

## Protein Misfolding and Aggregation in Neurodegenerative Disorders: Focus on Chaperone-Mediated Protein Folding Machinery

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### ABSTRACT

Proteins are structurally complex biomolecules which regulate fundamental processes of the cellular systems. An integrated network of molecular chaperones, ubiquitin proteasome system and autophagy regulate the cellular protein quality and maintain the balance between folded and unfolded proteins. However, several chronic challenges such as aging related physiological changes, diseases and certain stress conditions interfere with protein functioning. This leads to formation and accumulation of potentially toxic proteinaceous species in the cellular system. It is increasingly clear now that impairment(s) of protein quality control system is one of the common mechanisms, which leads to onset and progression of various human diseases including neurodegenerative disorders. Occurrence of cellular fibrillar protein aggregates marks the signature feature shared by most of these disorders. Molecular chaperones are the key regulators of the protein quality control machinery which have been identified as modulators of neurodegenerative diseases. They have been found to play essential role in eliminating toxic proteins by either inhibiting or promoting the process of aggregate formation. This review focuses on elucidating the role of chaperone-mediated protein folding and its application as a promising choice for development of novel therapeutic strategies against neurodegenerative disorders.

### INTRODUCTION

Proteins are delicate, versatile and structurally complex biomolecules which regulate fundamental processes of the cellular systems. They are synthesized as long stretches of amino acid chains and in order to achieve the functional state each polypeptide chain must be folded into a unique 3-dimensional structure<sup>[1]</sup>. However, proteins have a very narrow range of thermodynamically stable physiological environment inside the cells to achieve correct folding and to function<sup>[2]</sup>. In addition, several chronic challenges such as aging related physiological changes, diseases and certain stress conditions also interfere with protein functioning<sup>[2]</sup>. Therefore, how the cells manage their proteome and ensure their metastable conformations to retain the conformational flexibility in the ever changing cellular environment has emerged as a fundamental question in contemporary biomedical research.

Cellular system has developed a specialized folding machinery which has been optimised to function in co-operation with ribosome dependent protein synthesis system<sup>[3]</sup>. However, it has been estimated that only a fraction of the newly synthesized protein accomplish the functional folded state and others do not attain the ordered 3-dimensional structure, but have the potential to adopt folding conformation only after interaction with some folding substrates<sup>[4]</sup>. In a crowding environment where the cytosolic protein concentration

reaches upto ~300-400 gram/litre ( $g\ l^{-1}$ ), the folding becomes more challenging and the proteins are at great risk to adopt aberrant folding and aggregation<sup>[5]</sup>. This leads to formation and accumulation of potentially toxic proteinaceous species in the cellular system<sup>[6]</sup>. Therefore, cells have to routinely cope up with the problems of misfolded proteins owing to the rapidly changing cellular homeostasis associated with varying physiology and stressful conditions<sup>[7]</sup>. In view of the above, cellular system has developed a well-balanced protein quality control system to maintain proteome homeostasis which is essential for its normal functioning. An integrated network of molecular chaperones, ubiquitin proteasome system and autophagy system regulate the cellular protein quality and maintain the balance between folded and folding proteins<sup>[8]</sup>. Chaperones, in cooperation with their regulators assist de-novo or refolding of the nascent and denatured proteins respectively, along with selective removal of the irreversibly folded and aggregated proteins<sup>[9,10]</sup>. It has been well demonstrated that impairments of protein quality control system is one of the common mechanisms which leads to onset and progression of various human diseases including neurodegenerative disorders<sup>[11,12]</sup>. Present article has been divided in two sections: first section provides a brief overview of the chaperone based cellular protein folding and quality control machinery, and second part focuses on the impact of impaired folding machinery on onset and progression of neurodegenerative disorders. In addition, some recent findings on the ability of chaperones to suppress such fatal disorders have also been discussed.

## MOLECULAR CHAPERONS IN PROTEIN FOLDING AND QUALITY CONTROL

Physiological and environmental stress such as aging, diseases, heat, heavy metal ions and UV exposure induce damages to cellular proteins and accelerate imbalances in the cellular proteome<sup>[13]</sup>. Molecular chaperones are protective proteins which assist efficient folding/refolding of newly synthesized and denatured proteins, without being part of the final confirmation of the native protein<sup>[9]</sup>. Originally, chaperons were identified as proteins that were overexpressed in response to stress<sup>[14]</sup>. Later, functions of these proteins were also found to be essential to maintain the balance between de-novo folding and regulation of degradation pathways under normal physiological conditions. Nevertheless, despite of its constitutive levels, the expression and synthesis of these proteins greatly increases during the course of stress and therefore, these group of proteins are also known as heat shock proteins (Hsps)<sup>[7]</sup>. Classified according to the molecular weight and homologies, cells consist of several classes of chaperones such as Hsp40, Hsp60, Hsp70, Hsp90, Hsp100 and the small Hsps (sHsps)<sup>[15]</sup>. In the cellular system, three classes of chaperones - Hsp70, Hsp60 and Hsp90 - largely participate in de-novo folding and refolding of proteins in an ATP dependent manner<sup>[16]</sup>.

Chaperones start functioning from the point where stretches of nascent polypeptide chain get released from the polyribosomes<sup>[17]</sup>. Molecular chaperones recognize hydrophobic residues or unstructured peptide bonds exposed by non-native proteins. Such hydrophobic residues or unstructured peptide bonds are buried inside during native confirmation. Several chaperones such as trigger factors, ribosome binding Hsp70s, nascent chain associated complex (NAC), ribosome associated complex (RAC) etc. bind to the nascent proteins and assist co-translational folding<sup>[3]</sup>. However, co-translational folding is possible only for small and single domain proteins and majority of the cellular proteins are multi-domain and large. When such proteins are released in the cytoplasm, exposed non-native structures in the

nascent proteins trigger self-aggregation<sup>[6]</sup>. Hsp70 along with several other molecular chaperones plays a central role in identification and stabilization of such newly synthesized unfolded proteins. Hsp70 is a ubiquitously expressed, stress inducible multifunctional chaperone which mediates a variety of biological processes, including folding, targeting, degradation and interactions of proteins<sup>[16]</sup>. Hsp70 consists of a N-terminal ATP-binding domain (NBD) and a substrate binding domain (SBD) near the C-terminal lid region<sup>[18]</sup>. Co-factors of Hsp70 include Hsp40/DnaJ family proteins and nucleotide exchange factor which assist cycles of substrate binding and release in an ATP dependent manner<sup>[19]</sup>.

Successive rounds of Hsp70 mediated folding results in the formation of fully folded substrates. However, generation of terminally misfolded or slowly folding species is also possible during this phenomenon. In view of above, selective degradation of misfolded protein is needed to avoid accumulation of potentially toxic aggregates. The folding pathway continues to correct the slowly folding or misfolded species for which Hsp70-substrate complex recruits Hsp90 and other chaperones. Hsp90 is a crucial regulator of protein homeostasis which also functions via an ATP-dependent hydrolytic cycle<sup>[16]</sup>. Hsp90 acts downstream of Hsp70 and assists in achieving conformational maturation of various signal transduction proteins including kinases and many of the steroid hormone receptors<sup>[16]</sup>. Similar to Hsp70, Hsp90 has several regulators and co-chaperons such as Hop, CDC37, AHA1, p23 etc. which assist in attaining correct folding and stability of their substrates<sup>[20]</sup>. Hop allows substrate transfer through its tetratricopeptide repeat (TPR) domain and acts as a direct link between Hsp70 and Hsp90<sup>[21]</sup>. Moreover, Hsp40/Hsp70 and Hsp90 have also been reported to be involved in regulating ubiquitin proteasome system and autophagy<sup>[16]</sup>. This implies the fact that chaperones also facilitate timely removal of misfolded proteins. Quality control of misfolded proteins generated by Hsp70 and Hsp90 folding machinery is found to be further regulated by a different TPR domain-containing co-chaperones such as C terminus domain of Hsc70 interacting protein (CHIP)<sup>[22]</sup>. CHIP associates with Hsp70 or Hsp90 through its TPR domain and ubiquitinates misfolded substrates and target them for endoplasmic-reticulum-associated protein degradation pathway<sup>[22]</sup>. Interestingly, many of the Hsp90 substrates also include oncogenic proteins like kinases and transcription factors, documenting its role in tumor development<sup>[23]</sup>. Therefore, inhibition of Hsp90 has emerged as a novel therapeutic approach in cancer treatment.

As discussed earlier, protein folding in cytosol is generally achieved by Hsp70 along with downstream chaperones and chaperonins. Chaperonins are large multimember ring complexes that provide posttranslational folding of proteins in an isolated compartment, unimpaired by aggregation<sup>[24]</sup>. They promote folding of cytosolic proteins with size upto ~60 kDa; which are large, slow folding and aggregation sensitive in both prokaryotes and eukaryotes. It has been proposed that Hsp70 and Hsp60 chaperone machinery act sequentially, where the previous acts upstream with nascent polypeptide and the latter functions downstream and assists in folding of those substrates which have failed to reach to the final states by Hsp70 alone<sup>[16]</sup>.

Eukaryotic Hsp60 represents group I chaperonins which includes GroEL found in bacteria, mitochondria and chloroplast with its seven membered ring structure. GroEL functionally cooperates with GroES (Hsp10 in eukaryotes) which forms a lid on the folding cage and together they represent the most extensively studied protein folding machinery<sup>[25]</sup>. The encapsulated protein folds at the expense of ATP and the folded product is released from the cage again by ATP dependent dissociation of GroES<sup>[26]</sup>. Another group of eukaryotic

chaperonins known as TRiC/CCT, also exhibit an eight membered ring and function independent of the Hsp10 factor<sup>[27]</sup>. Interestingly, apical domain protrusion of TRiC replaces the role of lid during cage formation<sup>[27]</sup>. TRiC, with a longer folding time as compared to GroEL folds approximately 10% of the total cytosolic proteins including large multi-domain actin and tubulin proteins<sup>[28]</sup>. Interestingly, TRiC has also been found to prevent protein aggregation in some protein folding disease models<sup>[28]</sup>. One of the primary difference between GroEL and TRiC is that the previous acts strictly post-translationally while the later functions co-translationally to fold large proteins of 30 to 120 kDa size.

Role of sHsps and Hsp100 is also crucial in maintaining protein quality and cellular proteome homeostasis. sHsps similar to Hsp40 function independently during ATP reaction cycle<sup>[29]</sup>. sHsps bind to misfolded proteins and remain bound until they are released to the Hsp70/Hsp40 system, and consequently, prevent the unfolded proteins from aggregate formation<sup>[30]</sup>. Hsp100 family includes chaperones such as bacterial ClpB, yeast Hsp104 etc. which form another class of ATP driven folding system<sup>[31]</sup>. In co-operation with Hsp70/Hsp40, the hexameric ring like assemblies of these chaperones perform unfolding reaction followed by refolding of the substrate to attain native conformation<sup>[32]</sup>. Similar function is performed by Hsp110 in higher eukaryotes which refold and disaggregate the protein aggregates<sup>[32]</sup>.

## MOLECULAR CHAPERONES AND NEURODEGENERATIVE DISORDERS

Neurodegenerative disorders are a class of late-onset progressive disorders characterized by presence of misfolded protein aggregates in affected neurons and selective degeneration of the brain. Alzheimer's disease (AD), Parkinson's disease (PD) and polyglutamine (polyQ) disorders such as several Spinocerebellar ataxias, Huntington's disease (HD) etc. represent this category of neurodegenerative disorders. The protein aggregates in each case are formed as a result of protein misfolding which might occur due to genetic or environmental stresses. Most often the disease causing protein undergoes enrichment of the  $\beta$  sheet conformation during misfolding. Interestingly, none of these proteins exhibit  $\beta$  sheet conformation in their wild type state. For instance, Huntingtin (Htt) protein which is responsible for causing HD is a 3140 amino acid protein with a molecular mass of 349 kDa and has been reported to acquire an  $\alpha$ -helical/random coil conformation<sup>[33]</sup>. Similarly,  $\alpha$ -synuclein, the major component of Lewy bodies in PD is a 14 kDa protein which although has a low content of ordered structure but adopts a N-terminal  $\alpha$ -helical pattern otherwise<sup>[34]</sup>. In addition, A $\beta$  peptide which is involved in pathogenesis of AD is derived from the proteolytic cleavage of the  $\beta$ -amyloid precursor protein ( $\beta$ APP) and generally exists as a mixture of  $\beta$  sheet and random coil instead of a full-length  $\beta$  sheet conformer<sup>[35]</sup>.

## FORMATION OF PROTEIN AGGREGATES - A COMMON LINK BETWEEN VARIOUS NEURODEGENERATIVE DISORDERS

Conformational changes and protein misfolding result in amyloid-like aggregate formation which is a hallmark of most neurodegenerative diseases. For instance, HD is a dominantly inherited neurodegenerative disorder caused by expansion of CAG trinucleotide repeat in the coding region of the huntingtin (htt)

gene<sup>[36]</sup>. The exact cellular function of Htt is still debatable, however, it has been proposed to participate in vesicular transport and cell signaling<sup>[37]</sup>. Mutant Htt with expanded poly(Q) repeats tends to misfold and aggregates in the form of inclusion bodies in the cells<sup>[38]</sup>. Inclusion body formation results in pathological changes in the basal ganglia and cortical neurons of the brain<sup>[38]</sup>. PD on the other hand is a disorder of the central nervous system caused by mutations in the  $\alpha$ -synuclein gene. Types of mutations in cases of PD may range from missense mutations to gene duplication or triplication. These alterations lead to the formation of mutant  $\alpha$ -synuclein-containing aggregates known as Lewy bodies, the main pathological feature of PD<sup>[39]</sup>. Although the precise physiological function of  $\alpha$ -synuclein is undetermined, it has been found to be ubiquitously expressed in the entire central nervous system. Subsequently, Alzheimer's disease (AD) could be characterized as a typical protein misfolding disorder that leads to loss of neurons and synapses in the cerebral cortex and subcortical regions of the brain. AD pathogenesis is resulted by accumulation of misfolded A $\beta$  peptides and the tau protein<sup>[40]</sup>. While  $\beta$ APP, the precursor for A $\beta$ , is a transmembrane protein, tau is a microtubule-associated protein. Both undergo abnormal post-translational modifications to give rise to A $\beta$  and hyperphosphorylated tau and form major constituents of disease causing senile plaques and neurofibrillary tangles respectively<sup>[41]</sup>. Therefore, in view of the fact that protein misfolding serves as the root cause in most of neurodegenerative disorders, next section of the article discusses about major events which lead to disease pathogenesis and also provides a brief overview of the role of chaperones in alleviating aggregate-mediated cytotoxicity.

The presence of fibrillar protein aggregates marks the signature feature shared by most of the neurodegenerative disorders. Such aggregates were reported for the first time about a century ago by Alois Alzheimer in brain parenchymal tissues of mentally retarded patients<sup>[42]</sup>. Proteinaceous deposits or aggregates are considered to be the end products of "specific" interactions among partially folded but unstructured protein intermediates<sup>[43]</sup>. The process of polymerization involves explicit interactions between already deposited initial aggregates, the "seeds" and the newly emerged unfolded monomers<sup>[44]</sup>. In view of above, it has been proposed that misfolded protein monomers trigger the process of aggregate formation and disease pathogenesis<sup>[44]</sup>. Several studies suggest that undesirable conformational changes of corresponding proteins underlie the cause of disease pathogenicity<sup>[34]</sup>. One of the following reasons may result in transformation of a natively folded or even nascent polypeptide chain into a pathogenic misfolded conformation: (a) impairment of chaperone based protein folding machinery due to aging or environmental stresses (eg. wild type  $\alpha$ -synuclein in sporadic cases of PD) (b) genetic mutations in the protein coding region of the corresponding gene (eg. CAG expansion in exon 1 of htt) (c) stress mediated atypical post-translational modifications of the target protein (eg. hyperphosphorylation of the tau protein in AD) (d) precursor protein cleavage by proteolytic enzymes (eg. fragmentation of  $\beta$ -amyloid precursor protein in AD) (e) structural modifications driven by environmental changes such as oxidative stress, and (f) induced protein misfolding due to aggregation seeding, cross-seeding mechanisms<sup>[45]</sup>. The listed events, except for the occurrence of CAG expansion, entail changes in the secondary and/or tertiary structure of the target protein without any variation in the primary structure. This shows that the information needed for protein misfolding (except CAG expansion) is not encoded by the genome in sporadic cases of neurodegenerative disorders. Therefore, the protein aggregates found in neurodegenerative disorders share common structural and biochemical

features as per the altered confirmation of the constituent proteins.

## SHARED MECHANISM OF PROTEIN AGGREGATE FORMATION IN NEURODEGENERATIVE DISORDERS

As a nascent polypeptide chain emerges out of the translating ribosome, numerous hydrophobic amino acid stretches get exposed to the cellular milieu, which must be stabilized to ensure non-aggregated states of the protein. At this point, the polypeptide chain can theoretically adopt countless number of conformations out of which only one is sufficiently stable under physiological conditions and could take up the biologically active state. In the case of large and multidomain proteins, partially folded intermediates are produced during the folding process which often causes the “wrong” domains to interact with each other leading to the production of kinetically stable non-native species<sup>[43]</sup>. These species expose hydrophobic segments to the solvent, an event that sparks off the aggregation process<sup>[35]</sup>. The equilibrium between the native and non-native species shifts towards the non-native flank when the protein harbors mutations (like CAG repeats) or under conditions of cellular stress or aging.

As discussed earlier, the process of aggregate formation follows a “seeding nucleation” model consisting of two distinct phases: the slow progressing lag phase and the fast moving log phase<sup>[46,47]</sup>. The lag phase begins with the misfolded protein taking up an unusual  $\beta$  sheet conformation which initiates filament formation by cooperative aggregation of similar misfolded proteins. Such initial oligomeric assembly serves as a template or “seed” for the elongation process which is said to constitute the log phase of the aggregation process<sup>[46,47]</sup>. In the log phase, other misfolded protein molecules or even pre-formed seeds are recruited to the initial nuclei leading to rapid polymerisation of the filament and consequent formation of mature fibrillar structures<sup>[46,47]</sup>. These fibrillar deposits may then be further organized into larger aggregates or amyloid plaques. While aggregates are mostly sequestered and deposited intracellularly in the form of inclusion bodies, amyloid plaques are generally found outside the cell<sup>[47]</sup>. The intracellular inclusion bodies can be localized both in the cytoplasm as well as in the nucleus. Cytoplasmic inclusion bodies are most frequently deposited at the Microtubular Organisation Centre (MTOC or centrosome)<sup>[48]</sup>. The inclusion bodies are dense enough to sediment upon low speed centrifugation and could also be observed by light or electron microscopy<sup>[49]</sup>. Sometimes, the size of the inclusion bodies expands large enough to occupy approximately 30% of the cytosolic area. However, intra-nuclear protein inclusions are key features of most inherited neurodegenerative disorders and have been found to be more toxic than their cytoplasmic counterparts<sup>[49]</sup>. It has been proposed that neuronal inclusions sequester several key transcription factors through hydrophobic interactions and thus deplete the level of cellular transcriptional machinery<sup>[12]</sup>. Subsequently, a progressive increase in cellular toxicity may lead to activation of cell death pathways.

## MOLECULAR CHAPERONES – HANDY TOOLS TO MAKE OR BREAK CYTOTOXIC AGGREGATES

Compromised protein folding apparatus serves as one of the major cause for development of neurodegenerative disorders. Several

reports have indicated an early impairment of stress response in most of these disorders<sup>[50]</sup>. For instance, brain samples of sporadic AD and PD patients have been found to harbour an abnormally S-nitrosylated form of the endoplasmic reticulum (ER) chaperone protein disulphide isomerase (PDI)<sup>[51]</sup>. As a consequence, the anomalous chaperone is incapable of mounting an efficient ER stress response which is a basic necessity in all protein misfolding disorders. Similarly, a mutation in the gene encoding the co-chaperone for Grp78 (a crucial ER chaperone) has been shown to result in protein accumulation and neurodegeneration<sup>[52]</sup>. Moreover, mutations in genes encoding members of the Hsp family of chaperones that produce a dysfunctional protein have been found to be associated with several brain related disorders such as Hsp60-linked hereditary spastic paraplegia, Hsp27-linked distal hereditary motor neuropathy, Hsp22-linked distal motor neuropathy,  $\alpha$ B-crystallin-linked desmin-related myopathy, etc.<sup>[53]</sup>.

As discussed earlier, molecular chaperones represent key players of the protein quality control machinery and in this capacity they play essential role in eliminating toxic proteins by inhibiting or promoting the process of aggregate formation. Interestingly, molecular chaperones have been shown to be associated with Htt aggregates as well as with Lewy bodies in brain tissues of affected individuals<sup>[54,55]</sup>. Moreover, transgenic mouse models of SCA1, 3 and 7 have also been reported to exhibit such association<sup>[56]</sup>. It has been suggested that chaperones may either block the initial oligomerization of the misfolded proteins by preventing fibril formation or they may promote amyloid formation in an attempt to constrain the toxic species into benign aggregates<sup>[57,58]</sup>.

As discussed above, parts of the nascent polypeptides emerge out of the ribosome and associate themselves with Hsp70 chaperone to achieve favorable folding and to prevent undesirable interactions. Despite the available safeguards, if protein misfolding occurs due to one of the reasons discussed above, induction of additional Hsps takes place. For example, HeLa cells expressing ataxin-1 or ataxin-3 with expanded polyglutamine repeats exhibit induction of Hsp70<sup>[59,60]</sup>. Similarly, transgenic mouse models of HD also demonstrated upregulated Hsp levels<sup>[61]</sup>. In this context it is interesting to note that modulation of various Hsps has been found to function as excellent modifiers of protein misfolding neurodegenerative disorders. For example, overexpression of yeast Hsp70 homolog Ssa1 or Hsp40 homolog Ydj1 suppresses mutant Htt aggregate formation<sup>[11]</sup>. Similarly, Hsp70 and Hsp40 have been shown to reduce protein aggregation and alleviate neurotoxicity in animal and cell culture models of poly(Q) diseases<sup>[60,61]</sup>. Independent studies carried out on SCA3 *Drosophila* model also demonstrated inhibition of neurodegeneration upon targeted overexpression of Hsp70 and Hsp40<sup>[62]</sup>. Moreover,  $\alpha$ -synuclein-induced neuronal loss in a PD model of *Drosophila* is prevented by the expression of Hsp70<sup>[63]</sup>. Similarly, Hsp70 and Hsp40-mediated rescue of neurodegenerative phenotype has also been established in cases of familial amyotrophic lateral sclerosis (ALS)<sup>[64]</sup>.

In addition to Hsp70, other molecular chaperones have also been identified as modulators of neurodegenerative diseases. Hsp104, for instance, has been demonstrated to assist in solubilizing small amyloid-like aggregates in a yeast model of HD<sup>[65]</sup>. Furthermore, exogenous expression of Hsp104 in *C. elegans* and rats could also reduce aggregates and mitigates poly(Q) toxicity<sup>[66,67]</sup>. In addition to its own effect, small Hsps like Hsp26 in yeast and Hsp27 in rats have been shown to potentiate Hsp104-mediated suppression<sup>[65,67]</sup>. Hsp104 has also proved to be useful in PD rat model where it could exert its modifier effect by antagonizing  $\alpha$ -synuclein containing



oligomer formation leading to reduced dopaminergic degeneration<sup>[68]</sup>. Another co-chaperone to Hsp70 - CHIP (carboxy-terminus of Hsp70-interacting protein) is thought to act as an E3 ligase and target misfolded proteins for proteasomal degradation<sup>[57]</sup>. Over-expression of CHIP in HD and SCA3 cell lines exhibited increased ubiquitination and degradation of Htt and ataxin-3 respectively, which subsequently led to reduced poly(Q) aggregation and also averted cell death<sup>[69]</sup>. Similar kind of suppression was obtained in studies on primary neurons as well as with zebrafish models<sup>[70]</sup>. However, in spite of being marked for degradation, some mutant proteins often escape proteolysis and trap 19S subunit of the proteasome into the growing aggregates. This leads to inhibition of the ubiquitin-proteasome system and the eventual cellular dysfunction<sup>[71,72]</sup>. The cellular proteasomal machinery continues with its efforts to degrade the misfolded protein associated with it and as a result it may release more poly(Q)-containing fragments into the cytosol which in turn enrich the cellular pool of potentially more toxic oligomeric species.

Interestingly, several studies have reported that molecular chaperones could also promote protein aggregation in an attempt to minimize the presence of soluble oligomers in the cell. For example, HDJ2 (a human Hsp40 homologue), caused enhanced aggregation of mutant Htt in Cos-7 cells<sup>[73]</sup>. Likewise, chaperones belonging to the proteasomal machinery like Rpt4 and Rpt6 have also been shown to facilitate the process of aggregation for expanded-poly(Q) Htt as well as ataxin-3 proteins<sup>[74]</sup>. Similarly, TRiC (TCP-1 ring complex) chaperonin has been demonstrated to increase amyloid aggregation and suppress poly(Q) mediated toxicity in *C. elegans*, yeast and cell culture<sup>[75-77]</sup>. It has been demonstrated in yeast poly(Q) model that TRiC in conjunction with Ssa1 and Ydj1 could boost the conversion of low molecular weight soluble oligomers into high molecular weight aggregates which coincided with suppression of poly(Q) induced toxicity<sup>[76]</sup>.

Chaperones could also eliminate misfolded proteins by a unique type of autophagic mechanism known as Chaperone-mediated autophagy (CMA). CMA entails the use of a membrane translocation complex for delivery of the target proteins to the lysosomal lumen rather than canonical vesicles<sup>[78]</sup>. A recent study has found Hsp70 as an important player of this process<sup>[79]</sup>. Hsp70 may operate with other co-chaperones like Hsp40, Hsp90, hip, hop, and bag1 to facilitate aggregate clearance via CMA<sup>[79,80]</sup>. Among the proteins involved in neurodegenerative disorders,  $\alpha$ -synuclein was the first to be identified as a substrate for CMA<sup>[81]</sup>. These findings were later confirmed by studies on neuronal cell cultures as well as on model systems. Similarly, Htt cleavage products as well as mutant form of Htt were found to be the targets of CMA<sup>[82]</sup>. Intriguingly, CMA dysfunction has been shown to be associated with several neurodegenerative disorders<sup>[83]</sup>. In concordance with this observation, genetic upregulation of CMA proved to be protective against neurotoxicity<sup>[84,85]</sup>.

## CONCLUDING REMARKS

In brief, molecular chaperones not only assist in accurate folding of nascent polypeptide chains and denatured proteins but also participate in damage control in many neurodegenerative diseases. Several studies on model organisms including *Drosophila*, *C. elegans* and mice have shown that induction of chaperones can ameliorate disease pathogenicity either by preventing protein misfolding and aggregation or by promoting aggregate formation so that the abnormal proteins could be isolated in inclusions. In doing so, they may perform refolding events or direct members of the proteasome machinery to the site of damage. Therefore, molecular chaperones

occupy an important position in the list of probable genetic modifiers for neurodegenerative diseases, and therefore, are being considered as a promising choice for development of novel therapeutic strategies against neurodegenerative disorders. In fact, several studies have reported beneficial effects on disease symptoms upon administration of chaperone-inducible drugs. However, mechanistic details of chaperone action remain elusive. Further studies are needed to be carried out in the above area. Attempts should also be made to decipher the role of molecular chaperones as well as ubiquitin-proteasome system with respect to age-dependent progression of neurodegenerative phenotypes. Such a collective approach may help design effective treatment strategies against these devastating human disorders.

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## CONFLICT OF INTERESTS

The Author has no conflicts of interest to declare.

## REFERENCES

1. Dobson CM, Sali A, Karplus M. Protein folding—a perspective from theory and experiment. *Angew Chem Int Ed Engl* 1998; 37: 868-893.
2. Bartlett AI, Radford SE. An expanding arsenal of experimental methods yields an explosion of insights into protein folding mechanisms. *Nat Struct Mol Biol* 2009; 16: 582-588.
3. Preissler S, Deuerling E. Ribosome-associated chaperones as key players in proteostasis. *Trends Biochem Sci* 2012; 37: 274-283.
4. Frydman J. Folding of newly translated proteins in vivo: the role of molecular chaperones. *Annu Rev Biochem* 2001; 70: 603-647.
5. Zimmerman SB, Trach SO. Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli*. *J Mol Biol* 1991; 222: 599-620.
6. Ellis RJ, Minton AP. Protein aggregation in crowded environments. *Biol Chem* 2006; 387: 485-497.
7. Morimoto RI. The heat shock response: systems biology of proteotoxic stress in aging and disease. *Cold Spring Harb Symp Quant Biol* 2011; 76: 91-99.
8. Chen B, Retzlaff M, Roos T, Frydman J. Cellular strategies of protein quality control. *Cold Spring Harb Perspect Biol* 2011; 3: a004374.
9. Papsdorf K, Richter K. Protein folding, misfolding and quality control: the role of molecular chaperones. *Essays Biochem* 2014; 56: 53-68.
10. Nedelsky NB, Todd PK, Taylor JP. Autophagy and the ubiquitin-proteasome system: collaborators in neuroprotection. *Biochim Biophys Acta* 2008; 1782: 691-699.
11. Muchowski PJ. Protein misfolding, amyloid formation and neurodegeneration: a critical role for molecular chaperones? *Neuron* 2002; 35: 9-12.
12. Sakahira H, Breuer P, Hayer-Hartl MK, Hartl FU. Molecular chaperones as modulators of polyglutamine protein aggregation and toxicity. *Proc Natl Acad Sci USA* 2002; 99: 16412-16418.
13. Ashburner M, Bonner JJ. The induction of gene activity in *Drosophila* by heat shock. *Cell* 1979; 17: 241-254.

14. Morimoto RI. Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. *Genes Dev* 2008; 22: 1427-1438.
15. Li Z, Srivastava P. Heat-shock proteins. *Curr Protoc Immunol* 2004; Appendix 1: Appendix 1T.
16. Sarkar S, Singh MD, Yadav R, Arunkumar KP, Pittman GW. Heat shock proteins: Molecules with assorted functions. *Front Biol* 2011; 6: 312-327.
17. Wegrzyn RD, Deuerling E. Molecular guardians for newborn proteins: ribosome-associated chaperones and their role in protein folding. *Cell Mol Life Sci* 2005; 62: 2727-2738.
18. Zhu X, Zhao X, Burkholder WF, Gragerov A, Ogata CM, Gottesman ME, Hendrickson WA. Structural analysis of substrate binding by the molecular chaperone DnaK. *Science* 1996; 272: 1606-1614.
19. Mayer MP, Bukau B. Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol Life Sci* 2005; 62: 670-684.
20. Li J, Soroka J, Buchner J. The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. *Biochim Biophys Acta* 2012; 1823: 624-635.
21. Pratt WB, Toft DO. Regulation of signaling protein function and trafficking by the Hsp90/Hsp70-based chaperone machinery. *Exp Biol Med* (Maywood) 2003; 228: 111-133.
22. Cyr DM, Höhfeld J, Patterson C. Protein quality control. U-box-containing E3 ubiquitin ligases join the fold. *Trends Biochem Sci* 2002; 27: 368-337.
23. Calderwood SK. Molecular cochaperones: tumor growth and cancer treatment. *Scientifica* (Cairo) 2013; 2013: 217513.
24. Hartl FU, Hayer-Hartl M. Converging concepts of protein folding in vitro and in vivo. *Nat Struct Mol Biol* 2009; 16: 574-581.
25. Horwich AL, Fenton WA. Chaperonin-mediated protein folding: using a central cavity to kinetically assist polypeptide chain folding. *Q Rev Biophys* 2009; 42: 83-116.
26. Xu Z, Horwich AL, Sigler PB. The crystal structure of the asymmetric GroEL-GroES-(ADP)<sub>7</sub> chaperonin complex. *Nature* 1997; 388: 741-750.
27. Douglas NR, Reissmann S, Zhang J, Chen B, Jakana J, Kumar R, Chiu W, Frydman J. Dual action of ATP hydrolysis couples lid closure to substrate release into the group II chaperonin chamber. *Cell* 2011; 144: 240-252.
28. Kabir MA, Uddin W, Narayanan A, Reddy PK, Jairajpuri MA, Sherman F, Ahmad Z. Functional Subunits of Eukaryotic Chaperonin CCT/TRiC in Protein Folding. *J Amino Acids* 2011; 2011: 843206.
29. Jakob U, Gaestel M, Engel K, Buchner J. Small heat shock proteins are molecular chaperones. *J Biol Chem* 1993; 268: 1517-1520.
30. Sun Y, MacRae TH. Small heat shock proteins: molecular structure and chaperone function. *Cell Mol Life Sci* 2005; 62: 2460-2476.
31. Weber-Ban EU, Reid BG, Miranker AD, Horwich AL. Global unfolding of a substrate protein by the Hsp100 chaperone ClpA. *Nature* 1999; 401: 90-103.
32. Dragovic Z, Broadley SA, Shomura Y, Bracher A, Hartl FU. Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. *EMBO J* 2006; 25: 2519-2528.
33. Masino L, Pastore A. A structural approach to trinucleotide expansion diseases. *Brain Res Bull* 2001; 56: 183-189.
34. Winklhofer KF, Tatzelt J, Haass C. The two faces of protein misfolding: gain- and loss-of-function in neurodegenerative diseases. *EMBO J* 2008; 27: 336-349.
35. Barrow CJ, Yasuda A, Kenny PT, Zagorski MG. Solution conformations and aggregational properties of synthetic amyloid beta-peptides of Alzheimer's disease. Analysis of circular dichroism spectra. *J Mol Biol* 1992; 225: 1075-1093.
36. Huntington's disease Collaborative Research group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993; 72: 971-983.
37. Cattaneo E, Zuccato C, Tartari M. "Normal huntingtin function: an alternative approach to Huntington's disease". *Nat Rev Neurosci* 2005; 6: 919-930.
38. Legleiter J, Mitchell E, Lotz GP, Sapp E, Ng C, DiFiglia M, Thompson LM, Muchowski PJ. Mutant huntingtin fragments form oligomers in a polyglutamine length-dependent manner in vitro and in vivo. *J Biol Chem* 2010; 285: 14777-14790.
39. Lesage S, Brice A. "Parkinson's disease: from monogenic forms to genetic susceptibility factors". *Hum Mol Genet* 2009; 18: R48-R59.
40. Hashimoto M, Rockenstein E, Crews L, Masliah E. Role of Protein Aggregation in Mitochondrial Dysfunction and Neurodegeneration in Alzheimer's and Parkinson's Diseases. *Neuromolecular Medicine* 2003; 4: 21-36.
41. Tiraboschi P, Hansen LA, Thal LJ, Corey-Bloom J. The Importance of Neuritic Plaques and Tangles to the Development and Evolution of AD. *Neurology* 2004; 62: 1984-1989.
42. Alzheimer A. Über eine eigenartige der Hirnrinde Allg. Z. *Psych Gerichtl Med* 1907; 64: 146-148.
43. Speed MA, Wang DI, King J. Specific aggregation of partially folded polypeptide chains: the molecular basis of inclusion body composition. *Nat Biotechnol* 1996; 14: 1283-1287.
44. Lansbury PT Jr. Structural neurology: are seeds at the root of neuronal degeneration? *Neuron* 1997; 19: 1151-1154.
45. Uversky VN. Mysterious oligomerization of the amyloidogenic proteins. *FEBS Journal* 2010; 277: 2940-2953.
46. Jarrett JT, Lansbury Jr PT. Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell* 1993; 73: 1055-1058.
47. Jahn TR, Parker MJ, Homans SW, Radford SE. Amyloid formation under physiological conditions proceeds via a native-like folding intermediate. *Nat Struct Mol Biol* 2006; 13: 195-201.
48. Garcia-Mata R, Bebok Z, Sorscher EJ, Sztul ES. Characterization and dynamics of aggresome formation by a cytosolic GFP chimera. *J Cell Biol* 1999; 146: 1239-1254.
49. Scherzinger E, Lurz R, Turmaine M, Mangiarini L, Hollenbach B, Hasenbank R, Bates GP, Davies SW, Lehrach H, Wanker EE. Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. *Cell* 1997; 90: 549-558.
50. Batulan Z, Shinder GA, Minotti S, He BP, Doroudchi MM, Nalbantoglu J, Strong MJ, Durham HD. High threshold for induction of the stress response in motor neurons is associated with failure to activate HSF1. *J Neurosci* 2003; 23: 5789-5798.
51. Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, Masliah E, Nomura Y, Lipton SA. S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature* 2006; 441: 513-517.
52. Zhao L, Longo-Guess C, Harris BS, Lee JW, Ackerman SL. Protein accumulation and neurodegeneration in the woozy mutant mouse is caused by disruption of SIL1, a cochaperone of BiP. *Nat Genet* 2005; 37: 974-979.
53. Asea AAA, Brown IR. Heat Shock Proteins and the Brain: Implications for Neurodegenerative Diseases and Neuroprotection. 1st ed. Springer USA, 2008: 26-27.
54. Muchowski PJ, Schaffar G, Sittler A, Wanker EE, Hayer-Hartl MK, Hartl FU. Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc Natl Acad Sci USA* 2000; 97: 7841-7846.
55. McNaught KS, Shashidharan P, Perl DP, Jenner P, Olanow CW. Aggresome-related biogenesis of Lewy bodies. *Eur J Neurosci* 2002; 16: 2136-2148.
56. Everett CM, Wood NW. Trinucleotide repeats and neurodegenerative disease. *Brain* 2004; 127: 2385-2405.
57. Douglas PM, Summers DW, Cyr DM. Molecular chaperones antagonize proteotoxicity by differentially modulating protein ag-

- gregation pathways. *Prion* 2009; 3: 51-58.
58. Wolfe KJ, Cyr DM. Amyloid in neurodegenerative diseases: friend or foe? *Semin Cell Dev Biol* 2011; 22: 476-448.
  59. Cummings CJ, Mancini MA, Antalffy B, DeFranco DB, Orr HT, Zoghbi HY. Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. *Nat Genet* 1998; 19: 148-154.
  60. Chai Y, Koppenhafer SL, Bonini NM, Paulson HL. Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. *J Neurosci* 1999; 19: 10338-10347.
  61. Jana NR, Tanaka M, Wang G, Nukina N. Polyglutamine length dependent interaction of Hsp40 and Hsp70 family chaperones with truncated N-terminal huntingtin: their role in suppression of aggregation and cellular toxicity. *Hum Mol Genet* 2000; 9: 2009-2018.
  62. Bonini NM. Chaperoning brain degeneration. *Proc Natl Acad Sci USA* 2002; 99: 16407-16411.
  63. Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM. Chaperone suppression of alpha-synuclein toxicity in a *Drosophila* model for Parkinson's disease. *Science* 2002; 295: 865-868.
  64. Takeuchi H, Kobayashi Y, Yoshihara T, Niwa J, Doyu M, Ohtsuka K, Sobue G. Hsp70 and Hsp40 improve neurite outgrowth and suppress intracytoplasmic aggregate formation in cultured neuronal cells expressing mutant SOD1. *Brain Res* 2002; 949: 11-22.
  65. Cashikar AG, Duennwald M, Lindquist SL. A chaperone pathway in protein disaggregation. Hsp26 alters the nature of protein aggregates to facilitate reactivation by Hsp104. *J Biol Chem* 2005; 280: 23869-23875.
  66. Satyal SH, Schmidt E, Kitagawa K, Sondheimer N, Lindquist S, Kramer JM, Morimoto RI. Polyglutamine aggregates alter protein folding homeostasis in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 2000; 97: 5750-5755.
  67. Perrin V, Regulier E, Abbas-Terki T, Hassig R, Brouillet E, Aebischer P, Luthi-Carter R, Déglon N. Neuroprotection by Hsp104 and Hsp27 in lentiviral-based rat models of Huntington's disease. *Mol Ther* 2007; 15: 903-911.
  68. Lo Bianco C, Shorter J, Regulier E, Lashuel H, Iwatsubo T, Lindquist S, Aebischer P. Hsp104 antagonizes alpha-synuclein aggregation and reduces dopaminergic degeneration in a rat model of Parkinson disease. *J Clin Invest* 2008; 118: 3087-3097.
  69. Jana NR, Dikshit P, Goswami A, Kotliarova S, Murata S, Tanaka K, Nukina N. Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. *J Biol Chem* 2005; 280: 11635-11640.
  70. Miller VM, Nelson RF, Gouvion CM, Williams A, Rodriguez-Lebron E, Harper SQ, Davidson BL, Rebagliati MR, Paulson HL. CHIP suppresses polyglutamine aggregation and toxicity in vitro and in vivo. *J Neurosci* 2005; 25: 9152-9161.
  71. Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G. Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. *Mol Biol Cell* 2001; 12: 1393-1407.
  72. Bence NF, Sampat R M, Kopito RR. Impairment of the ubiquitin proteasome system by protein aggregation. *Science* 2001; 292: 1552-1555.
  73. Wyttenbach A, Carmichael J, Swartz J, Furlong RA, Narain Y, Rankin J, Rubinsztein DC. Effects of heat shock, heat shock protein 40 (HDJ-2), and proteasome inhibition on protein aggregation in cellular models of Huntington's disease. *Proc Natl Acad Sci USA* 2000; 97: 2898-2903.
  74. Rousseau E, Kojima R, Hoffner G, Djian P, Bertolotti A. Misfolding of proteins with a polyglutamine expansion is facilitated by proteasomal chaperones. *J Biol Chem* 2009; 284: 1917-1929.
  75. Nollen EA, Garcia SM, van Haften G, Kim S, Chavez A, Morimoto RI, Plasterk RH. Genome-wide RNA interference screen identifies previously undescribed regulators of polyglutamine aggregation. *Proc Natl Acad Sci USA* 2004; 101: 6403-6408.
  76. Behrends C, Langer CA, Boteva R, Böttcher UM, Stemp MJ, Schaffar G, Rao BV, Giese A, Kretzschmar H, Siegers K, Hartl FU. Chaperonin TRiC promotes the assembly of polyQ expansion proteins into nontoxic oligomers. *Mol Cell* 2006; 23: 887-897.
  77. Tam S, Spiess C, Auyeung W, Joachimiak L, Chen B, Poirier MA, Frydman J. The chaperonin TRiC blocks a huntingtin sequence element that promotes the conformational switch to aggregation. *Nat Struct Mol Biol* 2009; 16: 1279-1285.
  78. Kaushik S, Cuervo AM. Chaperone-mediated autophagy: a unique way to enter the lysosome world. *Trends Cell Biol* 2012; 22: 407-417.
  79. Agarraberes F, Dice JF. A molecular chaperone complex at the lysosomal membrane is required for protein translocation. *J Cell Sci* 2001; 114: 2491-2499.
  80. Arndt V, Dick N, Tawo R, Dreiseidler M, Wenzel D, Hesse M, Fürst DO, Saftig P, Saint R, Fleischmann BK, Hoch M, Höhfeld J. Chaperone-assisted selective autophagy is essential for muscle maintenance. *Curr Biol* 2010; 20: 143-148.
  81. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant alpha-synuclein by chaperone mediated autophagy. *Science* 2004; 305: 1292-1295.
  82. Schneider JL, Cuervo AM. Chaperone-mediated autophagy: dedicated saviour and unfortunate victim in the neurodegeneration arena. *Biochem Soc Trans* 2013; 41: 1483-1488.
  83. Koga H, Cuervo AM. Chaperone-mediated autophagy dysfunction in the pathogenesis of neurodegeneration. *Neurobiol Dis* 2011; 43: 29-37.
  84. Xilouri M, Brekk OR, Landeck N, Pitychoutis PM, Pappasilekas T, Papadopoulou-Daifoti Z, Kirik D, Stefanis L. Boosting chaperone-mediated autophagy in vivo mitigates alpha-synuclein-induced neurodegeneration. *Brain* 2013; 136: 2130-2146.
  85. Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N, Illes K, Khoshnan A, Martinez-Vincente M, Arrasate M, O'Rourke JG, Khashwji H, Lukacsovich T, Zhu YZ, Lau AL, Massey A, Hayden MR, Zeitlin SO, Finkbeiner S, Green KN, LaFerla FM, Bates G, Huang L, Patterson PH, Lo DC, Cuervo AM, Marsh JL, Steffan JS. IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. *J Cell Biol* 2009; 187: 1083-1099.

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