Unfolding the role of chaperones and chaperonins in human disease

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Molecular chaperones comprise several highly conserved families of related proteins, many of which are also heat shock proteins. Chaperone proteins are crucial for the maintenance of native protein conformation and recent research has demonstrated several mechanisms where defective chaperone proteins have pathogenic consequences. In this article, we describe the structure and function of chaperones in bacterial and eukaryotic cells, focusing on the chaperonin class of chaperones. We then summarize contemporary research concerning the role of these proteins in several human diseases, concentrating on the genes coding for chaperone and chaperonin proteins and the importance of chaperones in neurodegenerative diseases and as modifiers of amino acid substitution mutations in other proteins.

The native (correctly folded) state of a protein is required for biological activity and is predominantly determined by the primary structure, or amino acid sequence[1]. Successful protein folding is a multistep process of conformal fluctuation yielding transition states of lower free energy and generation of the native structure[2]. However, cellular conditions can favor non-productive folding and aggregation of polypeptide chains because of macromolecular crowding, incomplete availability of entire folding domains owing to the sequential nature of translation and the exposure of slow-folding, hydrophobic domains during translation[3]. To reduce protein aggregation, cellular mechanisms have evolved, such as co-translational domain folding for eukaryotic proteins with multiple domains[4] and the chaperone class of proteins[5].

Chaperones

A chaperone is a protein that selectively recognizes and binds to the exposed hydrophobic surfaces of a non-native protein in a noncovalent interaction in order to inhibit irreversible aggregation. Release of the polypeptide is commonly driven by an ATP-dependent conformational change of the chaperone, permitting subsequent folding of the non-native protein. Chaperones therefore prevent protein aggregation and facilitate the correct folding of non-native proteins through regulated binding and release *in vivo* (Refs 5–9).

There are several different classes of chaperones defined by molecular size, cellular compartment and function (Table 1). The largest chaperone families are Hsp90 (heat shock protein of apparent molecular weight 90 kDa), Hsp70 (70-kDa Hsps), Hsp60 (60-kDa Hsps), Hsp40 or DnaJ (40-kDa Hsps), and the small heat shock proteins (sHsps)[8]. The different chaperone systems cooperate in folding proteins[10].

Chaperone proteins can also participate in the degradation of misfolded proteins. Prolonged cycling through a chaperone system can occur when a substrate cannot be converted to the native state, enabling recognition of the incompletely folded protein by an E3 ubiquitin ligase and subsequent ubiquitination and targeting of the protein for degradation by the proteasome[11]. Co-chaperone proteins, such as the carboxy terminus of Hsp70-interacting protein (CHIP), could provide the physical and functional link between chaperone and protein degradation machinery in the cell[11]. Although specific estimates of the number of human chaperone and chaperonin proteins are not available, data from the Human Genome Project predict that more than 750 eukaryotic proteins are involved in protein folding and degradation[12].

Glossary

Amyloid-β peptide (Aβ): Alzheimer disease is characterized pathologically by cerebral plaques containing Aβ and neurofibrillary protein. Aβ is produced by cleavage of amyloid precursor protein by β-secretase enzymes into peptides of 42 and 40 amino acids in length, $A\beta_{42}$ and $A\beta_{40}$, respectively. $A\beta_{42}$ is highly amyloidogenic and prone to aggregation.
Desmin: A protein belonging to the type III intermediate filament family found in the sarcoplasma of skeletal and cardiac muscle.
Hydrometrocolpos: Hydrometrocolpos is a fluid-filled dilatation of the female genital tract caused by the retention of cervical secretions. Hydrometrocolpos usually results from obstruction of the female genital tract by vaginal agenesis or a transverse vaginal septum.
Inclusion bodies: Inclusion bodies are distinctive structures formed in the nucleus or cytoplasm of a cell. Inclusion bodies can contain aggregates of cellular material formed as a response to cellular injury or infection or in the process of cell degeneration.
Lewy bodies: A Lewy body is a cytoplasmic inclusion body composed of aggregates of abnormally accumulated proteins such as α-synuclein.
Polyglutamine (PolyQ) tract diseases: The polyQ tract diseases are a group of inherited neurodegenerative diseases characterized by gene mutations causing an expanded polyglutamine domain within the altered protein, resulting in protein misfolding and aggregation.
Post-axial polydactyly: Post-axial polydactyly can be defined as a supernumerary digit or digits on the ulnar side of the upper limb and the fibular side of the lower limb.

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Table 1. Major classes of chaperone and chaperonin proteins[a]

Eukaryotes[c]	Prokaryotes[c]	Interacting chaperones	Size (kDa)	Cellular compartment	Structure	Refs
Chaperone proteins[b]						
Hsp90α, Hsp90β, TRAP1	HtpG	Hsp70, Hsp40	90	C, ER	Dimer, α-helix/β-sheet layers	65
Hsp70, Hsc70, BiP, MHsp70	DnaK	Hip,Hop, GrpE	70	C, M, ER	N- and C-terminal domains	9
Hsp40	DnaJ	Hsp70, Hsc73	40	C, M, ER	Bi-helical	5
Small Hsps	IbpA, IbpB	Hsp70	12–43	C	Oligomers; α-crystallin domain	32
Chaperonin proteins[d]						
Group I						
Hsp60–Hsp10[e]	GroEL–GroES[e]	GimC	57	M	Two heptameric rings; homo-oligomeric	9
Group II						
TCP-1, or CCT	TF55,56 Thermosome		55	C, N	Octameric/nonameric rings; heteromeric	21

[a]Abbreviations: CCT, chaperonin-containing TCP-1; NNP, non-native protein; M, mitochondria; C, cytosol; N, nucleus; ER, endoplasmic reticulum.
[b]Does not include yeast or chloroplast chaperones.
[c]The different members in each chaperone or chaperonin class from eukaryotic and prokaryotic cells are not always homologous.
[d]Only co-chaperones and interacting proteins in eukaryotic systems have been listed.
[e]Hsp10 and GroES are co-chaperonin proteins.

Chaperonins and small heat shock proteins

The Hsp60 class of chaperones is also referred to as chaperonins. They are pre-existing complexes comprising two stacked rings of radially arranged subunits of approximately 60 kDa each (Table 1). Chaperonins facilitate the ATP-dependent folding of non-native proteins in addition to binding non-native substrates to prevent protein aggregation[9].

Small Hsps show an ATP-independent chaperone-like activity in cells *in vitro*. Non-native protein intermediates form a stable complex with the hydrophobic surfaces of sHsp complexes during cellular stress. This complex formation sequesters non-native substrates and prevents protein aggregation until the polypeptide can be folded under favorable conditions.

Chaperone and chaperonin structure and function

Chaperonins are double-ringed, multi-subunit complexes that primarily promote protein folding (Table 1, Fig. 1). Chaperonins are divided into Group I or Group II chaperonins based on the presence or absence of a co-chaperonin. We discuss the structure and function of the Group I and Group II chaperonins as models for the chaperone group, because the bacterial Group I chaperonins, GroEL and its co-chaperonin, GroES, have been studied extensively[13,14].

Group I chaperonins

Group I chaperonins [prokaryotic GroEL, mitochondrial Hsp60 and the Rubisco subunit binding protein (RBP) in plants] require a separate co-chaperonin or 'capping' molecule for function. The chaperonin GroEL is a homo-oligomer of 14 subunits, each of 57 kDa. These subunits have an apical, intermediate and equatorial domain and are arranged in two heptameric rings stacked back to back in a toroidal formation, defining two central cavities. The opened, apical domains of GroEL are lined with hydrophobic residues capable of nonspecific interaction with the exposed hydrophobic surfaces of the unfolded substrate. The binding of a non-native substrate, the co-chaperonin GroES, and adenosine 5′-triphosphate (ATP) molecules to a GroEL ring triggers a conformational change in GroEL that increases the volume of the central cavity, separating the hydrophobic apical residues[15]. This structural stretching allows partial unfolding of the substrate and culminates in substrate release into the central cavity of the chaperonin, allowing productive folding in a contained environment. Energy for the folding process is provided by ATP hydrolysis, which subsequently weakens the interaction between GroEL, the co-chaperonin GroES and substrate, leading to disassembly of the ring and substrate release.

Repeated rounds of insertion and expulsion from the chaperonin are usually required for a polypeptide to achieve the native state. Proteins up to 60–70 kDa in size with multiple domains can be folded in GroEL. In *E. coli*, approximately 20% of newly synthesized peptides are folded by GroEL–GroES *in vivo*, and substrates include the enzymes involved in transcription, translation and metabolism of amino acids and sugars[16]. There is no invariable 'consensus' sequence that facilitates substrate binding to GroEL, although preferred substrates have at least two domains with α/β folds and exposed hydrophobic surfaces[16].

Group II chaperonins

Group II chaperonins are found in Archaebacteria and Eukaryotes[17]. The Group II chaperonins were discovered after the Group I chaperonins and are highly related to each other, sharing up to 40% amino acid identity. The eukaryotic group II chaperonin is

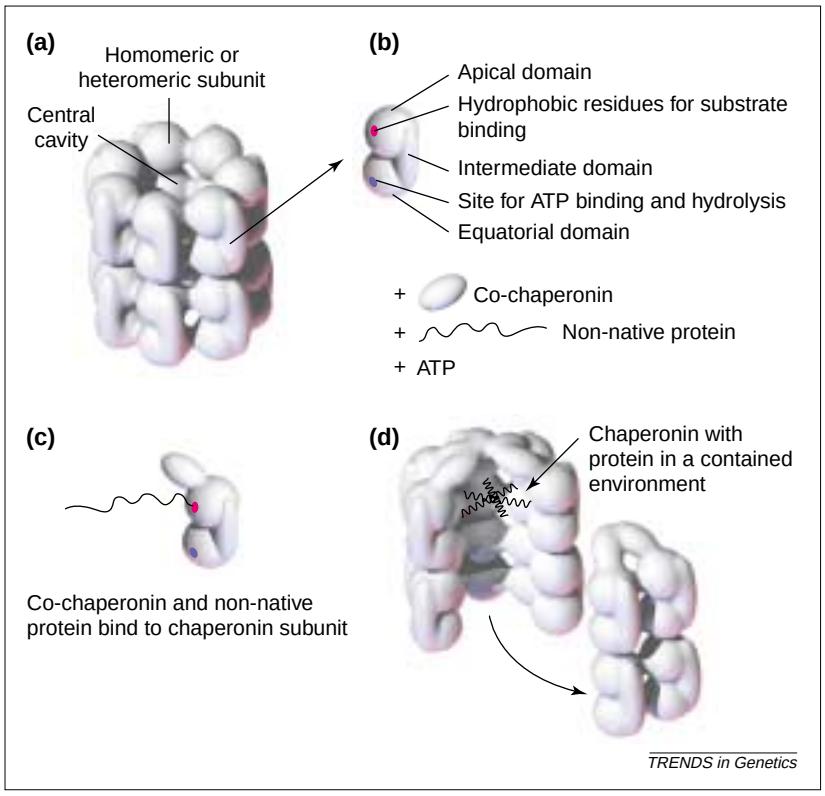

Fig. 1. Chaperonin structure and function. (a) Chaperonin structure. A chaperonin has two rings composed of homo- or heteromeric subunits that form two central cavities for unfolded substrate. (b) Each subunit has an apical domain (contains hydrophobic residues for substrate binding), an intermediate domain and an equatorial domain (contains site of ATP binding and hydrolysis). (c) Binding of non-native protein induces a conformational change in the chaperonin, resulting in structural stretching of the substrate. (d) The substrate is contained in the central cavity of the chaperonin, allowing folding in a protected environment. In some chaperonins, the co-chaperonin is replaced by a helical protrusion of the apical domain.

named T-complex-related polypeptide 1 (TCP-1; also called CCT or chaperonin-containing TCP-1) and has significant sequence similarity to GroEL (Ref. 18). Group II chaperonins consist of two stacked, octameric or nonameric rings and have a larger central cavity than Group I chaperonins. Archaebacterial Group II chaperonins have homomeric or alternating α/β subunits in each ring, whereas eukaryotic Group II chaperonins have six to nine different subunits of 55–60 kDa (Fig. 1, Ref. 17). Group II chaperonins have similar domain organization to Group I chaperonins, but have a helical protrusion that takes the place of the co-chaperonin GroES (Ref. 19). They have a similar mechanism to Group I chaperonins, but the mechanism of action for this group of chaperonins is less defined. Pulse-chase analysis has demonstrated that up to 15% of newly synthesized eukaryotic proteins interact with TCP-1, including actin, tubulin and myosin[20]. In contrast to the nonspecific binding found with GroEL, substrates interact with TCP-1 by subunit- or domain-specific mechanisms that are geometry dependent[20]. Determination of the crystal structure of the thermosome from *Thermoplasma acidophilum* shows that the helical protrusion of the apical domain can assume different conformations, including α-helical and β-sheet conformations, while in an open state ready for substrate binding[21]. This structural plasticity might confer the ability to interact with different types of substrate proteins[21].

Mutations in genes coding for chaperones, chaperonins and proteins with chaperone-like domains

Mutations in genes encoding proteins with chaperone-like domains and chaperonin proteins are important in at least five human diseases (Table 2). However, the number of conditions associated with dysfunctional human chaperone and chaperonin proteins will probably increase with further research.

Mutations in chaperonin proteins and human disease

McKusick–Kaufman syndrome (MKS; OMIM 236700) is inherited in an autosomal recessive pattern, and its main phenotypic features comprise HYDROMETROCOLPOS (see Glossary), POST-AXIAL POLYDACTYLY (PAP) and congenital heart disease[22]. Linkage to chromosome 20p12 was established in four sibships with MKS from the consanguineous Amish population and mutations in the causative *MKKS* gene were subsequently demonstrated in individuals from the Amish pedigree and a sporadic patient[23]. The *MKKS* gene encodes a protein that has homology to the Group II chaperonins (archebacterial chaperonins and T-complex-related proteins), including the thermosome from the Archebacteria, *Thermoplasma acidophilum* (Ref. 23). Modeling of the three-dimensional structure of the MKKS protein using data from the X-ray crystal structure of the *T. acidophilum* protein showed that the missense mutations in MKS are sited in the predicted equatorial domain of the protein, the conserved site of ATP hydrolysis that is known to be functionally important in *E. coli*[23]. MKS is rare, and further mutations in the *MKKS* gene in patients with the MKS phenotype have not yet been described.

Bardet–Biedl syndrome (BBS; OMIM 209901, 209900, 603650, 600374, 600151) is also inherited in an autosomal recessive pattern and is characterized by pigmentary retinopathy (RP), PAP, obesity, learning disability and hypogenitalism[24]. There is significant clinical overlap between BBS and MKS (Ref. 22), and mutations in the *MKKS* gene have subsequently been demonstrated in some patients with BBS (Refs 25,26). However, this gene is thought to account for a minority of the disease-causing sequence alterations in patients with BBS (Ref. 27). The difference in phenotypic severity in MKS and BBS has been hypothesized to result from quantitative or qualitative differences in the MKKS protein[25,26]. The retinal disease, obesity and learning disability in BBS are age-dependent, and it is proposed that these are caused by an inability of the putative MKKS chaperonin to maintain protein

Table 2. Diseases including chaperone and chaperonin proteins

Disease	Main phenotypic features	Gene/locus	Chaperone/chaperonin protein homology	Molecular mechanism of mutation	Refs
Human diseases caused by mutations in genes coding for chaperones and chaperonin proteins					
McKusick–Kaufman syndrome	Hydrometrocolpos Post-axial polydactyly Congenital heart disease	BBS6; 20p12; *MKKS* gene	Putative chaperonin	Disruption of ATP hydrolysis	23
Bardet–Biedl syndrome (BBS)	Pigmentary retinopathy	BBS1; 11q13			
	Post-axial polydactyly	BBS2; 16q21	No chaperone function		28
	Mental retardation	BBS3; 3p13			
	Obesity	BBS4; 15q21	Protein has TPR motif[a]		29
	Hypogenitalism	BBS5; 2q31			
	Renal abnormalities	BBS6; 20p12; *MKKS* gene	Putative chaperonin	Disruption of ATP hydrolysis	25,26
Autosomal recessive spastic ataxia of Charlevoix–Saguenay (ARSACS)	Motor neuropathy; sensory neuropathy cerebellar atrophy; retinal hypermyelination	13q11; *SACS* gene	DnaJ domain; domains similar to N-terminus of Hsp90	Deletion of gene	30
Desmin-related myopathy	Proximal/distal skeletal myopathy; cardiomyopathy; cataracts	11q21–23; *CRYAB* (αB-crystallin) gene	Small heat shock protein	Alteration in chaperone activity/tertiary structure of αB-crystallin	33
Congenital cataracts	Cataracts; microcornea/microphthalmia; nystagmus	21q22.3; *CRYAA* (αA-crystallin) gene	Small heat shock protein	Disruption of 'salt bridge' with destabilization of αA-crystallin protein	37
Neurological diseases modified by chaperone and chaperonin proteins					
Spinocerebellar atrophy (SCA) type 1	Cerebellar ataxia; bulbar dysfunction; motor deterioration	*SCA1*; 6p23			41
Spinocerebellar atrophy (SCA) type 3	Cerebellar ataxia; mental retardation	*SCA3/MJD*; 14q24.3			42
Spinobulbar muscular atrophy	Wasting and weakness of hip and shoulder girdle muscles; Male hypogonadism	*AR* gene; Xq11–12			43
Huntington disease	Progressive dementia; Choreiform movements; Psychiatric disturbance	*HD* gene; 4p16.3			44
Alzheimer disease (familial type 3 and familial type 4)	Progressive dementia Mood disturbance	*PSEN1*; 14q24.3 *PSEN2*; 1q31–42			54
Autosomal recessive juvenile Parkinson disease	Tremor; bradykinesia; increased tone	*PARK2*; 6q25.2–q27			55
Amyotrophic lateral sclerosis (ALS1)	Progressive muscle weakness; bulbar palsy	*SOD1*; 21q22.1			56
Diseases in which chaperone and chaperonin proteins may modify missense mutations					
Cystic fibrosis	Abnormal exocrine gland function; sinopulmonary infection; malabsorption; infertility	*CFTR*; 7q31.2			58
Phenylketonuria	Mental retardation; 'Mousy' odor; hypopigmentation	*PAH* gene; 12q24.1			60
Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	Hypoglycemia with stress	*MCAD* gene; 1p31			61,62

[a]TPR (tetratricopeptide repeat) motif; this motif is involved in protein–protein interactions.

integrity. However, the protein(s) folded by this chaperonin remain unknown.

Recently, two further genes mutated in BBS patients have been cloned[28,29]. Although the *BBS2* gene produces a protein with no known sequence homology or functional relationship to chaperone or chaperonin proteins[28], *BBS4* encodes a protein with a potential tetratricopeptide repeat (TPR) motif[29], which could be important in protein–protein interactions.

Mutations in chaperone proteins and human disease

Autosomal recessive spastic ataxia of Charlevoix–Saguenay (ARSACS; OMIM 270550) is an early-onset neurodegenerative disorder characterized by abnormal sensory and motor nerve conduction, hypermyelination of retinal-nerve fibers and atrophy of the cerebellar vermis[30]. The causative *SACS* gene has recently been cloned. The protein encoded by *SACS*, sacsin, has a

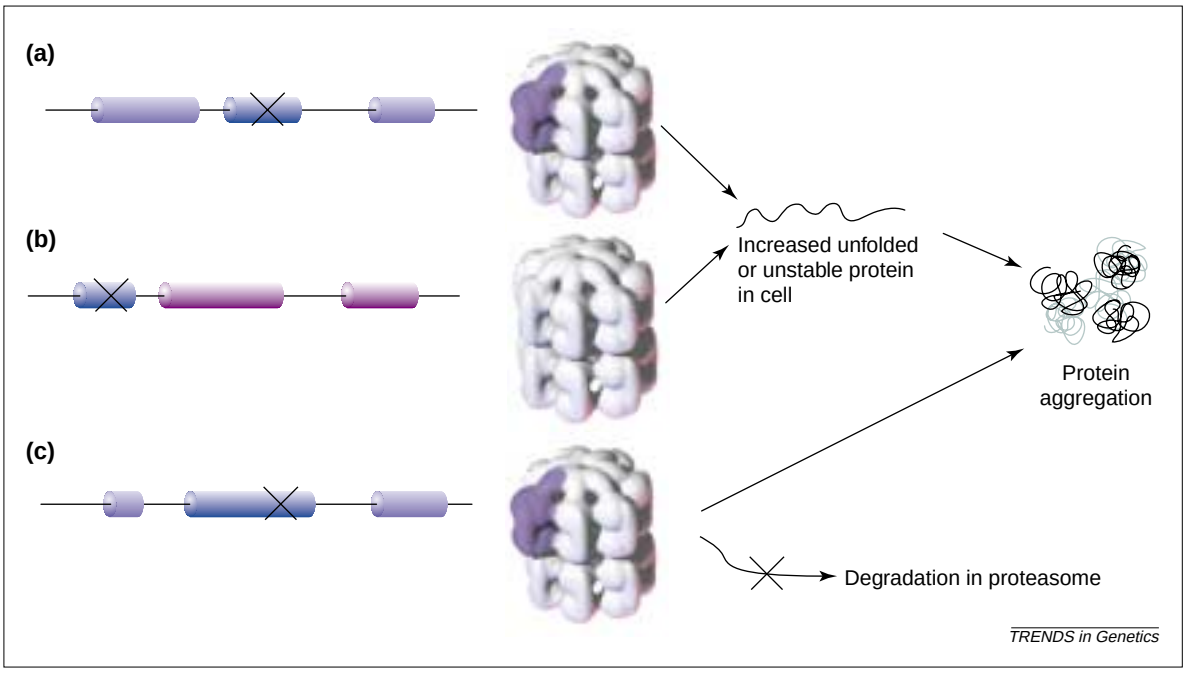

Fig. 2. Mechanisms of disease pathogenesis resulting from chaperone and chaperonin gene mutations or compromised chaperone function. (a) Mutations in chaperone or chaperonin genes create a defective subunit unable to bind substrate or create a subunit capable of binding substrate at reduced efficiency. (b) Mutations in a gene produce an unstable protein or an expanded polyQ tract prone to increased aggregation. (c) Mutations in a gene impair the ability of a chaperone to target misfolded protein for degradation in the proteasome. The above mechanisms create increased levels of unfolded or unstable protein in the cell, leading to increased protein aggregation.

'DnaJ' domain near the C-terminal portion of the protein and two domains with sequence similarity to the N-terminus of Hsp90 (Ref. 30). Mutations in the YDJ1 gene, a member of the DnaJ chaperone family in yeast, have been shown to alter the expression levels of three Hsp90 substrates in *Saccharomyces cerevisiae*[31]. High conservation of Hsp90 functions between humans and yeast suggest that sacsin could also interact with Hsp90 substrates and have a function in chaperone-mediated protein folding.

Small Hsps (Ref. 32) contain a characteristic, conserved 80–100 amino acid core named the α-crystallin domain. This domain is similar to the α-crystallin protein from the vertebrate eye lens that is thought to prevent protein precipitation in the lens. The α-crystallin domain of sHsps is composed of αA-crystallin and αB-crystallin subunits. The R120G mutation in the human αB-crystallin gene (*CRYAB*) causes DESMIN-related myopathy, an adult onset disorder characterized by cataracts and myopathy of the skeletal and cardiac muscle resulting from aggregates of the protein desmin[33]. Desmin-producing cell lines transfected with mutant R120G constructs and labeled with anti-αB-crystallin antibody show perinuclear and cytoplasmic aggregation of αB-crystallin compared with an even distribution of αB-crystallin throughout the cytoplasm in wild-type cells[33]. The aggregates of αB-crystallin protein are caused by a disturbance of chaperone activity and/or disruption to the tertiary structure of the αB-crystallin[34,35]. Interestingly, cultured lens epithelial cells from mice lacking the murine αB-crystallin gene are hyperproliferative with clones of abnormal ploidy[36], suggesting that the lens epithelial cells of the knockout mice are more prone to genomic instability. A similar mutation (R116C) in the human αA-crystallin gene causes congenital cataracts and microcornea[37]. Mutations in the human desmin gene (*DES*) also cause a desmin-related myopathy, hypothesized to be because of aggregation of intermediate filaments and impairment of filament assembly[38].

Chaperones and neurodegenerative diseases

The pathogenesis of many neurodegenerative diseases appears to involve aberrant protein folding that causes a conformational rearrangement and results in protein aggregation and subsequent tissue deposition of the protein or cell degeneration. These include the POLYGLUTAMINE (POLYQ) TRACT EXPANSION DISEASES, Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis and Creutzfeldt–Jakob disease[39,40]. Chaperones are implicated as modulators of disease pathology in the neurodegenerative diseases by their ability to modify protein aggregation (Fig. 2). However, the significance of protein aggregation in neuronal toxicity and cell death remains controversial.

PolyQ tract diseases

The PolyQ tract diseases are a group of inherited neurodegenerative disorders characterized by gene mutations causing an expanded polyglutamine domain within the mutant protein, resulting in

protein misfolding and aggregation. Examples of polyQ tract diseases include spinocerebellar atrophy (SCA), spinal bulbar muscular atrophy and Huntington disease[39].

Chaperones have increasingly been recognized as important in the polyQ tract diseases. The chaperone HDJ-2/HSDJ, a human DnaJ homologue, colocalized with ataxin-1 aggregates in the affected neurons in a brain tissue sample from a patient with SCA type 1 and in transgenic mice expressing mutant ataxin-1 (Ref. 41). The ataxin-1 aggregates were reduced by the overexpression of this chaperone in HeLa cells cotransfected with ataxin-1 and HDJ-2/HSDJ (Ref. 41). This finding implies that protein misfolding is responsible for the nuclear aggregates seen in SCA type 1, with chaperone overexpression allowing recognition of the abnormal polypeptide and then refolding or initiation of ubiquitin-dependent degradation.

Both Hsp40 and Hsp70 are found in intranuclear ataxin-3 aggregates in SCA type 3 (Machado–Joseph disease). Overexpression of the Hsp40 chaperones in transfected HeLa and COS7 cells suppressed aggregation of the mutant ataxin-3 (Ref. 42). There is some evidence to suggest that these responses are chaperone specific[42]. Chaperone overexpression also reduces protein aggregation in spinal bulbar muscular atrophy[43], and bacterial and yeast chaperones decrease aggregate formation and cell death in cell culture models of Huntington disease[44]. The length of the polyQ repeat directly correlated with the degree of protein aggregation in monkey kidney cells transfected with vectors containing full-length and truncated Huntington disease cDNA and in transgenic mice expressing the mutant huntingtin protein[45].

The precise relationship between the polyQ repeats, protein aggregation and the mechanism of cell toxicity is unknown. It has been hypothesized that polyQ tract expansions cause a conformational change in the mutant protein that promotes the aggregation of the protein into INCLUSION BODIES[46,47]. The abnormal aggregation increases chaperone expression as part of a protective cellular response to stabilize the mutant protein and reduce the cellular toxicity resulting from an inappropriate association of nuclear components[41]. The suppression of polyQ toxicity by chaperones Hsp70 and dHdj1 (Hsp40 homolog) in *Drosophila* has been associated with an alteration of the structure and, hence, a change in the solubility of the mutant protein[47]. However, cell toxicity can occur independently from protein aggregation, as overexpression of the chaperone HSPA1L (homologous to human Hsp40) modulates neurotoxicity without an observable effect on protein aggregation in a *Drosophila* model of human polyQ disease[48].

Alternatively, neurodegeneration in the polyQ tract expansion diseases could be due to a change in transcription or transcriptional regulation as a result of the mutant protein adopting a new conformation. Significant changes in transcription result from the expression of mutant ataxin-1 protein in mice[49]. It has also been suggested that the insoluble aggregates could cause neurodegeneration by interfering with axonal transport or apoptosis. Evidence for the involvement of apoptosis is provided by the generation of active caspases in primary rat neurons transfected with an expanded polyQ repeat involving the ataxin-3 gene (Q79) (Ref. 50). Lastly, the involvement of proteolytic pathways is supported by the finding of co-localization of the PA700 protein subunit with nuclear and cytoplasmic aggregates of an androgen receptor (AR) containing 48 glutamines (ARQ48) in transfected HeLa cells[51]. The PA700 protein subunit is a component of the proteasome involved in recognition of ubiquitin-modified substrates and targeting of the substrates for ATP-dependent degradation[51].

Other neurological diseases

Chaperones have also been demonstrated to be involved in neurological conditions other than the polyQ tract diseases. Mutations in the presenilin-1 (*PS-1*) and presenilin-2 (*PS-2*) genes cause early-onset Alzheimer disease by a mechanism that increases the production of AMYLOID-β PEPTIDE aggregates from amyloid precursor protein (APP) in the human cortex[52]. The chaperone BIP/GRP78 interacts with APP in the endoplasmic recticulum (ER) in mammalian cells[53]. The induction of chaperone BiP/GRP78 mRNA was significantly inhibited in transfected *PS1* mutant neuroblastoma cells exposed to tunicamycin, a drug that induces ER stress by preventing protein glycosylation[54]. *PS1* mutants might, therefore, downregulate chaperone function in the ER in mammalian cells, increasing the susceptibility of cells to ER stress and increasing the accessibility of APP to cleavage by secretase enzymes to generate shorter, more amyloidogenic peptides[52].

Aberrant protein degradation is also implicated in autosomal-recessive juvenile Parkinson disease (ARJP), a condition characterized by selective dopaminergic cell death and the accumulation of unidentified proteins without LEWY BODIES[55]. ARJP is caused by mutations in *PARK2*, rendering the gene product, parkin, unable to function as a ubiquitin-protein ligase and resulting in reduced protein degradation and subsequent protein accumulation[55].

Amyotrophic lateral sclerosis (ALS1) is caused by mutations in the Cu/Zn-superoxide dismutase (*SOD-1)* gene, resulting in misfolding and destabilization of the SOD-1 protein[56]. The unstable SOD-1 proteins form aggregates in the cytoplasm of cultured motor neurons expressing different SOD-1 mutations, in the motor neurons of transgenic mice,

and are found in the cells of affected patients at autopsy. The expression and activity of chaperone proteins Hsp70, Hsp27 and αB-crystallin was increased in NIH 3T3 cells stably expressing mutant *SOD-1*, thus depleting the cellular pool of chaperones available for the stress response. That ALS occurs only in the central nervous system (CNS) is attributed to non-CNS cells being able to upregulate chaperone expression, whereas CNS cells cannot[56].

Chaperones as modifiers of missense mutations that cause protein instability

Missense mutations can cause instability of the abnormal protein and/or defects in protein folding, with disease resulting from an inability of the protein to fold, a 'toxic' fold, or mislocalization of the misfolded protein[57]. Elevated levels of incorrectly folded protein can induce Hsp expression as part of a cellular response to increased levels of misfolded protein. For example, in cystic fibrosis, the common ΔF508 mutation results in a cystic fibrosis transmembrane conductance regulator (CFTR) protein that is unable to fold correctly and that is retained in the ER (Ref. 57). Hsp70 and Hsp90 chaperones have been shown to interact with the mutant CFTR protein in BHK cells[58] and Hsc70 inhibits the aggregation of mutant CFTR protein in *Escherichia coli* cells[59]. Modulation of the deleterious effects of mutations by chaperone proteins could explain phenotypic variability in individuals who have the same substrate gene mutation associated with different chaperone responses or variations in chaperone genes.

Several studies have examined the effects of chaperonin overexpression on missense mutations in cellular or bacterial systems. In phenylketonuria (PKU), reduced protein activity resulting from missense mutations in the phenylalanine hydroxylase (PAH) gene was rescued by chaperonin co-overexpression in *E. coli* cells[60]. The amount of mutant PAH protein was related to chaperonin activity and temperature conditions[60]. Similarly, in medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, several missense mutations (G447A, G799A and A1055G) resulted in abnormal proteins with decreased enzyme activity, and the enzyme activity of the mutant proteins could be partially recovered by co-overexpression of GroEL in *E. coli* cells[61,62]. These responses appear to be mutation specific, as two other mutations (A577G and G583A) produced a protein that showed little response to chaperonin co-overexpression[62]. In ethylmalonic aciduria, the mutant protein resulting from the G209S amino acid substitution in the short-chain acyl-CoA dehydrogenase (SCAD) could be folded into the correct conformation as efficiently as the wild-type protein in COS cells, but only in the presence of excessive amounts of chaperonins[63]. Together, these results suggest that chaperonin proteins could be significant modifiers of the phenotype of metabolic diseases.

The ability of chaperones to compensate for mutations in other genes has also been elegantly demonstrated in *Drosophila* (Ref. 64). In these experiments, Hsp90 was able to mask the effect of mutations, perhaps by facilitating correct protein folding despite an alteration in the amino acid sequence of the protein. However, when Hsp90 was diverted from this protective role by heat shock or inhibition with geldanamycin, the altered proteins were no longer stabilized and the phenotypic effects of the mutations were revealed[64]. The majority of the 'hidden' mutations were deleterious, but occasionally, the mutations produced an adaptive response. Hsp90 therefore conferred a capacity to store or buffer genetic changes and provided *Drosophila* with the ability to evolve rapidly in response to cellular stress.

Conclusion

The recent intense interest in chaperone proteins has provided significant advances in medical and scientific knowledge concerning these proteins. It is now clear that there are several diseases in which chaperones have a causative role. Mutations in genes for proteins with chaperone-like domains, in putative chaperonin proteins and in genes encoding small heat shock proteins can produce disease, most probably related to an inability of the abnormal chaperone protein to perform its designated function. In neurodegenerative diseases caused by expanded polyQ repeats or an accumulation of abnormal protein aggregates, chaperone overexpression has been shown to modify the disease phenotype in model systems, possibly as a consequence of the ability of the chaperone to influence protein aggregation and resulting in altered solubility of the mutant protein. However, the exact mechanism has not been fully determined. Chaperones and chaperonins could also prove to be important in human diseases caused by missense mutations that produce an unstable substrate protein, because of the potential of the chaperones to modulate the effects of these substrate mutations by repeated folding or stabilization of the substrate to prevent cellular toxicity. Future research could uncover therapeutic methods for influencing chaperone responses to exploit the potential of these proteins to fold non-native polypeptides or to initiate the degradation of unstable or abnormal proteins.

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