

Molecular chaperones: Multiple functions, pathologies, and potential applications

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1. ABSTRACT

Cell stressors are ubiquitous and frequent, challenging cells often, which leads to the stress response with activation of anti-stress mechanisms. These mechanisms involve a variety of molecules, including molecular chaperones also known as heat-shock proteins (Hsp). The chaperones treated in this article are proteins that assist other proteins to fold, refold, travel to their place of residence (cytosol, organelle, membrane, extracellular space), and translocate across membranes. Molecular chaperones participate in a variety of physiological processes and are widespread in organisms, tissues, and cells. It follows that chaperone failure will have an impact, possibly serious, on one or more cellular function, which may lead to disease. Chaperones must recognize and interact with proteins in need of assistance or client polypeptides (e.g., nascent at the ribosome, or partially denatured by stressors), and have to interact with other chaperones because the chaperoning mechanism involves teams of chaperone molecules, i.e.,

multimolecular assemblies or chaperone machines. Consequently, chaperone molecules have structural domains with distinctive functions: bind the client polypeptide, interact with other chaperone molecules to build a machine, and interact with other complexes that integrate the chaperoning network. Also, various chaperones have ATP-binding and ATPase sites because the chaperoning process requires as, a rule, energy from ATP hydrolysis. Alterations in any one of these domains due to a mutation or an aberrant post-translational modification can disrupt the chaperoning process and cause diseases termed chaperonopathies. This article presents the pathologic concept of chaperonopathy with examples, and discusses the potential of using chaperones (genes or proteins) in treatment (chaperonotherapy). In addition, emerging topics within the field of study of chaperones (chaperonology) are highlighted, e.g., genomics (chaperonomics), systems biology, extracellular chaperones, and anti-chaperone antibodies.

2. INTRODUCTION

2.1. Definitions

Molecular chaperones considered in this article are proteins that assist other proteins to fold, refold in case they have lost their native conformation, and travel to their destinations in the cell (including translocation across organellar membranes). Chaperones also participate in the dissolution of protein aggregates and in ushering damaged peptides toward degradation by proteolytic machines. A peptide in need of chaperone assistance constitutes the substrate for the chaperone and is called client polypeptide. The chaperones themselves are assisted by co-chaperones. These are also proteins that interact with the chaperone molecules but not with the client polypeptide.

In general, chaperone molecules have a modular structure with various segments that form functional domains, each domain in charge of one of the many functions of the entire molecule: binding client polypeptide, interacting with a cochaperone, and so on.

2.2. Scope

This article deals with anti-stress mechanisms evolved by living beings on planet Earth, focusing on one of the component of these mechanisms, the chaperoning systems. A general overview of molecular chaperones as defined above will be presented as an introduction for the non-specialist, and to ease the reading of other articles in this Special Issue. Important topics to be covered are the microanatomy of a chaperone molecule as the basis to understand the chaperones' structural alterations (e.g., mutations) leading to chaperone malfunction, and chaperoning teams and networks.

Structural features will be discussed pertinent to function and malfunction (chaperonopathies). Also, an overview will be presented of chaperonopathies (acquired and hereditary or genetic, including genetic polymorphisms of promoter-regulatory regions) and of their expected impact on the health of diverse cells and human populations. Lastly, the potential of using chaperones to prevent and cure the consequences of chaperonopathies and other abnormalities requiring chaperones for their correction will be outlined.

2.3. Objectives

The specific objectives of this article are to: (a) inform the non-specialized readers on chaperonology as an emerging scientific discipline with its distinctive methods, scope, objectives, and applications, including the roles of molecular chaperones in normal physiology; and (b) introduce the subdisciplines of chaperonomics and chaperonotherapy. The main goals are to prepare the reader for the other articles in this Special Issue, stimulate thought and research on the role of chaperones in health and disease, including the use of chaperones for preventing and curing disease.

3. PROTEIN HOMEOSTASIS

3.1. Life and death of a protein

The life of a protein molecule from parent gene to degradation, including folding, is schematically

represented in Figure 1. While virtually all protein molecules follow this route, what else happens to them depends on their functions and destinations: cytosol, organelle, cell membrane, or extracellular space.

Cellular stressors cause cell stress and have a deleterious impact at many stages of the protein's life. These effects of stressors are counteracted by anti-stress mechanisms, which are key players in the stress response. The chaperoning systems, of which molecular chaperones are the central components, are part of the cellular anti-stress mechanisms. Chaperones are also essential for normal cellular physiology, in the absence of stress, as they assist proteins throughout their lives. There is a certain parallelism between the immune and the chaperoning systems: both are ubiquitous and multifunctional, and both deal with potentially troublesome agents (e.g., microbial pathogens or unfolded polypeptides) but their realms are different. Lymphocytes and antibodies circulate in blood and lymph whereas chaperones remain inside cells. However, this latter point needs investigating since chaperones have been found in plasma and cerebrospinal fluid, in secretions and on the cell surface, and their presence in these locations could be more than simply the result of passive release by dying, disintegrating cells.

Nascent, not yet folded or only partially folded polypeptides, as well as partially denatured (e.g., unfolded due to cell stress) polypeptides must enter a folding or an elimination pathway. Likewise, unfolded or misfolded (e.g., structurally defective due to mutation or aberrant post-translational modification) polypeptides cannot remain as such in the intracellular environment because they have the tendency to entangle, aggregate with one another, and precipitate. Whatever the cause, denatured polypeptides lose solubility, which detracts from the pool of useful proteins available to the cell. This situation must be corrected to maintain the physiologic levels of native proteins and to avoid accumulation of useless ones in forms, e.g., aggregates and precipitates that are not directly accessible for refolding or for utilization as a source of the amino acids necessary for the synthesis of new proteins. Furthermore, protein precipitates may be deleterious to the cell. Figure 1 illustrates the main alternative pathways that an unfolded polypeptide (e.g., nascent, or genetically defective, or aged and damaged by aberrant post-translational modifications, or partially denatured by stress) will follow toward complete folding, or toward degradation.

3.2. The chaperone molecule

The many functions of chaperones are each primarily executed by a limited stretch of the molecule called functional domain (Figure 2, top line). A domain may be formed by more than one molecular segment that are not contiguous, which come together by bending and folding of the entire molecule to build a tertiary structure, and thus construct the functional domain (2). Each domain has a distinct function, but in addition it may participate in others pertaining primarily to other domains (interdomain interaction/cooperation).

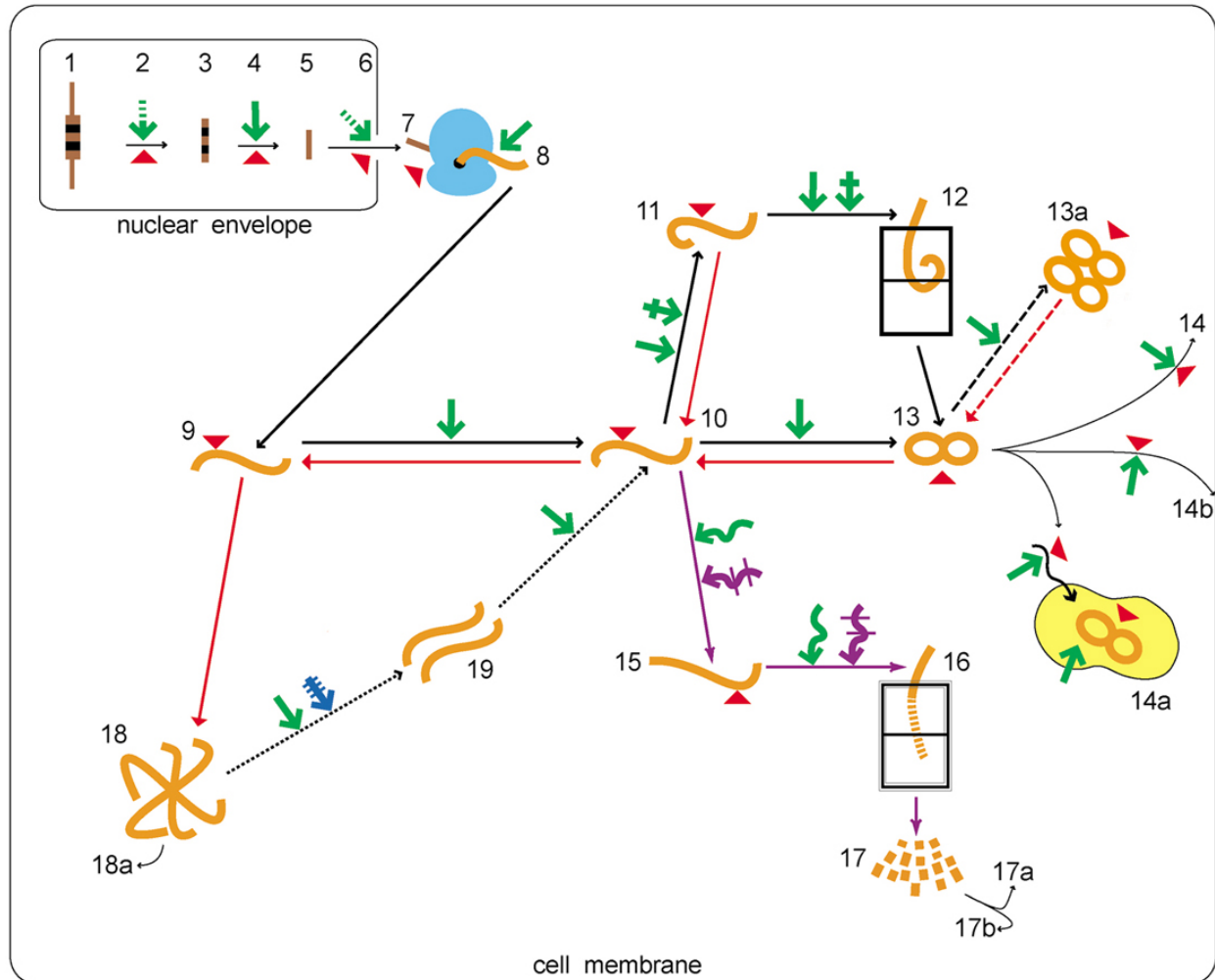


Figure 1. Cellular protein homeostasis: Impact of stressors and points of chaperone intervention. A protein is formed on the ribosome but its primary origin is in its gene. The gene is transcribed into primary transcript and processed into mRNA (in eukaryotes) and then translated on the ribosome. The nascent polypeptide chain emerges from the ribosome and folds to acquire secondary and tertiary structures (some proteins associate with others to form quaternary structures). Throughout these steps, molecular chaperones participate: they assist in the generation of proteins correctly folded, located in their places of residence. Stress can have a deleterious impact on all the main stages of protein development and life, from gene to mature molecule. Chaperones counteract the stress effects to enhance protein formation and to preserve the native, functional conformation of mature molecules. It follows that chaperone failure will have consequences unfavorable to cell physiology and survival. The first five steps in the life of a protein shown in the Figure occur inside the nucleus, and step 6 represents the transition from nucleus to cytosol. 1, nuclear DNA (line) gene (brown rectangle with two introns in black) encoding a protein. 2, gene transcription. 3, primary transcript (pre-mRNA) still containing the introns. 4, pre-mRNA processing, including intron removal and exon splicing. 5, mature mRNA (brown rectangle). 6, mRNA transport from nucleus to cytosol via nuclear pore complex. 7, mRNA translation on the ribosome (light blue). 8, the newly made polypeptide (orange, thick wavy line) is released by the ribosome, is met by ribosome-associated chaperones, and then proceeds on its way to post-translational folding. 9, the same polypeptide farther along the folding pathway, at which point it may aggregate and precipitate if not “protected” by chaperones. 10, the polypeptide interacts with the molecular chaperone machine, which is a team of several molecules, most importantly Hsp70(DnaK), Hsp40(DnaJ), and nucleotide-exchange factor, e.g., BAG-1 and HspBP1. This encounter may involve only Hsp70. 11, the complex polypeptide-chaperone machine is joined by cofactors for folding, e.g., Hip and Hop. The chaperone prefoldin may also play a role at this stage. 12, the polypeptide is freed from the chaperone-cofactor complex (whose components will be reused for another cycle starting at points 10 and 11), and handed over to the multimolecular folding machine (rectangle with solid-line sides), e.g., the cytosolic CCT (chaperonin-containing TCP-1, where TCP-1 stands for tailless complex polypeptide 1). 13, the fully folded protein emerges from the folding machine’s chamber in its native, functional conformation. Some proteins form functional oligomers (quaternary structure), 13a. 14, newly formed proteins are transported to the locales of their residence in the cytosol, others are translocated while folding is in progress into organelles (e.g., mitochondria; 14a) through the organellar

membranes, or are inserted into the cell membrane or are exported out of the cell (14b). Many proteins are probably folded without passing through the complex folding machine shown in 12, a possibility indicated by the black arrow from 10 to 13. Proteins accumulate structural damage as they age, and finally return to the pool of substrates (9) for chaperone and degradation systems. 15, when the polypeptide-chaperone complex (10) is met by a cofactor involved in the degradation pathway, e.g., BAG-1 and CHIP, the complex is diverted toward the proteolytic system, e.g., the ubiquitin-proteasome system (UPS). 16, the polypeptide is freed from the accompanying chaperone-cofactor complex (whose components are recycled back to 10 and 15 for reuse), linearized, threaded through the narrow entrance to the multimolecular proteolytic machine, e.g., the proteasome (rectangle with three-lined sides), and digested inside the proteolytic chamber. 17, the digestion products, oligopeptides 3 to 30-amino acids long, are released, possibly in a regulated fashion, into the intracellular milieu, where they will be further digested by carboxy- and amino-peptidases, yielding free amino acids. These latter residues will be reused to synthesize new proteins on the ribosomes (17a), which explains why under certain conditions of protein restriction some non-essential proteins become dispensable and are steered toward the proteolytic machine and degraded, as a source of amino acids to build other, essential, proteins. A variant of proteasome, the immunoproteasome (which is assembled *de novo* in cells undergoing inflammatory insult), digests proteins to yield antigenic peptides for antigen-presenting cells (17b). 18, partially unfolded polypeptides lose solubility and tend to aggregate and may proceed to the formation of precipitates or inclusion bodies, 18a; these bodies could be a mechanism for cleaning the intracellular milieu of potentially toxic, defective, molecules or, alternatively, the bodies could themselves be harmful; both alternatives are currently under active investigation. 19, chaperones, e.g., Hsp70(DnaK) and high molecular mass chaperones, and disaggregating enzymes, some of which are also members of the chaperone families, cooperate to dissolve the aggregates, rescue the polypeptides, and direct them toward a point, 10, at which time the polypeptides will have the chance to go in the direction of folding, or toward destruction and production of new amino acids if they are irreparable by the chaperoning-folding systems. Symbols key. Orange, thick wavy line, client polypeptide in need of assistance for folding or destined for degradation. Black thick straight arrows with angular heads, pathway toward folding for the production of healthy proteins with a native conformation, ready to function once they have reached the cell locales in which they reside. Black, thin, curved arrows, possible fates of mature proteins, precipitates, or oligopeptides and amino acids. Red arrows with triangular heads, pathway toward unfolding and aggregation, initiated by cell stressors, or by normal cellular processes when it is necessary to dismantle a normal multicellular complex, and to eliminate normal proteins, that have become dispensable (e.g., a gene repressor that must be removed at a certain point in the cell cycle to allow induction of the gene under its control). Purple arrows with elongated triangular heads, pathway toward degradation. Black wavy arrow, translocation pathway from cytosol into an organelle, for example a mitochondrion (14a). Black dotted arrows, recovery route from aggregate to free polypeptides to refolding. Possibly, this route is actively utilized in proteinopathies, also called protein misfolding diseases, i.e., pathologic disorders such as Alzheimer's, Parkinson's, and Huntington's diseases in which one or more proteins that are not chaperones are genetically defective and have a tendency to misfold and to form precipitates. There is evidence indicating that the chaperoning systems attempt to rescue misfolded polypeptides and return them to the pool of native, useful proteins. A similar process may take place during ageing when generation of damaged polypeptides (e.g., due to oxidative stress) increases. Unfortunately, ageing is accompanied by a decline in chaperone efficiency, which presumably leads to accumulation of precipitates and to a progressive dwindling of the pool of native, soluble proteins. Green solid arrows, points of chaperone intervention, i.e., chaperones providing essential assistance to vital cellular processes. Green broken arrows, critical processes for which chaperone assistance is most likely needed but not yet experimentally corroborated. Green solid, crossed arrows, cofactors (e.g., Hop) that interact with the complex chaperone-polypeptide and lead it towards the folding route. Red arrowheads, points of impact of stressors, i.e., molecule, multimolecular structure, function, or pathway adversely affected by stress. Green wavy arrows, chaperones that participate in protein-degradation pathways along with cofactors, e.g., CHIP, and ubiquitins (purple wavy, double-crossed arrows) that lead the chaperone-polypeptide complex (10) towards the degradation route. Blue triple-crossed arrow, disaggregating enzymes that interact with chaperones to dissolve protein aggregates; some of these enzymes are also chaperones. Portions of this figure are modified reproductions of portions of Figure 1 in reference 1, with permission from the copyright owner.

An important characteristic of chaperones is that they interact with other molecules (Figure 1), and these interactions occur via specific domains. Examination of Figures 2-4 allows one to envisage the consequences that structural modifications in certain segments of the chaperone molecule will have on chaperone functions, for example, interactions with other molecules (members of chaperone teams and chaperoning networks, and client polypeptides), as discussed below.

3.3. Chaperoning teams

Molecular chaperones form functional complexes with other molecules of the same chaperone group, or of another chaperone group, or with molecules that are not typical chaperones themselves (e.g., co-chaperones and cofactors) (Figure 4). These multimolecular complexes are

chaperone machines that carry out the chaperoning process, e.g., assist other molecules in their folding and re-folding pathways.

3.4. Chaperoning networks

The whole chaperoning process usually involves more than one chaperoning team in a coordinated succession of stages, each stage having a different team as the main player (Figures 1 and 4). The series of interactions between chaperones and chaperoning teams constitute a chaperoning network that assists protein formation and development from transcription through acquisition of the native configuration and degradation of aged or otherwise damaged molecules.

3.5. Protein folding vs. degradation

Damaged, structurally and functionally deficient protein molecules must be either restored to a normal,

Chaperonology, chaperonopathies, and chaperonotherapy

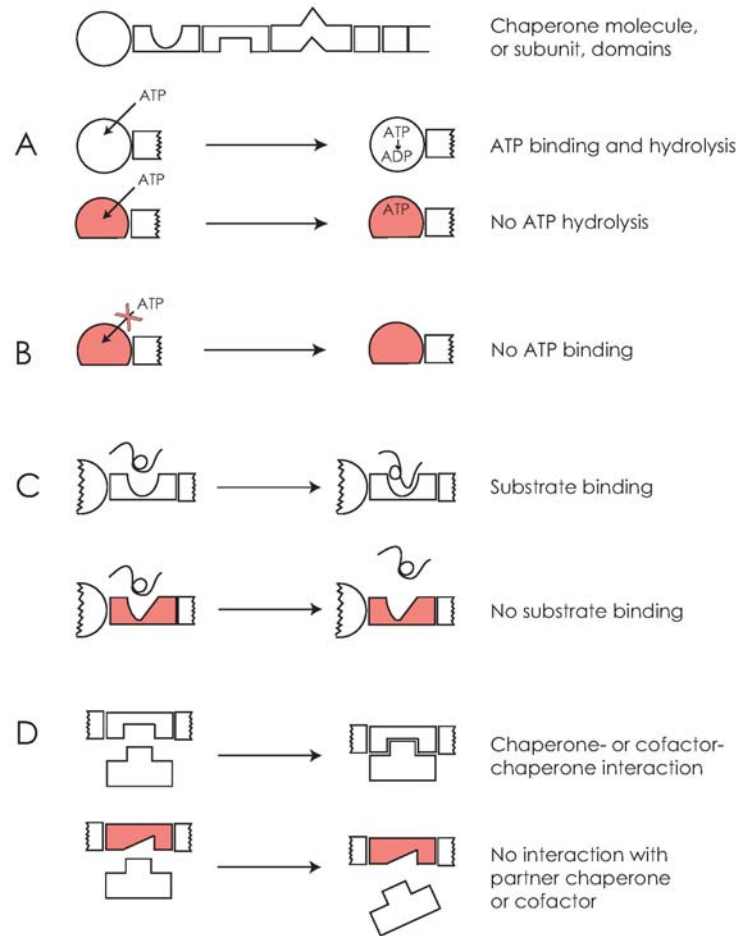


Figure 2. Submolecular basis for known or predicted structural chaperonopathies, Part I. Top line: Schematic of a chaperone molecule or subunit showing functional domains that can potentially be affected by mutations (and also by post-translational modifications). The domains represented from left to right are: ATPase/ATP-binding, circle; substrate-binding, rectangle with concave opening; networking, rectangle with rectangular opening; oligomerization, rectangle with angular opening and angular protrusion; hinge, square; ubiquitin-proteasome system (UPS)-interacting, rectangle with square opening to the right. Not all known functions of chaperones are represented, nor do all types of chaperone molecules have all of these domains. Also, the topology of the domains along the molecule varies in different chaperones, and hinge, flexible, regions may be numerous and distributed in various locations both between and within domains. The ATPase domain is involved in ATP binding and ATP hydrolysis, thus providing energy for other molecular work. The substrate-binding domain recognizes and binds a polypeptide (client polypeptide) in need of assistance for folding (*e.g.*, nascent protein as it emerges from the ribosome) or re-folding (*e.g.*, a protein partially denatured by cell stress). The networking domain mediates the recognition, binding, and interaction of a chaperone molecule or complex with another chaperone complex (interchaperone complex interactions), and with chaperone co-factors, and thus mediates the formation of the chaperoning network necessary for protein quality control. The oligomerization domain recognizes and interacts with the other components of the chaperone complex (intrachaperone complex interactions); some chaperone molecules associate with identical or different molecules (subunits) to form homo- or hetero-oligomers, which are in fact the machines that assist in the folding or re-folding of client polypeptides. Hinge regions are key to the allosteric changes that occur at various stages of the chaperoning process, including oligomerization, substrate recognition and binding, ATP binding and hydrolysis, ADP-ATP exchange, and release of the folded protein. A-D. Functions of molecular chaperone domains, and potential consequences of mutations on these functions (post-translational modifications could produce similar effects). The mutated domain is shaded in each case. A) Chaperones that have ATP-dependent functions rely on ATP hydrolysis generated by their own ATPase and ATP-binding abilities, or provided by a co-chaperone. In the case of a chaperone with its own ATPase, as represented here, a mutation that affects the ATPase domain and disrupts its enzymatic capacity will severely affect all ATP-dependent functions. B) Likewise, if the mutation precludes or impairs ATP binding, the ATP-dependent functions will also be disrupted. Mutations (or post-translational modifications) in substrate-binding, C, and networking, D, domains. C) A mutation in the substrate-binding domain can suppress or severely weaken the binding of the client polypeptide, and thus block the chaperoning process before it actually begins. D) The networking process may be eliminated, or slowed down considerably, if a chaperone molecule bears a mutation in the domain necessary for interaction with a co-chaperone or with other chaperone complexes, so as to integrate a complete chaperoning network.

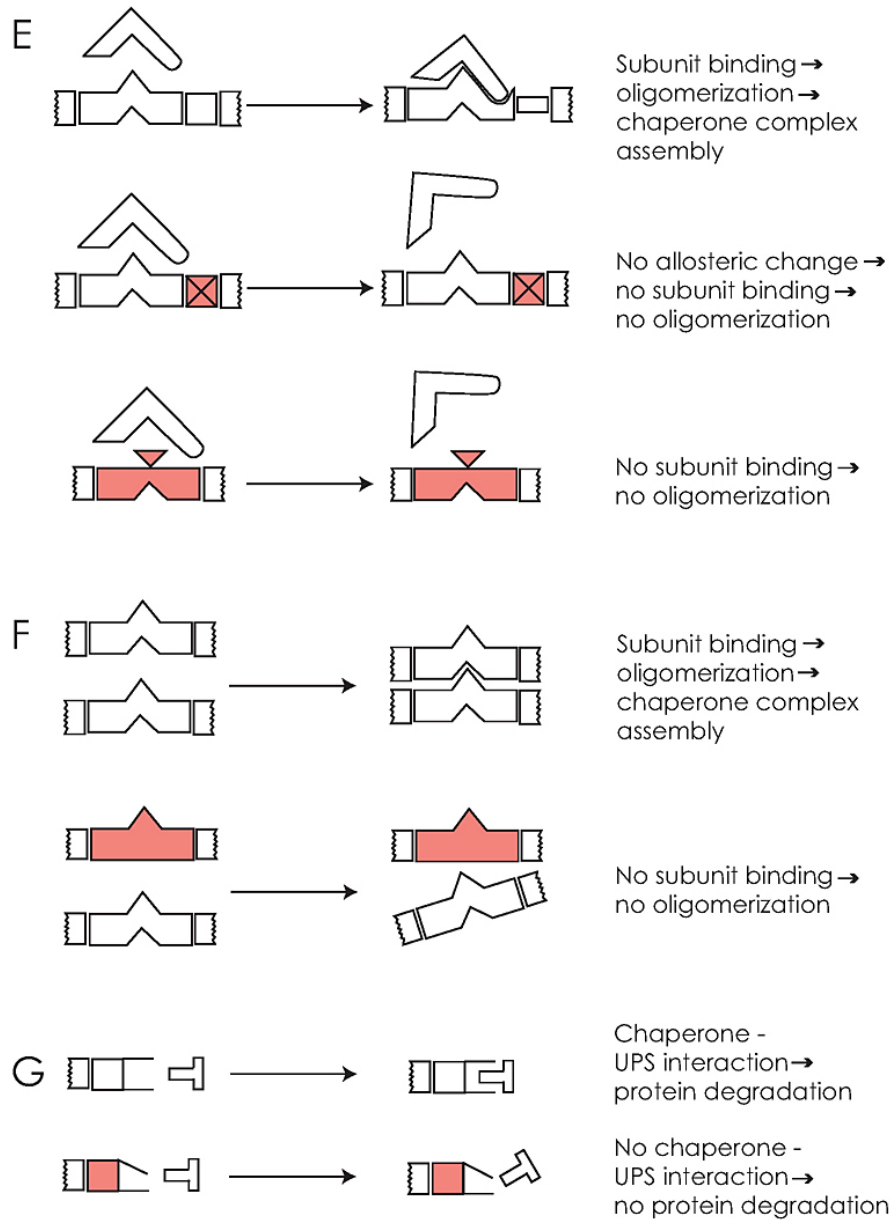


Figure 3. Submolecular basis for known or predicted structural chaperonopathies, Part II. Mutations in hinge region, E, (flexible to allow allosteric changes), and subunit-binding domain (hetero-oligomerization) (E bottom row). Mutations in hinge regions may severely affect chaperoning functions if they interfere with the necessary allosteric changes brought about by chaperone complex assembly and work, as illustrated in E (middle row). Mutations in subunit-binding (homo-oligomerization), F, and UPS-interacting, G, domains. (UPS stands for ubiquitin proteasome system). The oligomerization domain is essential for the formation of multi-subunit chaperone complexes such as those built by the chaperonins. Mutations that affect sites involved in the interaction between subunits to form the complex) and will impair or abolish chaperoning capacity. Multi-subunit chaperone complexes can be formed by different types of subunits (hetero-oligomeric chaperone complexes such as the eukaryotic cytosolic CCT) as represented in E, or they can be formed by identical subunits (like the GroEL and GroES complexes of bacteria) as shown in F. G) Chaperones interact with components of the UPS directly or through an intermediate (like CHIP or carboxy terminus of Hsp70-interacting protein), to facilitate degradation of defective and damaged proteins, and thus to avoid accumulation of irrecoverable molecules, which can aggregate, precipitate, and form pathological inclusion bodies. A mutation in a chaperone that affects the chaperone's ability to interact with the UPS can lead to pathology associated with inclusion-body formation. In addition to the possibilities displayed in the figure, other kinds of chaperone failure involving mutations are possible; these are not due to mutations in the chaperone molecule but rather to mutations in the client polypeptide that render it unrecognizable by the chaperone and, thus, precluding chaperone assistance.

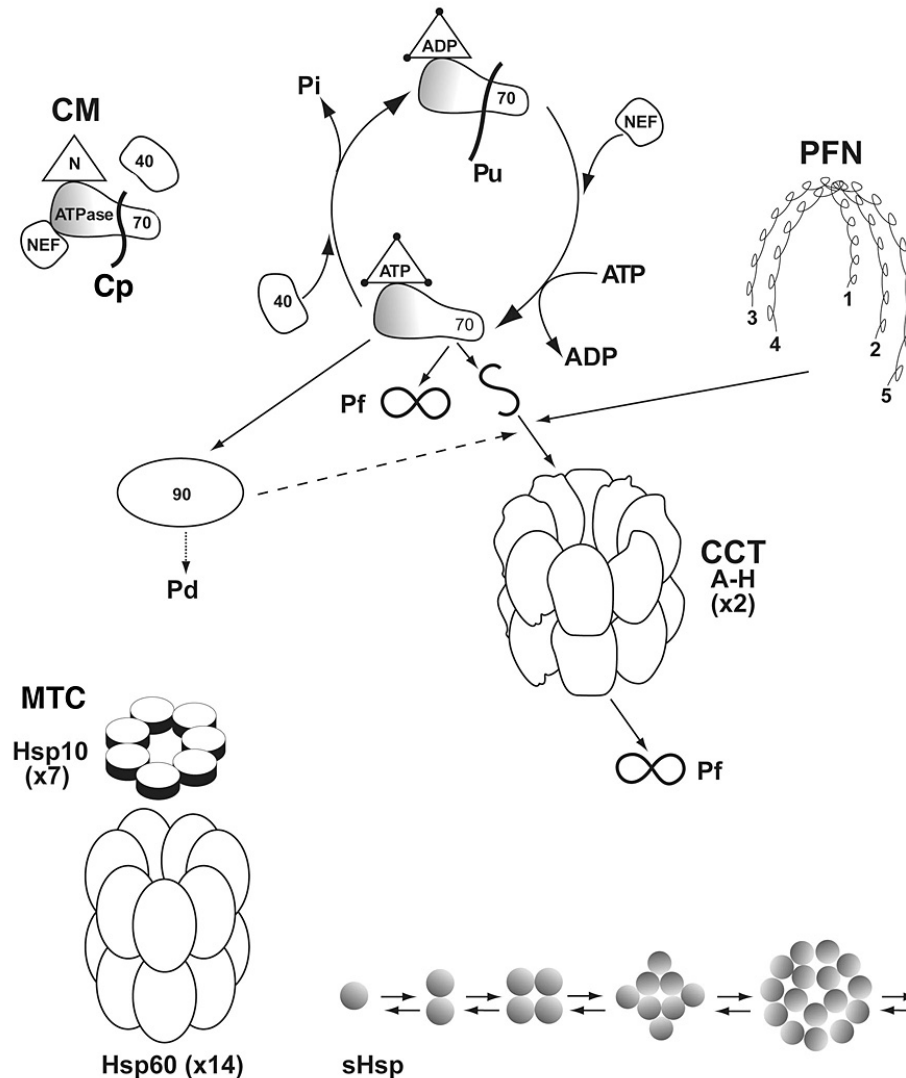


Figure 4. Chaperoning teams and networks. CM. The chaperone machine (CM) is a team of three proteins, Hsp70 (70), Hsp40 (40), and nucleotide (N; e.g., ATP) exchange factor (NEF). Hsp70 binds a client (e.g., unfolded or misfolded) polypeptide (Cp) and, in collaboration with the other members of the team, assists the polypeptide to fold correctly and thus achieve its native conformation. This chaperoning process involves ATP (adenosine triphosphate) hydrolysis mediated by the ATPase domain of Hsp70 and stimulated by Hsp40, and exchange of ADP (adenosine diphosphate) for ATP, an exchange that is induced by the nucleotide exchange factor (e.g., BAG-1, which means Bcl-2-associated athanogene 1, where Bcl-2 stands for B cell lymphoma 2). PFN. Prefoldin (PFN) is composed of distinct subunits (1 through 5) arranged in a medusa type of structure. MTC. The mitochondrial chaperonin (MTC) is a barrel shaped complex of two multimeric assemblages. The larger assemblage is formed of two superimposed rings, each with seven identical subunits (Hsp60 also named Cpn60, where Cpn stands for chaperonin), delimiting a central cavity, the folding chamber. The smaller assemblage is also ring shaped, being formed by seven identical subunits (Hsp10 also named Cpn10) that are considerably smaller than those around the folding chamber. The smaller ring serves as a lid to close the folding chamber while the polypeptide folding process is taking place inside. CCT. The chaperonin-containing TCP-1 complex (CCT), where TCP-1 means tailless complex polypeptide 1, is similar in overall structure to the mitochondrial chaperonin since it is also formed of two superimposed multisubunit rings and it has a central cavity. However, the CCT rings are formed of eight distinct subunits (A through H, also named alpha through theta) rather than seven identical subunits, and there is no third ring or lid. sHsp. The small heat-shock proteins (sHsp) are chaperones, such as the alpha-crystallins, that are usually 30 kD or less in mass and are normally present as monomers. However, in response to cellular stress, they form multimers of various sizes to participate in the protection of unfolded polypeptides. Various teams collaborate by forming a chaperoning network to assist protein folding, refolding, and degradation, and to participate in protein-precipitate dissolution, as suggested by the arrows between teams, and also indicated in Figure 1. Modified from Figure 2 in reference 1, with permission from the copyright owner.

functional conformation or eliminated. Molecular chaperones participate in the process of protein elimination by interaction with protein-degradation systems (Figure 1). Failure of chaperones will thus impair the process of eliminating defective protein molecules, which will accumulate in the cell and tissues and eventually cause problems. Some of these problems are due to the fact that the defective molecules will not enter the productive pathway conducive to protein homeostasis, perpetuating a situation leading to progressive protein depletion. In addition, some types of defective, misfolded proteins are toxic and/or tend to aggregate and precipitate, which can in itself be damaging to the cell, although the role of misfolded proteins and their aggregates in pathogenesis is still a matter of debate (3-11). Some chaperones are known to participate together with proteases in the dissolution of precipitates.

3.6. Systems biology: the chaperoning systems

Because of the ubiquity and multitude of interactions and functions of the chaperones, their failure is predicted to have a widespread impact on cells and organisms. However, the very intricate interrelationships among chaperones and chaperoning systems may provide a mechanism for counteracting the deleterious effects of a deficiency in a single chaperone. The complex network of interactions may have built in compensatory mechanisms, e.g., alternative routes, to preserve the functionality of the network when one or few network components fail. Nonetheless, failure of a chaperone is known to contribute significantly to developmental abnormalities, and to disease. Therefore, one can safely assume that at least some chaperone mutations or other structural defects with direct or indirect impairment of chaperoning functions have had during evolution (and will have in the future natural history of individuals and populations) an impact on the survival of cells and organisms. Since chaperones play a key role as components of anti-stress mechanisms, failure of chaperones in the face of environmental stress could have had a negative impact on the evolution of many species that today are extinct, or on the way to extinction. If so, there is promise in the use of chaperones (genes and proteins) to maintain and improve health and life.

4. STRESSORS AND STRESS

A great variety of agents have the potential to cause cell stress, i.e., the diversity and spread of cell stressors is considerable (12-14). Possibly, there is no ecological niche without stressors that at one time or another will cause stress to resident cells. Furthermore, it could be that stressors and stress are essential components of life. Thus, anti-stress mechanisms are predicted to be very ancient and widespread.

5. ANTI-STRESS MECHANISMS

Anti-stress mechanisms are varied (12-14). It is to be expected, from the key role that anti-stress mechanisms play in survival and from their predicted antiquity, that at least some of these mechanisms are virtually the same in all organisms while others differ

following evolutionary lineages. Thus, one may be able to elucidate the evolution of anti-stress mechanisms by identifying their conserved (universal) and divergent (particular or specific) components in representatives of various phylogenetic lineages.

6. CHAPERONES ACROSS ORGANISMS, CELLS AND TISSUES, CELLULAR COMPARTMENTS, AND THE EXTRACELLULAR SPACE

6.1. The three phylogenetic Domains

Molecular chaperones are good candidates for universal anti-stress mechanisms; they occur in the three phylogenetic Domains (12, 15). For example, the chaperone Hsp70(DnaK) has been found in all organisms investigated with very few exceptions within the phylogenetic Domain Archaea.

6.2. Archaea and eukaryotes

Interestingly, some types of chaperones, e.g., chaperonins of group II, occur in eukaryotes and in the prokaryotes archaea but not in the other prokaryotes, the bacteria (12, 15-17).

6.3. Bacteria and eukaryotic cell organelles

The eukaryotic cell is a complex assemblage of defined structures with components that betray their various origins; for example, mitochondria and plastids have chaperonins of group I, which are directly related to those of bacteria and that are absent from the eukaryotic cell cytosol.

6.4. Extracellular chaperones

Molecular chaperones have traditionally been considered to be confined inside cells. However, evidence is accumulating steadily showing that chaperones are also found on the cell surface, the extracellular space, and in serum and cerebrospinal fluid (18-21). The significance of extracellular chaperones from the biological-evolutionary, physiological, and pathological standpoints is under scrutiny at the present time. In this regard, it is noteworthy that circulating chaperones can elicit an autoimmune response with production of anti-chaperone antibodies (see the Chaperonopathies section, below).

7. CHAPERONOPATHIES

7.1. Definition

Abnormal chaperones have recently been grouped under the heading chaperonopathies, indicating a new pathological concept (22). The chaperone molecule itself may be structurally abnormal (qualitative chaperonopathy), or the levels of a structurally normal chaperone in the cell or extracellular fluids (e.g., intercellular space, blood, cerebrospinal fluid, and secretions such as saliva and urine) may be elevated or decreased (quantitative chaperonopathy) (1). Coexistence of both abnormalities, i.e., in structure and quantity, is also possible.

Structural defects may be due to mutations or to aberrant post-translational modifications, while abnormal quantities may be due to alterations in chaperone-gene

regulation and also to other factors. Gene dysregulation may be the consequence of genetic polymorphism in the promoter region of a chaperone gene.

7.2. Mutations

Mutations can in principle occur at any position along the chaperone molecule, affecting the structure and, possibly, also the function of the domain bearing the mutation (23). Thus a mutation can affect one or more of the properties of a chaperone molecule: interaction with another chaperone to form a team or with a chaperone of another team for networking, with a client polypeptide, and with a protein-degrading machine like the proteasome (Figures 2 and 3).

A number of experimental and clinical examples illustrate the effects of mutations in chaperone molecules (23). Other cases have been reported recently. In the bacterium *Escherichia coli*, mutations in the Hsp70(DnaK) ATPase domain affected interaction with the co-chaperone Hsp40(DnaJ) (Figure 4) and impaired the stimulatory effect that the latter has on ATP (adenosine triphosphate) hydrolysis by Hsp70(DnaK) (24). Also in *E. coli*, a mutant (with aspartic acid instead of glycine at position 122) of a nucleotide-exchange factor, the co-factor GrpE, did not form a stable complex with Hsp70(DnaK) detectable by size-exclusion chromatography as did the wild-type GrpE, and catalyzed ADP/ATP exchange (Figure 4) poorly as compared with the later wild-type co-factor (25). Both of these results suggest that the affinity of the mutant GrpE for the team partner Hsp70(DnaK) is lower than that of the wild-type molecule. Small heat-shock proteins (sHsp) are important components of the cell in organisms of the three phylogenetic Domains, and they form oligomers that regulate their chaperoning activities (Figure 4). A mutation (valine replaced by alanine at position 143) was found to affect oligomerization and, presumably, function (26).

A number of clinically important genetic disorders have been described that are associated with mutations in chaperones, as reviewed recently (23). In addition to those listed in this previous publication, others have recently been added to the inventory of genetic chaperonopathies. For example, mutations in a nucleotide-exchange factor were found to be associated with cases of Marinesco-Sjogren syndrome (27, 28). This autosomal recessive disorder is characterized chiefly by cerebellar ataxia, cataract starting in childhood, myopathy, short stature, and mental retardation. Other abnormalities have also been reported, e.g., skeletal malformations and hypogonadism. Several mutations were found in the gene *SIL1* that encodes the nucleotide-exchange factor (Figure 4, CM) SIL1 (also named BAP for BiP-associated protein) in cases of Marinesco-Sjogren syndrome. The exact mechanism responsible for the abnormalities observed in the patients studied and the role played in this mechanism by the mutated SIL1 protein were not elucidated. However, it is known that SIL1 acts as nucleotide-exchange factor for one of the members of the Hsp70 family, BiP (also named Grp78, GRP78, HSPA5, and Kar2p), which resides in the endoplasmic reticulum, an organelle present in all eukaryotic cells. The wide distribution of the endoplasmic

reticulum throughout tissues as well as the key role of SIL1 in all cells strongly suggest that mutations of this factor will have multisystemic manifestations, which is what happens in patients with Marinesco-Sjogren syndrome.

Williams syndrome (WS) is also a candidate to be included in the list of chaperonopathies in as much as a peptidyl-prolyl isomerase (PPIase) gene has been found deleted in some cases (29). WS is a relatively uncommon pathologic condition characterized by growth, cardiovascular and behavioral abnormalities, and mental underdevelopment. This neuropsychiatric disorder is associated with a DNA deletion (about 1.6 megabases) on chromosome 7. This deletion includes approximately 28 genes, among which there is *FKBP6*, which encodes FK506-binding protein 6. The latter belongs to the family of immunophilins, which together with the parvulins, constitute the superfamily of PPIases that play a role in protein folding.

Bardet-Biedl syndrome (BBS) is another complex pathologic disorder with various clinico-pathological manifestations apparent early in life: obesity, retinal dystrophy, polydactily, mental retardation, and renal dysplasia (23). Various genes (*BBS*) have been found compromised in this syndrome, two of which, *BBS6* and *BBS10* are related to the chaperonins of group II (30). Thus Bardet-Biedl syndrome is an example of complex neurodevelopmental disorder in which a defective molecular chaperone is likely to play a role and investigations in this direction seem justified and promising.

7.3. Post-translational modifications

Senescence implies a progressive decline of general vitality with age, including protein damage due to chemical modifications induced by stressors and oxidation (31). Consequently, the concentration of damaged, partially unfolded proteins increases with age resulting in an increased demand for chaperones. Because of this increased demand, it is likely that chaperones will not act as efficiently as required due to functional overload (32, 33). In addition, the chaperones themselves, being proteins, can be affected by age-related modifications, which may diminish their functional capabilities depending on which functional domain is most damaged, a situation similar to that due to mutations illustrated in Figures 2 and 3. For example, it was found that S-nitrosylated protein-disulphide isomerase is functionally deficient and may play a pathogenetic role in protein misfolding and neurodegeneration (34).

7.4. Expression modifications

The levels of chaperones in cells, tissues, and biological fluids have been found altered in many pathological disorders; a representative sample of them has been discussed recently (1). It is unclear why the levels of one or more chaperones are either increased or decreased in some diseases, but the fact remains that some quantitative profiles are promising indicators of certain diseases and/or of disease status, useful for diagnosis and prognosis. Moreover, it is likely that chaperones play a role in

pathogenesis; either enhancing disease progression or the reverse, thus quantitative changes in chaperones deserve investigation.

7.5. Anti-chaperone antibodies

The fact that chaperones are also found on the cell surface and outside cells altogether, in circulating biological fluids such as blood and cerebrospinal fluid, implies that the chaperones may be recognized as antigens by the immune system with subsequent immune reaction at the cellular and immunoglobulin levels. In fact, auto-immune reactions against chaperones have been shown in many instances (35). For example, plasma antibodies against Hsp60 and Hsp70 have been found associated with cardiac abnormalities in noise-exposed workers (36). In some patients with Guillain-Barre syndrome anti-Hsp antibodies have been detected in the cerebrospinal fluid (37). Likewise, anti-chaperone antibodies have been detected in patients with some type of cancers, diabetes, and HIV infection (38-40).

The above are just a minimal sample of the many publications reporting the presence of chaperones in biological fluids, which often are accompanied by anti-chaperone antibodies. Although some suggestive associations between anti-chaperone antibodies have been documented (for example in the publications cited above), it is not yet clear what role the antibodies play either in pathogenesis or, alternatively, in protecting the body against disease initiation and progression. Elucidation of these critical points, at least for a couple of systems-diseases, could greatly enhance future research to develop therapeutic means focusing on chaperones, either as targets of treatment or as means for treatment.

8. GENETIC POLYMORPHISM OF *hsp70* GENES AND SURVIVAL

Polymorphisms in *hsp70* genes have been investigated, and some have been found associated with longevity, ageing, and disease, as reviewed elsewhere (1). Recently, other examples have been reported. For example, five SNP (single nucleotide polymorphism) in the gene *STCH*, encoding STCH, an Hsp70-family member, were found to be significantly associated with the risk for gastric cancer, particularly the non-cardial type, in Japanese (41). Likewise, the genotypes *hsp70-2* B/B and *hsp70-hom* A/A and *hsp70-hom* B/B were significantly more frequent in patients with HAI (high altitude illness, an acute mountain sickness characterized by cerebral and pulmonary edemas occurring when susceptible persons climb to high altitude) than in matched controls, in a Chinese population (42). Exactly the reverse, i.e., significantly lower frequency, was observed for the genotype *hsp70-hom* A/B genotype in patients with HAI. These findings suggest that individuals with *hsp70-2* B/B, and *hsp70-hom* A/A and B/B genotypes are at higher risk for HAI than persons with the *hsp70-hom* A/B genotype.

The B/B genotype at position 1267 of the *hsp70-2* coding region is associated with NIDDM (non-insulin dependent diabetes mellitus) (43). Known complications of

NIDDM include atherosclerosis with atherosclerotic-plaque rupture in the carotid artery, and cerebral ischemia. It was found that patients with A/B and B/B genotypes had higher frequency of carotid plaques and cerebral ischemia (ictus or transient ischemic attacks) than patients with the A/A genotype. These results suggest that individuals with certain *hsp70-2* genotypes have a higher risk for some of the complications of NIDDM associated with atherosclerosis.

9. CHAPERONOTHERAPY

The role of chaperones in protecting against diseases of various kinds has been studied for years (44). Recently, several reports provide new evidence in favor of the potential of chaperones as therapeutic agents to control stress-related disorders and protein-misfolding diseases. For example, in the neonatal mouse, axotomy (a traumatic stressor) of the sciatic nerve (SN) was not accompanied by an increase in Hsc70 or Hsp70 levels, and caused cell death (45). Preparations of Hsc70 and Hsp70 in doses ranging from 5 to 75 micrograms applied to the SN stump improved significantly the survival of motor and sensory neurons. The data indicate that neurons in the neonatal mouse do not mount a stress response manifested by an increase of endogenous Hsc70 or Hsp70 chaperones in the face of a mechanical stressor (axotomy), and that the damaging effects of this stressor can be prevented by administering exogenous chaperones. Similarly, *hsp70*-gene overexpression was found to protect murine astrocytes submitted to stressors in an *in vitro* experimental system developed to simulate cell injury due to ischemia-hypoxia (46). Neurological protection was also observed *in vivo*, using rat brain as experimental model and plasmids carrying the chaperone genes for overexpression in the treated cells.

10. DISCUSSION

10.1. Yesterday and today

The history of chaperone science began many years ago, in 1962, with the discovery that fruit flies exposed to slightly higher temperature than the optimal for growth (heat shock) showed an increase in the puffs of certain chromosomes (47). These puffs were later shown to be mRNA that encoded proteins, which were called heat-shock proteins (Hsp) (references in 48). Since then, many Hsp have been identified, and a good portion of them was found to have chaperoning properties (49, 50). In parallel, biochemical and biophysical analyses have elucidated to various degrees the molecular mechanisms of the chaperoning process and the structure of the participants in this process (2, 51, 52). Also, phylogenetic analyses have revealed the evolution of chaperones and the ancestry of those present in bacteria, archaea, and eukaryotic cell compartments (53-61). An example is provided by the progressive elucidation of the distribution of the various chaperoning systems. GroEL/S was believed at first to occur only in bacteria, but it was later found in eukaryotic cell organelles, and lastly it was discovered in some archaea (16). The chaperonins Group II were thought initially to include eight subunits in the eukaryotic cell

cytosol and up to three subunits in archaea, but recently it was discovered that one archaeal species, *Methanosarcina acetivorans*, has five subunits (15, 17).

Lastly, the science of genomics has led to the discipline of chaperonomics, namely the identification of all chaperone genes in a genome, and their concerted analysis using proteomics and other high-throughput methods and bioinformatics (62).

10.2. Tomorrow: chaperonomics, systems biology, extracellular chaperones, and anti-chaperone antibodies

Genomics and chaperonomics are currently leading to systems biology (14, 33, 63, 64). The latter discipline will continue to expand and will provide in the very near future information on the interactions of any given chaperone and on all the molecules interacting with it. This will allow elucidation of complete chaperoning networks and their connections with other physiologically active molecular networks.

In addition, one can safely say that new chaperones will be discovered as well as new interactions and functions, some unveiled by systems biology as mentioned above. In parallel new chaperonopathies will be identified and the pathogenetic mechanisms involving chaperones will be revealed. In this regard, the frequency and implications of the existence of chaperones in the cell surface, the extracellular space, and in circulating fluids (blood, lymph, cerebrospinal fluid) will have to be assessed. This is an area that most likely will greatly expand in the immediate future, as well as the determination of the prevalence of anti-chaperone antibodies in humans and animal models of disease and the elucidation of the possible role of these antibodies in favor or against disease.

In applied fields, chaperones and anti-chaperone antibodies will most likely be found useful as biomarkers of disease (diagnosis), disease progression (or regression), and as prognostic indicators.

10.3. Beyond tomorrow: gene and protein therapies

It is expected from the pioneering work being reported in the last few years, that the use of chaperones, genes and proteins, in preventive and curative medicine, will become widespread, first in experimental models and then in real cases in humans, animals, and plants. Chaperonotherapy, by making cells more resistant to stressors and more efficient even under conditions of stress, may thus contribute to human health, species preservation, increased yields of bioreactors that use microbes or mammalian cells to generate useful products, and better crops even in harsh climates for agriculture.

11. ACKNOWLEDGMENT

We thank the members of our group for critical discussions, and the members of the Wadsworth Center's Photography and Illustrations Unit for help with the Figures. This paper is MS06-151 from the Center of Marine Biotechnology.

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Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; BAG-1, Bcl-2-associated athanogene 1, where Bcl-2 stands for B cell lymphoma 2; BBS, Bardet-Biedl syndrome; CCT, chaperonin-containing TCP-1, where TCP-1 stands for tailless complex polypeptide 1; CHIP, carboxy terminus of Hsp70-interacting protein; HAI, high altitude illness; Hip, Hsp70-interacting protein; Hop, Hsp70-Hsp90 organizing protein; Hsp, heat-shock protein; HspBP1, heat-shock protein-binding protein 1; NIDDM, non-insulin dependent diabetes mellitus; PPIase, peptidyl-prolyl isomerase; sHsp, small Hsp; SNP, single nucleotide polymorphism; UPS, ubiquitin-proteasome system; WS, Williams' syndrome.

Key Words: Molecular Chaperones, Structural Domains, Chaperone Teams, Chaperoning Networks, Stress, Chaperonopathies, Genetic Chaperonopathies, Acquired Chaperonopathies, Chaperone-Gene Dysregulation, Chaperone-Gene Polymorphisms, Extracellular Chaperones, Anti-Chaperone Antibodies, Chaperonomics, Chaperonotherapy, Review

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