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Perspective

THE CHAPERONING AND THE IMMUNE SYSTEMS WITH THE MICROBIOME INTEGRATE A MATRIX THAT SUPPORTS HEALTH: WHEN ONE OF THEM IS DISTURBED THE OTHERS SUFFER AND DISEASE ENSUES

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Abstract

Health, namely good health, depends on the interplay of factors pertaining to the body and to the environment in which the body has evolved and is currently immersed. Here, we consider three pillars of human health: the chaperoning and the immune systems (CS and IS, respectively) and the microbiome (ME). Evolutionarily, it appears that the CS preceded the IS both being essential to defend the organism against stressors and infectious agents, respectively. Presently, the two systems interact extensively, probably the result of millennia of parallel evolution in increasingly complex life forms requiring the cooperation of the two systems for survival. The ME in humans has established itself mainly in the body cavities such as the mouth and the genitourinary and gastrointestinal tracts. Therefore, the ME can be considered a portion of the environmental microbes that during evolution and development has also become a part of the body. Its role in health and disease is incompletely understood but it is already known that it interacts with the IS and, through the IS, it has an impact on the CS. In this article, we discuss diseases caused by failure of the CS. Molecular chaperones, the core components of the CS, can be abnormal and cause pathological conditions named chaperonopathies. These conditions may be genetic or acquired and can affect practically all types of cells, tissues, and organs. Consequently, the chaperonopathies are of interest to all medical specialties and we present here an update of this new field of Medicine.

I. Introduction

I.a. The triad chaperoning system, immune system, and microbiome: the human health pillars

Life Safety and Security includes a variety of issues and means among which the control of the personal health, of the body's integrity and functionality is paramount. It depends on environmental and bodily factors. Both interact inextricably and influence each other. This can be for good or for bad. Thus, the importance of keeping both, the environment and the body, in optimal conditions becomes central to Medicine and Public Health. A major element in the maintenance of the body's homeostasis is the chaperoning system. It is a master protector of protein shape and function. Its failure, represented by diseases named chaperonopathies, leads to discomfort and illness. For example, enzymes, many of which are essential for life, are physiologically active only if they exist in a functional shape, a tridimensional configuration named the native form. This form is achieved during the progression of a protein from its birth, so to speak, toward its maturity, namely its native status, with the help of components of the chaperoning system. Proteins are everywhere in the body. Therefore, components of the chaperoning system are present in all cells; tissues; and organs; and in the body fluids, such as blood, lymph, and

cerebrospinal fluid; and in the intercellular space. Inside cells they occur in all the compartments, for instance nucleus, cytosol, mitochondria, and endoplasmic reticulum. If the chaperones do not help proteins, for example the enzymes mentioned above, the proteins will not fold correctly and will not achieve their functional, native configuration and will malfunction, or will function only partially or not at all, Figure 1.

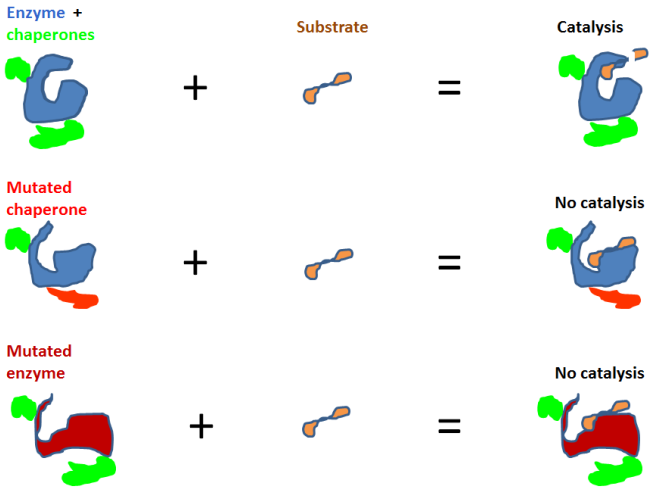

Figure 1. Schematics of some of the molecular mechanisms behind the failure of an enzyme to catalyze its substrate illustrating the key role of chaperones in protein homeostasis. In the top row, the enzyme is normal as is the chaperoning complex necessary to assist the enzyme to reach and maintain its native, functional conformation. Catalysis occurs normally and the substrate is modified. In the second row, the enzyme is normal but a chaperone, one of the members of the chaperoning complex, is abnormal due to a pathogenic mutation. Consequently, the enzyme cannot achieve its functional tridimensional conformation (it is incompletely folded or misfolded) and catalysis cannot occur. This is a situation amenable to positive chaperonotherapy, i.e., the defective chaperone can be replaced with a normal version via protein or gene administration, strategies that are under investigation. The bottom row illustrates the case in which the chaperoning complex is normal but the enzyme is mutated, it is abnormal to the point that even in the presence of a functional chaperoning complex it cannot function and, therefore, there is no catalysis of the substrate.

Consequently, pathologic conditions will occur, which because they are caused by "sick" chaperones, are called chaperonopathies.

The chaperoning system is, evolutionarily speaking, very ancient and must have evolved starting in the very primitive organisms that had to thrive under very harsh environmental conditions, such as those we imagine characterized the most primitive Earth. In today's organisms, chaperones are present in the three life Domains: Bacteria, Archaea, and Eukarya (eukaryotes); namely chaperones occur without exception in all organisms, even in what are considered to be the most ancient microbes that live in extreme environments. As a consequence of their being essential for life, chaperones are quite conserved in all life Domains; so for instance, Hsp70 in humans is very similar to Hsp70 from an archaeon. Likewise, human Hsp60 is very similar to that from bacteria. This similarity means that they are antigenically crossreactive and, therefore, it is no surprise that immunization with a bacterial chaperone, for example Hsp60, elicits antibodies that not only react with the bacterial immunogen, but also with the host's, i.e., the human's Hsp60. This situation occurs in real life because Hsp60 from bacteria colonizing the human body, e.g., the colon, invade the human tissues and circulation and, thereby, reach the immune system, which then produces antibodies against the foreign Hsp60. These antibodies can also react against the host's Hsp60 since, as said above, this is very similar to the bacterial counterpart.

It is clear then that there is interaction between the human chaperoning and immune systems and bacterial chaperones. Thus, a rational understanding of human physiology must include not only the environment and the chaperoning system, as outlined earlier, but also the microbiome present in humans.

The human microbiome, the new star in the scientific heavens of today, is constituted of bacteria, archaea, viruses, and fungi that inhabit mostly in the body cavities such as mouth, colon, and vagina. These organisms are symbiotic, commensal, or pathogenic. In other words, they play different roles, including causing disease if the conditions in the host are favorable to them and can, thus, become actively pathogenic. A graphic representation of the triad chaperoning system, immune system, and microbiome is shown in Figure 2. It can be seen that there is direct interaction between the microbiome and the immune system and that the interaction between the microbiome and the chaperoning system occurs through the immune system, as explained earlier. It remains to be established if there is a direct interaction between the microbiome and the chaperoning system. For instance, can bacterial and archaeal chaperones that invade the host tissues and blood exercise chaperoning functions on host's proteins? Can the foreign chaperones compete for substrate with the human counterparts? Can foreign chaperones perform any of the functions normally carried out by the host-cell's chaperones? These and many other questions can be asked at this time since knowledge of

the chaperoning system and of the microbiome is still scarce and one can foresee a progression toward finding more relationships between these two important components of the human body.

The chaperoning system is one of the anti-stress tools living organisms possess since very early in evolution. Stressors of various kinds have a deleterious impact on many components of cells, tissues, and organs, and one of the most noted is protein damage. This effect can be counteracted by the chaperoning system, which can refold denatured proteins and help them to regain their native configuration unless the damage is so advanced that it is irreversible.

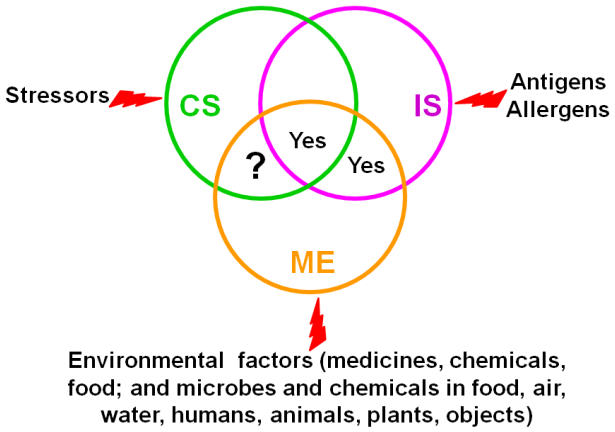

Figure 2. The three tenets of health discussed here with their overlapping functions and interactions. CS, chaperoning system; IS, immune system; ME, microbiome; Yes, shared functions, and interactions. CS is an essential anti-stress mechanism, probably developed since the origin of life precisely for the purpose of preventing, counteracting, and repairing the deleterious effects of stressors on molecules and cells. In today's life forms, including humans, the CS is still influenced by stressors of various types (physical, chemical, biological, psycho-chemical, mechanical) and it performs the same protective functions it did from primeval times but it is more sophisticated and complex as it developed during evolutionary time. Consequently, the CS plays a key role in the maintenance of health. The IS also plays a defensive role, especially against microbes and other noxae that may cause disease. Like the CS, the IS probably evolved from very primitive times but starting after the CS, when this was already present and well established. As a result, the two systems, i.e., the older CS and the IS, also evolutionarily old but not as much as the CS, started an interaction that has been developing over evolution to reach a high degree of complexity as we see today in humans, for instance. The ME in humans originated much later than the CS and IS and was sculpted through the centuries under the influence of the environment and factors that influence the life of its microbial components: for instance, food, chemicals, and medicines; microbes that entered the body with food, and water, and with human, animal, and plant secretions and products; and by skin-mucosa contacts with surrounding objects. The magnitude of its impact on health is only now beginning to be appreciated. It interacts with the IS, and through this interaction it has an impact on the CS and on health. Little is known on the possible direct interactions between the ME and the CS, as indicated with a question mark in the area of their overlap in the drawing. This area, shown with a question mark, appears as very attractive for research in the immediate future.

Thus stress and the chaperoning system play a major role in the status of human health, Figure 3.

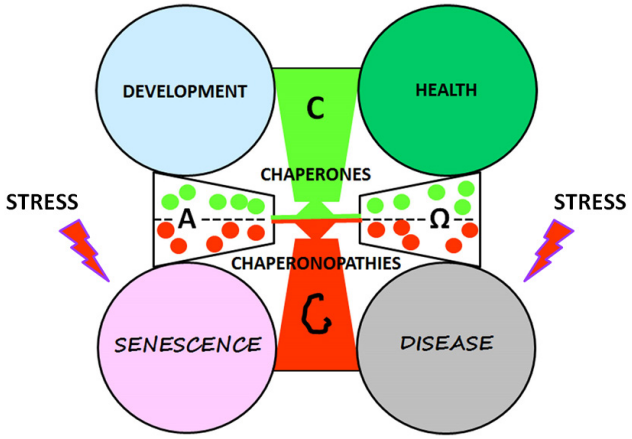

Figure 3. Schematics of the connections between the chaperoning system and the major developmental stages and health conditions that occur during life, and the environmental stressors. Chaperones (green and red circles) are the core components of the chaperoning system and play key roles in all cells and tissues, which is denoted by the alpha and omega symbols in the middle. Healthy, functional chaperones (green circles) are essential for development and health as shown in the upper half to the figure. However, in many situations chaperones are abnormal (red circles) because of inheritable mutations or post-translational modifications and can cause disease (as indicated by the red color in the lower half of figure). These diseases caused by defective chaperones are called chaperonopathies, which have a strong impact not only during senescence but also at any age from conception to birth, childhood, and adulthood. These diseases constitute the central topic of this article.

Chaperones play key roles during development and in maintaining protein homeostasis, as well as many others unrelated to protein homeostasis but that also are key to the preservation of health. If one or more chaperones are abnormal, namely if a chaperonopathy occurs, it is possible that pathology will develop, a disease will happen. Likewise, a decline with age in the efficiency of the chaperoning system can contribute to the process of aging in the entire organism, and also allow the initiation of age-related diseases, chronic inflammation, and autoimmunity.

I.b. Stress and disease

Cell stress can be caused by a variety of stressors and the consequences are thought to predispose to disease or to favor its initiation and/or progression (Epel and Lithgow 2014; Tarone and Brancaccio 2014). Organisms have evolved anti-stress mechanisms, among which the chaperoning system is one of the most ancients and widespread, contributing to protein homeostasis and maintenance of the physiological balance. However, chaperones can participate in disease in a way contrary to what is expected of them, namely they can be etiologic-

pathogenic factors by defect, excess, or mistake. These diseases in which one or more abnormal chaperone plays an etiologic-pathogenic role are the chaperonopathies.

I.c. Molecular chaperones pathology

Many authors began to recognize the role played by molecular chaperons in disease in the late XXth century (see references in Welch 1992; Macario 1995). Work continued in an increasing number of laboratories around the world and it became clear that chaperones can be involved in disease as etiologic-pathogenic factors. Abundant evidence for the pathology of molecular chaperones accumulated in the literature and a new field of Medicine slowly took shape. This field has been delineated in the course of the last several years (Slavotinek and Biesecker 2001; Macario and Conway de Macario 2005; Magen et al. 2008; Almeida-Souza et al. 2010; Kakkar et al. 2014; Roos et al. 2014) and was depicted in some detail in a recent book (Macario et al. 2013) and in a dedicated Website (Molecular chaperones pathology). Work continues steadily in many laboratories with a variety of approaches and more cases are continuously detected and reported. New information is made public constantly, and here we intend to present some of it as a brief update. We give preference to work with human materials, in which a direct association between chaperone pathology and disease is depicted, providing mechanistic insights. In general we use the chaperone names used by the authors communicating the chaperonopathies, names that are not always in agreement with the guidelines on chaperone nomenclature (Kampinga el al. 2009), which should be consulted by the reader to avoid confusion.

I.d. Chaperonopathies

The chaperonopathies encompass the pathologic conditions that involve one or more components of the chaperoning system as part of the pathogenic mechanism. The list of diseases that are chaperonopathies and candidates that need further analysis for confirmation is growing steadily. Since this is an emerging field, the list of chaperonopathies is subject to changes and updates. Many of these diseases have been discussed in previous publications (see for instance Macario et al., 2013). Here we deal with other examples not mentioned before.

The field includes a number of apparently unrelated pathological conditions, very diverse, but that share an important feature: they all have the same type of etiologic-pathogenic factor, namely a defective chaperone or co-chaperone. For instance, some diseases that are dissimilar in clinico-pathological signs and symptoms are due to defects in Hsp60 with impact on mitochondrial physiology.

Conversely, many patients may show similar signs and symptoms and may be diagnosed with the same disease, e.g., distal motor neuropathy, but in some of them a chaperonopathy may contribute to the mechanism of disease while not in others. It follows, that the patients should be grouped into two different categories and managed accordingly. In this instance, the chaperonopathy concept can serve as a flashlight that helps in sorting out different entities that otherwise would remain in the dark and taken to be copies of one and the same condition.

There are various classes of chaperones and members of any of these classes can be pathogenic if abnormal in structure-function or apparently normal (considering our current means for studying molecules from clinical specimens) but involved in a pathogenic pathway as seen, for instance, in some types of cancer, Table 1.

The chaperonopathy concept can show relationships between different conditions (patients) that may otherwise be at first invisible. This concept ought to help scientists and clinicians to unveil the etiopathogenesis of apparently very diverse diseases that will, thus, be seen as essentially similar in terms of pathogenic mechanism, or at least in terms of one of the mechanisms leading to pathology. Consequently, these conditions will be approached in research, diagnosis, prevention, and therapeutics in a coherent manner and patient management will improve. The chaperonopathy concept is like a master key useful to open a variety of doors.

The detailed molecular pathogenic mechanisms in which defective chaperones are the protagonists, and that are at the basis of the lesions observed at the tissue and organ levels in patients with chaperonopathies, have for the most part not yet been elucidated. We hope that by realizing that these diseases share physiopathologic features more research on mechanisms at the cellular and molecular levels will be undertaken: it is likely that knowledge on

Table 1. Subpopulations of Hsp-chaperones by molecular weight		
kDa	**Classical family**	**Others causing chaperonopathies**
≥200	None	Sacsin
100-199	Hsp100-110	VCP
81-99	Hsp90	Paraplegin [SPG7]; UNC45B
65-80	Hsp70/DnaK	Spastin [SPG4]
55-64	Hsp60 (chaperonins Groups I and II, e.g., Cpn60 and CCT-BBS	BAG3; immunophilins FKB-type: peptidyl-prolyl cis-trans isomerases (PPI; FKBP5; FKBP10); myocilin; protein disulfide-isomerases (PDI; ER); SERPINH1 (Hsp47)
35-54	Hsp40/DnaJ	AIP; AIPL1; BCS1L; CALR; clusterin; DNAJC19; FOXRED1; melusin; morgana; SIL1; TBCE; torsin A
≤34	sHsp (crystallins)	Alpha Hemoglobin-Stabilizing Protein (AHSP); alpha-synuclein; BCAP31; frataxin; Hsp10 (Cpn10); HSPB11; immunophilin cyclophilin-type: peptidyl-prolyl cis-trans isomerase (PPI; PPIB); LRPAP1; RP2; SOD1-2

one condition will enhance understanding of the others, and will also encourage research on them.

I.e. Protein folding in vitro vs. in vivo

It is increasingly clear that many of the details that we have learned about protein folding using experimental systems in vitro do not reproduce accurately those that actually happen in vivo, under natural conditions (Choi et al. 2013; Hingorani and Gierasch 2014). This situation is not unexpected but it should be emphasized once and again so the reality does not elude us altogether by forgetfulness. Thus, interpretation of the consequences that any given chaperonopathy might have upon protein folding and other cellular functions in vivo must be cautious.

We are beginning to realize that protein folding might occur with or without chaperone assistance, co-translationally, post-translationally very near the ribosome, and in the midst of a myriad of intracellular molecules with possible multiple interactions. It is difficult to ascertain for any given protein all the mechanisms or modes involved in its correct folding, whether these occur co- and/or post-translationally, to what extent chaperones participate, and which chaperones contribute to the whole process in vivo, physiologically. Therefore, the impact of any given chaperone that is defective on the folding of its substrate(s) can vary depending on the folding mode and microenvironment components and conditions.

In addition, defective chaperones may have an impact on a variety of cellular molecules and pathways unrelated to protein folding, since chaperones do have functions other than those pertaining to protein quality control.

In any case, it has to be borne in mind that the chaperoning system closely interacts with the ubiquitin proteasome system (UPS) and with the chaperone-mediated autophagy (CMA) machinery, and also with other body systems, such as the immune system (the latter especially in what pertains to chronic inflammatory and autoimmune conditions) (for reference see Macario et al. 2013). Furthermore, the activities of the chaperoning system overlap with those characteristic of carcinogenesis and senescence, and its components are affected by both. Therefore, it is expected that chaperonopathies will have an impact on the UPS, CMA, the immune system, tumor growth and dissemination, ageing processes, and apoptosis. This prediction is being confirmed by the new data on molecular aspects of chaperonopathies discussed in this review, as it will be seen in the following sections.

II. Structural hereditary chaperonopathies

Chaperonopathies can be classified according to diverse criteria. For example, considering their origin they can be sorted into genetic and acquired. The former are due to mutations while the latter are due to other mechanisms that do not involve genetic defects as, for example, chaperonopathies due to aberrant post-translational modifications. All these have been

Table 2. Illustrative examples of genetic chaperonopathies			
Gene Name / HGNC / ID / UniProtKB/Swiss-Prota	**Synonyms**	**Total aa**	**Chaperonopathies. Mutations**
B-cell receptor-associated protein 31. BCAP31 (BAP31) / 16695 / 10134 / P51572	6C6-AG, BAP31, CDM, DDCH, DXS1357E	246	OMIM: 300398 BCAP31, located in human Xq28. Loss of function mutations *300398 - B-cell receptor-associated protein 31; bcap31. #300475 - deafness, dystonia, and cerebral hypomyelination.
Calreticulin. CALR / 1455 / 811 / P27797	CRT, HEL-S-99n, RO, SSA, cC1qR	417	OMIM: 109091- calreticulin; calr. myeloproliferative neoplasms with nonmutated jak2; essential thrombocythemia or primary myelofibrosis not associated with a jak2 or mpl.
p97 protein/ valosin-containing protein. p97/CVP / 12666 / 7415 / P55072	ALS14, HEL-220, HEL-S-70, IBMPFD, IBMPFD1, TERA, p97	806	OMIM: 601023. #613954 - Amyotrophic lateral sclerosis 14, with or without frontotemporal dementia; als14. #167320 - inclusion body myopathy with early-onset paget disease with or without frontotemporal dementia 1
Low density lipoprotein receptor-related protein associated protein 1. LRPAP1 / 6701 / 4043 / P30533	Alpha-2-macroglobulin receptor-associated protein, RP11-529E10.3, A2MRAP, A2RAP, HBP44	357	OMIM: 601023. #613954 - Amyotrophic lateral sclerosis 14, with or without frontotemporal dementia; als14. #167320 - inclusion body myopathy with early-onset paget disease with or without frontotemporal dementia 1
Protein unc-45 homolog 1. UNC45B protein [Homo sapiens] / 14304 / 146862. / Q8IWX7	(unc-45 homolog B (C. elegans), CMYA4, SMUNC45, UNC45	850	MIM: 611220. UNC45B is a co-chaperone of Hsp90 dedicated to myosin folding (myoblast fusion and sarcomere organization). An UCS domain mutation might cause the development of juvenile cataracts.
HSPB1 / 5246 / 3315 / P04792	HHSP27, HSP28	205	OMIM: 606595.CHARCOT-MARIE-TOOTH DISEASE, NEURONAL, TYPE 2F OMIM: 608634 Neuropathy, distal hereditary motor, type IIB Amyotrophic Lateral Sclerosis (ALS)
DNAJB13. 30718 / 374407 / P59910	TSARG5; TSARG6; RSPH16A	316	OMIM: *610263. Cause primary ciliary dyskinesia and male infertility.
DNAJC13 / 30343 / 23317 / O75165	DnaJ homolog subfamily C member 13, KIAA0678, RME8	2,243	OMIM: 614334. The mutation Asn855Ser segregated with Parkinson's disease with Lewy bodies in members of the affected families.
DNAJC19 / 30528 / 131118 / Q96DA6	DnaJ homolog subfamily C member 19, Mitochondrial import inner membrane translocase subunit TIM14, isoform 1	116	OMIM: 608977. Early onset dilated cardiomyopathy syndrome (DCMA).
HSPA9 / :5244 / 3313 / P38646	Heat shock 70 kDa protein 9; GRP75; GRP-75; HSPA9B; MTHSP75; mortalin	679	OMIM: 616854. Epiphyseal and vertebral dysplasia, microtia, and flat nose, plus associated malformations

[a]HGNC, human genome nomenclature committee; see references in the text.

discussed in some detail previously (Macario et al. 2013) and here we will add some that were not mentioned before. Recent examples of genetic chaperonopathies are displayed in Table 2, and a brief discussion follows.

II.a. Endoplasmic reticulum (ER) chaperones

II.a.1. BCAP31. This is an abundant membrane protein of the ER and acts as a chaperone in processes such as protein degradation (ERAD, i.e., ER-associated degradation), export of proteins into the Golgi, and programmed cell death. Mutations were identified in the gene encoding this chaperone that caused loss of function and the patients affected showed motor and intellectual defects with dystonia, sensorineural deafness accompanied by white matter changes (Cacciagli et al. 2013). Affected cells presented altered ER morphology and Golgi disorganization. The underlying mechanism

seems to be a profound disturbance of the ER-Golgi interaction.

II.a.2. Calreticulin (CALR). Several myeloproliferative malignancies are associated with somatic mutations in the Janus kinase 2 gene (JAK2) and it was found that many of those in which there is no mutation of the JAK2 gene do have mutation in the CALR gene (Klampfl et al. 2013; Nangalia et al. 2013). The manifestations and clinical course of essential thrombocythemia were different in patients with the JAK2 mutations as compared with those with the CALR mutations (Rumi et al. 2014).

II.a.3. Protein p97. This protein is an AAA ATPase (ATPase associated with a variety of cellular activities) that forms hexameric rings and functions as a molecular chaperone in the cell. A polymorphic pathologic condition characterized by the presence of inclusion body myopathy, Paget's disease, and frontotemporal dementia (IBMPFD) has been attributed to single amino-acid mutations of p97. It was found that part of the pathology in IBMPFD caused by some mutations in p97 was the consequence of alteration in ERAD (endoplasmic reticulum associated degradation) (Weihl et al. 2006). More recently, it was found that some of the pathologic mutations disrupt the ability of inter-subunit communication and, thereby, the formation of functional p97 hexamers (Tang and Xia 2013). This would be the mechanism that causes alterations in many tissues leading to the various clinical manifestations of IBMPFD.

II.b. Mitochondrial chaperones

II.b.1. HSPA9. This chaperone is frequently called HSPA9B, or GRP75, or mortalin. A mutation in the gene encoding this chaperone has been found associated with a syndrome that shares some characteristics with the so called cerebral-ocular-dental-auricular-skeletal syndrome (CODAS) but that includes other anomalies, such as severe microtia and nasal hypoplasia, and other malformations (Royer-Bertrand et al. 2015). For this reason the syndrome has been dubbed EVEN-PLUS syndrome for epiphyseal, vertebral, ear, nose, plus associated findings.

Quantitative changes of HSPA9B (e.g., decreased in affected cells) have been found in certain cases of Parkinson's disease (PD), and qualitative changes (i.e., mutations) have been identified that were associated with some types of PD, showing mitochondrial functional defects (see references in Macario et al., 2013). However, little is known on the molecular mechanisms responsible for the chaperone abnormalities observed in PD. In a recent work, it was investigated the effects of reduced levels of mortalin on mitochondrial quality control, focusing on its interaction with Parkin and PINK1, two PD-related proteins pertinent to mitochondrial homeostasis (Burbulla et al. 2014). The observations reported suggest an increase in the vulnerability of cells with low levels of mortalin to apoptosis, and shed some light on molecular pathways that might participate in its pathogenic role in PD. A reduction of mortalin was accompanied by unfolded protein response in mitochondria, proteolytic stress, autophagic degradation of mitochondrial fragments, and overall decrease in mitochondrial mass. Interestingly, these pathologic alterations were corrected by complementation with wild-type (normal) mortalin but not by mortalin variants known to be associated with PD. Pathological mortalin phenotypes were rescued by Parkin and PINK1 with direct participation of lysosome-mediated mitochondrial clearance and of autophagic mechanisms. Clearly, all these observations considered together indicate that there is a physiopathogenic link between mortalin, or its pathogenic variants, with the unfolded protein response in mitochondria and the autophagic machinery, and that this link is determinant in the pathogenesis of PD. Most importantly, the results offer stimulus to investigate therapeutic strategies based on the removal of pathologic, malfunctioning, mitochondria, using PINK1 and Parkin, or chemical compounds that might correct, at least partially, mitochondrial dysfunction via these two molecules and mortalin.

II.b.2. DNAJC19. A few years ago it was reported that a mutation in the mitochondrial co-chaperone DNAJ19 is associated with an autosomal recessive condition similar to the Barth syndrome (Davey et al. 2006). This condition named dilated cardiomyopathy with ataxia (DCMA) syndrome is characterized by early onset dilated cardiomyopathy with conduction defects, non-progressive cerebellar ataxia, testicular dysgenesis, growth failure, and 3-methylglutaconic aciduria. Some of these characteristics are also observed in the Barth

syndrome, also known as 3-Methylglutaconic aciduria type II, which is an X-linked genetic disorder occurring only in males and affecting various organs and systems. More recently, another yet undescribed mutation was discovered that was associated with a similar syndrome, including dilated cardiomyopathy, anemia, ataxia, and male genital anomalies (Ojala et al. 2012). No information was provided regarding the molecular mechanisms causing the observed tissue lesions and clinico-pathological findings. However, since a deficiency of DNAJC19 is manifested in methylglutaconic aciduria type V, which is quite characteristic, the report alerts to the possibility of early diagnosis via perinatal, or early childhood screening, before cardiac symptoms are manifest and before resorting to laborious and time-consuming molecular genetic analyses.

II.c. DNAJB6

Mutations in the HSP40 chaperone DNAJB6 causes limb-girdle muscular dystrophy type 1D (LGMD1D) (Sarparanta et al. 2012). These pathogenic mutations localize to the G/F domain, whose functions are not well understood. In a recent work, it was shown that LGMD1D mutations in a yeast model disrupt the processing of specific conformers of two yeast prions, [RNQ+] and [PSI+] and the processing of the nuclear TDP-43 stress granules in mammalian cells (TDP-43 is a protein that forms nuclear inclusions in LGMD1D) (Stein et al. 2012). These new data indicate that the G/F domain is crucial for chaperone-substrate interaction, not only for the recognition of a specific client, but also for interacting with various client conformers. It was proposed that disruption of the G/F domain by the pathogenic mutations leads to aggregation of conformers, which are toxic and at the basis of the pathogenic mechanism underlying development of LGMD1D.

II.d. DNAJB13

A mutation in DNAJB13 was found associated with primary ciliary dyskinesia (PCD) and male infertility (El Khouri et al., 2016). PCD is an autosomal-recessive disease characterized by functional and structural defects of motile cilia that, clinically, is manifested as recurrent respiratory-tract infections that, in males, are frequently accompanied by infertility due to sperm flagellar dysfunction. Most genes identified that are associated with PCD code for components of dynein arms or for proteins involved in dynein assembly. This new publication now adds DNAJB13 to the list of genes that can cause, if mutated, PCD. This is another example of very similar abnormal phenotypes, all due to gene mutations, of which only a part are chaperonopathies, namely caused by a mutation in a chaperone gene. Many patients show very similar signs and symptoms but only some of them are chaperonopathies, which would never be detected if the clinicians do not have in mind the chaperonopathy concept (see earlier, Section I.d. Chaperonopathies).

II.e. DNAJC13

A mutation in the gene encoding DNAJC13, which belongs to the family of Hsp40/Dnaj co-chaperones, was found to be associated with familial Parkinson's disease and Lewy body pathology (Vilariño-Güell et al. 2014). This protein, also called 8/RME-8 (for receptor-mediated endocytosis 8) regulates clathrin coating of early endosomes, was found to bear the mutation Asn855Ser that segregated with the disease in various members of the affected families. The mutation resulted in gain of function, impairing endosomal transport. This finding is important since it connects various aspects of vesicle trafficking and degradation pathways with the genetics of the parkinsonism spectrum associated with Lewy body pathology.

II.f. Hsp27 (HSPB1)

Many among all the hereditary motor neuropathies grouped under the name Charcot-Marie-Tooth disease are caused by mutations in the small heat-shock protein Hsp27 (HSPB1) (for review see Macario et al. 2013). In recent work it was investigated the impact of the pathogenic mutations (T164A, T180I, P182S, and R188W) on the quaternary structure and biophysical and functional properties of the chaperone (Chalova et al. 2014). The methods used were fluorescence spectroscopy, dynamic light scattering, size-exclusion chromatography, and measurement of chaperone-like activity. The main observations were: a) the T164A mutation caused destabilization of the quaternary structure and decrease of thermal stability but did not affect chaperoning activity; b) P182S was accompanied by large oligomers of 1,000 kDa or larger. These oligomers had very low thermal stability; and c) R188W

caused a considerable decrease in chaperoning activity but no detectable changes in quaternary structure or thermal stability. Noteworthy is that although all the mutations studied are associated with Charcot-Marie-Tooth disease type 2, they do not have the same impact on the structure and function of the affected chaperone, HSPB1. These observations emphasize once again the need for a detailed differential diagnosis in diseases that might be caused by defective chaperones. Another example of this situation has recently been reported in which HSPB1 mutations were associated with Amyotrophic Lateral Sclerosis (ALS) in some patients but not in others (Capponi et al., 2016). In a large population (247 unrelated Italian patients) two variants were found (Gln190His and Ala204Glyfs*6). The mutant HSPB1 protein carrying the latter variant caused an alteration of the molecular dynamic equilibrium, in turn causing the sequestration of the wild type HSPB1 in a stable dimer with loss of chaperoning capacity. The same clinical disease in a variety of patients does not always have the same mechanism: in some cases there is a chaperonopathy that distinguishes them from the rest. Therefore, a precise diagnosis of pathogenic mechanism is mandatory, including the search for a mutant chaperone, if a proper treatment is to be implemented. If it is established that a defective chaperone is part of the pathogenesis and it is known that different mutations of the same gene can cause disease, the differential diagnosis must proceed further to elucidate precisely which mutation is present in any given patient: patient and family management and treatment might differ considerably between the various mutations.

II.g. CCT

Two recent publications describe alterations in the CCT subunit 5 molecule carrying a mutation that is pathogenic in humans. CCT5 mutant was reported to be less efficient in chaperoning activity with regard to γD-crystallin, used as a model substrate, and in suppressing aggregation of mutant huntingtin than the wild type counterpart (Sergeeva et al. 2014). Another work carried out with an archaeal model established that the pathogenic mutation impairs ATPase and various chaperoning activities, response to temperature changes, and capacity to dissolve protein aggregates (Min et al. 2014). Detailed analysis of purified preparations showed that most if not all these deficiencies of the mutant were due to impaired formation of the hexadecameric functional chaperonin complex.

II.h. Prefoldin

To our knowledge there are no reports of human diseases associated with mutations of prefoldin. However, it is probable that these diseases do occur but have not yet been identified. We hope that as physicians become familiar with the chaperonopathies they will look for them and make proper diagnoses, including conditions due to prefoldin abnormalities, which in turn will lead to a search for efficacious treatments.

That mutations in prefoldin may cause disease in humans can be predicted from research done in mice. It was found that mice with a mutation in the subunit one (Pfdn1) showed phenotypes reflecting cytoskeletal defect, such as ciliary dyskinesia, neuronal loss, and B and T cell developmental and functional failure (Cao et al. 2008). Later, it was reported that a mutation in subunit 5 (Pfdn5) causes photoreceptor degeneration, central nervous system abnormalities, and male infertility (Lee et al. 2011). It is now necessary, as for many other chaperonopathies, to elucidate the cellular and molecular mechanisms responsible for the observed abnormalities at the tissue and organ levels. These mechanistic studies will hopefully be encouraged when it is realized that all these diseases, involving defective chaperones of one sort or another, form a coherent group and share many features so that when the mechanism is elucidated for one the knowledge will illuminate the search on others.

II.i. Dedicated chaperones

II.i.1. LRPAP1 mutation and myopia. The protein LRP1, encoded in the gene LRPAP1 (low density lipoprotein receptor-related protein associated protein 1), is considered to be a chaperone for TGF-β (transforming growth factor β), which plays roles in various important physiological functions such as cell proliferation and differentiation and in pathologies, including cancer, heart disease, diabetes, hereditary hemorrhagic telangiectasia, Marfan and Ehlers-Danlos and Loeys–Dietz syndromes, Parkinson's disease, and other abnormal conditions. For example, it was reported that truncated variants of the LRPAP1 gene are associated with myopia, which parallels the high frequency of this eye abnormality

Table 3. Human chaperone-gene polymorphisms: Illustrative examples	
Genetic polymorphism[a]	**Abnormality / disease**
Hsp60 (HPD1) (rs72466451)	Increased in frequency in a subgroup of sudden infant death syndrome.
FK506-binding protein 51 (FKBP5)	T allele of rs1360780 was more frequent in patients with both, major depressive disorder (MDD) anxiety disorder than in patients with MDD only.
Hsp70 (HSPA1A)	The frequency of +190CC genotype and +190C allele was increased in patients with schizophrenia. These traits represent risk of developing paranoid schizophrenia.
Hsp70 (HSPA1B, HSPA1L; and HSPA1)	HSPA1B (rs1061581), HSPA1L (rs2227956) and HSPA1 (rs1043618) polymorphisms are associated with a decreased risk of idiopathic pulmonary fibrosis.

[a]See references in the text.

observed in Marfan sydrome (Aldahmesh et al. 2013).

II.i.2. UNC45B mutation and juvenile cataracts. UNC45B is a co-chaperone of Hsp90 dedicated to the proper folding of myosin, including myoblast fusion and sarcomere organization. A mutation in the UCS domain is suspected to be involved in the development of juvenile cataracts (Hansen et al. 2014).

III. Genetic polymorphism

Some recently published examples of the association of polymorphism of chaperone genes with pathological conditions are displayed in Table 3. This is an area in which the search for molecular mechanisms is much needed. The information available is scarce and precisely for this reason we dedicate space to these associations: the aim is to direct the attention of scientists in basic and clinical research to a field full of promises in need of exploration.

III.a. Polymorphism of Hsp60. Chaperonopathies in pediatrics. A significant increase in the frequency of the Hsp60 single-nucleotide variant (rs72466451) was found in a subgroup (4.5%) of sudden infant death syndrome (SIDS) (Courts et al. 2013).

III.b. Polymorphism of FK506-binding protein 51 (FKBP5) gene in anxiety disorders.

Chaperonopathies in psychiatry. Major depressive disorder (MDD) is usually accompanied by anxiety disorder. Since both pathologies are related to psychological stress, which may act as a determinant factor, it is likely that molecules and pathways pertinent to the stress response participate in pathogenesis. An indication that it might be so, is the finding that a genetic variant (T allele of rs1360780) of the chaperone gene FK506-binding protein 51 (FKBP5) was found to be present in patients with both, MDD and anxiety disorder, at higher frequency than in patients with only MDD (Minelli et al. 2013). Furthermore, among the controls the T allele occurred more frequently in persons with personality characteristics reflecting an increased vulnerability to anxiety.

III.c. Polymorphism of Hsp70 gene (HSPA1A) in paranoid schizophrenia.

Chaperonopathies in psychiatry. The search for genetic markers that might help in elucidating pathogenic mechanisms of psychiatric disorders, and become useful in diagnostics and as therapeutic targets has been active for years with some promising results. One of the latest examples of this research established that for the gene Hsp70 (HSPA1A), the +190CC genotype and +190C allele were over-represented in patients with paranoid schizophrenia and significantly increased the risk for developing schizophrenia in females more than in males (Kowalczyk et al. 2014). This kind of data is encouraging in general in what concerns the search for chaperonopathies in psychiatric disorders and, in particular, because it suggests that certain Hsp70 polymorphisms could be associated with increased risk for developing paranoid schizophrenia.

III.d. Polymorphism of Hsp70 genes (HSPA1B, HSPA1L; and HSPA1) in idiophatic pulmonary fibrosis. HSPA1B (rs1061581), HSPA1L (rs2227956) and HSPA1 (rs1043618) polymorphisms are associated with a decreased risk of idiopathic pulmonary fibrosis (Aquino-Gálvez et al., 2015).

syndromes. Many of these conditions were considered previously (Macario et al. 2013) but new cases were reported afterwards, and illustrative examples of these new cases, pertaining to cancer, are listed in Table 4, and briefly discussed below.

Table 4. Illustrative examples of chaperonopathies by mistake: Cancer

Hsp-chaperone[a]	Increased and tumorigenic in:	Tumorigenic effect
Hsp27 (HSPB1)	Prostate cancer	Required for IL-6-mediated EMT (Epithelial to Mesenchymal Transition) and metastasization.
αB-crystallin	Head and neck squamous cell carcinoma (HNSCC)	Stimulates vascular endothelial growth factor (VEGF) secretion and is associated with distant metastases formation.
Calreticulin (ER chaperone)	Adrenocortical carcinomas (ACC)	Calreticulin increase, assessed by immunohistochemistry in tumor tissue, was significantly associated with tumor progression.
Grp78	Gastric cancer	Favors tumor cell proliferation, local tissue infiltration. and lymph node metastasization
TRAP1 (TNF receptor-associated protein 1). Other names: HSP 75, HSP75, HSP90L, TRAP-1. (Mitochondrial chaperone)	Various cancers	Binds and inhibits succinate dehydrogenase. It favors the succinate-dependent stabilization of the proneoplastic transcription factor HIF1α.
Co-chaperone small glutamine-rich TPR-containing protein alpha (SGTA)	Prostate cancer	SGTA interacts with the androgen receptor (AR) and other cancer-related proteins favoring tumor cell proliferation.
CDC37 (co-chaperone for Hsp90)	Hepatocellullar carcinoma	Overexpressed in the tumor tissue, favoring tumor cell proliferation and invasiveness and counteracting apoptosis of the tumor cells.
CCT (TRiC) complex	Acute myeloid leukemia (AML)	Oncoprotein AML1-ETO maturation is enhanced by CCT.
CCT2 and TCP1 (CCT1)	Breast cancer	Expression of the *cct2* and *tcp1* genes is essential for breast cancer cell growth.

[a]See references in the text.

IV. Chaperonopathies by mistake

Chaperonopathies by mistake are those in which a chaperone is normal, or apparently normal according to the methods currently available to study molecules in vivo, but its activity favors initiation and/or progression of a pathologic condition, for instance certain types of cancer and chronic inflammatory and autoimmune

IV.A. Cancer

IV.A.a. Hsp90 and tumor growth and angiogenesis. Hsp90 has been dubbed the oncogenic chaperone considering its active participation in tumor growth and dissemination. Negative chaperonotherapy, consisting of blocking Hsp90 pro-tumor activities, has been tested in a variety of experimental systems using Hsp90-blocking compounds as anti-cancer

treatment, and some of these compounds are ready for advanced phases of clinical trials. However, there are still several aspects of the Hsp90 participation in carcinogenesis that are not fully understood. In this regard, it has been found that Hsp90 binds and stabilizes the kinase PRKD2, which is a key factor in the process of tumor cell-endothelial cell communication in some types of intestinal and nervous system tumors (Azoitei et al. 2014). Inhibition of Hsp90 with a range of chemical compounds of diverse structures elicited proteasome-dependent degradation of PRKD2 and lead to an increase of apoptosis of cancer cells. Also, it was found that hypoxia-induced accumulation of HIF-1α and activation of NF-κB in tumor cells depend on the presence of functional PRKD2. The levels of HIF-1α and secreted VEGF-A were restored by expression of the kinase in hypoxic cancer cells treated with Hsp90-blocking compounds. The data indicate that hypoxia and Hsp90 are actively associated with carcinogenesis and interconnected at the level of PRKD2 to modulate NF-κB/ VEGF-A, and thus promote angiogenesis and growth of tumor cells.

IV.A.b. Hsp27 (HSPB1) in epithelial-mesenchymal transition. The mechanism of metastasization is central to cancer disease and has been the subject of many studies but there still remain key aspects that are not well understood. One of these facets of cancer dissemination that is incompletely understood is epithelial-to-mesenchymal transition (EMT). A progress in this direction is exemplified by data showing that the chaperone Hsp27 (HSPB1) is important for EMT in prostate cancer (Shiota et al. 2013). Presence of Hsp27 favored EMT whereas a reduction of the chaperone decreased cell migration, tissue invasion, and the activity of matrix metalloproteinase. It would appear that Hsp27 is necessary for a series of molecular interactions that precede and induce EMT, including IL-6- dependent STAT3 phosphorylation, nuclear translocation, and STAT3 binding to the Twist promoter.

IV.A.c. VEGF stimulation. It is well established that the vascular endothelial growth factor (VEGF) plays a central role in tumor growth and dissemination. For instance, new data implicate αB-crystallin in promoting distant metastases in head and neck squamous cell carcinoma (van de Schootbrugge et al. 2013). It was suggested that this pro-tumorigenic effect of αB-crystallin can be attributed to its chaperoning of VEGF so it folds correctly and is subsequently secreted.

IV.A.d. ER chaperones

IV.A.d.1. Calreticulin. Mutations in the CALR gene and malignancies. The association of CALR gene mutation and myeloproliferative disorders was discussed earlier (see II. A. Calreticulin, and Table 2). Here, we add information implicating CALR in adrenocortical carcinomas (ACC). Comparative proteomics showed 20 up-regulated and 9 down-regulated proteins in ACC tissues compared with paired normal controls (Yang et al. 2013). Calreticulin was one of the overexpressed proteins and its levels paralleled ACC stages; the more advanced the ACC the higher the levels of calreticulin.

IV.A.d.2. Grp78. Grp78 overexpression helps tumors cells to proliferate. The levels of this chaperone were determined by immunohistochemistry in gastric tissue of 160 patients with gastric cancer who underwent D2 radical gastrectomy and adjuvant chemotherapy (Yang et al. 2014b). A detailed evaluation of Grp78 levels was carried out, considering various parameters: age and sex of the patients; disease stage; degree of tumor-cell differentiation; depth of tumor penetration in the gastric wall; presence of metastasis in lymph nodes; and time to recurrence. The observations reported provide insights into how Grp78 "mistakenly" helps the disease rather than the patient. Grp78 was elevated specially in tumors from patients with deep infiltration, poor differentiation, and lymph node metastasis. Also, Grp78 levels were clearly increased at late disease stages, and in patients showing shorter times of recurrence. Furthermore, it was observed that Grp78 expression favored cell proliferation, since down regulation of the chaperone resulted in arrest of gastric cancer cells in G1 phase. Contrariwise, overexpression of the chaperone stimulated cell-cycle progression.

IV.A.e. Mitochondrial chaperones

IV.A.e.1. TRAP1 (the correct name HSPC5; other names used are HSP90L and HSP75). Involvement of TRAP1 in tumorigenesis has been studied in various laboratories but the mechanism or mechanisms by which this

mitochondrial chaperone favors tumor growth have not been completely elucidated. In this regard, some progress was lately achieved as it was found that the chaperone bind succinate dehydrogenase and inhibits it (Sciacovelli et al. 2013). This effect of TRAP1 on the succinate dehydrogenase enzyme causes respiratory chain downregulation, which in turn would favor tumorigenesis by priming the succinate-dependent stabilization of the proneoplastic transcription factor HIF1α independently of hypoxic conditions.

IV.A.f. Co-chaperones

IV.A.f.1. SGTA. It is well established that a variety of solid cancers require the chaperones Hsp70 and Hsp90 for growth. It is then pertinent to wonder about the role in tumorigenesis of co-chaperones relevant to Hsp70 and Hsp90 such as those bearing the tetratricopeptide repeat (TPR). One candidate in prostate cancer (PCa) is small glutamine-rich TPR-containing protein alpha (SGTA) that interacts with the androgen receptor (AR) and with other cancer-relevant molecules. Knockdown of SGTA lowered expression of many genes in PCa cells and restricted their proliferation (Trotta et al. 2013). In addition, the regulation of one third of 5α-dihydrotestosterone target genes was affected by SGTA knockdown. The ratio AR:SGTA was significantly increased in PCa as compared with matched benign prostatic hyperplasia tissue, by immunohistochemistry. Thus it became clear that SGTA affects the genome-wide AR transcriptional activity and signaling pathways, which in the absence of SGTA turn toward a tumorigenic mode.

IV.A.f.2. CDC37. The protein CDC37 (cell division cycle 37) interacts with Hsp90 and also with various protein kinases such as CDK4, CDK6, SRC, RAF1, and MOK, and with elF-2 alpha kinases. CDC37 acts as a co-chaperone for Hsp90 directing it to its target kinases. Because of its known functions and interactors it is assumed that CDC37 is very useful to tumor cells, representing an example of a normal but "mistaken" co-chaperone that is involved in pathways favorable to disease rather than the contrary. In a recent study, the anti-tumor effects of CDC37 inhibition were investigated in human hepatocellular carcinoma (HCC) (Wang et al. 2014). The study was carried in 91 patients by measuring CDC37 mRNA using real-time PCR, and the effects of silencing CD37 by RNA interference on cell proliferation, gene expression levels, and tumorigenicity were measured, using the hepatoma cell lines HepG2 and Huh7. CDD37 was overexpressed at the transcript and protein levels in HBV-associated HCC patients. Inhibition of in vitro cell proliferation with cell cycle arrest at the G1 phase and apoptosis stimulation resulted from silencing the expression of CDC37 in the cell lines. Several genes known to be involved in cell proliferation, cell cycle progression, and apoptosis and to be regulated by CDC37 were found downregulated. Furthermore, the in vitro colony-forming and the in vivo xenograft growth abilities of Huh7 cells with Cdc37 gene knockdown were significantly decreased. These data help understand the mechanisms involved in the pro-carcinogenic properties of Hsp90 and its associated co-chaperone and, consequently, open new roads for devising chaperonotherapy strategies targeting the chaperone and/or its co-chaperone in those cancers which, like HCC, depend heavily for growth and progression on these two molecules.

IV.A.g. CCT

The fusion oncoprotein AML1-ETO is determinant in the etiology of acute myeloid leukemia (AML) by altering the expression of genes necessary for myeloid cells to differentiate. It was found that to achieve its active configuration AML1-ETO requires the assistance of the CCT complex and the participation of Hsp70 (Roh et al., 2015).

In breast cancer as in many other malignancies gene amplification and overexpression drive carcinogenesis. Among the genes found altered in breast cancer those encoding the CCT subunits TCP1 (CCT1) and CCT2 were identified (Guest et al., 2015). These genes were necessary for tumor cell growth in vitro.

These findings point to the CCT chaperone as a collaborator with cancer and, therefore, its subunits can be considered possible targets for negative chaperonotherapy (see later the section on chaperonotherapy).

IV.B. Viral infection

IV.B.a. CCT and rabies. Viruses are known to take over host-cell mechanisms for their own benefit, and molecular chaperones do no escape this alienation. In a mouse model, it was demonstrated that a neurotropic rabies virus (RABV) altered expression of over 20 host-cell proteins; the great majority of them were upregulated (Zhang

et al. 2013). One of the latter was CCTγ, which colocalized with the viral proteins N and P and formed Negri bodies in the nucleus. Knockdown of CCTγ resulted in a significant inhibition of viral replication. In another report, the role of CCTα in virus replication was described (Zhang et al. 2014). Thus rabies can be considered a chaperonopathy by mistake or collaborationism, in as much as a normal chaperone works in favor of a pathogen rather than protecting its own cell against the invading enemy. This concept, in turn, suggests that a therapeutic strategy for rabies could be directed to inhibit the CCT chaperone in infected cells.

IV.B.b. Hsp90 and enterovirus 71 (EV71). Another example of chaperonopathy by collaborationism was recently reported, pertaining to the role of Hsp90 in the assembly of virus particles (Wang et al. 2013b). It was found that Hsp90β (but not Hsp90α) specifically associated with EV71 virus particles. Furthermore, by reducing expression of Hsp90β it was observed a decrease in particle formation.

IV.B.c. Hsp70 and Crimean-Congo hemorrhagic fever virus (CCHFV). The Bunyaviridae family includes the Nairovirus genus, which comprises serious human and animal pathogens of various species and serogroups. To one of these serogroups, the Crimean-Congo hemorrhagic fever virus (CCHFV) serogroup, belong the CCHFV and Hazara viruses (HAZV). The former is a zoonotic agent that causes hemorrhagic fever in humans with high mortality rates whereas HAZV is nonpathogenic and can be used as model to understand the biology and pathogenicity of CCHFV. It was demonstrated that the viral nucleocapsid protein (N) forms a complex with HSP70 (Surtees et al. 2016). Interference with the formation of the N-HSP70 complex by reducing the active intracellular HSP70 using HSP70 inhibitors produced a decrease in virus titers. This is another example of chaperonopathy by mistake in which a human chaperone works for the enemy, a deadly virus in this case. Furthermore, the work also illustrates the potential of negative chaperonotherapy with chaperone inhibitors to treat fatal diseases in which chaperones are essential for survival of the infectious agent.

IV.C. Bacterial toxins

IV.C.a. CCT and bacterial toxins. Bacterial toxins can gain the intracellular environment by various mechanisms, some of which include recruitment of host cells molecules. An example of this mechanism, involving chaperones, was recently reported (Slater et al. 2013). The CCT complex was necessary for the intracellular delivery of anthrax lethal factor. The toxin was active after its translocation into the cell through the plasma membrane with the assistance of the CCT complex, which assisted in the translocation and in maintaining the functional conformation of the toxin and, thus, preserving its toxic potency throughout the entire process. A therapeutic strategy can be envisioned of the negative chaperonotherapy type, in which anti-CCT agents could be devised to block the assistance provided by this chaperone to the toxin, for instance agents that would block its translocation into the cell.

IV.D. Autoimmunity

The role of chaperones in the development of autoimmune conditions has been investigated for many years, particularly for Hsp60 (Cappello et al., 2009; 2010; Marino Gammazza et al., 2012; 2014; Juwono and Martinus, 2016).

IV.D.a. Hsp60 in Hashimoto's thyroiditis. We recently reported data from studies of serum and tissue samples from patients with Hashimoto's thyroiditis which suggest that Hsp60 plays a role as autoantigen in the pathogenesis of this autoimmune condition (Marino Gammazza et al. 2014). Hsp60 was elevated in pathologic thyroid tissue, and present in thyrocytes and also in oncocytic metaplasia cells, with the latter cells showing the chaperonin not only inside but also in the plasma membrane. This would facilitate antigen (Hsp60)-antibody reaction on the cell surface and cell damage or lysis, with the antibodies being of various origins.

For example, several amino-acid sequence segments were identified that were very similar in Hsp60, thyroglobulin (TG), and thyroid peroxidase (TPO); these similar segments could very well serve as crossreactive epitopes for the three proteins when confronted with antibodies made against any of them.

Other findings were a tendency to high levels of circulating Hsp60 in patients and the antibodies against TG and TPO cross-reacted with human

Hsp60. Taken together all the observations indicated that Hsp60 may be involved as autoantigen with a role in the initiation and/or perpetuation of Hashimoto's thyroiditis.

IV.D.b. Chronic inflammatory bowel diseases, chaperones, and methanogens. The question of whether the archaeal organisms (methanogens) inhabiting the human body play a pathogenic role has been in the minds of researchers for a long time. The answer has always been negative: methanogens do not seem to play a direct role in pathogenesis. However, over the last several years it has become evident that methanogens in humans can play an indirect role in pathogenesis. How? One way is by favoring the growth of pathogenic organisms (bacteria) with which they share human ecosystems, for example, the large bowel, vagina, and periodontal space (Conway de Macario and Macario, 2009). More recently, it has been observed that human methanogens have an impact on the immune system (Bang et al., 2014; Bang and Schmitz, 2015). All these findings suggest that a comprehensive strategy should be implemented to elucidate the syntrophic associations that are essential for a healthy relation among microbes (including methanogens) and between them and the host organism (including its immune and chaperoning systems, see Figure 2), and to unveil those associations that lead to disease.

We proposed that chaperones, particularly Hsp60, play a pathogenic role in inflammatory bowel diseases, for example ulcerative colitis (UC), and, therefore, should be given protagonism along with the microbiota and probiotics in future studies and in patient management (Bellavia et al. 2013). Hsp60 is very abundant in the affected intestinal mucosa with quantities increasing in parallel with severity of disease and decreasing to normal levels in remission induced by treatment. Therefore, UC could be an example of chaperonopathy by mistake in which Hsp60 acts as a pathogenic autoantigen and/or stimulator of secretion of pro-inflammatory cytokines.

Another avenue toward clarifying the association of human methanogens and disease is that suggested by the occurrence of antibodies cross-reactive with human and archaeal chaperones. For instance, antibodies were found in sera of humans harboring in the periodontal space the methanogenic archaeon *Methanobrevibacter oralis*, and showing signs and symptoms of periodontitis and autoimmunity; these antibodies reacted against human and archaeal CCT subunits (Hirai et al. 2013). Antibodies from patients with periodontitis had antibodies predominantly against CCT3 and CCT4 subunits, whereas antibodies from patients with autoimmune syndromes reacted preferentially with CCT3 and CCT8. These findings do not demonstrate a role of archaeal chaperones as antigens in the development of disease in humans, but they send an alert signal to physicians and other health professionals in research and practice. Archaeal antigens, particularly chaperones that can invade the human organism from the intestine, vagina, or periodontal space, could very well elicit antibodies crossreactive with human molecules and, thereby, favor the development of autoimmune syndromes. This would be comparable to what happens with similar molecules, i.e., chaperones, from pathogenic and non-pathogenic bacteria that inhabit the same human ecosystems as the methanogens.

IV.D.c. Hsp47 and Crohn's disease. Hsp47 is a chaperone dedicated to collagen folding and assembly that has been implicated in some pathological conditions in which collagen is affected. This view has gained further support stemming from new data on the participation of Hsp47 induced by interleukin (IL)-17A in the intestinal fibrosis observed in Crohn's disease (Honzawa et al. 2014). The levels of expression of the IL-17A and Hsp47 genes were elevated in the intestinal tissue of patients. Hsp47 was present in α-smooth muscle actin-positive cells as determined by immunohistochemistry, with the number of Hsp47-positive cells being considerably increased in the pathologic intestinal tissue. It was suggested that this association between elevated Hsp47 levels and pathological fibrosis indicates that the chaperone might be implicated in the generation of excess intestinal fibrosis in Crohn's disease. This would be another example of chaperonopathy by mistake, in which a normal chaperone contributes significantly to the pathogenic mechanism of a human disease.

IV.D.d. Hsp60 in schizophrenic psychosis. The risk of developing schizophrenic psychosis is increased in children born to mothers with gonoccocal infection. While there is some disagreement on this concept, a new study, seems to support it (Reuss and Asif 2014). A rabbit antiserum against *Neisseria gonorrhoeae*

(α-NG) was found to recognize Hsp60, and this anti-α-NG antiserum, also reactive with human Hsp60, reduced neurite growth in an in vitro system involving human NTera2-D1 cells. These findings are intriguing and, once again, point toward participation of Hsp60 as an autoantigen because of its sharing of epitopes with other classes of human molecules that can be recognized by antibodies originally elicited by any of the crossreactive molecules: one class of molecule, for example the bacterial Hsp60, is the initial immunogen but the resulting antibodies react also with the other classes of molecules (not necessarily chaperones), leading to disease. No doubt this is a very attractive research field, in view of the fact that it can provide mechanistic information on chaperonopathies in psychiatric pathology, which would benefit from molecular information necessary to develop treatment agents.

IV.D.e. CCT6A and autoimmunity in systemic lupus erythematosus (SLE)

Renal proximal tubular epithelial cell line HK-2 were found to express CCT6A on their surface and it was involved in the binding of synthesized SL2 peptides (Chen et al., 2016). Anti-CCT6A antibody blocked the cytotoxicity on HK-2 cells mediated by Vδ2 γδ T cells. CCT6A concentration was elevated in plasma of SLE patients. The results implicate CCT6A as autoantigen recognized by Vδ2 γδ T cells and, consequently, makes SLE a candidate to be included in the list of pathologic conditions in which a chaperonopathy by mistake contributes to the mechanism of disease.

V. Acquired chaperonopathies: Post-translational modifications of chaperone molecules that can contribute to disease

V.a. Hsp70. A methyltransferase METTL21A was identified that trimethylates lysine residues present in members of the human Hsp70 (HSPA) family (Jakobsson et al. 2013). The enzyme, named HSPA lysine (K) methyltransferase (HSPA-KMT), was found to function in vitro and in vivo and to methylate exclusively 70 kDa proteins in mammalian protein extracts. Trimethylation of Hsc70 (HSPA8) by this enzyme had an impact on the properties of the chaperone: it modified its affinity for monomeric and fibrillar forms of α-synuclein.

V.b. PDI. Aggregation of misfolded proteins with intracellular body formation occurs in familial amyotrophic lateral sclerosis (FALS) associated with mutant superoxide dismutase (mSOD1). These protein aggregates could be implicated in the neuronal cell death observed in FALS. An anti-aggregation mechanism involves the chaperone protein disulfide isomerase (PDI). It was observed that S-nitrosylation of PDI inhibited its activity as indicated by increased mSOD1 aggregation and neuronal cell death (Jeon et al. 2014). Similarly, it was suggested that abnormal S-nitrosylation of PDI contributes to cellular and tissue pathology in neurodegenerative conditions (Halloran et al. 2013). Both reports re-emphasize the importance of studying post-translational modification of chaperones in health and disease: it is likely that the various destinations and functions of any given chaperone depend on post-translational modifications, and that when the modification is abnormal pathology can ensue.

VI. Chaperonopathies in which the chaperone is normal but its interactor is not: A comment on chaperone-mediated autophagy (CMA) defective due to LAMP2 mutation

Some chaperonopathies (chaperone failure) are caused by mutation of substrates that because of the mutation suffer modifications of their structures and can no longer be recognized by the pertinent, normal chaperones (Macario et al. 2013). Likewise, mutations in molecules that interact physiologically with chaperones, for instance, in the formation of chaperoning teams and networks, may result in structural changes that impede interaction. In these instances, the chaperoning team or machine does no assemble correctly and/or interaction between teams, i.e., networking, does not occur. A similar situation may explain some cases of failure of CMA, if the interaction of Hsc70 (HAPA8) with its receptor on the lysosome, LAMP-2 (Lysosomal-associated membrane protein 2), does not occur because of a mutation of the latter. This is a possibility that ought to be investigated in pathologies attributed to mutations in LAMP-2, such as Danon's disease (Fidzianska et al. 2013).

VII. Epigenetics and chaperonopathies

VII.a. Epigenetic regulation of Hsp-chaperone genes in cancer: Hsp27 (HSPB1). Epigenetic mechanisms are likely to regulate chaperones and to play a role in many chaperonopathies

(Macario et al. 2013). One more example of participation of epigenetics in chaperonopathies by mistake or collaborationism has recently been published (Wang et al. 2013a). The expression and methylation of the Hsp27 (HSPB1) gene was studied in samples of oral squamous cell carcinoma. Expression was weak and the promoter was hypermethylated in tumor cells and the situation was reversed by a DNA methyltransferase inhibitor. This and other similar examples point to the tumorigenic role of promoter methylation of anti-cancer genes, some of which could be chaperone genes. The findings also emphasize the potential role of chaperonopathies related to epigenetic mechanisms in cancer and, by extension, to other pathological conditions.

VIII. Chaperonotherapy

Chaperonotherapy consists of using chaperones as therapeutic agents or targets. This form of therapeutics encompasses at least three modalities, positive, negative, and combined. The former includes replacement of a defective chaperone using gene therapy procedures or administering the chaperone protein. Negative chaperonotherapy involves the suppression of a chaperone gene or inhibition of the activity of a chaperone protein when it plays a pathogenic role (chaperonopathies by mistake or collaborationism) and its action must be stopped, for example in certain cancers that need chaperones for growth. Lastly, combined chaperonotherapy is characterized by the administration of a compound that will induce some chaperone genes and down-regulate others or inhibit their protein products. In all cases the goal is to maintain or restore the physiologic integrity of the chaperoning system and the optimal balance between stress and anti-stress mechanisms.

New information on negative chaperonotherapy applied to human acute myeloid leukemia (AML) has become available (Reikvam et al. 2013). Leukemic cells were treated with the Hsp70 inhibitor VER-155008, or with the Hsp90 inhibitor 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), or with both simultaneously. The effects observed with VER-155008 were a dose-dependent inhibition of cytokine-dependent AML cell proliferation and promotion of apoptosis. Inhibitors anti-Hsp70 and anti-Hsp90 added simultaneously had increased anti-proliferative and pro-apoptotic effects as compared with addition of only the anti-Hsp70 inhibitor. Similarly, inhibition of Hsp70 has recently been proposed to treat certain tumors that for growth and infiltration depend on this chaperone (Gabai et al., 2016). The chaperone helping the tumor is located in the tumor-stromal cells, i.e., the host's tissue; this situation is a good example of chaperonopathy by mistake or collaborationism, requiring negative chaperonotherapy targeting Hsp70. Combined chaperonotherapy is being applied more often than before and offers some hopes for the treatment of certain diseases in which some chaperones help the cell and the organism while, in contrast, other chaperones are pathogenic. For example, in a recent work, Celastrol was tested as a potential therapeutic agent for Gaucher's disease and its effects on glucocerebrosidase (GCase) were examined (Yang et al. 2014a). The disease is caused by mutations in the Glucosidase beta gene, and the resulting protein misfolds and has impaired enzymatic activity. Celastrol on the one side inhibited Hsp90-driven protein degradation and on the other stimulated production of Hsp70 1A and 1B, and modulated expression of BAG-3, which helped in folding and maturation of a mutant (pathological and pathogenic) GCase that without chaperone assistance was very defective in form and function.

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