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Structure, Function, and Clinical Relevance of Molecular Chaperones – A Review Article

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Abstract

Proteins known as molecular chaperones are essential components of cellular protein homeostasis since these proteins assist others in folding and stabilizing or degrading the polypeptides. The principle is that if misfolded or aggregated proteins—common in many pathologies such as cancer, neurodegenerative, and genetic diseases—are inhibited from forming deposits, then normal pathology can be restored. In general, chaperones have been recognized as significant targets and tools in clinical therapies due to their centrality towards proteostasis and thus provide room for innovation treatment approaches such as pharmacological chaperone therapy, chaperone inhibition, and gene-based enhancement strategies. Therefore, a deep insight into the structure and function of these proteins is paramount for safe and target-specific intervention development. The paper should try to convince the readers regarding the fact that though molecular chaperones possess all the requirements idealized theoretically to be applied clinically as therapeutic agents, there exist several practical obstacles that make this successful clinical application dubious.

Keywords

Molecular chaperones, protein folding, proteostasis

Introduction

Proteins enable nearly all cellular activities-catalysis, signaling, information transfer, structural and dynamic control-dependent on their acquiring and maintaining exact three-dimensional shapes within the crowded and chemically varied intracellular environment. Folding does not occur efficiently in vivo; hydrophobic stretches and reactive side chains make the newly synthesized or stress-damaged polypeptides more likely to misfold, bind aberrantly, and aggregate. The integrated proteostasis network of core operational molecular chaperones that conformational surveillance helps folding/refolding assist route malfunctioning protein degradation pathways as needed to prevent these fates. Chaperones are not mere disaster relievers; they act co- and post-translationally on an appreciable part of the proteome thereby ensuring cellular health and organism viability (Hartl, 2011; Kim et al., 2013; Bukau et al., 2006).

Molecular chaperones first were popularized by studies on the heat shock response in which specific proteins strongly induced by thermal and other environmental

stresses were found to protect against proteotoxic damage (Lindquist & Craig, 1988). The work later extended: many chaperones are constitutively expressed, involved in the essential housekeeping function of nascent chain folding, and recovery pathways for various forms of insult—oxidative, metabolic, pathological—across cytosolic and organellar compartments (Morimoto, 2008; Bukau et al., 2006). The inducible heat shock network is therefore appreciated as one dynamically regulated arm from within a continuum of systems that offer surveillance through even basal growth conditions (Lindquist & Craig, 1988; Morimoto, 2008; Bukau et al., 2006).

Molecular chaperones belong to several conserved families structurally and mechanistically. The Hsp70 (DnaK) system—regulated by J domain proteins and nucleotide exchange factors—binds short hydrophobic motifs exposed on nascent or misfolded chains in an ATP regulated cycle central to early folding decisions (Mayer & Bukau, 2005). Hsp90 is an ATP dependent dimeric machine acting downstream that stabilizes a defined "client" subset enriched for signaling proteins, kinases, and hormone receptors with specificity tuned by the largest cohort of co chaperones (Pearl, 2016). Chaperonins are double ring nanocages—for example bacterial GroEL/GroES; eukaryotic TRiC/CCT—that encapsulate folding intermediates for iterative cycles of ATP driven annealing (Horwich et al., 2006). Small heat shock proteins (sHSPs) as holdases capture destabilized proteins and cooperate with the main ATPase systems in refolding or clearance (Carra et al., 2017).

Chaperones function in the broader PQC circuit and work at an individual level of folding. By transient binding non-native conformers, they may direct substrates to cycles for productive refolding, direct more recalcitrant species to the ubiquitin–proteasome system or autophagic pathways, and integrate signaling cascades that appropriately scale proteostasis capacities across physiological demands (Bukau et al., 2006; Hartl, 2011). The Hsp90 example in regulating signaling networks illustrates this aspect of systems biology: stability modulation of client proteins and their activities feeds back onto control of the cell cycle and other stress responses in developmental programs (Pearl, 2016). Conceptually, the PQC network increasingly becomes regarded as a tunable proteostasis "boundary" whose manipulation may restore balance in disease states (Balch et al., 2008).

Deficits in proteostasis and limitations in the capacity of available chaperones are, therefore, integral to many degenerative diseases. The central pathology of these conditions is characterized by the misfolding and aggregation of proteins; however, other pathologies also bear relevance. Diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and Amyotrophic Lateral Sclerosis fall in this category. Chaperones when experimentally upregulated have the potential to ameliorate proteotoxicity and improve cell survival within model systems (Morimoto, 2008; Balch et al., 2008; Kim et al., 2013). Small heat shock proteins cooperate with downstream ATP driven machineries to hold, disaggregate and refold metastable proteins. Therein lie prescription opportunities to enhance such pathways as organismic age is achieved and cellular stress mounts (Carra et al., 2017).

Cancer presents the opposite problem because malignant cells frequently upregulate and rely on chaperone networks—"chaperone addiction"—to stabilize mutated, overexpressed, or otherwise metastable oncoproteins while buffering proteotoxic stress from aneuploid genomes and hostile microenvironments (Calderwood & Gong, 2016). Hsp90 and Hsp70 disease expression has been validated in multiple tumor types with disease progression, and Hsp90 emerged as a validated therapeutic target because its inhibition promotes coordinated degradation of numerous oncogenic clients (Pearl, 2016; Rastogi et al., 2024). Clinically validated that single agent Hsp90 inhibitors have toxicity and efficacy hurdles to cross but combination regimens and next-generation compounds informed by mechanistic insights are advancing, continue to advance (Hong et al., 2013; Rastogi et al., 2024).

High resolution structural studies have defined the mechanism of chaperone action. The GroEL/GroES chaperonin is known to participate in the dynamic equilibrium between an open state that binds non native substrates and a closed state that encapsulates them inside the GroES cap and drives their folding by iterative ATP dependent conformational changes crystallography combined with cryo EM and kinetic dissection has been used to develop current mechanistic models (Horwich et al., 2006). Eukaryotic group II chaperonins, TRiC/CCT, which are required for folding of cytoskeletal proteins such as actin and tubulin, have built in lid domains which enclose their substrate and exhibit subunit diversity

conferring substrate specificity (Spiess et al., 2004; Hartl, 2011). Cryo EM analysis of archaeal and eukaryotic systems also revealed more details about dynamics of lid closing and chamber architecture which refine our understanding about possible encapsulated folding landscapes (Zhang et al., 2010).

Chaperone biology translation is already an active frontier. Pharmacological chaperones are small, highly specific target ligands that bind to and stabilize destabilized proteins; clinical development is underway in a few indications related to lysosomal storage disorders and genetic diseases (Parenti et al., 2015). Structure guided correctors like lumacaftor and tezacaftor exemplify this strategy binding to CFTR stabilizes thermodynamically fragile transmembrane domains trafficking promotion of disease variants provides the Mechanism of CFTR Correction…, 2021). Chaperone-based immunomodulation falls within the investigative activities as an ancillary oncology therapeutic agent. It can involve extracellular or tumor-derived heat shock proteins influencing antigen presentation and immune activation (Calderwood & Gong, 2016). Theoretically, such targeted ligands expand the earlier definition of pharmacological chaperones as different from non-specific chemical osmolytes since they provide specificity at therapeutic concentrations (Parenti et al., 2015; Calderwood & Gong, 2016).

The chaperone systems are, first of all, evolutionary conserved and, second, interdependent. They underscore cellular life with its foundational roles. From bacteria to mammals in modular sequential pathways chaperones operate engaging nascent chains buffering environmental insults maintaining proteome integrity over the lifespan perturbations in any node reverberate across the proteostasis network with physiological and pathological consequences (Hartl, 2011; Kim et al., 2013; Lindquist & Craig, 1988; Carra et al., 2017).

This review integrates the knowledge currently available regarding the structure, function, and clinical relevance of major molecular chaperones. Architectural diversity and mechanistic cycling of major chaperones are proteostasis pathways through which diseases can attack cells; their discussion is followed by a brief description of neurodegeneration, cancer, and inherited protein folding disorders. The paper finally summarizes novel therapeutic strategies that either harness or modulate chaperone systems toward restoring cellular homeostasis.

Structure of chaperons

The molecular chaperones show a rather impressive structural variation whose architecture matches their particular functions in the proteostasis network. Among the best characterized chaperone complexes belong to the family of chaperonins represented by the bacterial GroEL–GroES system. GroEL represents a large double-ring structure built from 14 subunits (~57 kDa each) arranged as two heptameric rings, while GroES acts as a heptameric “lid” capping the central cavity. Subunits in GroEL display three clearly separated domains: an equatorial domain for ATP binding and inter-ring contacts, an apical domain which binds unfolded polypeptide and GroES, and an intermediate hinge domain that is always implicated in allosteric communication between ATP binding and hydrolysis. Conformational transitions induced by ATP binding result in rotation followed by elevation of apical domains thus increasing the volume of internal cavity opening access to isolated hydrophilic environment facilitating folding. Cryo-electron microscopic and crystallographic structural studies have worked out the stepwise mechanism of substrate encapsulation and release thereby highlighting the cooperative transitions that underlie chaperonin function (Horwich et al., 2006; Clare et al., 2012).

Other chaperone families have evolved entirely different, though crucially important, structural schemes. Hsp70 is a protein of about 70 kDa mass, comprising an approximately 43 kDa nucleotide-binding domain connected with a substrate-binding domain of about 27 kDa by a conserved interdomain connector whose existence permits allosteric regulation between the domains. The binding and hydrolysis of ATP induce conformational changes in the SBD to change from an open state that allows for fast exchange of substrates to a closed-lid state which binds unfolded polypeptides strongly. The Hsp90 chaperone operates as a homodimer with each protomer containing three discrete domains: an N-terminal ATPase domain, a middle domain capable of interaction with client proteins, and a C-terminal dimerization domain. Conformational cycling between open and closed states modulated ultimately by ATP and cochaperones is required during stabilization and maturation processes concerning client proteins. (Pearl, 2016; Mayer & Gierasch, 2019).

Functions of Molecular Chaperones

Molecular chaperones assist in correctly folding newly synthesized polypeptides and prevent misfolding or aggregation with others that may be functionally damaging to the cell. During translation, emerging chains of polypeptides from the ribosome are in an unfolded state and quite vulnerable to incorrect interaction either with other proteins or with themselves. Chaperones of the type Hsp70 bind transient hydrophobic segments on these chains and prevent nonspecific aggregation while directing the polypeptide toward attaining its native conformation (Kim et al., 2013). The cycle is also ATP-dependent, binding, and release; it is regulated by co-chaperones for example Hsp40 and nucleotide exchange factors in maintaining as well as immediately post-synthesis protein quality control (Mayer & Bukau, 2005). The substrate is isolated from the crowded cytosolic milieu inside a box provided by chaperonins like GroEL/GroES, further facilitating efficient accurate folding (Horwich et al., 2006).

<start_of_text>Molecular chaperones play critical roles in assisting the folding of newly synthesized polypeptides and in the refolding of denatured or misfolded proteins under stress conditions like heat shock, oxidative stress, and toxin assault. Heat shock proteins induced during such stresses constitute proteotoxic stress the first line of defense. Members of Hsp70 and Hsp90 bind partially unfolded intermediates either by refolding them to their native state or targeting them for degradation through the ubiquitin-proteasome pathway thereby ensuring on one hand repair as well as on the other hand degradation so that no damaged protein accumulates within a cell which may be highly toxic due to aggregate formation or loss of important cellular function.

Molecular chaperones assist the assembly and disassembly of large macromolecular complexes. For instance, Hsp90 is essential to support the maturation process for a broad diversity of signaling proteins among which kinases, steroid hormone receptors, and transcription factors (Pearl, 2016).This function is mostly controlled by co-chaperones that regulate the ATPase cycle of Hsp90 as well as client specificity. By stabilizing those regulatory proteins, signaling pathways regulating cell growth and differentiation, and apoptosis that Hsp90 controls.

Another major role that chaperones play is in the translocation of proteins across cellular membranes. Most proteins are synthesized in the cytoplasm and then need to be targeted to organelles like mitochondria, chloroplasts, or the endoplasmic reticulum (ER). Chaperones such as mitochondrial Hsp70 (mtHsp70) prevent aggregation by maintaining precursor proteins in an unfolded state that is competent for translocation and import into mitochondria through channels on these membranes. Similarly, BiP (an Hsp70 homolog located in the lumen of ER) assists in folding and quality control of proteins inside this organelle.

Chaperones play a role in the prevention of protein aggregation, one of the key features in many neurodegenerative disorders such as Alzheimer's, and Parkinson's and Huntington's diseases. Chaperones bind to intermediates that are prone to aggregation and in this way suppress the formation of toxic oligomers, and even disaggregate aggregates when cooperating with disaggregases such as Hsp104 in yeast or the Hsp70/Hsp40/Hsp110 complex in mammals (Mogk et al., 2018). It is increasingly perceived that this anti-aggregation function is a treatable activity by enhancing it pharmacologically through chaperones or chemical modulators.

Chaperones can function as signal transducers and stress sensors too. For example, Hsp70 and Hsp90 control the stability of different transcription factors in which heat shock factor 1 (HSF1) essentially controls the expression of stress-inducible genes (Morimoto, 2008). It gives a regulatory feedback mechanism such that chaperone levels are dynamically upregulated in proteotoxic stress to maintain proteostasis and ultimately cell health.

Molecular chaperones carry out a broad essential function that includes support of folding protein, the refolding of misfolded proteins, prevention of aggregation, and mediation degradation among others including helping in complex assembly and protein translocation. Proteostasis places them centrally not only under normal cell physiology but also in diseases and further, they may be applied therapeutically.

Clinical Applications of Molecular Chaperones

Molecular chaperones have increasingly assumed the role of major targets and agents in clinical therapies across a wide spectrum of diseases—from cancers to genetic disorders involving protein misfolding, besides neurodegenerative diseases. Inhibition of chaperones, pharmacological chaperone therapy, and enhancement of chaperones or gene-based approaches can broadly classify the clinical applications.

Cancer Therapeutics: Hsp90 Inhibition

Heat shock protein 90 (Hsp90) belongs to the highly conserved molecular chaperone family whose members participate in the stabilization of a broad spectrum of oncogenic client proteins comprising kinases, hormone receptors, and transcription factors. Tumor cells frequently demonstrate an apparent dependency on Hsp90 as coined with the term “chaperone addiction” since there is a high level of proteotoxic stress, in addition to the genetic instability found within cancer cells. This condition makes Hsp90 an attractive target for anticancer therapy (Wei, 2024; García-Carbonero et al., 2013).

Some of the Hsp90 inhibitors that have made their way into the clinical pipeline include 17-AAG, 17-DMAG, ganetespib, and AUY922 for indications such as HER2-positive breast cancer, pancreatic cancer, multiple myeloma, melanoma, and renal cell carcinoma. These agents demonstrated limited activity when used as single agents in clinical settings compared with strong preclinical data due to toxicity issues and poor bioavailability-in addition to intrinsic or acquired resistance (García-Carbonero et al., 2013; Wei, 2024). Such combination therapy provided improved efficacy because already approved cancers also showed improvement when attacked by multiple fronts simultaneously. For example, efficacy was notably improved for metastatic HER2-positive breast cancer by combining both 17-AAG and trastuzumab than using either agent alone (García-Carbonero et al., 2013). Precursors like ganetespib further bring treatment enhancement on combination with chemotherapy or immune checkpoint inhibitors in both preclinical setups and clinical trials (Wei, 2024).

Also, newer Hsp90 inhibitors like pimitespib have gotten approvals for select uses, one of them being against gastrointestinal stromal tumors (GIST) and has improved progression-free as well as overall survival with side effects that can be managed (Wei, 2024). Studies going on will probably help in the discovery of biomarkers to stratify patients more aptly who will gain most from therapies targeted at Hsp90inhibitors since over 20 different inhibitors continue to be tested in numerous oncology trials (García-Carbonero et al., 2013).

Pharmacological and Chemical Chaperone Therapy

Pharmacological chaperones are a hopeful new class of small molecules that will bind select and stabilize mutant proteins tending toward misfolding, in this way rescuing their function. The approach applies most directly to inherited lysosomal storage diseases and other genetic disorders where the problem is one of protein misfolding.

A leading example for a pharmacological chaperone is Migalastat which has gained approval in the management of Fabry Disease. Thus, selective stabilization of amenable mutant forms increases its trafficking and enzymatic activity meantime reducing clinical manifestations like renal dysfunction and cardiac hypertrophy (Benjamin et al., 2016). Agents like ambroxol and isofagomine have demonstrated optimistic results in increasing activity as well as improving patient symptoms relating to Gaucher disease during early-phase clinical studies (Benjamin et al., 2016).

Pharmacological chaperones such as deoxynojirimycin (DNJ) co-administered with enzyme replacement therapy in Pompe disease have elicited encouraging results about improved stability and activity of acid α-glucosidase. However, some patients developed adverse effects on muscles at higher doses. These examples underscore the potential of pharmacological chaperones as adjuncts or alternatives to enzyme replacements. Chemical chaperones include agents 4-phenylbutyrate (PBA), trehalose, and mannitol. They are less specific but work by stabilizing protein conformations and alleviating cellular stress. PBA has restored proteostasis in addition to cognitive improvement possible late in the course of pathology, thereby instilling hope not only for Alzheimer’s disease but also for all other Protein Aggregation Disorders (Hetz & Mollereau, 2014; Perlmutter, 2002). PBA treatment has also been protective in models of amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) in addition to Huntington’s disease through lowering ER Stress as well as enhancement of protein folding (Hetz & Mollereau, 2014).

Chaperone Enhancement and Gene-Based Strategies

Endogenous chaperone upregulation or direct delivery of chaperone genes constitutes another promising strategy. Since HSF1 is one way through which to activate the expression of protective chaperones, particularly Hsp70, efforts directed toward its activation are attractive. Preclinical studies show that some small molecules related to celastrol and carbenoxolone have

indicated the expression of Hsp70 as theoretically possible for therapeutics; however, their toxicity has made clinical development impossible.

Viral vector-based gene therapies delivering Hsp70 or associated chaperones are being preliminarily explored in the context of neurodegeneration, with particular focus on Parkinson's disease. While initial clinical safety data is promising, more evidence supporting therapeutic efficacy is needed. (Kalmar & Greensmith 2009) Moreover, extracellular chaperones like clusterin have shown the ability to convey neuroprotection in animal models of Alzheimer's disease through mitigation of amyloid-beta toxicity and resultant neuroinflammation (Nuvolone et al., 2016).

Emerging and Future Directions

The clinical chaperonotherapy discipline continues to grow with the development of positive (chaperone replacement or enhancement) and negative (chaperone inhibition) therapies in the management of particular diseases. For example, while some chaperones are pathogenic in ALS and MS—e.g., mutated valosin-containing protein or extracellular Hsp60 small heat shock proteins need upregulation to achieve effective disease modification—positive support is constantly needed. Improved efforts toward drug delivery, especially passage through the blood-brain barrier, and more specific chaperone modulators that would carry minimal off-target effects are also highly prioritized for achieving clinical success. Mutation-specific responsiveness to pharmacological chaperones discovered under precision medicine will further enable patient selection to improve clinical outcomes.

Conclusion

Molecular chaperones are major components of the cellular machinery that assure and control quality at different levels of protein folding inside the cell, as well as inhibit toxic aggregation. The complicated multidomain assembly enables these proteins to serve numerous variants of functionally related client proteins during stress conditions and contribute to proteostasis. Clinically speaking, they are viewed as shining targets for therapy development in cancer, genetic diseases, and neurodegenerative disease—either by inhibition, pharmacological stabilization, or gene-based approaches. Though there are still delivery, specificity and side effect obstacles in harnessing Chaperones as treatment across so much disease diversity, research continues to unlock great potential when it becomes personal.

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