in Hsp60 as the age progresses make the autologous chaperonin immunogenic, thus stimulating autoantibody production. Hence, although the number of Hsp60 molecules decreases with age, their immunogenicity-antigenicity would increase.

An open question is how and when (i.e., during fetal development and after birth) Hsp60 is released outside the cell. It has recently been shown that Hsp60 is secreted from normal adult rat cardiomyocytes via the exosomal pathway (58) but, to our knowledge, no other similar observations have been reported regarding normal human cells. We recently demonstrated with cultured cell lines that viable tumor, but not normal cells secrete Hsp60 into the extracellular milieu by the lipid rafts-exosomal pathway (54). The Hsp60 secretion pathway(s) of human senescent cells are currently poorly known.

Independently from the via of release, it is well established that presence of Hsp60 in the extracellular space facilitates the contact with the immune system cells, promoting their activation; for example, we recently showed that Hsp60 is present in macrophages of colon mucosa from patients with ulcerative colitis and its levels decreased after an effective therapy, thus suggesting its role in maintaining mucosal inflammation in these patients (62). Analogously, in another work on bronchial mucosa from chronic obstructive pulmonary disease patients we found increased levels of Hsp60, compared to smoking and non-

smoking controls, both in epithelium and lamina propria, and, in the latter, this chaperonin localized into neutrophils (manuscript submitted). In both cases, Hsp60 binding with inflammatory cells may trigger or perpetuate immune system activation and, thus, disease progression. Although it is possible that inflammatory cells in the airways are able to produce their own Hsp60, we cannot exclude that, at least in part, Hsp60 reaches inflammatory cells after release from other cytotypes (e.g., epithelial cells) and interact with receptors localised on the inflammatory-cell surface.

However, there is some controversy concerning whether or not immune-cell activation by receptor-binding Hsp60 (e.g., using toll-like receptors) requires bacterial-derived products such as LPS, flagellin, or lipoprotein (15, 63), a concern that has been ruled out for other Hsps (as Hsp70) by the use of recombinant proteins isolated from insects and of other appropriate controls (64).

From the interstitium, Hsp60 can reach the bloodstream. When in circulation, Hsp60 appears to be a key endogenous inflammatory mediator by causing the release of pro-inflammatory cytokines and nitric oxide by immune competent cells (65). In contrast, other studies have demonstrated that induction of immunity to Hsp60 can attenuate inflammatory diseases (65, 66). It is very difficult, if not impossible, at the present time to always distinguish between situations in which Hsp60 plays a passive role as an autoantigen and situations in which the chaperonin has an active role as a chaperokine (endocrine-like or signalling function), inducing inflammation and/or an immune response to other antigens. What happens and when may depend on a variety of factors, not completely understood. In addition, the role of Hsp60 during inflammation seems to depend on the subtypes of activated lymphocytes infiltrating tissues. For example, in atherosclerosis (ATS) Hsp60 would have pro-inflammatory effects (Figure 2A) (67), whereas in rheumatoid arthritis, it could have an anti-inflammatory action (Figure 2B) (68).

Table 1. Aging-related pathologies in which Hsp60 is believed to play a pathogenetic role

Damaged tissue/organ	Cells involved in physiopathogenesis	Disease	References
Vessels	Endothelial cells, macrophages	Vasculitis, atherosclerosis	84-86, 88, 91
Heart	Myocardocytes	Myocarditis, infarct, heart failure	18, 58, 59, 80, 87, 89, 90, 92-94, 95-98, 100, 101
	Conducting system cells	Atrial fibrillation	110-112
Brain	Neurons, glia	Neurodegenerative disorders	103-105
Joints	Synoviocytes	Degenerative joint disease, rheumatoid arthritis	66, 107-109
Bone	Osteoblasts, osteoclasts	Osteoporosis	117, 118
Pancreas	Beta-cells	Type II Diabetes	119
Bronchi	Epithelial cells	Chronic obstructive pulmonary disease	120, 121
Eye	Trabecular endothelial-like cells of the iridocorneal angle	Glaucoma	113
Oral cavity	Gingival cells	Periodontitis	115, 116

In any case, given the data available at present, one can assume that the changes in the immune system response observed during aging are correlated, at least partially, to the decline in Hsp60 levels and/or to structural changes in this chaperonin due, for example, to post-translational modifications. Abnormal levels and/or structural damage in Hsp60 will most likely lead to scrambling of its interactions with immune system components and, consequently, to pathogenesis (27). Hence, a future challenge will be to shed more light into the nature and types of interactions between Hsp60 and the immune system during aging.

6. HSP60 IN CELL AGING

Cell aging includes morphological, structural, and biochemical changes as part of a complex biologic program whereby old individuals accumulate senescent cells in their bodies. Aging cells in culture become flat and enlarged, developing extensive vacuolization and, *in vitro* as well as *in vivo*, they show modified secretory pathways and a reduced ability to respond to stressors and to divide, with growth arrest in late stages (69).

Some key components of the senescence process may be regulated by chaperones (70). Analogously to what happens during the normal-dysplasia-carcinoma transition in various anatomical sites (71-75), levels of Hsp60 increase in human skin fibroblasts during replicative senescence (69, 76) (Figure 3), and the rapid increase in the levels of this chaperonin was positively correlated with cell cycle progression (69). A correlation between increased levels of Hsp60 and senescence in human skin fibroblasts was shown to involve interaction between Hsp60 and mtHsp70 (77). These *in vitro* results are in agreement with *in vivo* studies that reported an increased Hsp60 expression in the forearm skin of elderly subjects in comparison with young individuals, while other Hsps did not show differences (78). The authors postulated that the chaperonin-level changes occurring with age are the consequence of the mitochondrial oxidative stress characteristic of cell senescence.

Certain chaperones can inhibit caspase-dependent apoptosis, conferring immortality to the cell (79). Hsp60 is one of these caspase-dependent apoptosis inhibitors (80). Moreover, exogenous Hsp60 produced by a persistent infection with *Chlamydia trachomatis* can also block the

anti-apoptotic and the pro-senescence effects of the host's (endogenous) Hsp60, and in turn favour the active proliferation of damaged cells (81). Finally, chronic infection with *C. trachomatis* with host invasion by the bacterial Hsp60 generates anti-chaperonin antibodies that crossreact with the host's counterpart and, thereby, causes a variety of lesions in those locations in which the host's Hsp60 happens to reside (61).

In summary, the pioneering studies discussed in the preceding paragraphs suggest that Hsp60 plays a role in the cell senescence process but the precise molecular mechanisms are not yet fully elucidated and deserve further analysis, because their elucidation will provide useful clues for developing preventive and treatment means applicable to Hsp60 chaperonopathies.

7. HSP60 IN AGING RELATED DISEASES

The number of papers showing the participation of Hsp60 in aging-related disease development is constantly growing. The involvement of Hsp60 in the pathogenesis of aging-related diseases has been studied with a number of approaches, both *in vivo* and *in vitro*, and at tissue, cellular, and subcellular levels, as summarized in Table 1. Some of these papers are mainly based on observational studies and a cause-effect relationship has not been yet properly investigated. Hsp60 in aging individuals seems to have variable roles, which most likely depend on conditions in the cell, tissue, and organism affected. Hsp60 may metamorphose from a cell-protecting molecule to a dangerous one, thus being the Proteus of human disease pathogenesis. This is possibly due to largely unknown mechanisms that regulate its gene expression and to variables in the translation process as well as to post-translational modifications. In addition, the roles of Hsp60 may be influenced by its interactions with other intracellular proteins and other processes, including secretion into the extracellular space with the chaperonin becoming an extracellular chaperone, signal molecule, autoantigen, or cytokine-like or endocrine-like molecule.

The involvement of Hsp60 in age-related tumor pathogenesis has been extensively discussed elsewhere (82, 83). In the following subsections we will briefly discuss other widespread aging-related pathological conditions that reduce significantly the quality and extent of life in Western countries.

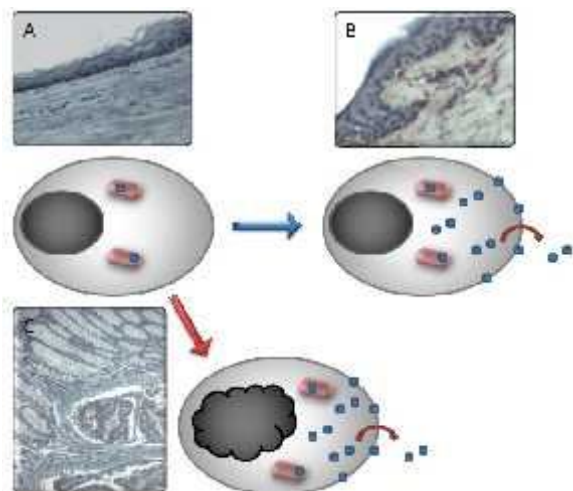


Figure 3. Hsp60 expression during senescence and carcinogenesis. Intracellular Hsp60 (small blue squares) levels can increase during cell senescence (blue arrow) as well as during carcinogenesis (red arrow), as demonstrated, for example, by immunostaining (performed by LSAB2 Kit, Dako, Carpinteria, CA, USA, Cat. No. K677; human primary monoclonal anti-Hsp60 antibody: SIGMA, Milan, Italy, Cat. No. H4149, dilution: 1:400). Skin specimens of young human subjects (A) are commonly negative for Hsp60, while dermal fibroblasts of old subjects (B) show a diffuse positivity (red positivity) (unpublished data; see also Ref 69 for an *in vitro* demonstration). Hsp60 positivity (red) is commonly present in colon cancer cells (C, right-lower corner), while normal colonic mucosa is negative or low-positive (C; left-upper corner) (see Ref 83 for a review). Hsp60 overexpression may be associated to cell secretion (curved red arrow) as already demonstrated for tumor cells, see Ref 54). Mitochondria are shown as small pink capsules. Original pictures, magnification: 200X in A; 400X in B, and 100X in C.

7.1. Atherosclerosis and heart failure

Although atherosclerosis (ATS) has been proposed to be an “autoimmune disease due to an immune reaction against Hsp60” (67), the exact involvement of Hsp60 in the pathogenesis of ATS is still confused by contradictory results. Therefore, further studies are necessary to definitively clarify the roles of Hsp60 as etiologic factor and of the antibodies against it in sustaining the inflammation underlying the pathogenesis of ATS (84-87). Also, it is pertinent to consider the contribution of molecular mimicry between human and microbial Hsp60 in generating an autoimmune response that in turn causes endothelial damage and artery inflammation (88-94). A number of *in vivo* data strongly support the involvement of Hsp60 in the pathogenesis of coronary artery disease (59, 87, 95-98), but this view was not supported by data from others (99).

With regard to heart failure, Hsp60 is gaining ranks as a clinical marker for monitoring this condition (58, 80, 100). Hsp60 is overexpressed in the cytosol, localises in the cell membrane, and can also be secreted by stressed

myocardiocytes (58, 80). Higher than normal anti-Hsp60 antibody levels were correlated with higher levels of brain natriuretic peptide, left ventricular end-diastolic dimension and with the extent of cardiac dysfunction (101). If confirmed, these data may become of clinical utility for heart failure management.

7.2. Neurodegenerative disorders

Several kinds of stresses, among which nitrosidative stress, may cause accumulation of aberrant proteins and neuronal cell damage or death (102). Thus, stress-induced proteins like some of the chaperones have been proposed as protective molecules for the nervous system cells. For example, overexpression of the *hsp60* gene was observed in experimental subarachnoidal haemorrhage in rats, possibly induced as a protective mechanism (103).

Hsp60 has been proposed as a good histological marker of the normality of unaffected cells in not damaged areas in pathological brains (104), although it is extremely difficult to value its levels *in vivo* in patients with Alzheimer's, Parkinson's, Huntington's, and prion diseases in clinical practice.

Hsp60 levels, as well as those of other stress proteins, were found elevated in lymphocytes from Alzheimer's disease patients when compared to controls (105). It could be of some utility to test levels of Hsp60 in patients with mild cognitive impairment (a clinical condition that precede Alzheimer's disease arise), for assessing the potential value of this protein as an early marker of the disease.

Lastly, since Hsp60 plays a role in the pathogenesis of ATS as discussed above, it can be inferred that by similar mechanisms this chaperonin is implicated in cerebrovascular disorders of the central nervous system, such as stroke, as also proposed by others (106).

7.3. Degenerative joint diseases

Hsp60 has been implicated in the pathogenesis of degenerative joint disease, both in young and elderly people (66, 107, 108). It has been postulated that a humoral response against bacterial Hsp60 (exogenous chaperonin) could elicit a cross-reaction against the infected-host's Hsp60 (endogenous chaperonin) and other antigens in the synovial tissue in rheumatoid arthritis, thus perpetuating the local inflammatory and destructive processes (108, 109).

7.4. Other pathologies

Hsp60 levels and the presence of anti-chaperonin auto-antibodies have been studied in relation to onset and progression of other aging-related diseases different from those already discussed above, such as atrial fibrillation (110-112), glaucoma (113, 114), periodontitis (115, 116), osteoporosis (117, 118), type II diabetes (119), and chronic obstructive pulmonary disease (120, 121). In these instances it is of the essence to determine to what extent Hsp60 contributes to pathogenesis when defective or to protection when normal, for elucidating the molecular mechanisms by which this chaperonin might trigger the

initiation, and/or modify the course, of these age-related disorders.

8. SUMMARY AND PERSPECTIVES

A positive correlation between longevity and capacity for mounting a strong heat-shock response has been reported (122, 123), implying that chaperones are key components of adaptive mechanisms for survival. Unfortunately, chaperone levels and/or functionality generally decrease with age. For example, Hsp60 could be overloaded by the increasing demand due to the accumulation of damaged proteins that occurs during senescence, which could result in a widespread chaperoning deficiency and lead to the onset of aging-related disorders (30, 124). A shift in the balance between misfolded proteins and available chaperonins, and other Hsps, in aging organisms, can bring about defects in signal transduction, protein transport, cellular organization, and immune functions (30, 32).

All the information available at the present time indicates that Hsp60 is a key factor for protein homeostasis and cell survival, and that this chaperonin is involved in various ways in the onset and progression of aging-related diseases. We have surveyed in this review some of the pathological conditions in which Hsp60 is implicated but there are many others which could also be listed here. In addition, one has to assume that other syndromes and diseases will in the near future be found to have a pathogenetic component involving Hsp60. In all these conditions, already described or to be found, it is necessary to investigate in depth the mechanism of Hsp60 participation and, thus, uncover clues useful for the development of diagnostic and therapeutic means, which ought to help considerably the management of aging-related disorders.

9. ACKNOWLEDGEMENTS

This study was supported by funds of the Istituto Euro-Mediterraneo di Scienza e Tecnologia, Palermo, Italy.

10. REFERENCES

1. F. Ritossa: A new puffing pattern induced by heat shock and DNP in *Drosophila*. *Experientia* 18, 571 (1962)
2. A. Tissières, HK Mitchell, UM Tracy: Protein synthesis in salivary glands of *Drosophila melanogaster*: relation to chromosome puffs. *J Mol Biol* 84(3), 389-98 (1974)
3. F.C. Neidhardt, R.A. VanBogelen, V. Vaughn: The genetics and regulation of heat-shock proteins. *Annu Rev Genet* 8, 295-329 (1984)
4. J.R. Subjeck, T. Shyy: Stress protein system of mammalian cells. *Am J Physiol* 250, C1 (1986)
5. K. Watson: Microbial stress proteins. *Adv Microbial Physiol* 31, 183 (1990)
6. R.J. Ellis, S.M. Van der Vies: Molecular chaperones. *Annu Rev Biochem* 60, 321-47 (1991)
7. C. Georgopoulos, W.J. Welch: Role of the major heat shock proteins as molecular chaperones. *Annu Rev Cell Biol* 9, 601-34 (1993)
8. B.M. Sanders: Stress protein in aquatic organisms: an environmental perspective. *Crit Rev Toxicol* 23, 49 (1993)
9. A.J.L. Macario, C.B. Dugan, E. Conway de Macario: A *dnaK* homolog in the archaeobacterium *Mathanosarcina mazei* S6. *Gene* 108, 133 (1991)
10. J. Frydman: Folding of newly translated proteins *in vivo*: the role of molecular chaperones. *Annu Rev Biochem* 70, 603-47 (2001)
11. F.U. Hartl, M. Hayer-Hartl: Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 295, 1852-8 (2002)
12. J. Haak, K.C. Kregel: A cell stress odyssey. *Novartis Found. Symp* 291, 3-15 (2008)
13. F. Cappello, E. Conway de Macario, L. Marasa, G. Zummo, A.J.L. Macario: Hsp60 expression, new locations, functions, and perspectives for cancer diagnosis and therapy. *Cancer Biol, Ther* 7, 801-809 (2008)
14. B. Henderson: Integrating the cell stress response: a new view of molecular chaperones as immunological and physiological homeostatic regulators. *Cell Biochem Funct* 28, 1-14 (2010)
15. B. Henderson, S.K. Calderwood, A.R. Coates, I. Cohen, W. Van Eden, T. Lehner, A.G. Pockley: Caught with their PAMPs down? The extracellular signaling actions of molecular chaperones are not due to microbial contaminants. *Cell Stress Chaperones* 15, 123-41 (2010)
16. H. Luo, F.T. Robb: A modulator domain controlling thermal stability in the Group II chaperonins of Archaea. *Arch Biochem Biophys* (2011) (In press.)
17. N.R. Douglas, S. Reissmann, J. Zhang, B. Chen, J. Jakana, R. Kumar, W. Chiu, J. Frydman: Dual action of ATP hydrolysis couples lid closure to substrate release into the group II chaperonin chamber. *Cell* 144(2), 240-52 (2011)
18. J. Zhang, B. Ma, F. Dimaio, N.R. Douglas, L.A. Joachimiak, D. Baker, J. Frydman, M. Levitt, W. Chiu: Cryo-EM structure of a Group II chaperonin in the prehydrolysis ATP-bound state leading to lid closure. *Structure* 19(5), 633-9 (2011)
19. A.J.L. Macario, E. Conway de Macario: The pathology of anti-stress mechanisms: A new frontier. *Stress* 7, 243-249 (2004)

20. A.J.L. Macario, E. Conway de Macario: Sick chaperones, cellular stress, and disease. *N Engl J Med* 353, 1489-1501 (2005)
21. A.J.L. Macario: The chaperonopathies: a new field of medicine for study by physicians and all others involved in medical sciences and public health. Medicine & Health 2012 published by Going International, Vienna, Austria.
22. A.J.L. Macario, E. Conway de Macario: Chaperonopathies and chaperonotherapy. *FEBS Letters* 581, 3681-3688 (2007a)
23. A.J.L. Macario: Heat-shock proteins and molecular chaperones: Implications for pathogenesis, diagnostics, and therapeutics. *Intl J Clin Lab Res* 25, 59-70 (1995)
24. A.J.L. Macario, T.M. Grippo, E. Conway de Macario: Genetic disorders involving molecular-chaperone genes: A perspective. *Genet Med* 7, 3-12 (2005)
25. A.J.L. Macario, E. Conway de Macario: Molecular chaperones and age-related degenerative disorders. *Adv Cell Aging Gerontol* 7, 131-162 (2001a)
26. A.J.L. Macario, E. Conway de Macario: Sick chaperones and ageing: A perspective. *Ageing Res Rev* 1, 295-311 (2002)
27. A.J.L. Macario, F. Cappello, G. Zummo, E. Conway de Macario: Chaperonopathies of senescence and the scrambling of the interactions between the chaperoning and the immune systems. *Ann N Y Acad Sci* 1197, 85-93 (2010)
28. R. Njemini, C. Demanet, T. Mets: Aging-related differences in basal heat shock protein 70 levels in lymphocytes are linked to altered frequencies of lymphocyte subsets. *Aging Cell* 7, 498-505 (2008)
29. C. Soti, A.S. Sredhart, P. Csermely: Apoptosis, necrosis and cellular senescence: chaperone occupancy as a potential switch. *Aging Cell* 2, 39-45 (2003)
30. C. Soti, P. Csermely: Aging and molecular chaperones. *Exp Gerontol* 38, 1037-1040 (2003)
31. C. Soti, P. Csermely: Aging cellular networks: chaperones as major participants. *Exp Gerontol* 42, 113-119 (2007a)
32. C. Soti, P. Csermely: Protein stress and stress proteins: Implication in aging and disease. *J Biosci* 32, 511-515 (2007b)
33. J. Bhattacharyya, E.V. Shipova, P. Santhoshkumar, K.K. Sharma, B.J. Ortwerth: Effect of a single AGE modification on the structure and chaperone activity of human alphaB-crystallin. *Biochemistry* 46, 14682-14692 (2007)
34. P.A. Kumar, M.S. Kumar, G.B. Reddy: Effect of glycation on alpha-crystallin structure and chaperone-like function. *Biochem J* 408, 251-258 (2007)
35. A.J.L. Macario, E. Conway de Macario: Chaperonopathies by defect, excess, or mistake. *Ann N Y Acad Sci* 1113, 178-191 (2007b)
36. E.T. Soo, Y.K. Ng, B.H. Bay, G.W. Yip: Heat shock proteins and neurodegenerative disorders. *Scientific World J* 8, 270-274 (2008)
37. A.J.L. Macario, E. Conway de Macario: The molecular chaperone system and other anti-stress mechanisms in archaea. *Front Biosci* 6, d262-283 (2001b)
38. A.J.L. Macario, M. Lange, B.K. Haring, E. Conway de Macario: Stress genes and proteins in the Archaea. *Microbiol Mol Biol Reviews* 63, 923-967 (1999)
39. A.J.L. Macario, M. Malz, E. Conway de Macario: Evolution of assisted protein folding: the distribution of the main chaperoning systems within the phylogenetic domain archaea. *Front Biosci* 9, 1318-1332 (2004)
40. A.L. Horwich, W.A. Fenton, E. Chapman, G.W. Farr: Two families of chaperonins: physiology and mechanism. *Annu Rev Cell Dev Biol* 23, 115-45 (2007)
41. S. Jindal, A.K. Dudani, B. Singh, C.B. Harley, R.S. Gupta: Primary structure of a human mitochondrial protein homologous to the bacterial and plant chaperonins and to the 65-kilodalton mycobacterial antigen. *Mol Cell Biol* 9, 2279-2283 (1989)
42. S.K. Sadacharan, A.C. Cavanagh, R.S. Gupta: 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* 116, 507-517 (2001)
42. J.J. Hansen, P. Bross, M. Westergaard, M.N. Nielsen, H. Eiberg, A.D. Borghlum, J. Mogensen, K. Kristiansen, L. Bolund, N. Gregersen: Genomic structure of the human mitochondrial chaperonin genes: HSP60 and HSP10 are localised head to head on chromosome 2 separated by a bidirectional promoter. *Hum Genet* 112, 71-77 (2003)
44. J.L. Blanchard, M. Lynch: Organellar genes: why do they end up in the nucleus? *Trends Genet* 16, 315-320 (2000)
45. K. Braig, Z. Otwinowski, R. Hegde, D.C. Boisvert, A. Joachimiak, A.L. Horwich, P.B. Sigler: The crystal structure of the bacterial chaperonin GroEL at 2.8 Å. *Nature* 371, 578-586 (1994)
46. N.A. Ranson, H.E. White, H.R. Saibil: Chaperonins. *Biochem J* 333, 233-242 (1998)
47. J.F. Hunt, A.J. Weaver, S.J. Landry, L. Gierasch, J. Deisenhofer: The crystal structure of the GroES co-chaperonin at 2.8 Å resolution. *Nature* 379, 37-45 (1996)
48. G. Levy-Rimmler, R.E. Bell, N. Ben-Tal, A. Azem: Type I chaperonins: not all are created equal. *FEBS Lett.* 529, 1-5 (2002)
49. K.L. Nielsen, N.J. Cowan: A single ring is sufficient for productive chaperonin-mediated folding *in vivo*. *Mol Cell* 2, 93-99 (1998)

50. K.L. Nielsen, N. McLennan, M. Masters, N.J. Cowan: A single-ring mitochondrial chaperonin (Hsp60-Hsp10) can substitute for GroEL-GroES *in vivo*. *J Bacteriol* 181, 5871-5875 (1999)
51. Y. Dubaquié, R. Looser, U. Funfschilling, P. Jenö, S. Rospert: Identification of *in vivo* substrates of the yeast mitochondrial chaperonins reveals overlapping but non-identical requirement for hsp60 and hsp10. *EMBO J* 17, 5868-5876 (1998)
52. J.D. Cechetto, B.J. Soltys, G.S. Gupta: Localization of mitochondrial 60-kD heat shock chaperonin protein (Hsp60) in pituitary growth hormone secretory granules and pancreatic zymogen granules. *J Histochem Cytochem* 48, 45-56 (2000)
53. B.J. Soltys, R.S. Gupta: Immunoelectron microscopic localization of the 60-kDa heat shock chaperonin protein (Hsp60) in mammalian cells. *Exp Cell Res* 222(1), 16-27 (1996)
54. A.M. Merendino, F. Bucchieri, C. Campanella, V. Marcianò, A. Ribbene, S. David, G. Zummo, G. Burgio, D.F. Corona, E. Conway de Macario, A.J.L. Macario, F. Cappello: Hsp60 is actively secreted by human tumor cells. *PLoS ONE* 5, e9247 (2010)
55. D. Chandra, G. Choy, D.G. Tang: Cytosolic accumulation of HSP60 during apoptosis with or without apparent mitochondrial release: evidence that its pro-apoptotic or pro-survival functions involve differential interactions with caspase-3. *J Biol Chem* 282, 31289-301 (2007)
56. H. Taguchi, Y. Makino, M. Yoshida: Monomeric chaperonin-60 and its 50-kDa fragment possess the ability to interact with non-native proteins, to suppress aggregation, and to promote protein folding. *J Biol Chem* 269, 8529-8534 (1994)
57. G. Levy-Rimler, P. Viitanen, C. Weiss, R. Sharkia, A. Greenberg, A. Niv, A. Lustig, Y. Delarea, A. Azem: The effect of nucleotides and mitochondrial chaperonin 10 on the structure and chaperone activity of mitochondrial chaperonin 60. *Eur J Biochem* 268, 3465-3472 (2001)
58. S. Gupta, A.A. Knowlton: HSP60 trafficking in adult cardiac myocytes: role of the exosomal pathway. *Am J Physiol Heart Circ Physiol* 292, H3052-3056 (2007)
59. A. Shamaei-Tousi, A. Steptoe, K. O'Donnell, J. Palmen, J.W. Stephens, S.J. Hurel, M. Marmot, K. Horner, F. D'Aiuto, A.R. Coates, S.E. Humphries, B. Henderson: Plasma heat shock protein 60 and cardiovascular disease risk: the role of psychosocial, genetic, and biological factors. *Cell Stress Chaperones* 12, 384-392 (2007)
60. I.M. Rea, S. McNerlan, A.G. Pockley: Serum heat shock protein and anti-heat shock protein antibody levels in aging. *Exp Gerontol* 36, 341-35 (2001)
61. F. Cappello, E. Conway de Macario, V. Di Felice, G. Zummo, A.J.L. Macario: *Chlamydia trachomatis* infection and anti-Hsp60 immunity: The two sides of the coin. *PLoS Pathog* 5(8), e1000552 (2009)
62. G. Tomasello, V. Rodolico, M. Zerilli, A. Martorana, F. Bucchieri, A. Pitruzzella, A.M. Gammazza, S. David, F. Rappa, G. Zummo, P. Damiani, S. Accomando, M. Rizzo, E. Conway de Macario, A.J.L. Macario, F. Cappello: Changes in immunohistochemical levels and subcellular localization after therapy and correlation and colocalization with CD68 suggest a pathogenetic role of Hsp60 in ulcerative colitis. *Appl Immunohistochem Mol Morphol*, (2011)
63. M.F. Tsan, B. Gao: Heat shock proteins and immune system. *J Leuk Biol* 85, 905-910 (2009)
64. A. De Maio: Extracellular heat-shock proteins, cellular export vesicles and the stress observational system. A form of communication during injury, infection and cell damage. *Cell Stress Chaperones* 16, 235-249 (2011)
65. W. Van Eden, G. Wick, S. Albani, I. Cohen: Stress, heat shock proteins, and autoimmunity: how immune responses to heat shock proteins are to be used for the control of chronic inflammatory diseases. *Ann NY Acad Sci* 1113, 217-237 (2007)
66. J.A. van Roon, W. van Eden, J.L. van Roy, F.J. Lafeber, J.W. Bijlsma: Stimulation of suppressive T cell responses by human but not bacterial 60-kD heat-shock protein in synovial fluid of patients with rheumatoid arthritis. *J Clin Invest* 100, 459-463 (1997)
67. G. Wick: Atherosclerosis—an autoimmune disease due to an immune reaction against heat-shock protein 60. *Herz* 25, 87-90 (2000)
68. F.J. Quintana, A. Momran, P. Carmi, F. Mor, I.R. Cohen: Hsp60 as a target of anti-ergotypic regulatory T cells. *PLoS ONE* 3(12), e4026 (2008)
69. V. Di Felice, N. Ardizzone, V. Marcianò, T. Bartolotta, F. Cappello, F. Farina, G. Zummo: Senescence-associated HSP60 expression in normal human skin fibroblasts. *Anat Rec* 284A, 446-453 (2005a)
70. C.C. Deocaris, S.C. Kaul, R. Wadhwa: On the brotherhood of the mitochondrial chaperones mortalin and heat shock protein 60. *Cell Stress Chaperones* 11, 116-128 (2006)
71. F. Cappello, M. Bellafiore, A. Palma, V. Marciano, G. Martorana, P. Bellafiore, A. Martorana, F. Farina, G. Zummo, F. Bucchieri: Expression of 60-kD heat shock protein increases during carcinogenesis in the uterine exocervix. *Pathobiology* 70, 83-88 (2002)
72. F. Cappello, M. Bellafiore, A. Palma, S. David, V. Marcianò, T. Bartolotta, C. Sciumè, G. Modica, F. Farina, G. Zummo, F. Bucchieri: 60KDa chaperonin (HSP60) is over-expressed during colorectal carcinogenesis. *Eur J Histochem* 47, 105-110 (2003a)

73. F. Cappello, F. Rappa, S. David, R. Anzalone, G. Zummo: Immunohistochemical evaluation of PCNA, p53, HSP60, HSP10 and MUC-2 presence and expression in prostate carcinogenesis. *Anticancer Res* 23, 1325-1331 (2003b)
74. F. Cappello, S. David, F. Rappa, F. Bucchieri, L. Marasà, T.E. Bartolotta, F. Farina, G. Zummo: The expression of HSP60 and HSP10 in large bowel carcinomas with lymph node metastases *BMC Cancer* 5, 139(2005a)
75. F. Cappello, A Di Stefano, SE D'Anna, CF Donner, G Zummo: Immunopositivity of heat shock protein 60 as a biomarker of bronchial carcinogenesis. *Lancet Oncol* 6, 816 (2005b)
76. Y.H. Lee, J.C. Lee, H.J. Moon, J.E. Jung, M. Sharma, B.H. Park, H.K. Yi, E.C. Jhee: Differential effect of oxidative stress on the apoptosis of early and late passage human diploid fibroblasts: implication of heat shock protein 60. *Cell Biochem Funct* 26, 502-508 (2008)
77. Z. Kaul, T. Yaguchi, S.C. Kaul, R. Wadhwa: Quantum dot-based protein imaging and functional significance of two mitochondrial chaperones in cellular senescence and carcinogenesis. *Ann NY Acad Sci* 1067, 469-473 (2006)
78. P. Gromov, G.L. Skovgaard, H. Palsdottir, I. Gromova, M. Østergaard, J.E. Celis: Protein profiling of the human epidermis from the elderly reveals up-regulation of a signature of interferon-gamma-induced polypeptides that includes manganese-superoxide dismutase and the p85beta subunit of phosphatidylinositol 3-kinase. *Mol Cell Proteomics* 2, 70-84 (2003)
79. J. Nylandsted, M. Rohde, K. Brand, L. Bastholm, F. Elling, M. Jäättelä: Selective depletion of heat shock protein 70 (Hsp70) activates a tumor-specific death program that is independent of caspases and bypasses Bcl-2. *Proc Natl Acad Sci USA* 97, 7871-7876 (2000)
80. L. Lin, S.C. Kim, Y. Wang, S. Gupta, B. Davis, S.I. Simon, G. Torre-Amione, A.A. Knowlton: HSP60 in heart failure: abnormal distribution and role in cardiac myocyte apoptosis. *Am J Physiol Heart Circ Physiol* 293, H2238-H2247 (2007)
81. V. Di Felice, S. David, F. Cappello, F. Farina, G. Zummo: Is chlamydial heat shock protein 60 a risk factor for oncogenesis?. *Cell Mol Life Sci* 62, 4-9 (2005b)
82. F. Cappello, A.M. Czarnecka, G. La Rocca, A. Di Stefano, G. Zummo, A.J.L. Macario: Hsp60 and Hsp10 as antitumor molecular agents. *Cancer Biol Ther* 6, 487-489 (2007)
83. F. Cappello, S. David, G. Peri, F. Farina, E. Conway de Macario, A.J.L. Macario, G. Zummo: Hsp60: molecular anatomy and role in colorectal cancer diagnosis and treatment. *Front Biosci* 3, 341-51 (2011)
84. Q. Xu, G. Luef, S. Weimann, R.S. Gupta, H. Wolf, G. Wick: Staining of endothelial cells and macrophages in atherosclerotic lesions with human heat-shock protein- reactive antisera. *Arterioscler Thromb* 13, 1763-1769 (1993)
85. G. Schett, Q. Xu, A. Amberger, R. Van der Zee, H. Recheis, J. Willeit, G. Wick: Autoantibodies against heat shock protein 60 mediate endothelial cytotoxicity. *J Clin Invest* 96, 2569-2577 (1995)
86. Q. Xu, G. Schett, H. Perschinka, M. Mayr, G. Egger, F. Oberhollenzer, J. Willeit, S. Kiechl, G. Wick: Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. *Circulation* 102(1), 14-20 (2000)
87. Z. Prohaszka, J. Duba, L. Horvath, A. Csaszar, I. Karadi, A. Szebeni, M. Singh, B. Fekete, L. Romics, G. Fust: Comparative study on antibodies to human and bacterial 60 kDa heat shock proteins in a large cohort of patients with coronary heart disease and healthy subjects. *Eur J Clin Invest* 31, 285-292 (2001)
88. M. Mayr, B. Metzler, S. Kiechl, J. Willeit, G. Schett, Q. Xu, G. Wick: Endothelial cytotoxicity mediated by serum antibodies to heat shock proteins of *Escherichia coli* and *Chlamydia pneumoniae*: immune reactions to heat shock proteins as a possible link between infection and atherosclerosis. *Circulation* 99, 1560-1566 (1999)
89. A. Ciervo, P. Visca, A. Petrucca, L.M. Biasucci, A. Maseri, A. Cassone: Antibodies to 60-kilodalton heat shock protein and outer membrane protein 2 of *Chlamydia pneumoniae* in patients with coronary heart disease. *Clin Diag Lab Immunol* 9, 66-74 (2002)
90. L.M. Biasucci, G. Liuzzo, A. Ciervo, A. Petrucca, M. Piro, D.J. Angiolillo, F. Crea, A. Cassone, A. Maseri: Antibody response to chlamydial heat shock protein 60 is strongly associated with acute coronary syndromes. *Circulation* 107, 3015-3017 (2003)
91. M. Knoflach, B. Mayrl, C. Mayerl, R. Sedivy, G. Wick: Atherosclerosis as a paradigmatic disease of the elderly: role of the immune system. *Immunol Allergy Clin North Am* 23, 117-132 (2003)
92. S. Hoshida, M. Nishino, J. Tanouchi, T. Kishimoto, Y. Yamada: Acute *Chlamydia pneumoniae* infection with heat-shock-protein-60-related response in patients with acute coronary syndrome. *Atherosclerosis* 183, 109-112 (2005)
93. L.G. Spagnoli, S. Pucci, E. Bonanno, A. Cassone, F. Sesti, A. Ciervo, A. Mauriello: Persistent *Chlamydia pneumoniae* infection of cardiomyocytes is correlated with fatal myocardial infarction. *Am J Pathol* 170, 33-42 (2007)
94. V.Y. Hoymans, J.M. Bosmans, P.L. Van Herck, M.M. Ieven, C.J. Vrints: Implications of antibodies to heat-shock proteins in ischemic heart disease. *Int J Cardiol* 123, 277-282 (2008)
95. A. Veres, G. Füst, M. Smiela, M. McQueen, A. Horváth, Q. Yi, A. Bíró, J. Pogue, L. Romics, I. Karádi, M.

- Singh, J. Gnarp, Z. Prohászka, S. Yusuf: Heart Outcomes Prevention Evaluation (HOPE) Study Investigators. Relationship of anti-60 kDa heat shock protein and anti-cholesterol antibodies to cardiovascular events. *Circulation* 106, 2775-2780 (2002)
96. J. Wysocki, B. Karawajczyk, J. Gorski, A. Korzeniowski, Z. Mackiewicz, G. Kupryszewski, R. Glosnicka: Human heat shock protein 60 (409-424) fragment is recognized by serum antibodies of patients with acute coronary syndromes. *Cardiovasc Pathol* 11, 238-243 (2002)
97. C. Lenzi, A. Palazzuoli, N. Giordano, G. Alegente, C. Gonnella, M.S. Campagna, A. Santucci, M. Sozzi, P. Papakostas, F. Rollo, R. Nuti, N. Figura: *H. pylori* infection and systemic antibodies to CagA and heat shock protein 60 in patients with coronary heart disease. *World J Gastroenterol* 12, 7815-7820 (2006)
98. G. Novo, F. Cappello, M. Rizzo, G. Fazio, S. Zambuto, E. Tortorici, A. Marino Gammazza, S. Corrao, G. Zummo, E. Conway de Macario, A.J.L. Macario, P. Assennato, S. Novo, G. Li Volti: Hsp60 and heme oxygenase-1 (Hsp32) in acute myocardial infarction. *Transl Res* 157(5), 285-92 2011
99. A. Steptoe, A. Shamaei-Tousi, A. Gylfe, L. Bailey, S. Bergström, A.R. Coates, B. Henderson: Protective effect of human heat shock protein 60 suggested by its association with decreased seropositivity to pathogens. *Clin Vaccine Immunol* 14, 204-207 (2007)
100. N. Latif, P.M. Taylor, M.A. Khan, M.H. Yacoub, M.J. Dunn: The expression of heat shock protein 60 in patients with dilated cardiomyopathy. *Basic Res Cardiol* 94, 112-119 (1999)
101. D. Giannessi, C. Colotti, M. Maltinti, S. Del Ry, C. Prontera, S. Turchi, A. Labbate, D. Neglia: Circulating heat shock proteins and inflammatory markers in patients with idiopathic left ventricular dysfunction: their relationships with myocardial and microvascular impairment. *Cell Stress Chaperones* 12, 265-274 (2007)
102. T. Nakamura, Z. Gu, S.A. Lipton: Contribution of glutamatergic signaling to nitrosative stress-induced protein misfolding in normal brain aging and neurodegenerative diseases. *Aging Cell* 6, 351-359 (2007)
103. M. Satoh, J. Tang, A. Nanda, J.H. Zhang: Heat shock proteins expression in brain stem after subarachnoid hemorrhage in rats. *Acta Neurochir Suppl* 86, 477-482 (2003)
104. J.E. Martin, M. Swash, K. Mather, P.N. Leigh: Expression of the human GroEL stress-protein homologue in the brain and spinal cord. *J Neurol Sci* 118, 202-206 (1993)
105. V. Calabrese, R. Sultana, G. Scapagnini, E. Guagliano, M. Sapienza, R. Bella, J. Kanski, G. Pennisi, C. Mancuso, A.M. Stella, D.A. Butterfield: Nitrosative stress, cellular stress response, and thiol homeostasis in patients with Alzheimer's disease. *Antioxid Redox Signal* 8, 1975-1986 (2006)
106. Q. Xiao, K. Mandal, G. Schett, G. Mayr, F. Oberhollenzen, J. Willeit, S. Kiechl, Q. Xu: Association of serum-soluble heat shock protein 60 with carotid atherosclerosis: clinical significance determined in a follow-up study. *Stroke* 36, 2571-6 (2005)
107. B. Wilbrink, M. Holewijn, J.W. Bijlsma, J.L. van Roy, W. den Otter, W. van Eden: Suppression of human cartilage proteoglycan synthesis by rheumatoid synovial fluid mononuclear cells activated with mycobacterial 60-kd heat-shock protein. *Arthritis Rheum* 36, 514-518 (1993)
108. V. Krenn, H.P. Vollmers, P. von Landenberg, B. Schmausser, M. Rupp, A. Roggenkamp, H.K. Müller-Hermelink: Immortalized B-lymphocytes from rheumatoid synovial tissue show specificity for bacterial HSP60. *Virchows Arch* 427, 511-518 (1996)
109. U. Rudolph, R. Rzepka, S. Batsford, S.H. Kaufmann, K. von der Mark, H.H. Peter, I. Melchers: The B cell repertoire of patients with rheumatoid arthritis. II. Increased frequencies of IgG+ and IgA+ B cells specific for mycobacterial heat-shock protein 60 or human type II collagen in synovial fluid and tissue. *Arthritis Rheum* 40, 1409-1419 (1997)
110. A.E. Schäfler, K. Kirmanoglou, J. Balbach, P. Pecher, A. Hannekum, B. Schumacher: The expression of heat shock protein 60 in myocardium of patients with chronic atrial fibrillation. *Basic Res Cardiol* 97, 258-61 (2002)
111. M. Yang, H. Tan, L. Cheng, M. He, Q. Wei, R.M. Tanguay, T. Wu: Expression of heat shock proteins in myocardium of patients with atrial fibrillation. *Cell Stress Chaperones* 12, 142-50 (2007)
112. H. Cao, L. Xue, X. Xu, Y. Wu, J. Zhu, L. Chen, D. Chen, Y. Chen: Heat shock proteins in stabilization of spontaneously restored sinus rhythm in permanent atrial fibrillation patients after mitral valve surgery. *Cell Stress Chaperones* 2011 Apr 1. [Epub ahead of print]
113. M.B. Wax, G. Tezel, I. Saito, R.S. Gupta, J.B. Harley, Z. Li, C. Romano: Anti-Ro/SS-A positivity and heat shock protein antibodies in patients with normal-pressure glaucoma. *Am J Ophthalmol* 125, 145-157 (1998)
114. M.B. Wax, G. Tezel, J. Yang, G. Peng, R.V. Patil, N. Agarwal, R.M. Sappington, D.J. Calkins: Induced autoimmunity to heat shock proteins elicits glaucomatous loss of retinal ganglion cell neurons via activated T-cell-derived fas-ligand. *J Neurosci* 28, 12085-12096 (2008)
115. S.W. Chung, H.S. Kang, H.R. Park, S.J. Kim, S.J. Kim, J.I. Choi: Immune responses to heat shock protein in *Porphyromonas gingivalis*-infected periodontitis and atherosclerosis patients. *J Periodontal Res* 38, 388-93 (2003)

116. J. Choi, S.Y. Lee, K. Kim, B.K. Choi: Identification of immunoreactive epitopes of the *Porphyromonas gingivalis* heat shock protein in periodontitis and atherosclerosis. *J Periodontol Res* 46, 240-5 (2011)

117. S. Meghji, M. Lillicrap, M. Maguire, P. Tabona, J.S. Gaston, S. Poole, B. Henderson: Human chaperonin 60 (Hsp60) stimulates bone resorption: structure/function relationships. *Bone* 33, 419-425 (2003)

118. Y.S. Kim, J.M. Koh, Y.S. Lee, B.J. Kim, S.H. Lee, K.U. Lee, G.S. Kim: Increased circulating heat shock protein 60 induced by menopause, stimulates apoptosis of osteoblast-lineage cells via up-regulation of toll-like receptors. *Bone* 45, 68-76 (2009)

119. A. Mostafazadeh, C. Herder, B. Haastert, P. Hanifi-Moghaddam, N. Schloot, W. Koenig, T. Illig, B. Thorand, R. Holle, M.B. Eslami, H. Kolb, KORA Group: Association of humoral immunity to human Hsp60 with the IL-6 gene polymorphism C-174G in patients with type 2 diabetes and controls. *Horm Metab Res* 37, 257-263 (2005)

120. F. Cappello, A. Di Stefano, S. David, F. Rappa, R. Anzalone, G. La Rocca, S.E. D'Anna, F. Magno, C.F. Donner, B. Balbi, G. Zummo: Hsp60 and Hsp10 down-regulation predicts bronchial epithelial carcinogenesis in smokers with chronic obstructive pulmonary disease. *Cancer* 107, 2417-2424 (2006)

121. C.A. Carter, M. Misra, S. Pelech: Proteomic analyses of lung lysates from short-term exposure of Fischer 344 rats to cigarette smoke. *J Proteome Res* (2011) (In press.)

122. D. Garigan, A.L. Hsu, A.G. Fraser, R.S. Kamath, J. Ahringer, C. Kenyon: Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation. *Genetics* 161, 1101-1112 (2002)

123. A.L. Hsu, C.T. Murphy, C. Kenyon: Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* 300, 1142-1145 (2003)

124. P. Csermely: Chaperone overload is a possible contributor to 'civilization diseases'. *Trends Genet* 17, 701-704 (2001)

Abbreviations: Hsps: heat shock proteins; ATS: atherosclerosis; COPD: chronic obstructive pulmonary disease

Key Words: Hsp60, Chaperoning system, Chaperonin, Hsp10, Senescence, Chaperonopathies, Chaperonotherapy, Review

Send correspondence to: Francesco Cappello, Department of Experimental Biomedicine and Clinical Neurosciences, Human Anatomy Section, Emerico Luna, Via del Vespro 129, 90127 Palermo, Italy, Tel: +390916553505, Fax: +390916553580, E-mail: francapp@hotmail.com

Fig. 1

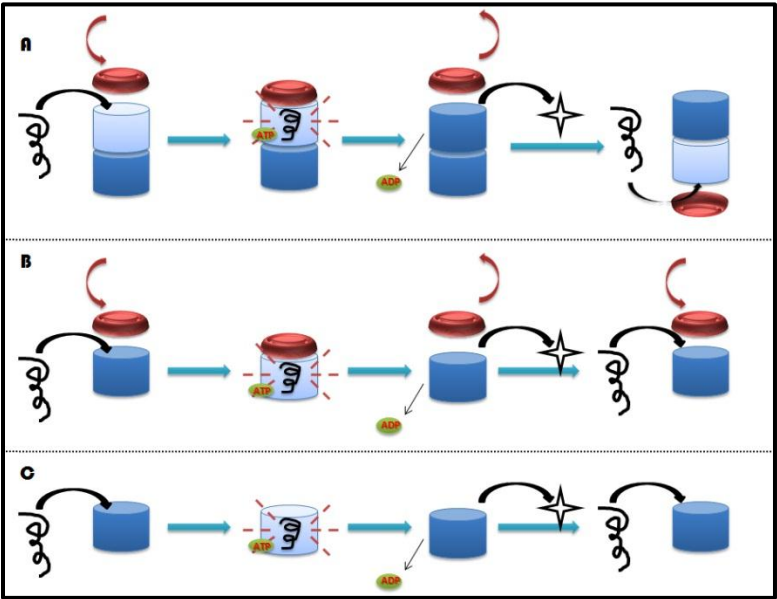


Fig. 2

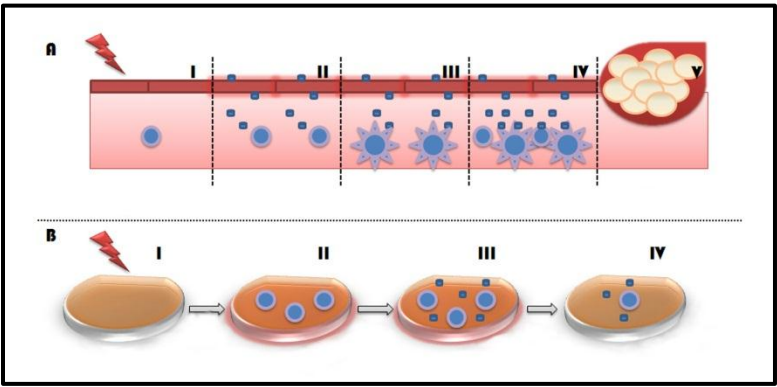


Fig. 3

