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The Structural Mechanisms of Chaperones and Their Applications in Medicine and Production

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Abstract. The structural formation and metabolic activities of organisms are inseparable from precise regulations of appropriate protein expression, processing, and modification. However, new-synthesized polypeptides or incompletely folded proteins in cells are prone to undirected misfolding and then aggregation, under external stress conditions. Many neurodegenerative diseases are intimately related to the aggregation of misfolded proteins in nerve cells. Importantly, molecular chaperones can specifically bind to substrates (including peptides and proteins) to stabilize their conformations to prevent aggregating, direct correct folding or refolding, promote disulfide-bond formation, and also regulate appropriate modifications such as phosphorylation. In addition, some chaperones mediate the localization of membrane proteins and extracellular secretion of proteins. Through a complete and thorough study of the complex interaction network between molecular chaperones and intracellular molecules, it is possible to provide reliable avenues and effective immune targets for therapies for cellular pathogen diseases. Some of them also serve as auxiliary factors for the efficacious delivery of protein drugs. Moreover, molecular chaperones play an important role in the artificial production of recombinant proteins, or rather, even indispensable. The artificial synthesis of chemical nanochaperones is a highly promising direction nowadays.

Keywords: Chaperones, HSPs, domains, disease therapy, recombinant-protein production

1. Introduction

The molecular chaperone serves an essential role in the protein metabolic network, which has been applied in various areas such as the production of the recombinant protein and disease therapy, especially for certain kinds of cancers. As chaperones accompany with the entire metabolism of proteins , a certain space to research still exists in the fields of complex interaction networks, especially for the potential interactions including orchestrating protein-regulated gene expression as well as the protein cooperation and antagonism. Thereby, there are noticeable challenges about how to reasonably transfer the extracellular experiment conclusions to complex intracellular circumstances. Chaperones significantly take effect on a big network of protein processing, folding, disulfide-bond formation, protein modification, renaturation as well as degradation, etc. Therefore, chaperones have effects on building organismic structures, metabolic modulation, as well as immune regulation. Some chaperone proteins interact to cooperate in some cases of protein metabolism.

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This article summarizes the major chaperone categories and explicitly clarified their functional structures. Chaperones are generally localized in cytosol, ribosomes, endoplasmic reticulum (ER) and mitochondria. In addition, there also exist chaperones in bacterial periplasm, which particularly function in disulfide-bond formation and membrane-protein localization.

Molecular chaperones have been mainly applied in producing recombinant proteins through decreasing aggregates. Under stress conditions or the lack of ER (in bacteria), proteins are much more liable to be misfolded or aggregated with low solubility. Hence, chaperones increase the yield of folded proteins and their solubility. Researchers verified co-expression of the signal peptide and the chaperone can restore the yield suppressed by the signal peptide. Moreover, predecessors proved that salt inhibits the chaperone-like activity of camel α-S_1 casein [1], which indicates that the inorganic condition of the substrate should be considered to enhance the chemical stability.

Necessary to be concerned, many wild chaperone proteins have indeterminacy and degeneracy while functioning in protein modification. Hence, to enhance the functional directivity of chaperones is a currently worth-exploring issue to mediate proper and correct modification. And some periplasmic chaperones mediate the formation of disulfide bonds, which is adverse to proteins not expected to form disulfide bonds. Artificial chemical nanochaperones show potential in exhibiting highly specific regulatory functions [2]. Nanochaperones are devised for the requirement of human production and research.

2. Chaperone categories and fundamental functions

Chaperones' functions mainly include taking effect on avoiding protein misfolding, dissolving and degradation of misfolded protein, folding protein correctly, maintaining the folded structure, modification, transportation, and structural protein localization. Hence, cytoplasmic chaperones are usually located in mitochondria and the structures in the protein generation-transportation pathway. Certain cytoplasmic chaperones transport target proteins and specifically bind to the transport protein (on membranes of organelles or the cell) or nuclear import receptor, directing the process of trafficking. Periplasmic chaperones are located in the periplasm to typically function in disulfide-bond formation, protein assembly and transportation. Finally, chemical chaperones are invaluable to explore and devise.

2.1. Families of HSPs

HSPs exist inside the prokaryotic or eukaryotic cells, serving as conserved and cytoplasmic chaperones. Categories of HSPs are classified based on their molecular weights.

Primarily, they are significantly expressed under mainly heat stress in order to dissolve aggregates and mediate protein folding correctly, which is a well-known aspect but not only. In eukaryotes, the external stress induces protein misfolding and accumulation, which triggers the Endoplasmic Reticulum Stress (ERS), typically including Unfolded Protein Response (UPR) to restore homeostasis and autophagy initiated. In UPR, relative downstream chaperones' transcriptions are activated by the original three membrane-embedded sensor proteins that are PKR-like ER Kinase (PERK), Inositol-requiring Enzyme 1α (IRE1α), and Activating Transcription Factor 6 (ATF6).

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2.1.1. HSP110

Family HSP110 is also known as HSPH. Their molecular sizes are within the range of 105~110 kilodaltons (kDa). They exist mainly in various eukaryotic organisms, being localized in nuclei and cytosol. HSP110s show relatively high homology to HSP70s, which are regarded as the members of HSP70 superfamily.

Family HSP110 is recognized by the typical functional structure. The nucleotide-binding domain (NBD) possesses ATP-binding activity but not hydrolysis, which contains the N-terminal. The β-sandwich peptide (SBDβ) and α-helical bundle (SBDα) both serve as the substrate-binding subdomains, which collectively compose the substrate-binding domain (SBD). There is a unique loop region within SBDβ, which has been demonstrated to determine the selectivity for binding to substrates, especially aromatic residues. A hydrophilic inter-domain linker within NBD connects NBD and SBDβ [3]. HSP110s individually serve as the holdase chaperones. A part of them can dissociate the protein aggregates as ATP-dependent. C-terminal extension connected with SBDα lacks of research conclusions. Some HSP110s function as ATP-independent passive chaperones in maintaining the peptide's unfolded state [3]. Furthermore, HSP110s perform as the nucleotide exchange factor (NEF) for HSP70s and also help refold client proteins.

2.1.2. HSP100

The size of HSP100s remain in the range of 100~104 kDa in common. Typically, protein HSP104 exists in mitochondria of the *Saccharomyces cerevisiae* and ClpA/B/C are localized in cytosol of *Escherichia coli* (E.coli). Some of the HSP100s are traced in ER or the chloroplast.

HSP100s work as the hexamer in the conformation of a channel loop. The HSP100 monomer has three key domains which include AAA+ module(s) (one AAA+ module containing at least one AAA domain), N-terminal domain (NTD), and the intermediate coiled-coil domain (ICCD) (figure 1) [4]. Every AAA domain with ATPase activity, has both a nucleotide-binding motif and an ATP hydrolysis motif [5]. NTD contains the substrate-binding groove in order to unfold the misfolded protein and dissolve aggregates (figure 1). ICCD participates in modulating the chaperone activity by its conformational change. In some cases, HSP100s are orchestrated by specific co-chaperones while disaggregating inclusion bodies (IBs). Researchers demonstrated the protein HSP100 to act in the malaria protein secretion [4]. In mammalian cells, ClpB of HSP100 in bacteria can elicit an immune response by activating immunocytes, which displays an ideal target for the bacterial disease therapy. ClpB may also serves as the signal factor.

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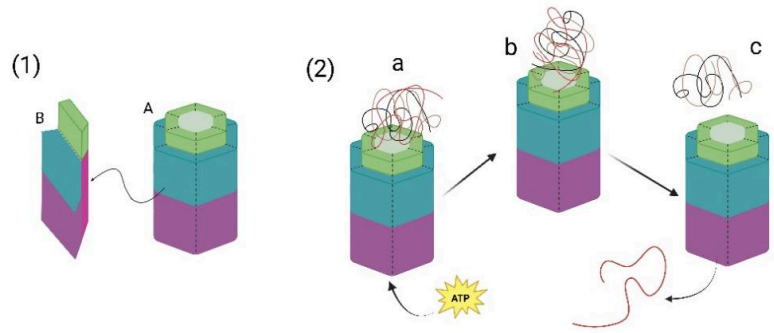


Figure 1. (1). (A) The brief model picture of an entire HSP100 hexamer. (B) One HSP100 monomer with three main functional regions, devided from a hexamer. The region of AAA+ Module(s) in purple and wine red, NTD in light green and ICCD in Tiffany blue conjoin as the spatial distribution. (2). The flow diagram of dissolving aggregates. (a) For the first step, the aggregate binds randomly to the top entrance of HSP100. Following that, the ATP binds to AAA+ module. (b) Secondly, certain protein(the red chain shown) residues are bound to form commited complex. (c) Leaning on the energy supply from ATP hydrolysis, accumulated protein in red gets unfolded and obtains the proper conformation to cross the oligomeric barrel, exiting from the bottom

2.1.3. HSP90

The molecular weight of family HSP90 is about 90 kDa. Most of HSP90 proteins exist in mammalian cells and a unique protein HtpG located in the cytoplasm which belongs to the E.coli [5].

HSP90s work as dimers in common. Every single monomer has the typical structure of three domains as well as a flexible linker between N-terminal domain (NTD) and middle domain (MD) (figure 2). C-terminal domain (CTD) is verified to act as the co-chaperone-binding region and also dimerize HSP90s. MD governs substrate bonding. NTD takes charge of ATP bonding and hydrolysis [6]. Moreover, on the NTD, there exists the small lid region (LR) like a lid that changes conformation to facilitate NTD for ATP bonding and release. LR closes after ATP bonding and opens while releasing ADP [7].

The cycle of HSP90s utilizing ATP contains six periods (figure 2). The HSP90 dimer in the initial Stage I binds to two ATP molecules on two NTDS, becoming as the ATP-bound Stage II. Then the CTD captures co-chaperone-client protein complex, entering Stage III. Subsequently, the linker folds inwards and two ATP-bound NTDs stick down. Meanwhile, two MDs simultaneously capture one client protein (CP) delivered by the co-chaperone (C-C), as the Stage IV. In the Stage V, the linker further folds inwards and two NTDs embrace tightly. Meanwhile, MDs become intertwined inwards and tightly fasten the client inside. Along with ATP hydrolysis, MDs accomplish folding protein correctly (as an example), entering the Stage VI. Complex of Stage VI approximately resembles the Stage IV. Finally, ADPs, the client and co-chaperone are completely released, and HSP90 dimer expands as the open initial state. On the whole, family HSP90 functions as ATP-dependent in directing protein folding, assembly of polymers and mediating receptor bonding [7].

DAF-21 of family HSP90 in *Caenorhabditis* has been postulated as the 'holdase' chaperone of kinase WEE-1,3, according to that the absence of DAF-21 results in the defect of ovocyte diakinesis arrest with Polar-body-like membrane-bound organelles appearing [8]. And some of HSP90s hold repressor proteins to participate in gene silencing. In plants, the nuclear-localized HSP90 can hold and maintain brassinosteroid-insensitive 2 (BIN2) [9].

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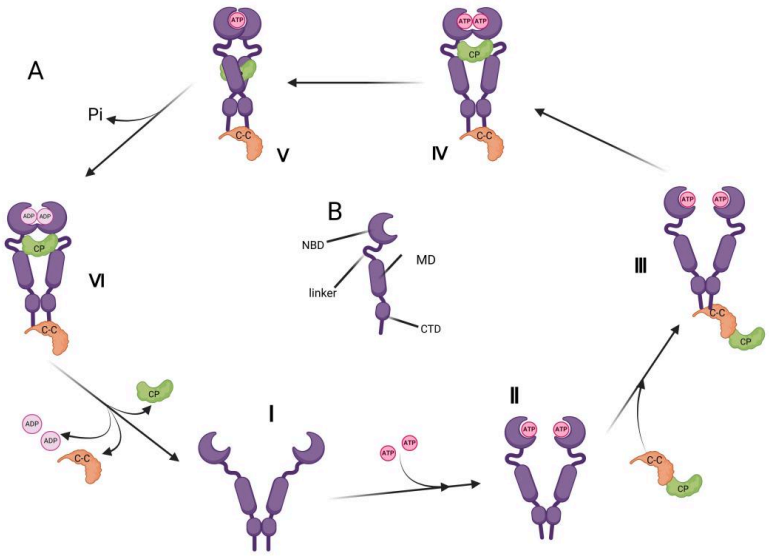


Figure 2. (A) A functional cycle of the HSP90 dimer. Once the complex in Stage IV is interacted with by HSP90 inhibitor, it triggers the substrate degraded by lysosomes and ubiquitinated protease pathways [6]. (B) A model of HSP90 structural distribution [7]

2.1.4. HSP70

The family HSP70 mainly weighs 68~78 kDa. HSP70s widely exist in kinds of organisms. They have three essential domains homology to HSP110s' at a certain level.

Remarkably, the C-terminal extension of HSP70s is shorter than HSP110s. The HSP70 usually works as a monomer (figure 3). SBDα next to C-terminal extension, serves as a lid to control the substrate bonding and release [10]. And the SBDβ connected with SBDα, takes charge of client bonding by loop regions in order to maintain the unfolded conformation and also mediate the correct folding. The inter-domain linker within NBD which includes N-terminal, connected to the SBDβ, can build connections with NBD's subdomains to achieve the interaction between ATP hydrolysis and substrate release [10].

In certain cases, HSP70s operate with auxiliary chaperones such as the HSP110 and HSP40 to function in dissolving aggregates. Notably, some HSP70s are related to the organelle protein trafficking, for example, protein Mortalin mediates the import of mitochondrial proteins [11]. Furthermore, Mortalin also binds to and inhibits the tumor suppressor p53 [11].



Figure 3. A distribution model of Hsp70's key regions

2.1.5. HSP60

Family HSP60/HSPD is generally acknowledged with the weight within 52~65 kDa. The majority of HSP60s are localized in mitochondria of mammalian cells. And some exist in chloroplast. Remarkably, GroEL is one type of HSP60s, located in cytosol of E.coli [5].

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Family HSP60 usually works as the oligomer, which consists of two heptameric barrels [12, 13]. Every HSP60 monomer HSP60 has three main functional domains (figure 4). The apical domain (AD) governs the client bonding as well as co-chaperone combination. The intermediate domain (ID) modulates the hydrophilicity of AD. The equatorial domain (ED) binds to the ATP molecule [13]. N-terminal HSP60 connected with ED is free, exhibiting 100% conservation. The N-terminal directs the HSP60 to be localized in mitochondria. The C-terminal as a sequence of G-repeat has unknown functions at present.

The radical efficacy of family HSP60 is to orchestrate peptides folding correctly and to facilitate protein refolding (figure 3). Researchers propounded that the HSP60 exerts an important impact on the oocyte development, with high expression-level area gradually migrating into the oocyte [13]. HSP60s also take specific effect in tumor cells on promoting metastasis or triggering immune response [13]. And researchers established that the absence of HSP60 induces the blocked cholesterol synthesis, which serves as one factor that causes hypo- or de-myelinating diseases [12].

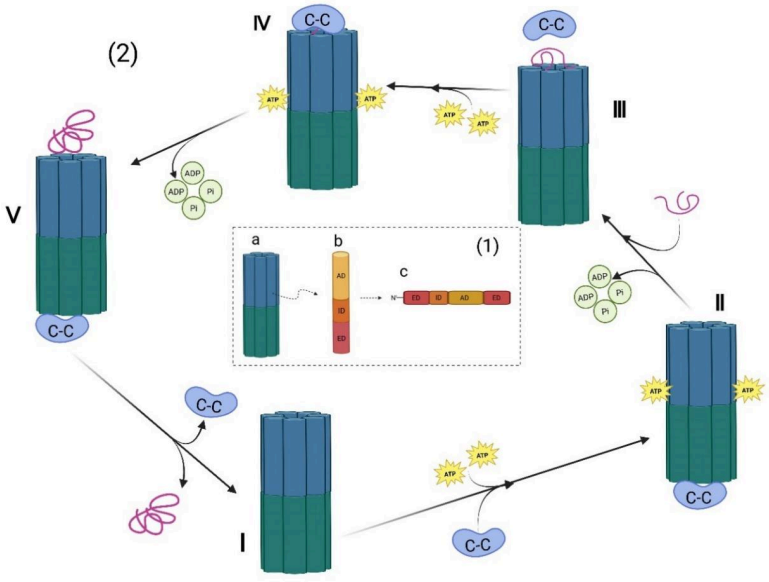


Figure 4. (1). (a) A HSP60 tetradecamer assembled from two heptamers. Every upper monomer concatenates with the corresponding lower one, by virtue of their individual EDs. (b) Domain arrangement of single HSP60 monomer. (c) Functional region arrangement of HSP60 amino acid chain. (2). The flow diagram of HSP60 tetradecamer on folding protein. (I) Initial open tetradecamer. (II) ATP molecules are bound to EDs of the upper heptamer and co-chaperone(C-C) binds to ADs beneath. (III) One peptide chain is bound on upper opening. (IV) The co-chaperone binds to upper ADs and covers the opening up. Meanwhile, the client accesses into the barrel for folding. (V) Folded protein floats up to the opening and co-chaperone is bound to the lower heptamer again. Then they are both released

2.1.6. HSP40/JDP

HSP40s are known as the name 'JDP', mainly within the size range of 40~47 kDa. The typical HSP40 called DnaJ is expressed in E.coli, located in cytosol and ER [5].

Family HSP40 takes effect as dimers. The wild HSP40 chaperones of Type I (including DnaJ) have three conserved regions (figure 5). The N-terminal J-domain (JD) binds to co-chaperones [14]. The flexible linker Gly/Phe-rich sequence (G/F) after JD, participates in the process of substrate

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bonding and specific interaction with co-chaperone HSP70. Two zinc finger motifs (ZFMs) exist in a certain sequence connected with G/F, mediating the substrate bonding and delivery. The C-terminal fragment (CTF) contains substrate-binding domain(s) (CTDx) as well as dimerization domain (D) in certain HSP40s [15]. The Type II with longer linker lacks ZFMs and Type III lacks all domains except JD that may be located anywhere [14,15]. A dimerization domain is localized in the end of CTF in certain HSP40s. Researchers presented some homologs to DnaJ act as the factor in mediating the endocytosis [14].

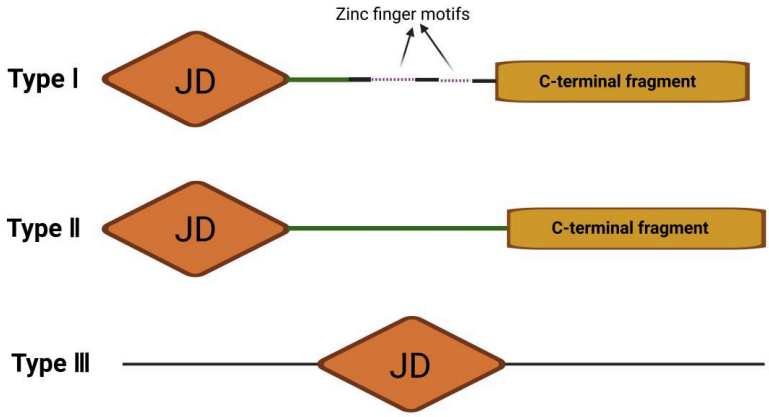


Figure 5. Models of Hsp40 funtional regions, and the contrast of three types

2.1.7. Small HSPs

Small HSPs (sHSPs) encompass the HSPs which weigh about 15~30 kDa. There are two sHSP chaperones known in genome of *Saccharomyces cerevisiae* [16]. The GroES that has been deeply studied exists in cytosol of E.coli, which notably interacts with GroEL as a typical co-chaperone system. The sHSPs in humans are mainly active in cytosol, nuclei and mitochondrial intermembrane space.

Various sHSPs have the conserved and characteristic α-crystallin domain (ACD), which is located in the middle fragment of sHSPs (figure 6). The N-terminal region (NTR) exhibits highly hydrophobic feature and the C-terminal region (CTR) is rich in hydrophilic residues. Remarkably, these regions have complex interactions with each other as well as client proteins [16].

Human sHSPs function as the polymer with uncertain number of subunits, which are polymerized by their ACD β-sandwich structures. The subunit number of oligomers is modulated by the solution condition and sHSP's modification. Nevertheless, the assembly of oligomers is orchestrated by all the functional regions. Furthermore, it is noticed that subunit-exchange phenomenon appears frequently between different oligomers, which exhibits positively correlated with temperature [16, 17].

The sHSPs function as ATP-independent in preventing the intermediate-state protein from generating prefibrillar and amorphous aggregates [17], or postponing the aggregation of misfolded proteins. HSPB4 and HSPB5 are both the small HSPs, which can co-associate to maintain the solubility of lens proteins to refrain from lens cataract [16, 17]. Lens sHSPs also function in the apoptosis pathway to control cell apoptosis. Worthy to be concerned, sHSPs can interact with actins to maintain cytoskeletal integrity [16].

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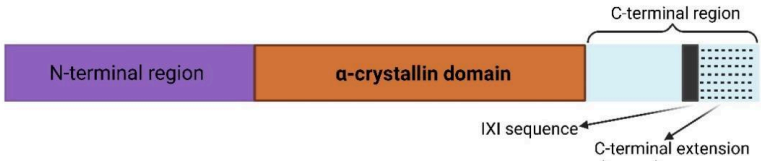


Figure 6. A schematic model of HSPB5/αB-crystallin (αBC) molecular structures

Different kinds of sHSP have mutable lengths of various regions and only partial sHSPs feature IXI sequence and C-terminal extension. Two unfolded flanking regions (terminals) exhibit significant dynamism as well as low similarity among mutants. Flanking regions of sHSPs (not only αBC's) can trigger molecular crowding while binding to the intermediate-state protein, and also prevent central domain from aggregation. IXI sequence mediates the interaction between oligomer subunits. N-terminal region of αBc has been verified to coordinate binding to amorphously aggregated lysozyme. Moreover, all sHSPs are prone to form amyloid fibril due to their highly β-sheet property, which is inhibited by C-terminal extension that exhibits capability of solubilizing sHSP-client complex aggregates [17].

2.2. Group Thioredoxin

Group Thioredoxin (Trx) of thioredoxin (or -like) proteins mainly exist in cytosol. They all possess the typical and functional sequence of 'Cys-X-X-Cys' (X referring to a random amino acid residue) which orchestrates the disulfide bond exchange reaction, presenting their disulfide reductase activity [18]. Worthy to be mentioned, the active sequence of YbbN in E.coli serves as 'Ser-X-X-Cys'. Significantly on the other side, Trxs can also promisingly function as ATP-independent in maintaining clients' stable state to decrease aggregate formation.

It is verified in E.coli that TrxA's C-terminal domain interacts with ADP-ribosyltransferase toxin (Trex) to exhibit the chaperone activity [18]. TXNL1 as a Trx protein, possesses capability of preventing the insulin from aggregation under heat stress. Trx2 of *Trypanosoma brucei* is mainly located in mitochondria, having been proven to inhibit the insulin from aggregate formation by changing conformations under heat stress, and additionally, Trx2 has not effective disulfide reductase activity [19]. It can be postulated that Trx2 functions as the holdase chaperone by potential client-binding domain.

2.3. Family Dsb

Family Dsb is localized in periplasm or the inner membrane of Gram-negative bacteria. They mainly function in forming disulfide bonds in periplasmic space by the multi-Dsb system (figure 7).

DsbB exists in the inner membrane, accepting electrons from the reaction of DsbA and delivering them to cytochromes. DsbA exists in cytosol and functions in disulfide-bond formation for target proteins. DsbC in cytosol, orchestrates incorrect disulfide bonds cleavage. DsbD is located in the inner membrane to deliver electrons from thioredoxin to DsbC. The Dsbs that regulates disulfide bonds in periplasm, lean on the functional sequence 'Cys-X-X-Cys' same to the Trx's. In addition, DsbD also has that sequence in structure. Moreover, DsbE constructively regulates the maturity of Cytochrome C. And DsbD can bind to DsbE or DsbC, to maintain their reducibility via its reductase activity [5].

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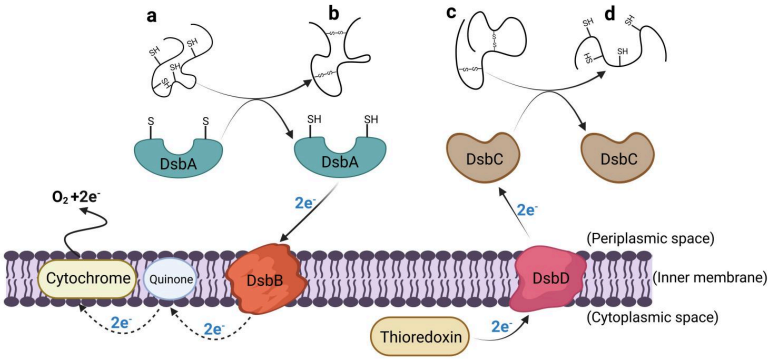


Figure 7. The multi-Dsb system of regulating disulfide bonds (a) The primary protein without disulfide bonds. (b) The protein with disulfide bonds correctly formed. (c) The protein with incorrect disulfide bonds. (d) The status after cleaving incorrect disulfide bonds up

2.4. PPIases

Peptidyl-prolyl isomerases (PPIases) exist in periplasm and the outer or inner membrane of prokaryotic organisms [5]. Notably, PpiA serves as an important chaperone of PPIases in humans, which is localized in nuclei and verified to maintain stem cells' vitality.

The following text concentrates in detail on SurA as an important chaperone in E.coli. ATP-independent SurA specially binds to the outer membrane proteins (OMP) to maintain them for delivering OMP to β-barrel assembly machinery (BAM), as well as folding it. SurA is the unique chaperone that interacts with POTRA (polypeptide transport-associated) domain of OMP BamA [20]. Furthermore, SurA has been revealed that it additionally expands OMP and enhances OMP's solubility, in order to facilitate OMP achieving favorable folding and assembly by BAM.

Protein SurA has three functional regions, significantly working with a dynamic conformational change (figure 8) which is examined by techniques of solutional nuclear magnetic resonance (SNMR) and single-molecule fluorescence resonance energy transfer. The NC core module (NCM) consists of the C-terminal helix tail and N-terminal region that fold together. PPIase domain 1 (P1) domain incorporates the peptide-binding site. And P1 is also acknowledged as the regulator on OMP assembly [20]. Necessary to be mentioned, researchers detected that P1 domain itself has not active isomerase activity. PPIase domain 2 (P2) enhances the OMP assembly by BAM [20].

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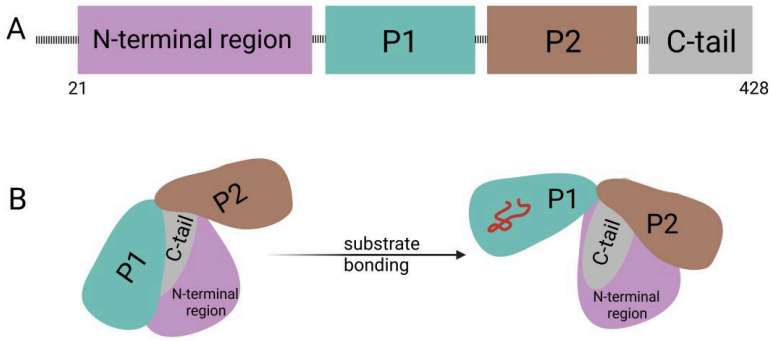


Figure 8. (A) A distribution model of SurA's structural regions, analyzed from the crystal structure. (B) The schematic model of SurA's dynamic conformational change. In the left resting period, P1 closely adheres to the NCM and P2 shows comparatively less combination level with them. Along with binding to the client, P1 changes into the open state, while P2 changes the conformation and approaches the NCM. Two PPIase domains play counteracting roles by competitively occupying the space on NCM, to orchestrate client bonding

2.5. Certain proteins with extra chaperone activities

Some families generally lack chaperone activities. But some certain proteins within these families have been observed to exhibit certain chaperone activities, in addition to performing their primary functions. This is a field well worth discussing.

2.5.1. SecB

Protein SecB weighs 69kDa, active in cytosol of Gram-negative bacteria. SecB always exerts efficacy as a tetramer, on binding to the ribosome-nascent chain (RNC) on the residue of the generated peptide chain. SecB has high-level anti-folding activity and also facilitates the co-translational folding of peptides unreleased from ribosomes. On the other aspect, SecB participates in the inner membrane protein (IMP) localization, as well as the trans-membrane transport of the periplasmic protein or OMP. Moreover, it is noticeable that the other members of family Sec have no such chaperone activity.

2.5.2. Trigger factors

Trigger factors (TFs) are around 48kDa of weight. TFs remarkably exist on ribosomes in bacteria, and additionally be traced in chloroplasts [21].

ATP-independent TFs possess three key domains as a CTD-back-folded conformation (figure 9). All domains contain sites for nascent-chain interactions (the PPIase domain has only a single one) to bind to peptides in order to maintain them [21,22]. The linker connects PPIase domain (PD) and N-terminal domain (NTD). NTD additionally functions in ribosome bonding and PD takes effect on co-chaperone bonding. C-terminal domain (CTD) homology to SurA's, has the most client-binding sites [22]. Furthermore, TFs bind to certain partially folded proteins in the intermediate state, and shield their oligomeric regions. As exemplified, researchers detected that TFs suppress the *Vibrio harveyi* luciferase dimerization [21, 22]. In some cases, TFs also trigger proteins into intermediate state with aggregation resistance [21]. Super notably, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is folded while binding to TFs and rapidly self-associates after release [22], which is still ATP-independent as ground-breaking.

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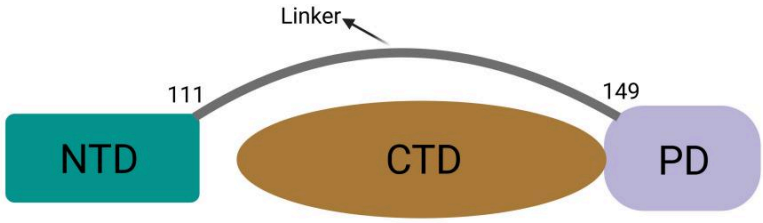


Figure 9. A schematic model of TFs' structural distribution

2.6. Chemical chaperones

Certain chemical molecules display chaperone activity as well, which shows significant potential in disease therapy. They are divided into categories of specific and non-specific chaperones.

As constructively exemplified, chemical chaperones can effectively alleviate the intensity of ERS. In the case that the free fatty acid palmitate results in the inhibition of glucose-stimulated insulin secretion (GSIS), applying 4-phenylbutyric acid (4-PBA) can alleviate the phenomenon of ERS by restoring effective insulin concentration and controlling early death of islet cells [23]. Tauroursodeoxycholic Acid (TUDCA) inhibits the phosphorylation of PERK and IRE1 in ERS pathway to restrain cell damage.

Li, et al. designed a kind of nanochaperone that consists of three compounds (not of amino acids) that conjoin together [24]. This nanochaperone stimulates the functional HSP70, to bind to incompletely folded insulin and prevent aggregation or hydrolysis. It serves as an ideal avenue for efficient insulin delivery. Li, et al. devised a series of artificial chemical nanochaperones with tunable and highly specific functions in efficiently refolding denatured biological enzymes [2].

3. Chaperones in disease treatment

HSPs endow cellular pathogens with resistance to certain stresses, and many HSPs have been confirmed to be immunogenic. Therefore, targeting specific HSPs by inhibitors shows a promising effect on treating cellular pathogen-mediated diseases. The following text also discusses cancer's therapeutic strategies related to HSP90 and the role of HSPs in tauopathies.

3.1. In bacterial diseases

Under the stresses of oxidation, heat, etc. in bacteria, ClpB individually exerts an effect on dissociating aggregates, but cooperation with DnaK presents stronger specificity and efficiency. DnaK recognizes aggregates and recruits ClpB for disaggregation and monomer extraction. ClpB macroscopically increases bacterial intracellular fission and inextricably bounds up with Type VI Secretion System about infectivity. And ClpB has been proposed to likely facilitate *Mycobacterium tuberculosis* virulence restoration.

Guanidinium chloride (GdmHCl) serves as the specific inhibitory for ClpB, which interferes with UPR in bacteria by stably binding to ATP and inhibiting ATP hydrolysis. As experimentally confirmed, low-concentration GdmHCl efficiently constrains over half of *Ehrlichia* that have invaded macrophages, without affecting activity of macrophages [25]. And many sHSPs possess high immunogenicity and they conservedly maintain virulence of pathogenic spirochetes of *Leptospira*.

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Furthermore, analyzing chaperonome has been regarded as a valuable approach to researching HSPs' evolution as well as their functional universality and specificity in *Leptospira*. Chaperonomics might supports the breakthrough in disease combat.

3.2. In unicellular eukaryotic organism disease

In fungal pathogens, the HSP90 serves as the super crucial chaperone in ERS. Targeting HSP90 is regarded as the first approach worthy to explore, but which runs the risk of affecting HSP90 of host cells. O'Meara, et al. proposed that targeting the interacting proteins of HSP90 by their selective inhibitor deserves adequate attention for Candida therapy [26], which requires the complete exploration on the whole HSP90 regulatory network.

*Pf*Grp94 in *Plasmodia* is a significant ER chaperone that takes effect as ATP-dependent on maintaining conformation and solubility by binding to malate dehydrogenase, but not refolding it. Researchers hypothesized that the mild inhibitor NECA can change *Pf*Grp94's conformation, to inhibit *Pf*Grp94 from ATP bonding, under the experimental result that NECA-ATP binding stability shows greater than *Pf*Grp94-ATP [27].

Many HSPs are found as antigenic active substances to activate lymphocytes, which has been experimentally demonstrated in *Taenia dolium*'s sHSP35.6, *Ostertagia ostertagi*'s HSP18 (with bovine serum), *Leishmania*'s HSP20 (with dog serum), etc.

3.3. In cancers

HSP90 participates in the process of the cell cycle and cooperates with chaperone Cd37 to activate the signaling kinase in anti-apoptotic pathway. It facilitates the tumor cell in proliferation as well as adhesiveness weakened [28]. Notably, tumor cells' demand for HSP90 exhibits explosive growth, along with the process of the cell cycle. As confirmed, HSP90 up-regulates the expression of NF-kB and survivin, and also down-regulates the expression of pro-apoptotic proteins. Protein NF-kB serves as the multi-function transcriptional activator in tumor cells [28].

Moreover, as experiments showed, inhibiting HSP90 presents remarkable disruption in tumor cells, compared with suppressing IkB (inhibitor of kappa B) kinase that functions in the anti-apoptotic pathway. Significantly, targeting HSP90 is regarded as an efficacious approach to cancer combat. Utilizing some compounds that bind to and disrupt NTD of the HSP90 shows feasible, on the other side, compounds DDO-5936 and Novobiocin can bind to CTD of HSP90, to influence interactions between the HSP90 and its co-chaperones. DDO-5936 specifically targets the HSP90 of tumor cells but not of native cells [28].

3.4. In tauopathies

The tau protein exerts an effect on stabilizing microtubules. In the brain, misfolded tau (as the 'seed') can trigger inert monomers aggregating into fibrous amyloid which features many β-sheets. In the process of aggregation, tau 'seeds' make inert tau partially change conformation to reveal hydrophilic amyloid motifs which mediate fibril formation. The tau-fibril appearance induces the tauopathies including certain neurodegenerative diseases such as Alzheimer's. And different conformations of monomeric 'seeds' and aggregates correspond to different kinds of tauopathies.

Tauopathies can be recovered and prevented by the cooperation system of the HSP40/70/90. Various HSP40s take charge of recognizing and binding to kinds of tau monomers as well as aggregates and transport them to HSP70s, assisting HSP70 in directing refolding. Essentially,

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HSP90s are regulated by HSP70s to bind to repeat domains of intermediately folded tau, and finally fold tau into inert conformation [29].

DnaJA1/2 of Type I HSP40, takes the monomeric tau as substrates and be recruited by co-chaperone for correct folding or refolding. As the research indicated, DnaJA1/2 have capacity of binding to aggregates indeed, but cannot facilitate tauopathy combat. Thus, DnaJ1/2 are hypothesized as incompetent to cooperate with co-chaperones for further folding. Type II HSP40 undertakes binding to monomeric 'seeds' and tau fibrils. For details, DnaJB1/4 have been proven to bind to fibrils and assist in refolding and disaggregating. DnaJC7 of Type III is concluded to bind to natively folded tau with great ease and forestall triggering denaturation. Additionally, sHSPs perform as ATP-independent holdase and the HSPB1 hinders tau's phosphorylation to avert relevant diseases [29].

4. Application of chaperones in production

In the industrial scale-up production of recombinant proteins such as insulin, the insoluble IBs formed by misfolded proteins appear frequently, which critically declines the production efficiency. Especially in E.coli as a system widely utilized, recombinant proteins are usually folded belatedly and also typically lack significant protein-process organelles such as ER which can directedly facilitate correct folding. In such a case, chaperones show enormous potential in decreasing IBs to stimulate the field with ideal functions and solubility. Moreover, protein modification driven by chaperones is crucial as well.

Shanmugasundaram, et al. devoted efforts in seeking highly efficient chaperones from species such as *archaea* rather than the E.coli-native, to achieve IB prevention. They confirmed exogenous application of chaperones significantly attenuates aggregation degree of IBs, under in vitro experiment. As results indicated, a chaperonin species can reduce IBs roughly by half in both height and width but cannot elevate active functional proteins correctly folded, which is detected by Atomic Force Microscopy. And Shanmugasundaram, et al. employed two types of plasmids corresponding to introduced genes of the target protein Green Fluorescence Protein (GFP) and the chaperone from *archaea*. GFP shows fluorescence leaning on its correct folding and it usually undergoes misfolding in bacteria as temperature (37 ℃)-driven. As experimentally confirmed, intracellular co-expression can enhance the stronger fluorescence signal which means increased content of soluble GFP. From these results, it is demonstrated that co-expression serves as an important approach to enhancing the industrial efficiency of recombinant proteins [30].

5. Conclusion

Molecular chaperones have been emphasized and continuously examined since the last century. This review clearly described the main classes of chaperones and their significant functional structures. Conspicuously, there exist 'elegant' similarities and abundant variations among chaperones which are intertwined into a grand-scale metabolic network that remains far from fully elucidated. The research on chaperone-related diseases and protein production still show significant potential and room for development. The artificial synthesis of molecules with specific chaperone activities represents an important breakthrough, making the utilization of chaperones highly convenient, efficient and controllable.

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References

[1] Badraghi, J., Yousefi, R., Saboury, A. A., Sharifzadeh, A., Haertlé, T., Ahmad, F., & Moosavi-Movahedi, A. A. (2009). Effect of salts and sodium dodecyl sulfate on chaperone activity of camel alphaS(1)-CN: insulin as the target protein. *Colloids and surfaces. B, Biointerfaces, 71*(2), 300–305. https: //doi.org/10.1016/j.colsurfb.2009.03.008

[2] Li, Y., Yin, D., Lee, S. Y., & Lv, Y. (2024). Engineered polymer nanoparticles as artificial chaperones facilitating the selective refolding of denatured enzymes. *Proceedings of the National Academy of Sciences of the United States of America, 121*(19), e2403049121. https: //doi.org/10.1073/pnas.2403049121

[3] Yakubu, U. M., & Morano, K. A. (2018). Roles of the nucleotide exchange factor and chaperone HSP110 in cellular proteostasis and diseases of protein misfolding. *Biological chemistry, 399*(10), 1215–1221. https: //doi.org/10.1515/hsz-2018-0209

[4] Zolkiewski, M., Zhang, T., & Nagy, M. (2012). Aggregate reactivation mediated by the HSP100 chaperones. *Archives of biochemistry and biophysics, 520*(1), 1–6. https: //doi.org/10.1016/j.abb.2012.01.012

[5] Mamipour, M., Yousefi, M., & Hasanzadeh, M. (2017). An overview on molecular chaperones enhancing solubility of expressed recombinant proteins with correct folding. *International journal of biological macromolecules, 102*, 367–375. https: //doi.org/10.1016/j.ijbiomac.2017.04.025

[6] Peng, C., Zhao, F., Li, H., Li, L., Yang, Y., & Liu, F. (2022). HSP90 mediates the connection of multiple programmed cell death in diseases. *Cell death & disease, 13*(11), 929. https: //doi.org/10.1038/s41419-022-05373-9

[7] Schopf, F. H., Biebl, M. M., & Buchner, J. (2017). The HSP90 chaperone machinery. *Nature reviews. Molecular cell biology, 18*(6), 345–360. https: //doi.org/10.1038/nrm.2017.20

[8] Inoue, T., Hirata, K., Kuwana, Y., Fujita, M., Miwa, J., Roy, R., & Yamaguchi, Y. (2006). Cell cycle control by daf-21/HSP90 at the first meiotic prophase/metaphase boundary during oogenesis in Caenorhabditis elegans. *Development, growth & differentiation, 48*(1), 25–32. https: //doi.org/10.1111/j.1440-169X.2006.00841.x

[9] Samakovli, D., Margaritopoulou, T., Prassinos, C., Milioni, D., & Hatzopoulos, P. (2014). Brassinosteroid nuclear signaling recruits HSP90 activity. *The New phytologist, 203*(3), 743–757. https: //doi.org/10.1111/nph.12843

[10] Mayer M. P. (2013). HSP70 chaperone dynamics and molecular mechanism. *Trends in biochemical sciences, 38*(10), 507–514. https: //doi.org/10.1016/j.tibs.2013.08.001

[11] Amick, J., Schlanger, S. E., Wachnowsky, C., Moseng, M. A., Emerson, C. C., Dare, M., Luo, W. I., Ithychanda, S. S., Nix, J. C., Cowan, J. A., Page, R. C., & Misra, S. (2014). Crystal structure of the nucleotide-binding domain of mortalin, the mitochondrial HSP70 chaperone. *Protein science : a publication of the Protein Society, 23*(6), 833–842. https: //doi.org/10.1002/pro.2466

[12] Cömert, C., Kjær-Sørensen, K., Hansen, J., Carlsen, J., Just, J., Meaney, B. F., Østergaard, E., Luo, Y., Oxvig, C., Schmidt-Laursen, L., Palmfeldt, J., Fernandez-Guerra, P., & Bross, P. (2024). HSP60 chaperone deficiency disrupts the mitochondrial matrix proteome and dysregulates cholesterol synthesis. *Molecular metabolism, 88*, 102009. https: //doi.org/10.1016/j.molmet.2024.102009

[13] Singh, M. K., Shin, Y., Han, S., Ha, J., Tiwari, P. K., Kim, S. S., & Kang, I. (2024). Molecular Chaperonin HSP60: Current Understanding and Future Prospects. *International journal of molecular sciences, 25*(10), 5483. https: //doi.org/10.3390/ijms25105483

[14] Qiu, X. B., Shao, Y. M., Miao, S., & Wang, L. (2006). The diversity of the DnaJ/HSP40 family, the crucial partners for HSP70 chaperones. *Cellular and molecular life sciences : CMLS, 63*(22), 2560–2570. https: //doi.org/10.1007/s00018-006-6192-6

[15] Li, J., Qian, X., & Sha, B. (2009). Heat shock protein 40: structural studies and their functional implications. *Protein and peptide letters, 16*(6), 606–612. https: //doi.org/10.2174/092986609788490159

[16] Janowska, M. K., Baughman, H. E. R., Woods, C. N., & Klevit, R. E. (2019). Mechanisms of Small Heat Shock Proteins. *Cold Spring Harbor perspectives in biology, 11*(10), a034025. https: //doi.org/10.1101/cshperspect.a034025

[17] Carver, J. A., Grosas, A. B., Ecroyd, H., & Quinlan, R. A. (2017). The functional roles of the unstructured N- and C-terminal regions in αB-crystallin and other mammalian small heat-shock proteins. *Cell stress & chaperones, 22*(4), 627–638. https: //doi.org/10.1007/s12192-017-0789-6

[18] Dumont, B., Terradot, L., Cascales, E., Van Melderen, L., & Jurėnas, D. (2024). Thioredoxin 1 moonlights as a chaperone for an interbacterial ADP-ribosyltransferase toxin. *Nature communications, 15*(1), 10388. https: //doi.org/10.1038/s41467-024-54892-w

[19] Currier, R. B., Ulrich, K., Leroux, A. E., Dirdjaja, N., Deambrosi, M., Bonilla, M., Ahmed, Y. L., Adrian, L., Antelmann, H., Jakob, U., Comini, M. A., & Krauth-Siegel, R. L. (2019). An essential thioredoxin-type protein of

DOI: 10.54254/2753-8818/2025.AU25737

Trypanosoma brucei acts as redox-regulated mitochondrial chaperone. *PLoS pathogens, 15*(9), e1008065. https: //doi.org/10.1371/journal.ppat.1008065

[20] Soltes, G. R., Schwalm, J., Ricci, D. P., & Silhavy, T. J. (2016). The Activity of Escherichia coli Chaperone SurA Is Regulated by Conformational Changes Involving a Parvulin Domain. *Journal of bacteriology, 198*(6), 921–929. https: //doi.org/10.1128/JB.00889-15

[21] Hoffmann, A., Bukau, B., & Kramer, G. (2010). Structure and function of the molecular chaperone Trigger Factor. *Biochimica et biophysica acta, 1803*(6), 650–661. https: //doi.org/10.1016/j.bbamcr.2010.01.017

[22] Wu, K., Minshull, T. C., Radford, S. E., Calabrese, A. N., & Bardwell, J. C. A. (2022). Trigger factor both holds and folds its client proteins. *Nature communications, 13*(1), 4126. https: //doi.org/10.1038/s41467-022-31767-6

[23] Choi, S. E., Lee, Y. J., Jang, H. J., Lee, K. W., Kim, Y. S., Jun, H. S., Kang, S. S., Chun, J., & Kang, Y. (2008). A chemical chaperone 4-PBA ameliorates palmitate-induced inhibition of glucose-stimulated insulin secretion (GSIS). *Archives of biochemistry and biophysics, 475*(2), 109–114. https: //doi.org/10.1016/j.abb.2008.04.015

[24] Li, C., Liu, X., Zhang, Y., Lv, J., Huang, F., Wu, G., Liu, Y., Ma, R., An, Y., & Shi, L. (2020). Nanochaperones Mediated Delivery of Insulin. *Nano letters, 20*(3), 1755–1765. https: //doi.org/10.1021/acs.nanolett.9b04966

[25] Kędzierska-Mieszkowska, S., & Zolkiewski, M. (2021). Hsp100 Molecular Chaperone ClpB and Its Role in Virulence of Bacterial Pathogens. *International journal of molecular sciences, 22*(10), 5319. https: //doi.org/10.3390/ijms22105319

[26] O'Meara, T. R., Robbins, N., & Cowen, L. E. (2017). The HSP90 Chaperone Network Modulates Candida Virulence Traits. *Trends in microbiology, 25*(10), 809–819. https: //doi.org/10.1016/j.tim.2017.05.003

[27] Muzenda, F. L., Stofberg, M. L., Mthembu, W., Achilonu, I., Strauss, E., & Zininga, T. (2025). Characterization and Inhibition of the Chaperone Function of Plasmodium falciparum Glucose-Regulated Protein 94 kDa (Pf Grp94). *Proteins, 93*(5), 957–971. https: //doi.org/10.1002/prot.26779

[28] Basset, C. A., Conway de Macario, E., Leone, L. G., Macario, A. J. L., & Leone, A. (2023). The chaperone system in cancer therapies: HSP90. *Journal of molecular histology, 54*(2), 105–118. https: //doi.org/10.1007/s10735-023-10119-8

[29] Ryder, B. D., Wydorski, P. M., Hou, Z., & Joachimiak, L. A. (2022). Chaperoning shape-shifting tau in disease. *Trends in biochemical sciences, 47*(4), 301–313. https: //doi.org/10.1016/j.tibs.2021.12.009

[30] Shanmugasundaram, M., Pavlova, N. V., Pavlov, A. R., Lednev, I. K., & Robb, F. T. (2021). Improved folding of recombinant protein via co-expression of exogenous chaperones. *Methods in enzymology, 659*, 145–170. https: //doi.org/10.1016/bs.mie.2021.09.001