Int J Clin Lab Res (2000) 30:49–66

REVIEW

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Stress and molecular chaperones in disease

Received: 3 April 2000 / Accepted: 21 April 2000

Abstract Stress, a common phenomenon in today's society, is suspected of playing a role in the development of disease. Stressors of various types, psychological, physical, and biological, abound. They occur in the working and social environments, in air, soil, water, food, and medicines. Stressors impact on cells directly or indirectly, cause protein denaturation, and elicit a stress response. This is mediated by stress (heat-shock) genes and proteins, among which are those named molecular chaperones because they assist other proteins to achieve and maintain a functional shape (the native configuration), and to recover it when partially lost due to stress. Denatured proteins tend to aggregate and precipitate. The same occurs with abnormal proteins due to mutations, or to failure of post-transcriptional or post-translational mechanisms. These abnormal proteins need the help of molecular chaperones as much as denatured molecules do, especially during stress. A cell with normal anti-stress mechanisms, including a complete and functional set of chaperones, may be able to withstand stress if its intensity is not beyond that which will cause irreversible protein damage. There is a certain threshold that normal cells have above which they cannot cope with stress. A cell with an abnormal protein that has an intrinsic tendency to misfold and aggregate is more vulnerable to stress than normal counterparts. Furthermore, these abnormal proteins may precipitate even in the absence of stress and cause diseases named proteinopathies. It is possible that stress contributes to the pathogenesis of proteinopathies by promoting protein aggregation, even in cells that possess a normal chaperoning system. Examples of proteinopathies are age-related degenerative disorders with protein deposits in various tissues, most importantly in the brain where the deposits are associated with neuronal degeneration. It is conceivable that stress enhances the progression of these diseases by facilitating protein unfolding and misfolding, which lead to aggregation and deposition. A number of reports in the last few years have described research aimed at elucidating the role of heat-shock proteins, molecular chaperones in particular, in the pathogenesis of neurodegenerative disorders. The findings begin to shed light on the molecular mechanism of protein aggregation and deposition, and of the ensuing cell death. The results also begin to elucidate the role of molecular chaperones in pathogenesis. This is a fascinating area of research with great clinical implications. Although there are already several experimental models for the study of proteinopathies, others should be developed using organisms that are better known now than only a few years ago and that offer unique advantages. Use of these systems and of information available in databases from genome sequencing efforts should boost research in this field. It should be possible in the not-too-distant future to develop therapeutic and preventive means for proteinopathies based on the use of heat-shock protein and molecular chaperone genes and proteins.

Key words Stress · Heat-shock proteins · Molecular chaperones · Proteinopathies · Neurodegeneration

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Introduction

This brief overview is intended to update a previous article published in this journal in 1995 [1]. At the time it was stated: "The era of Hsp (heat-shock protein) and molecular chaperone pathology has dawned. It is likely that genetic and acquired defects of Hsp and molecular chaperone structure and function will be identified, and will play a primary, or auxiliary but determinant, role in disease."

In the 5 years or so that have elapsed since then, a great deal of information on Hsp and molecular chaperones has accumulated. Data and hypotheses on their role in health and disease, and on their potential use in diagnostics and therapeutics, have also been published, particularly in the last couple of years.

Objective and scope

In this article, selected examples will be used to illustrate the scientific progress mentioned above. Emphasis will be placed upon disorders in which protein abnormalities leading to protein deposits (proteinopathies) are pathological landmarks, such as a variety of neurodegenerative diseases.

Attempts were made to consult printed and electronic literature, and colleagues, so a rather complete panorama might be depicted. However, the field has become too big for a short review. This is one of the reasons why only illustrative cases will be presented pertinent to a limited area of medicine, hopefully in a manner understandable also to the non-specialist, and giving enough information to stimulate further literature searches by interested readers.

In addition to those areas of progress mentioned in the Introduction, others will also be given preference in this article. It has become clear that: "Today, a well-rounded treatment of Hsp, molecular chaperones, and heat-shock genes pertinent to human medicine must include a parallel discussion of data from humans, eukaryotes other than humans, eubacteria, and archaea. This type of comprehensive approach provides the best standpoint to understand the evolution and contemporary structure and function of the heat-shock genes and Hsp" [1]. Therefore, a brief overview of Hsp evolution will be included. Since at the beginning of the third millennium stress is the scourge of our time, stress should also be considered as a potential factor in pathogenesis.

Additional information and bibliography

This overview is a part of a series of interconnected articles on stress genes and molecular chaperones [2–5]. They complement each other in explaining the basic concepts, surveying recent findings, presenting the practical and biotechnological applications of stress genes and proteins, describing the implications of research with these genes and molecules for pathology and medicine, and assembling a comprehensive bibliography. For example, one article is a comprehensive review dealing with stress genes and proteins in archaea, but it also provides extensive information on anti-stress mechanisms pertinent to bacteria and eukaryotes [3]. Another, shorter publication discusses the distribution in nature of an important family of stress genes and proteins, which are molecular chaperones [2]. It also discusses the evolution of these proteins and the important questions raised by their discontinuous occurrence in living cells. A third article emphasizes the potential applications of stress genes and proteins, and other anti-stress mechanisms, in the biotechnology industry [4]. Lastly, and very pertinent to this overview, another article reviews diseases characterized by protein deposits and neurodegeneration [5]. This latter publication provides the foundations of the present paper and its list of references complements that of the present work. Many statements here are based on references cited in that publication, which are not listed in this overview to save space and avoid repetition. Readers who are interested in background information, more bibliography, and more details are encouraged to consult these complementary articles.

Stress

Stress is caused by a stressor (Table 1) impacting on an object, specifically for the purposes of this article, on a cell or organism [6]. Stress is characterized by the stress response, which represents an attempt by the cell to counteract the deleterious effects of the stressor. The central effect of stressors is protein denaturation, and the stress response consists, among other things, of mechanisms aimed at preventing protein damage, restoring altered proteins to their native, functional configuration, and eliminating molecules irreversibly denatured.

House-keeping genes are down-regulated or shut off as a consequence of stress, whereas stress genes are up-regulated, or called into action if they were inactive before the stressor impacted on the cell. The products of stress genes are called stress proteins or, for historical reasons [1], Hsp [7] (Table 2). Some stress proteins function as molecular chaperones, i.e., they assist other polypeptides to fold correctly, to refold in case they were partially unfolded (reversibly denatured) by stress, assemble into higher order structures (oligomers), and translocate to the cell's locales where they reside [8-10]. Chaperones also participate in the elimination of abnormal polypeptides, due to mutations or damage by stress, if they are beyond repair [11, 12].

It is pertinent to ask to what extent psychological stress, which significantly impacts the whole body via endocrine responses, plays a role in proteinopathies [5]. Most likely,

Table 1 Cell stressors[a]

Type	Name, description
Physical	Heat (including fever); several types of irradiation, including ultraviolet light
Oxygen	Oxygen-derived free radicals (ROS); hydrogen peroxide; anaerobiosis to aerobiosis shift (e.g., reperfusion); hypoxia-anoxia (ischemia)
pH	Alkalosis; acidosis; pH shift
Biological	Infection; inflammation; fever
Psychological	Emotion; hormonal imbalance due to HPA axis dysfunction
Osmotic	Changes in the concentration of salt, sugars, other osmolytes (hyper- or hypo-osmotic shock)
Nutritional	Starvation: multiple; specific (carbon, glucose, nitrogen, phosphate, nitrate)
Antibiotics	Puromycin; tetracycline; nalidixic acid
Alcohols	Ethanol; methanol; butanol; propanol; octanol
Metals	Cadmium; copper; chromium; zinc; tin; aluminum; mercury; lead; nickel
Insecticides, pesticides	Lindane; diazinon; paraquat; thiram; tributyltin
Mechanical	Compression; shearing
Other	Benzene and derivatives; phenol and derivatives; teratogens; carcinogens; mutagens; arsenite; arsenate; amino-acid analogues; nicotine; anesthetics; desiccation

[a] Modified from reference [6], with permission from the copyright owner. These agents cause stress in cells from the three phylogenetic domains, Bacteria, Archaea (both prokaryotes), and Eucarya (eukaryotes). ROS, reactive oxygen species; HPA, hypothalamic-pituitary-adrenal.

Table 2 Stress (heat-shock) proteins[a]

Group	Examples
1	Molecular chaperones and chaperonins in organisms of the three phylogenetic domains: Bacteria, Archaea, and Eucarya. Eukaryotic representatives are listed in Table 3
2	Regulators of stress (heat-shock) genes: positive regulators or activators (e.g., heat-shock factor or HSF, in eukaryotes); negative regulators (e.g., HSF4 in eukaryotes; HrcA and HspR in bacteria); sigma factors (*e.g.*, σ^{32}, σ^{E}, σ^{B}) in bacteria
3	Proteasome components and ubiquitin in archaea and eukaryotes. Other proteases: Clp family; Lon/La; HtrA in bacteria
4	Proteins in the intercellular connective material of multicellular structures formed as a response to stress (e.g., in the archaeon *Methanosarcina mazeii*)
5	Transport proteins (e.g., TrkA in the archaeon *Methanosarcina mazeii*)
6	Enzymes and co-factors involved in the synthesis of other stress proteins listed in 1-5 above), and other molecules such as thermo-protectants or "chemical" chaperones (e.g., trehalose in the yeast *Saccharomyces cerevisiae*; di-*myo*-inositol phosphate and cyclic diphosphoglycerate in some archaea)
7	All proteins, enzymes, co-factors, and gene-regulatory factors involved in sporulation (e.g., in the bacterium *Bacillus subtilis*)

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stress does influence intracellular parameters (e.g., pH, electrolytes) in a way that promotes protein denaturation. These effects on normal proteins are probably efficiently counteracted by the physiological battery of Hsp and chaperones in most cases. However, if the tendency of a protein to unfold and aggregate is intrinsically high due to structural defects (mutation), any stressor, including psychological ones, may tilt the balance towards irreversible denaturation, beyond the re-folding power of the cellular chaperoning system. How much does psychological stress, a very common occurrence in the life of many individuals, change the odds in the direction of protein deposition? A related ques-

tion pertains to the role of psychological stress caused by the disease itself on the progression of proteinopathies. Chronic ailments, such as Parkinson's disease, are a major determinant of psychological stress [13]. It is, therefore, conceivable that a self-perpetuating mechanism might be established in which the succession stress-disease-stress, and so on, accelerates protein deposition, aging, and death.

Protein balance

Cell physiology under normal conditions, and survival under stress and recovery after it, require a balanced protein content. The cell needs a set of proteins in the proper, functional, shape, i.e., the native configuration, each within a physiological range of concentrations. The critical effect of stressors is protein denaturation, whereby the native configuration is lost. As a consequence, the polypeptides tend to aggregate and then to precipitate. If these events become pronounced and irreversible cell death ensues.

To counteract the effect of stressors, the cell mounts the stress response, which consists of several anti-stress mechanisms. Foremost among these mechanisms is the chaperoning process.

The eukaryotic cell compartments

The stress response of the eukaryotic cell must be analyzed in relation to its various compartments: cytosol, nucleus, mitochondria, endoplasmic reticulum (ER), Golgi apparatus, cytoplasmic membrane, and peroxysome. These compartments have a long evolutionary history, with different origins, which results in a complex array of constituents with distinctive properties. They all might have the same or very similar families of Hsp at first glance, but this does not necessarily mean that they are indeed identical (Table 3). A given Hsp of the ER might not be functionally identical to its most-similar counterpart of the cytosol, and it might not interact with the same co-factors for assisting protein folding.

Proteins destined for the membrane, or for secretion, are usually synthesized on the ER membrane and then translocated through it into the lumen. This is an oxidizing environment with enzymes and molecular chaperones (Table 3) that receive the new polypeptides and change them into mature proteins. In the ER lumen several events take place: *N*-linked glycosylation, signal peptide cleavage, and disulfide bond formation, just to name a few. The latter is mediated by a family of enzymes belonging to the small Hsp, called protein disulfide isomerase (PDIase).

The ER is like a central station in protein biogenesis. Consequently, it is also a sensor of proteins in trouble, or troublesome. If an abnormal protein, e.g., a denatured one, accumulates in the lumen, a stress response is elicited. This response has been called the unfolded protein response (UPR) [27, 40]. A signal transduction mechanism is triggered by the presence of unfolded proteins in the ER that informs genes in the nucleus of this abnormality and that ER chaperones are in demand above the usual levels. The pertinent nuclear genes are activated and chaperones residing in the ER are synthesized so they can meet the needs at the troubled spot.

Important ER chaperones are BiP(GRP78) and GRPO94 (Table 3). The genes encoding these proteins are called into action during the UPR. They are regulated mostly at the transcriptional level. The *cis*-acting DNA site responsible for the UPR-induced activation is called unfolded protein response element (UPRE) in yeast [27, 40]. Another *cis*-acting site with similar function exists in higher eukaryotes and is called the ER stress response element (ERSE) [27, 40, 41]. This element is involved in the transcription of GRP78, GRP94, and calreticulin, which are important ER chaperones. Recent work has unveiled critical points in the transcription of these genes and their regulation, which are involved in the pathogenesis of some disorders associated with protein deposition and neurodegeration.

Protein folding and building versus destruction

Protein balance is a major determinant of cell survival and evolutionary success. It entails mechanisms for generating new, functionally optimal molecules, in the necessary concentrations, and for the elimination of molecules in excess, or functionally inadequate, the accumulation of which might interfere with intracellular processes. The latter may be a simple mechanical burden that is usually associated with a toxic effect. Hence, the cell has evolved mechanisms for eliminating useless, potentially harmful polypeptides, and for making them the source of building blocks for generating new molecules. Key players in these mechanisms are the enzymes that degrade proteins, i.e., proteases, including the proteasome complex [11, 12, 42].

Other anti-stress mechanisms

The cell's response to stress encompasses various events in addition to those associated with protein re-folding or degradation. The range of anti-stress mechanisms may be inferred from the variety of stress proteins listed in Table 2. Numerous Hsp are involved in cellular processes that do not pertain to polypeptide re-folding but protect the cell as a whole, while others are relevant to the maintenance of pro-

Table 3 Stress (heat-shock) proteins and chaperones in eukaryotes[a]

Family		Examples[b]		
Name(s)	Mass (kDa)	Cytosol; nucleus	Mitochondria	Endoplasmic reticulum (ER)
Heavy; high molecular weight; Hsp100	100 or higher	Hsp101(Hsp102); Hsp104;Hsp105α & β; Hsp110[c]		Hsp170(Grp170)
Hsp90	81–99	Hsp90(Hsp82;Hsp83; Hsp90α & Hsp90β) Grp94[d]		Hsp90(GRP94; Grp94; endoplasmin; gp96)[e]
Hsp70 Chaperones	65–80	Hsp70(Hsp72) Hsc70(Hsp73) SSA1-4; SSB1-2[f]	mhsp70(SSC1) mt-Hsp70(Grp75)[g]	BiP(Grp78) SSi1p; Kar2p; Lhs1p[h]
Hsp60 Chaperonins	55–64	CCT(TRiC; TCP-1 complex) subunits (up to 9)[i]	Hsp60(Cpn60)[j]	
Hsp40 DnaJ	35–54	Hsp40; HDJ-1(MAS5) HDJ-2(HSDJ) Sis1p; Ydj1p[k]	Hsp40; Mdj1p Mdj2p	Hsp40; Hsp47 Sec63p; Scj1p; JEM1p
Small Hsp sHsp	34 or lower	Hsp10; PPIase; PDIase Prefoldin(Gim) subunits (up to 6) α-crystallin family[l]	Hsp10(Cpn10) PPIase; mt-GrpE (Yge1p;Mye1p)	Cpn20 PPIase PDIase

[a] Reproduced from reference [7], with permission of the copyright owner.

[b] Synonyms are within parentheses. Stress proteins and molecular chaperones of the chloroplast, typical of plants, are not included. General and specific references pertinent to Hsp families (members, function, mechanism of action, partners, evolution) are given in [3, 5].

[c] See references in [5, 14, 15].

[d] See references in [5, 16–18].

[e] Grp94 (Glucose-Regulated Protein 94) interacts with one or more of these other chaperones: BiP(Grp78), ERp72 (a PDIase), calnexin, calreticulin, Hsp47, and hsp170. Interactions and participants vary with different Grp94 functions. See references in [5, 19–22].

[f] The SSA and SSB groups are found in yeast and are also written Ssa and Ssb. Hsc70 (and/or Hsp70) interacts with co-factors Hip (for Hsc70-interacting protein, named also p48) and Hop (for hsp70/hsp90-organizing protein, named also IFF-SSP-3521, extendin, or p60 in mammals, or STI1 in yeast), and with CHIP (for carboxyl terminus of Hsc70-interacting protein), Hsp40, and BAG1. See references in [5, 15, 17, 23–26].

[g] Mitochondrial Hsp70 interacts with mGrpE(Mge1p; Yge1p), Mdj1p, and members of the translocase system of the inner membrane (Tim proteins) for protein folding and transport. It also interacts with Mdj1p and proteases (e.g., PIM1) for degradation of misfolded proteins. See references in [5, 9].

[h] BiP (binding protein or immunoglobulin heavy chain-binding protein) interacts with Sec63p, and other components of the ER for protein translocation; also other interactions occur between the ER chaperones. See references in [5, 9, 22, 27].

[i] CCT, for chaperonin containing TCP-1; also named TRiC for TCP-1 ring complex; TCP-1 stands for tailless complex protein 1. CCT interacts with Hop, and possibly with prefoldin/Gim, to assist in protein folding. See references in [4, 5, 28, 29].

[j] See references in [5, 30, 31].

[k] Human and eukaryotic (mostly studied in yeast) homologues of the Hsp40 family are numerous, and they interact with other chaperones in the different cell compartments. See references in [5, 32, 33].

[l] References pertinent to: PPIase for peptidyl-prolyl *cis-trans* isomerase; PDIase, for protein-disulfide isomerase; prefoldin(Gim); α-crystallin family, and other sHsp can be found in [3–5, 10, 34–39].

teins in the native configuration, but indirectly. Examples of these Hsp are those responsible for the synthesis of the non-proteinic chaperones (also named chemical chaperones) such as trehalose, glycerol, and other osmolytes, and other small non-sugar compounds [3, 4]. All these non-proteinic anti-stress molecules play a crucial role in avoiding cell damage in the face of stress caused by different stressors.

The newcomers

Over the past couple of years, in addition to reports describing new members of well-established Hsp families, there have been reports of other molecules with properties that make them candidates for inclusion among the chaperones. One such newcomer is the protein clusterin [43, 44]. It is a 75- to 80 kilodalton (kDa) heterodimer with about 30% of its mass being *N*-linked carbohydrate. It is the product of a single gene. The translated polypeptide is internally cleaved with generation of two subunits, which are then secreted from the cell.

Clusterin is present in virtually all tissues, and is widespread among mammals, with amino-acid sequence conservation in the range of 70%–80% similarity. Evidence is accumulating that supports the notion that the clusterin gene is a stress gene and that its product functions as a molecular chaperone. Furthermore, preliminary data suggest that clusterin expression correlates with some pathological processes observed in Alzheimer's disease (AD) and ischemic tissues.

Lipids have been implicated in the stress response and in protein folding [45]. Lipid-assisted protein folding in certain locations, such as the cell membrane, is perhaps a phenomenon more common and important than previously surmised. The question of whether lipids play a key role in protein folding and assembly in the cell membrane has not been answered in detail, but deserves investigation. The large family of lipids encompasses many different molecules, among which sphingolipids are known to have a variety of functions. Ceramide, a sphingolipid, has recently been considered a candidate for a stress response molecule [46]. Several stressors have been found to cause accumulation of ceramide. Among these stressors are heat, chemotherapeutic agents, and tumor necrosis factor-α. Ceramide accumulation induced by these stressors occurs via effects on the enzymes that produce the sphingolipid. The latter molecule modulates apoptosis, cell cycle arrest, and senescence, cellular events associated with stress. Hence, the enzymes, cofactors, etc., that participate in the synthesis, storage, and transport of ceramide can be included under the umbrella of stress molecules, like those listed in Table 2.

Recent news on Hsp and molecular chaperones pertinent to human disease or models of human disease

Protein disulfide isomerase

PDIase is a well-known enzyme that catalyzes disulfide bond formation and is present in organisms of the three phylogenetic domains (Table 3, [3]). Recently, the genes for this chaperone in *Kluyveromyces lactis* (a type of yeast) were cloned and sequenced [36]. This organism is used for overproduction of recombinant proteins, some of which may be for clinical application. It is, therefore, pertinent to try to elucidate how *K. lactis* accomplishes protein biogenesis under physiological circumstances, and when it is transformed with a vector bearing a foreign gene that is expressed and produces recombinant protein in large quantities. Aggregation and precipitation must be avoided, and proper folding must be optimized in these systems designed for industrial production of useful molecules. Manipulation of chaperone genes, such as those that encode PDIase, has potential for improving the final shaping of recombinant proteins. Manipulation of genes for specific biotechnological purposes must be preceded by cloning, sequencing, and testing, to elucidate expression patterns and regulatory mechanisms [4].

Peptidyl-prolyl *cis-trans* isomerase (PPIase)

This chaperone belongs to a large family of enzymes with a very similar function, the isomerization of the peptide bond at the amino-terminal side of proline (Table 3, [3]). They can be sorted into three main groups. Two are composed of enzymes that bind immunosuppresants and, thus, the cyclosporin-binding (also known as cyclophilins) and the FK506-binding PDIases can be distinguished. The third group includes the parvulins. In mammals, over 15 different PDIases with strong affinity for FK506 have been identified. The majority are present at high concentrations in the brain. In addition to the recognized, typical function of PDIase, namely isomerization of the peptide bond, other roles have been demonstrated or are suspected for these ubiquitous molecules, for example: as yet uncharacterized influence on gene transcription, protein secretion from the ER, release of intracellular Ca^{2+}, cell signalling, formation of the steroid-hormone/receptor complex and of the collagen helix. Most pertinent to this article, the role of a parvulin in Alzeimer's disease and the possible therapeutic applications of ligands for PDIases in the treatment of neurological disorders are being investigated [38]. Preliminary data indicate that inhibitors of the FK506-binding PDIases

have a promising future as mediators of neuroprotection against stressors and in nerve regeneration.

Hsp26

This sHsp (small Hsp) has recently been studied in the yeast *Saccharomyces cerevisiae* [34]. It forms large oligomeric complexes, with up to 24 monomers. These complexes are present in the absence of stress. However, they dissociate in response to heat shock, a phenomenon apparently necessary for efficient chaperoning. The unfolded polypeptides, which are in need of assistance for re-folding, bind more easily and strongly to the dissociated monomers than to the complex. The binding of the substrate (denatured polypeptide) to dissociated Hsp26 generates larger, globular assemblies, the chaperoning complex, with a stoichiometry of one Hsp26 dimer binding one molecule of substrate. These results reveal a novel feature of molecular chaperoning. When not needed, Hsp26 is "stored" in a 24-meric complex, but in the presence of substrates, the complex dissociates for chaperoning, disassembled dimers bind the substrate, and then reassemble to build the chaperoning complex.

Hsp27

The anti-stress effects of this sHsp have been known for some time from studies with human and mouse cells [47, 48]. More recently, expression of the gene encoding Hsp27 was examined, along with that of the *hsp32* gene, in the rat brain in response to heat shock [37]. Both chaperones increased in response to hyperthermia, and were found to localize to the synaptic sites. These findings suggest that the two sHsp play a role in the protection of synapses against the effects of stressors.

In another study, the influence of phosphorylation and oligomerization of Hsp27 on its protective function against the protein-denaturing effects of stressors was studied in rat cardiomyocytes [49]. It was known that Hsp25 in rodents and Hsp27 in humans have protective effects agaist stress caused by hyperthermia, chemotherapeutic agents, and oxidizing reagents. This study [49] showed that Hsp27 administered to rat cardiomyocytes via an adenovirus vector protected the transformed cells from the effects of ischemia – as had been previously found for another chaperone, Hsp70(DnaK). The protective action of Hsp27 did not depend on its phosphorylation. Moreover, non-phosphorylated Hsp27 formed larger oligomers than the phosphorylated molecules, a relevant observation since the protective, chaperoning functions of sHsp involve the formation of large oligomeric complexes.

Hsp32 (stress-inducible heme-oxygenase or heme oxygenase 1)

The activation of the genes encoding Hsp32 and Hsp70(DnaK) was studied in endothelial cells lining blood vessels [26]. These cells are believed to play a critical role in the anti-stress mechanism set in motion by inflammation, a potent cell stressor (Table 1). It was found that the anti-oxidant pyrrolidinedithiocarbamate (PDTC) produces a time- and dose-dependent activation of the two genes. This effect was mediated by activation of the heat-shock factor 1 (HSF 1). It is clear from these findings that the stress response in endothelial cells is a prominent feature during inflammation, and that there is room for investigating possible ways to improve the anti-stress mechanisms of these cells using Hsp32, Hsp70(DnaK), and HSF 1, or their genes.

α-crystallin

This chaperone constitutes a large family of proteins all characterized by the α-crystallin sequence signature. In the past it was believed that α-crystallin was present only in the crystallin of the eye. This is no longer an accepted idea. Crystallin molecules occur in lenticular and non-lenticular tissues. They are widespread throughout the body. In a recent study, recombinant αA-crystallin and αB-crystallin were analyzed using light-scattering and spectroscopic methods [39]. The chaperone activity of αA-crystallin increased in response to heat shock, but that of αB-crystallin did not. The latter was found to be more active than αA-crystallin at the physiological temperature, whereas αA-crystallin was more active during hyperthermia. These changes in chaperoning activity correlated with the magnitude of exposure of surface hydrophobic sites. The more sites exposed, the stronger the chaperoning activity. αA-crystallin underwent conformational changes as the temperature increased, with more hydrophobic sites becoming exposed on its surface. These transitions did not occur in αB-crystallin. The results also showed once again that the ability of chaperones to bind substrate (i.e., denatured polypeptides) depends on surface hydrophobic patches.

The secondary, tertiary, and quaternary structures of αB-crystallin were investigated to assess the effect of mutations on the conformation of this chaperone [35]. A mutant αB-crystallin, typical of desmin-related myopathy and cataract (the *R120G* mutation), was examined. This disease is characterized by the co-aggregation of intermediate filaments with αB-crystallin. It was found that the mutant was altered at its three levels of structure, secondary, tertiary, and quaternary. It was also less stable in

the face of heat stress and considerably less efficient for chaperoning in vitro than the wild-type molecule. The mutant did not prevent the filament/filament interaction that leads to aggregation. The loss of this protective, chaperoning capacity probably plays a significant pathogenetic role. The findings reported, as well as the inferences from the data, open avenues for designing strategies to cure the disease by manipulating the crystallin molecules and/or their genes.

Hsp40(DnaJ)

A new member of the hsp40(DnaJ) family of Hsp (Table 3) has recently been described [32]. The new Hsp, named Hsc40 (for heat-shock cognate 40), was identified in human and mouse cells. The gene consisted of five exons and four introns. This is in contrast to other family members, which consist of three exons and two introns. The Hsc40 molecules from human and mouse cells shared 95% identity, but were only 60% identical to the other known Hsp40 proteins in these two species. The new human and mouse *hsc40* genes responded to heat shock with an increase in their mRNA above the basal levels typical of non-stressful conditions. The expression pattern of *hsc40* was very similar to that of the *hsc70* gene, both under basal and stressful conditions. This is another line of evidence supporting the authors' proposal that the newly cloned gene is different from *hsp40(dnaJ)*, in the same way that makes *hsc70* distinct from the *hsp70* genes. The newly described Hsc40 protein possesses a J domain, which is characteristic of this family of chaperones [33], and bears witness to the function and interaction of Hsc40 with other chaperones that are known to be mediated by the J domain.

Pathological protein deposits

A number of pathological disorders show abnormal protein deposits inside and/or outside cells in one or more tissues. These deposits are the anatomical manifestation of protein abnormalities that cause aggregation and precipitation of the affected molecules. These disorders are grouped under the name of proteinopathies, many of which affect the central nervous system and are accompanied by neurodegeneration. A sample of these proteinopathies is given in Table 4. The following will focus on those neurodegenerative disorders listed in the Table 4 that have been studied recently, with the aim of establishing whether or not stress, Hsp, molecular chaperones, and proteolytic systems play a role in pathogenesis, either as enhancers of disease progression or as safeguards of health.

Alzheimer's disease (AD)

Stress response failure

Mutations in presenilin-1 or -2 seem to play a critical role in the pathogenesis of AD, particularly the early-onset form. Certain mutations increase production of the pathogenic Aβ peptides. A recent report indicates that several presenilin-1 mutants contribute to the pathogenesis of AD in at least two ways: by increasing the production of Aβ peptides, and by subverting the UPR [55]. Neuroblastoma cells harboring mutant presenilin-1 showed a weak UPR. It was known that generation of Aβ peptides is kept in check by a mechanism involving BiP(GRP78) [56]. When abnormal or denatured polypeptides reach a level in the ER that is beyond that which can be dealt with by the chaperones in this organelle, the UPR is elicited. In yeast, the central known players that participate in the UPR are the transmembrane protein Ire1 (or Ern1), which signals from the ER to the genes in the nucleus that more chaperones are needed, and the transcription factor Hac1 [27, 40]. Hac1 interacts with UPRE, and initiates transcription of those genes that are controlled by this element. Ire1 and Hac1 are required for yeast survival under stressful conditions. In higher eukaryotes, the equivalents of the yeast's Ire1 and Hac1 are Ire1α and Ire1β, respectively [27, 40, 41]. These molecules regulate UPR genes via ERSE. Katayama et al. [55] showed that in neuroblastoma cells with mutant presenilin-1, the gene encoding BiP is not activated by UPR to the same levels observed in cells without the mutant. The levels of BiP mRNA were lower in cells with the abnormal protein than cells with the normal presenilin-1 molecule. This finding paralleled other results also indicating that the major players in the UPR are not performing normally in AD cells or in mouse models of the disease. For example, primary cultures of neurons from mice harboring a mutant presenilin-1 gene had lower levels of BiP mRNA than normal controls, and the chaperones BiP and GRP94 were considerably reduced in the brain of patients with familial or sporadic AD compared with age-matched controls. These findings taken together indicate that presenilin mutations enhance the accumulation of pathogenic peptides and also disrupt the UPR. Both alterations in the normal functioning of the mechanisms to keep protein balance within a physiological range appear to complement each other in causing disease.

PPIase

Recent work has focused on the interaction of a parvulin named Pin1 with the protein tau, which is another landmark of AD and other neurological disorders [57]. Tau is a microtubule-associated protein (MAP) that plays a role in the sta-

Table 4 Protein deposits in degenerative diseases ("proteinopathies")[a]

Deposit		Disease
Type	Component(s)	
Amyloid plaque; neuritic plaque (extracellular)	Aβ; NACP (α-synuclein fragment); ubiquitin; activated C' components; α_1-chymotrypsin; apolipoprotein	Alzheimer's
Neurofibrillary tangles (intracellular)	Tau (in PHF); ubiquitin; MAP_2	
Pick body (intracellular)	Neurofilaments; Aβ; tau	Pick's
Lewy body (intracellular)	α-synuclein; ubiquitin; UCH-L1; neurofilaments; Cdk5	Parkinson's
		Occasionally in Shy-Drager syndrome, Alzheimer's, and dementia with Lewy bodies (DLB)
Cytoplasmic inclusions or aggregates (intracellular)	Tau; ubiquitin; αB-crystallin; α-synuclein	Multiple systems atrophy
	Superoxide dismutase; ubiquitin; α-synuclein	Amyotrophic lateral sclerosis (ALS) and familial ALS (FALS)
	Mitochondria; SRC-1; Hsp70; Hsp90; HDJ-2; proteasome components	Bulbospinal atrophy (Kennedy's)
	Neurofilaments; Cdk5	Parkinson's; ALS
Straight filaments (intracellular)	Tau; ubiquitin	Progressive supranuclear palsy (PSP)
Nuclear inclusion (intranuclear)	Protein with poly-Q tract expansion (*e.g.*, huntingtin, ataxins); ubiquitin; Hsp	Huntington's and other poly-Q tract expansion disorders[b]
Amyloid plaque (extracellular)	PrP^{sc}; ubiquitin	Prion diseases (transmissible spongiform encephalopathies), particularly kuru
Neuroserpin inclusion, or Collins, body (intra- and extracellular)	Neuroserpin	Familial encephalopathy with neuroserpin inclusion bodies (FENIB)
Corneal opacity (extracellular)	α-crystallin	Cataract
Filaments (intracellular)	Intermediate filaments; αB-crystallin; desmin	Desmin-related myopathy (DRM)
Rosenthal fiber (intracellular)	Intermediate filaments; Hsp27; αB-crystallin	Alexander's
Mallory body (intracellular)	Intermediate filaments; sHsp	Alcoholic hepatitis

[a] Modified from reference [5] with permission from the copyright owner. Abbreviations are: Aβ, amyloid β peptide, C', complement; PHF, paired helical filament; MAP_2, microtubule-associated protein 2; UCH-L1, ubiquitin carboxy-terminal-hydrolase-L1; SRC-1, steroid-receptor coactivator 1; Hsp, heat-shock protein; sHsp, small Hsp; HDJ-2, human Hsp40(DnaJ) homolog; Cdk5, cyclin-dependent kinase 5; PrP^{sc}, prion protein scrapie (or res, i.e., resistant to digestion with proteinase K). See references in [5, 50–54, 60].

[b] Spinocerebellar ataxia type 1 (SCA1), SCA2, SCA3 (Machado-Joseph's disease), SCA6, SCA7; bulbospinal atrophy (spinobulbar muscular atrophy, Kennedy's disease); and dentatorubral-palidoluysian atrophy.

bilization of vesicles, regulates their transport and that of organelles within them, anchors enzymes, and supports axonal growth [58]. Six isoforms of tau are known to occur in the human central nervous system that originate in a single *tau* gene by alternative splicing. The various proteins contain several (up to 17) serine/threonine-proline (C/T-P) sites that are phosphorylation targets. Hyperphosphorylated tau is typically found in the brain of AD patients. Normally, tau becomes hyperphosphorylated during mitosis. Lu at al. [57] showed that Pin1 binds to hyperphosphorylated tau, and that phosphorylation of T231 is necessary and sufficient for microtubule binding. T231 is phosphorylated in the normal brain and hyperphosphorylated in that of AD patients. Pin1 was found to bind hyperphosphorylated tau from AD brains but not tau from matched controls. Pin1 was detected in the paired helical filaments (PHF), which typically occur in AD. PHF are composed of microtubules and tau and make up the neurofibrillary tangles (Table 4). Tau is hyperphosphorylated in AD and cannot bind microtubules to promote their assembly. However, these properties were regained by tau bound to Pin1. Peptide bond isomerization on the amino-terminal side of P232, brought about by Pin1, restored the ability of tau to bind microtubules. It was also found that soluble Pin1 was very low in AD brains. Possibly, Pin1 was sequestered in the neurofibrillary tangles attached to tau, and thus was no longer part of the soluble pool. This would lead to mitotic arrest, which in turn may unchain the pathway leading to cell death.

Poly-Q tract expansion diseases

Spinocerebellar ataxia type 1

In neurons from patients with spinocerebellar ataxia type 1 (SCA1), and from transgenic mouse models, ataxin-1 aggregates into a nuclear inclusion (NI). This inclusion contains ubiquitin-proteasome components and HDJ-2/HSDJ (a member of the Hsp40(DnaJ) family, Table 3). In a recent study, HeLa cells transfected with mutant ataxin-1 developed NI that also contained proteasome components and HDJ-2/HSDJ [59]. Overexpression of HDJ-2/HSDJ decreased the frequency of ataxin-1 aggregation. This anti-aggregation effect depended on the presence of the J domain in the HDJ-2/HSDJ molecule (a result that differs from that obtained with the same Hsp and ataxin-3 mutants). No increase in the Hsp70(DnaK) chaperone was observed in cells with NI. It was concluded that ataxin-1 aggregates do not induce a stress response involving the *hsp70(dnaK)* gene, in contrast to what happens in spinocerebellar ataxia type 3. The NI in the transfected HeLa cells did not contain Hsp25/27, Hsp60, Hsp90(hsp90α), or Hsp110 (Table 3), which is the same as in the aggregates produced by ataxin-3.

SCA3 or Machado-Joseph's disease

Demonstration of molecular chaperones in NI

Neuronal cells expressing normal or mutant ataxin-3 were investigated to determine the components of the NI and the effect of chaperones on their formation [60]. A fragment of ataxin with 78 Q residues formed NI. The following Hsp were detected using specific antibodies: Hsc70 [or Hsp73, *i.e.*, the product of the constitutively-expressed *hsp70(dnaK)* gene], Hsp70 [or Hsp72, i.e., the product of the inducible *hsp70(dnaK)* gene], HDJ-1, and HDJ-2 [the latter two members of the Hsp40(DnaJ) family] (Table 3). The other Hsps investigated, Hsp27, Hsp60, Hsp90, and Hsp110, were not detected in the NI. The same results were obtained with NI in transfected neuronal and non-neuronal cells expressing the full-length ataxin-3 protein, and with brain cells from SCA3 patients.

Stress response

Hsp70(DnaK) and Hsc70 increased in transfected cells compared with non-transfected controls [60]. The results suggested that ataxin-3 aggregates induce a stress response in affected cells, and consequently the effect of Hsp overexpression was also examined.

Effect of Hsp expression

Fusion proteins containing green-fluorescence protein (GFP, a convenient tag to visualize the labelled molecule of interest, in this case PQ tracts of various lengths) and 15, 35, 56, or 80 Q residues were expressed in neural transfected cells [60]. The longer the Q tract, the more efficient the formation of aggregates. These aggregates contained or lacked the same Hsp detected or missing in the NI discussed above. Likewise, the transfected cells showed manifestations of a stress response similar to that described for cells expressing an ataxin-3 fragment with 78 Q.

Effects of Hsp40(DnaJ)

It was known that HDJ-2 suppresses aggregation of androgen receptor in Kennedy's disease bulbospinal atrophy [61], and of ataxin-1 in SCA1 [59]. The authors [60] performed experiments to investigate whether ataxin-3 aggregates were also affected by Hsp40(DnaJ). The results showed that while HDJ-1 and HDJ-2 suppressed aggregation by truncated and full-length ataxin-3, Hsp70(DnaK) and Hsp27 had no effect. Interestingly, an HDJ-2 molecule lacking the amino-terminal J domain [typical of the Hsp40(DnaJ) family] [33] was more

effective at suppressing aggregation than the complete HDJ-2 protein. These results differ from those obtained with ataxin-1 aggregates, which showed that the anti-aggregation effect of HDJ-2/HSDJ was entirely dependent on the presence of the J domain [59]. It is difficult to explain these contradictory results. The J domain is an essential component of the Hsp40(DnaJ) proteins, necessary for interaction with the other member of the chaperoning complex, Hsp70(DnaK) [33]. Thus, one must conclude that for the anti-aggregation effect to take place, an Hsp40(DnaJ)-Hsp70(DnaK) interaction is not required if the aggregates are formed by ataxin-3 mutants, whereas such an interaction would be necessary if the aggregates are formed by ataxin-1 mutants.

Drosophila model

Expression of a fragment of ataxin-3 with expanded PQ tract in the fruit fly *Drosophila* causes production of NI and neurodegeneration [62]. The pathological protein with expanded PQ tract was directed to the eye using a genetic strategy, and appearance of NI was followed in this organ. Hsp70 was found to localize in the inclusions. Overexpression of a human gene encoding Hsp70 in the fly (the human and *Drosophila* Hsp70 proteins share 74% and 85% identity and similarity, respectively) did not affect NI formation, but suppressed neurodegeneration. The conclusions were that Hsp70 diminishes the toxicity of the abnormal protein, and that this toxicity is not related directly to the intracellular inclusions. These inclusions may, therefore, have little to do with pathogenicity, which is contrary to what has been proposed by others.

Another Drosophila model

A genetic approach was followed in an attempt to detect enhancer and suppressor genes for the PQ tract expansion toxicity [63]. Two polypeptides were generated, one with 20 and the other with 127 Q. These poly-Q peptides were expressed in the eye of transgenic flies, and their effects on eye morphology and pigmentation were recorded. Flies with the 20-Q peptide were normal, but those expressing the 127-Q protein developed abnormal eyes. Pathological protein aggregates were found in remnants of the degenerated retina. Thirty fly lines were established by means of crossings that suppressed eye degeneration in heterozygous descendants from transgenic ancestors, and 29 lines were also obtained with enhanced eye damage. Two of the suppressor lines were analyzed in detail. In one line, the suppressor effect could be assigned to a gene encoding a predicted protein of 334 amino acids with a calculated molecular weight of 37 kDa. This protein had an amino-terminal domain similar to that of the human Hsp40(DnaJ) named HDJ-1, with 54% and 72% identity and similarity, respectively. Consequently, the new protein was considered to be a member of the Hsp40(DnaJ) family, and was named dHDJ-1 ("d" for *Drosophila*). In the other fly line with a suppressor effect, suppression was assigned to a gene encoding a putative protein of 508 amino acids and a calculated molecular weight of 58 kDa. Sequence analysis showed that this new protein had seven tetratricopeptide repeats [64, 65] and a carboxy-terminal J domain [33], and that it shared 46% and 67% identity and similarity, respectively, with the human tetratricopeptide repeat protein 2 (TPR2). Hence the *Drosophila* protein was called dTPR2. In summary, both newly found molecules, dHDJ-1 and dTPR2, possess a J domain of approximately 70 amino acids. This domain is typical of J proteins, among which the most characteristic are the Hsp40(DnaJ) molecules that interact with Hsp70(DnaK), induce ATP hydrolysis, and thereby enhance substrate (abnormal or denatured peptide in need of assistance for folding)-Hsp70(DnaK) binding for subsequent folding and release.

The results added to what was already known about the role of Hsp40(DnaJ) in preventing or reducing aggregate formation in PQD. dTPR2 may also act in another way. TPR domains are made of 3–16 degenerate repeats of 34 amino acids [64, 65]. These repeats may be contiguous or separated by short stretches of amino acids without TPR. Repeats form two anti-parallel α helices, and multiple TPR in tandem assemble into right-handed suprahelical structures. These are optimal for protein-protein contact. Thus, one may assume that the anti-toxic effect of dTPR involves protein-protein interaction. dTPR and/or dHDJ-1 may bind to the mutant, toxic PQ tract and somehow abate toxicity.

Huntington's disease

Huntington's disease (HD) is characterized by protein deposits in the nucleus that contain the mutant protein huntingtin and components of the ubiqutin-proteaseome system (Table 4). These histological findings suggest that Hsp and molecular chaperones might be involved. To test this hypothesis, the effects of heat shock and inhibition of the Hsp40(DnaJ) protein and the proteasome on cytological manifestations of HD were assessed in cell lines of various types [66]. The systems used were the human neuroblastoma (SH-SY5Y), rat pheochromocytoma (PC12), and monkey kidney (COS-7) cell lines. Cells were transfected with constructs bearing poly-Q tracts of various lengths. Intranuclear inclusions appeared in transfected cells expressing 43-74 CAG repeats (Q residues), but not in cells expressing 23 repeats. Heat shock increased the proportion of transfected cells expressing the mutant construct with inclusions. The same effect was caused by proteasome inhibition with lactacystin. Overexpression HDJ-2/HSDJ [a member of the

Hsp40(DnaJ) family in humans, Table 3] had no effect on inclusion formation in PC12 and SH-SY5Y cells, but increased inclusion formation in COS-7 cells. The protein deposits contained 20-S proteasome components, ubiquitin, Hsp70, and Hsp40, as demonstrated by immunohistochemistry with specific antibodies. Presence of the 20-S proteasome in the inclusions correlated with its absence in the cytosol, as shown previously [59].

Yeast model

Experiments were carried out with the yeast *Saccharomyces cerevisiae* to explore the effects of poly-Q tracts of different lengths on aggregate formation and cell damage, and to standardize the yeast system for studying the pathogenetic mechanisms of PQD [67]. This yeast system offers some advantages since it is rather well known from the genetic standpoint and is amenable to genetic manipulations. The amino-terminal portion of huntingtin (the pathological peptide typical of HD) was fused with the green fluorescent protein. Poly-Q with 25, 47, 72, or 103 residues were tested. As expected, the longest produced the most-pronounced aggregates, the shortest produced no aggregates, and those in between produced intermediate-size aggregates in relation to their lengths. Mutation in the yeast that inhibited the ubiquitin-proteasome pathway had no effect on aggregate formation, suggesting that ubiquitination of huntingtin is not required for aggregation in yeast, in contrast to what seems to be the case in humans.

Hsp26

Lack of Hsp26 (an important Hsp in yeast), increased or reduced expression of Hsp90 (in yeast Hsc/p82). Elimination of the members of the hsp70(DnaK) family (constitutively induced Ssa1 and Ssa2, and stress-inducible Ssa3 and Ssa4, Table 3), and elimination or increased expression of Ydj1 [a yeast member of the Hsp40(DnaJ) family], did not have significant effects on the distribution of the pathogenic peptides within the cell. Deletion of the gene for Hsp35 (a heat-inducible, very abundant Hsp in *S. cerevisiae*, which is a member of the glyceraldehyde-3-phosphate dehydrogenase family, an enzyme associated with HD) also had no effect. In contrast, overexpression of Sis1 [a member of the yeast's Hsp40(DnaJ) family] caused the appearance of two inclusions per cell rather than the usual single aggregate. Overexpression of *hsp104* increased the number of aggregates formed by the longest poly-Q tracts tested (i.e., tracts with 72 and 103 Q), while deletion of the gene encoding this Hsp abolished aggregation, regardless of the peptide used. The effects of Hsp104 were similar to those observed for the yeast prion protein Sup35, which is the soluble form of the prion element [PSI^+]. Overexpression and deletion of Hsp104 cures cells of the [PSI^+] element and returns Sup35 to a soluble state [67, 68]. Perhaps Hsp104 is required to maintain the Q-rich prion domain of Sup35 in an aggregation-prone state, and thus to enhance prion formation. A similar mechanism might operate in HD that would promote aggregation of huntingtin.

Prion disease

A few fungal models of prion disease have been characterized. Examples of protein-based genetic elements (or prion-type genetic elements) include the *S. cerevisiae* [*URE3*] and [*PSI*], and *Podospora anserina* [*Het-S*] [68]. The yeast [*PSI*] is a prion-type protein derived from the polymerization of the ribosome-release factor Sup35 (also called eRF3). The Sup35 polymer ($Sup35^{PSI}$) is defective in translation termination, which causes translational readthrough of termination codons (nonsense suppression). Aggregates of $Sup35^{PSI}$ (prion aggregates) have properties similar to those of the mammalian PrP^{sc} (prion protein scrapie), the pathological version of PrP^{c}, which is also called PrP^{res} (res meaning resistant) to indicate its resistance to digestion with proteinase K compared with that of the non-pathological form. Formation and propagation of the [*PSI*] element in vivo is mediated by the amino-terminal region of Sup35. This region, which is also involved in Sup35 polymerization in vitro, has equivalents in PrP and huntingtin. Several Hsp and chaperones have been investigated in search of their possible roles in prion formation, propagation, and cure. It has been found, for instance, that overproduction of Hsp104 cures [*PSI*]. Surprisingly, the same effect is obtained by eliminating Hsp104. Thus, identical effects are caused by excess or total lack of Hsp104. Attempts have been made to explain these observations. Excess Hsp104 enhances solubilization of $Sup35^{PSI}$, but induction of the *hsp104* and other *hsp* genes by heat shock does not cure cells of [*PSI*]. The reason for this lack of curing effect may be the chaperone action of Ssa1, a member of the Hsp70(DnaK) family in *S. cerevisiae* (Table 3). Overproduction of Ssa1 (which is stress inducible) would protect [*PSI*] from the curing action of excess Hsp104. This observation is important since it indicates that prions can commandeer cellular mechanisms for their benefit and against the cell's own interests.

Another group of yeast chaperones is the Ssb subfamily with two members, Ssb1 and Ssb2, which are very similar to each other [68]. They are collectively designated as Ssb protein, and have been found also in another model system of human disease, the nematode *Caenorhabditis elegans*. Ssb is not required for viability, whereas Ssa is. Furthermore, Ssb is

not part of the response elicited by heat shock in yeast. Ssb seems to be involved in the folding of nascent polypeptides as they are being synthesized on the ribosome. Interestingly, Ssb enhances [*PSI*] curing, in sharp contrast to Ssa. Excess Ssb increases [*PSI*] curing by excess Hsp104, while lack of Ssb has the opposite effect. In yeast cells lacking Ssb, spontaneous [*PSI*] formation and [*PSI*] induction by excess Sup35 are augmented. Also, the toxicity of excess Sup35 is decreased in the absence of Ssb and the [*PSI*] curing effect of guanidine hydrochloride is greatly enhanced.

The results summarized above emphasize once again the need for research aimed at pinpointing exactly which molecular chaperones are involved in proteinopathies, either as pathogenetic factors or as protective means against the toxicity and aggregation tendency of abnormal peptides. Moreover, these findings show that the action of molecular chaperones may vary significantly depending on their concentrations, and whether they act alone or in conjunction with other chaperones, co-chaperones, and cofactors. This in turn highlights important considerations pertaining to the auxiliary factors in a cell, which will determine the direction a chaperone will take in its activity, and thereby the outcome of such activity. These auxiliary factors (some of which may also be chaperones) probably play a key role in determining the selective toxicity of pathological polypeptides, which is characteristic of the proteinopathies. Certain types of cells (e.g., those of the central nervous system) are affected seriously, whereas others are not, despite the fact that they also harbor the pathological polypeptide. In this regard, it is pertinent to mention that co-factors might also be involved in other neurodegenerative diseases such as AD [69].

Progressive supranuclear palsy

Progressive supranuclear palsy (PSP), also named Steele-Richardson-Olszewski syndrome, is in most cases a sporadic disease with gait ataxia, rigidity, and impairment in eye movement control. It is most common after 60 years of age. The brain is affected, including the pons, and shows neurofibrillary tangles composed of microtubules and tau (Table 4). These tangles are also present in AD, but while in PSP they consist of straight filaments, in AD the tangles are made of paired helical filaments. Neurofibrillary tangles in AD contain ubiquitin and tau protein (Table 4). They also contain an abnormal form of ubiquitin named UBB, which is the product of the ubiquitin-B gene UBB^{+1}. In a recent study it was demonstrated that the abnormal ubiquitin B is a component of the neurofibrillary tangles present in PSP [52]. It was proposed that the formation of protein deposits in PSP might be due to aggregation of multiubiquitinated polypeptides that cannot be degraded due to failure of the ubiquitin-proteasome system. This failure was attributed to an inhibitory effect caused by the defective ubiquitin B molecule.

Other developments of interest

Heme oxygenase

Two heme oxygenase isoforms are known: heme oxygenase-1 (also called Hsp32) and heme oxygenase-2, abbreviated HO1 and HO2, respectively. HO1 is an Hsp whose gene is induced by a variety of stressors and has a potent cell protection effect. This effect has been recently characterized in some detail and seems to be due to cellular iron regulation [70]. The expression of the HO1 gene increases considerably in response to stress in various tissues, but not as much in the brain. In contrast, the HO2 gene is constitutively expressed, more in the brain than in other tissues, and does not respond to stressors. Recent work has shown that HO2 has neuroprotectant effects [71]. In mice lacking the HO2 gene, occlusion of the middle cerebral artery caused focal ischemia and stroke, which was considerably more severe than in mice bearing the gene, or lacking instead the HO1 gene. These results indicate the potential of these genes for protecting brain cells from environmental stressors (the HO1 gene) and from the devastating consequences of cerebral stroke (the HO2 gene).

Heat-shock factor

Stress genes in eukaryotes are regulated at various levels. Transcription initiation depends on heat-shock factor (HSF), which constitutes a family of related proteins [72]. They interact with heat-shock elements, which are *cis*-acting sites usually located upstream of stress genes. In vertebrates, at least four HSF occur, HSF1–4. The first three are active regulators, whereas HSF4 seems to be a negative regulator. The mechanism of action of these factors in various organisms such as humans, rodents, birds, insects, yeasts, and plants, has been studied. Recently, an investigation revealed altered levels of HSF1 in human prostate cancer cells [73]. The gene for this factor was overexpressed in the cell line PC-3M, which is the metastatic variant of the prostate carcinoma PC-3 line. Overexpression of the gene for Hsp27 was found to coexist with overexpression of the gene for HSF1. The clinical and pathological significance of these findings remains to be assessed. However, they represent a pioneering effort that opened the route to further investigate if, when, and how stress-gene regulation is altered in disease, and what are the consequences of this alteration for the stress genes and the cells.

Seeing the whole picture

A complete understanding of disease and of the pathogenic or protective roles of Hsp can only be achieved if the diversity and evolution of these molecules are studied. Moreover,

studies of the evolution of Hsp have shown that the human cell is a mosaic of structures derived from prokaryotes [3].

All living cells have been divided into three main evolutionary lines or phylogenetic domains, Bacteria, Archaea (both prokaryotes), and Eucarya (eukaryotes) [74, 75]. Bacteria are also known as eubacteria, while archaea were originally called archaebacteria. Archaea and eubacteria share many characteristics, since both are prokaryotes, but they also differ in important aspects, for example in the mechanisms of initiation of gene transcription, DNA replication and repair, and translation. Most importantly, while many bacteria are pathogens for humans, animals, and plants, no pathogens have yet been discovered among the archaea.

The classification into domains is based primarily on comparative analyses of the sequences of small subunit rRNA (16-S in prokaryotes and 18-S in eukaryotes). Examination of the amino-acid sequences of conserved proteins, e.g., Hsp, has also lead to the construction of phylogenetic trees that would explain the evolutionary relationships among organisms of each domain and across domains [3]. These sequence comparisons clarified that the organelles typical of the eukaryotic cell (e.g., mitochondria, hydrogenosome, chloroplast) are of prokaryotic origin and that the Hsp in these organelles are similar to either those of bacteria or to those of archaea. Thus, while the Hsp70(DnaK) molecule present in the cytosol of the eukaryotic cell is the equivalent of that in Gram-negative bacteria, the Hsp70(DnaK) of archaea is similar to that of Gram-positive bacteria (Table 5). These similarities between Hsp go beyond the primary structure; they also pertain to higher levels of structure with functional implications. Structural similarities pertaining to the whole molecule or to portions of it (functional domains) reflect the sharing of common evolutionary ancestors and indicate similarities of function and mechanism of action. Hence, when one studies the biochemistry and mechanism of action of an Hsp from the Gram-negative bacterium *Escherichia coli*, for example, one is gathering information that in part is directly applicable to eukaryotes.

These evolutionary considerations that impact on structure and function of Hsp in different organelles highlight the importance of the molecular, cytological, and anatomical

Table 5 Molecular chaperones and chaperonins in the three phylogenetic domains[a]

Bacteria	Archaea	Eucarya
GroEL (Hsp60)	No[b]	mt, chl: Hsp60[c] (Rubisco subunit binding protein) ct, ER: No
GroES (Hsp10)	No	mt, chl: Yes ct, ER: No
No	TF55; thermosome subunits	ct: TRiC (CCT; TCP-1) subunits mt, chl, ER: No
G+, DnaK[d] (Hsp70)	Hsp70(DnaK)[e] No-hyp.[f]	No
G-, DnaK[d] (Hsp70)	No	mt, chl: Hsp70 ct, ER: Hsp70 para.[g]
DnaJ (Hsp40)	Hsp40(DnaJ)[e] No-hyp.	ct, mt, chl, ER: Yes
GrpE	GrpE No-hyp.	mt, chl: Yes ct, ER: No
G-, HptG	No	ct, ER: Hsp90 para. mt, chl: No

[a] Reproduced from reference [3], with permission from the copyright owner.

[b] No, not yet investigated or not well characterized, or investigated but not yet found.

[c] Abbreviations are: mt, mitochondria; chl, chloroplast; ct, cytosol; ER, endoplasmic reticulum.

[d] G+ and G-, Gram-positive and negative type of DnaK, respectively.

[e] Protein similar to Gram-positive bacterial homologues but transcription initiation mechanism similar to that of eucarya.

[f] No-hyp., not yet investigated, or investigated but not yet found in hyperthermophiles.

[g] Para., paralogous.

precision that must be expected from studies aiming at elucidating the role of Hsp in disease. If one examines whether an Hsp is present in a pathological protein deposit, one ought to establish which Hsp it is. Is the Hsp co-precipitated with the abnormal (mutant) peptide (e.g., ataxin-1, or Aβ-peptide, or tau, or α-synuclein) one that normally resides in the cytosol, or is it from the mitochondria, or from the ER? The distinction is important. An Hsp in the cytosol interacts with co-chaperones and cofactors that are different from those that the equivalent Hsp in the ER interacts with (Table 3). Interactions are also different in the mitochondria as compared with those that take place in the cytosol and the ER. It follows that if one wishes to discover the role of a given Hsp in a specific proteinopathy, one must examine the various cellular compartments separately and, within each compartment, study the various Hsp and co-factors involved in protein folding and refolding. The complexity of the problem is compounded by the fact that the protease system must be analyzed together with the chaperones and their interactions. Until such comprehensive studies are completed, only a partial picture of the role of Hsp and the protease system will be available, and preventive or therapeutic strategies and means will most likely not be as effective as one would wish. The results of recent work described in this brief overview demonstrate, however, that significant progress has already been made, and that the type of research described is very promising.

Conclusions, questions, and perspectives

The concept of proteinopathy is now well established (however the sound of the word might not be pleasing to the ear!). It encompasses disorders that display as a prominent feature an abnormal polypeptide, the product of a mutant gene. The abnormality in the primary structure of the polypeptide usually leads to misfolding, and this in turn results in a malformed protein molecule. Hence, proteinopathies are sometimes also called conformational disorders.

Malformed proteins suffer from two main defects: they do not function or they function less efficiently than the normal version of the same molecule, and they tend to aggregate and form deposits intra- and/or extracellularly. At the very least these deposits represent a mechanical obstacle to many cellular processes. In addition, malformed mutant polypeptides and their accumulation can be toxic to the cell.

In parallel with the development of the proteinopathy concept, and the conformational disorder idea, there has been considerable progress in the understanding of protein folding, namely the mechanism by which a polypeptide achieves its final, functional, native conformation. It has been known for a long time that a polypeptide has the code for its proper folding written in its amino-acid sequence (primary structure). In the last several years it has been learned that many polypeptides need assistance from other cellular components to achieve the native configuration encoded in their primary structures. Molecular chaperones, many of which are Hsp, are among the most-important cellular components that provide assistance to nascent polypeptides so they can fold correctly. Chaperones also participate in the refolding of partially denatured polypeptides and in the dissolution of protein deposits. It is not surprising then that Hsp and molecular chaperones have been the focus of research in many laboratories aimed at elucidating their role in proteinopathies. Can chaperones assist in the folding of a mutant polypeptide? Can they avoid formation of protein deposits? Can they abate the toxicity of abnormal polypeptides? Can they dissolve protein deposits? If so, which chaperones are primarily involved and which are secondary but still necessary players? Can chaperones and/or their genes be manipulated and used to prevent the manifestations of disease, or slow down or cure a disease already on its course, or eradicate the abnormality from a cell lineage? These, and other related questions have been addressed by the authors of the papers discussed in this overview and by other scientists. The results obtained have been briefly described here. There are no definitive answers to the questions, but the information accumulated provides hints that indicate in which direction one may continue the research efforts. In practice, there are essentially two roads one can follow: study the abnormal protein itself and its gene (sequence, inheritance, transcription, regulation, mRNA processing) or analyze the protein-folding process and its players. The latter could very well be abnormal in an otherwise normal cell, and thus cause misfolding and aggregation in the absence of an abnormal polypeptide outside the chaperone system itself. It is conceivable that failure of folding, or misfolding, may be due to a deficient or absent molecular chaperone (due to gene mutation, loss, or inactivation/suppression). A combination of factors is also possible.

Future research ought to be based on considerations such as those in the preceding paragraphs, which take into account the complexity of the normal and pathological processes involved in proteinopathies. Direct studies with pathological materials and use of animal models will continue to be of great value in elucidating etiology and pathogenesis. Microbes should also be utilized as experimental models. In addition to the fungal systems already investigated, described briefly in this overview, others should be standardized with bacteria and archaea. The latter are of particular interest because they have a chaperoning machinery quite similar to that of the cytosol of the eukaryotic cell. In contrast, the bacterial chaperoning machinery resembles that of the mitochondria.

A recent study illustrates the usefulness of a prokaryotic model in the understanding of the molecular mechanism involved in amyloidogenesis [76]. Amyloid is a deposit of abnormal protein in the extracellular space of brain and other tissues, which accompanies aging and is more pronounced in

a variety of diseases such as AD. This pathological condition is named amyloidosis. Amyloid is made of fibrils, which are unbranched, and assembled into a cross-β-fiber structure, with β-strands perpendicular and β-sheets parallel to the fiber's axis, a structural detail reflected in a typical X-ray diffraction pattern. Another distinctive characteristic of amyloid is that it stains with Congo red. A number of different proteins can form amyloid fibrils if given the required conditions. There does not seem to be anything in common among the proteins that have been found capable of forming amyloid fibrils. One may conclude that although certain mutations may favor amyloidogenesis, any protein, normal or abnormal, may be caused to form amyloid fibrils by factors in its microenvironment.

It was found that a methionine aminopeptidase from the hyperthermophilic archaeon *Pyrococcus furiosus* can form amyloid fibrils [76]. This organism's optimal temperature for growth is 100°C and all its components are conditioned to function at this temperature. Methionine aminopeptidase was more resistant to denaturation by heat and guanidine hydrochloride than molecules from other organisms that grow optimally at much lower temperatures (e.g., 37°C), but it was denatured by the latter agent when its concentration was increased above certain levels. At these high concentrations, guanidine hydrochloride caused disappearance of α-helices and an increase of β-sheets with fibril formation and amyloidogenesis. The amyloid so generated met the histochemical, structural, and microscopic criteria usually required to identify typical amyloid in other, already well-characterized systems. These findings illustrate from another angle the importance appreciating the "whole picture". The results also encourage the development of prokaryotic models to study proteinopathies.

Acknowledgements Work performed in the authors' laboratories was supported by grants from the USA Department of Energy, and from the National Science Foundation.

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