

13

The Work of Chaperones

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13.1

From The Levinthal Paradox To The Anfinsen Cage

The classic experiment of Anfinsen showing that the unfolded ribonuclease folds spontaneously *in vitro* established the thermodynamic hypothesis of protein stability, i.e., that a protein's primary sequence dictates three essential and partially overlapping features of proteins, viz. the assembly pathway, the structure and function, without need of any further genetic information [1]. However, how the correct folding of a protein is selected among an astronomically large number (10^{16}) of possible conformations to give the native active state was enigmatic for a long time, a problem known as the Levinthal paradox. This was particularly true for large proteins, but it is now clear that folding pathways *guide* the protein, along energy landscapes, towards the unique (lowest energy) native conformation, through a series of partially folded intermediate states known as molten globules [2]. In other words, it is possible to arrive at the native state of a protein after having searched through *only* a minute fraction of the total number of conformations [3, 4]. The transition from the molten globule state (which contains elements of secondary structure such as α -helices and β -sheets, but lack well-defined, unique tertiary interactions) to the native state is often the rate-limiting step (Fig. 13.1).

In vitro, self-folding gives low yields and slow rates, particularly at temperatures above 15–20°C and at high protein concentrations, i.e., under situations of incredibly high macromolecular crowding as is the case in living cells [4b]. Also, unfavorable side-reactions, such as misfolding or aggregation of partially folded intermediates (Fig. 13.2), often compete with the correct assembly pathway [5]. Therefore, self-assembly is not the predominant form of protein assembly *in vivo*, and proteins will be assisted by a particular class of proteins, sequestering them during folding in a safe environment protected from aggregation, sometimes referred to as the Anfinsen cage [6] or box of infinite dilution. These folding or assembly helpers have been termed molecular chaperones. More than just playing the role of a passive cage, chaperones also decrease the roughness of the energy landscape of the substrate protein. After assisting the correct folding and assembly of other proteins, they are not themselves components of the final functional structures, nor do they cause covalent modifications of the target protein or protein complex, for example, the *Escherichia coli* GroEL/GroES chaperonin, which participates in bacteriophage

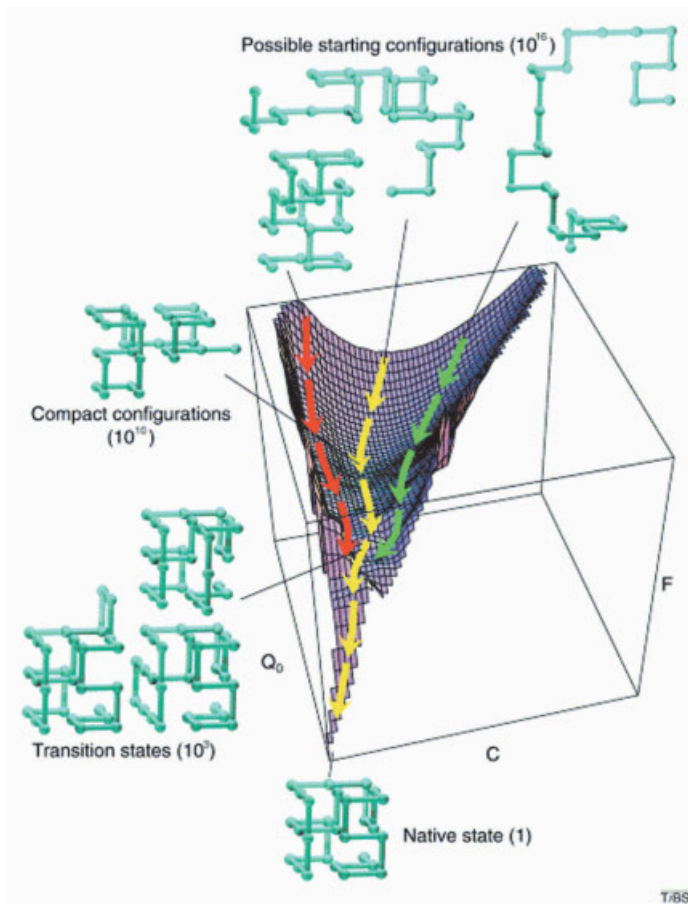


Figure 13.1 Free-energy landscape of the fold-ing of a protein. The free energy (F) surface or free energy landscape of a protein is represented as a function of the number of native contacts (Q) and the total number of (native and nonnative) contacts (C). Native and non-native contacts refer to contacts bringing or not towards the native state, respectively. The surface shown in the figure illustrates that at the beginning of the folding reaction there are many conformations of similar free energy, so that the accessible surface is very broad. As folding progresses, the energy of the system decreases with the formation of native contacts that are generally

more stabilizing than the non-native ones. Thus, the entropy decreases as the native state is approached, and the free-energy surface has a funnel-like shape that guides the system towards the unique (lowest energy) native conformation. Among the intermediate folding species are the molten globules, which are generally close to the native state. The yellow trajectory shows the average folding pathway, and the other two trajectories (green and red) show a range of two standard deviations around the average and are thus expected to include 95% of the trajectories. Reprinted with per-mission from Dinner et al. [4].



Figure 13.2 Formation of a domain-swapped aggregate in the process of protein folding. A newly synthesized protein molecule in the process of folding seeks a thermodynamically stable structure. Among possible low-energy structures that it may seek are a monomer, a domain-swapped dimer, and a domain-swapped aggregate. Reprinted with permission from Eisenberg [298].

λ head assembly, is not found in the assembled head structures. Molecular chaperones also act as refolders of misfolded or aggregated substrates, probably through substrate unfolding (either local or global) [7, 8].

Chaperones are ubiquitous, universal, highly conserved throughout evolution in both structural and functional properties: HSP70 is the most conserved protein known to date that is found in all biota, i.e., eubacteria, eukaria and some of the archaea [9]. This high conservation across the phylogenetic domains [10, 11] has provided support for a phylogenetic classification of all living cells [12–14] that intriguingly differs from that based on comparative analyses of 16S rRNA sequences: the HSP70-based phylogenies predict a specific evolutionary relationship between the archae and Gram-positive bacteria on the one hand, and between the Gram-negative bacteria and eukaryotes on the other [15].

The myriad of functions of the molecular chaperones [16–18] can be summarized as follows:

1. *De novo* protein folding, i.e., co- or post-translational folding of ribosome-bound nascent polypeptide chains.
2. Refolding and prevention and reversion of aggregation of misfolded or denatured proteins.
3. Protein translocation across membranes.
4. Post-translational quality control, to detect and eliminate, in co-operation with proteases, the proteins irreversibly unfolded.
5. Assembly and disassembly of protein and nucleoprotein complexes.
6. Modulation of the heat-shock response.

In summary, two assignments can be made for the molecular chaperones: house-keeping and defence against stress functions. It is therefore not surprising that, to fulfill all their roles, the chaperones function with a cohort of accessory factors [19a].

13.2

The Folding Machines

13.2.1

The Trigger Factor (TF)

The trigger factor (TF) is an ATP-independent chaperone. It displays both chaperone and peptidyl-prolyl-*cis-trans*-isomerase (PPIase) activities [19b]. It is not a heatshock protein, but is induced upon cold shock and enhances *E. coli* viability at low temperatures [20a]. The lack of TF (deletion of the gene *tig*) has almost no effect on *E. coli* growth, but a strain with deletions of both *tig* and *dnaK* can survive only at low temperature [20b, 20c].

The trigger factor interacts with the ribosome [21–23] and is, along the chaperone pathway, the first that affects the folding of newly formed protein chains, scanning for prolyl bonds that need catalysis of isomerization. However, the binding of TF to peptides is not dependent on the presence of proline residues, and it is not known whether PPIase activity is required for the TF chaperoning of nascent chains. Binding of TF to the ribosome is important for creating a high local concentration of substrates [24, 25].

The trigger factor is composed of three domains: an N-terminal domain (NTD), which mediates association with the large ribosomal subunit, a central substrate binding and PPIase domain, and a C-terminal domain (CTD). It is monomeric in its ribosome-associated state, but uncomplexed TF is in monomer–dimer equilibrium, with two-thirds existing in a dimeric state [26].

13.2.2

The DnaK/DnaJ/GrpE System

The control of protein folding by DnaK is coupled to its ATPase activity, and these two activities correspond to two functional domains: the 44 kDa NTD (residues 1–385) which binds and hydrolyses ATP; a CTD (residues 390–638) which consists of (i) a 18 kDa β -sandwich subdomain which binds and releases polypeptide targets (substrate-binding domain) [27] and (ii) another subdomain (10 kDa) composed of five α -helices (residues 537–638) [28a] that acts like a lid [286] over the β -sandwich subdomain to encapsulate the bound peptide in the ADP-bound state; the bound peptide contacts the β -sandwich but not the lid.

The DnaK-binding motif in substrates consists of a core of up to five large hydrophobic or aromatic residues, and flanking regions enriched in positively charged residues which are of decreasing importance with increasing distance from the core [29]. As both denatured proteins and folding intermediates display hydrophobic surfaces, DnaK and the various eukaryotic HSP70s stabilize non-native polypeptides through the binding and release of these extended hydrophobic peptide segments that are normally buried in the fully folded form, but are exposed during protein synthesis, protein translocation and protein degradation. However, *E. coli* DnaK also binds some native proteins such as λ P, λ O, λ CIII, RepA, heat-shock

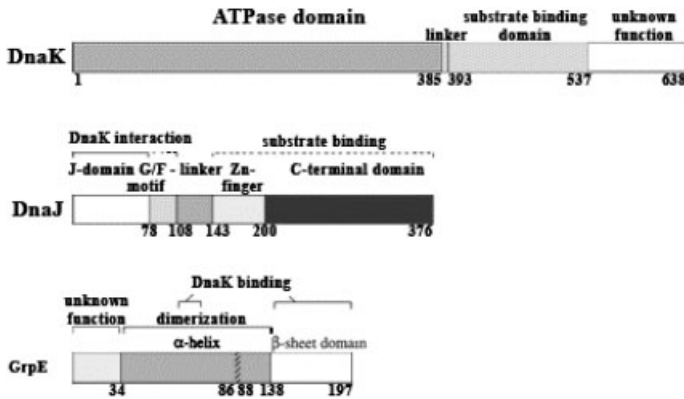


Figure 13.3 Domain organization of the *E. coli* chaperones DnaK, DnaJ and GrpE. Residue numbers define the approximate individual domain borders. Residues 386–392 of DnaK constitute a linker between the ATPase and the substrate-binding domain. Residues 86–88 of GrpE constitute a break of the long N-terminal α helix in the GrpE monomer that interacts with DnaK. Reprinted with permission from Bukau and Horwich [299].

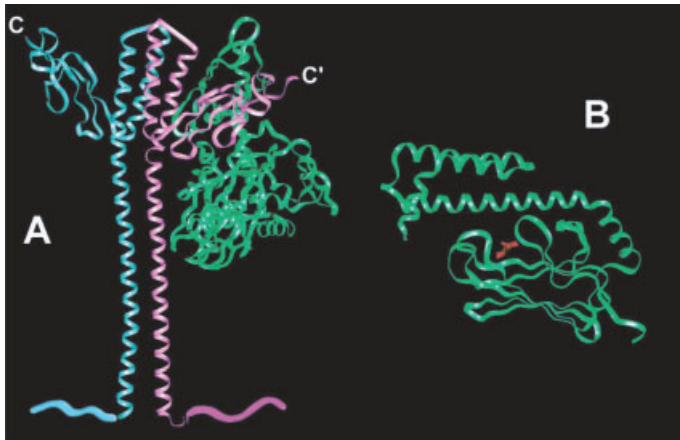


Figure 13.4 Structures of the *E. coli* chaperones GrpE and DnaK. (A) Structure of the GrpE homodimer complexed to the ATPase domain of DnaK. The proximal and distal GrpE monomers are shown in purple and light blue, respectively. The ATPase domain is shown in green. In total, there are six contact areas between DnaK and GrpE. (B) Structure of the C-terminal substrate binding and lid domains of DnaK. DnaK is shown in green and the bound peptide in red. Reprinted with permission from Chesnokova et al. [300].

transcription factor σ^{32} [30], the tumor suppressor protein p53 [31]. BIP, a resident endoplasmic reticulum HSP70, associates with immunoglobulin heavy and light chains [32] and human immunodeficiency virus envelope glycoprotein gp160 [33].

There is a mutual stimulation of ATPase activity and substrate release [34–40]. Interestingly, interdomain coupling occurs even when the lid is deleted, but potassium ions are indispensable for the mutual functional control of the two domains [41a]. The ATP-bound form of DnaK is characterized by high on- and off-rates of substrate interactions (fast peptide binding and release) and a low affinity, whereas the ADP-bound form is characterized by low on- and off-rates (slowly binding and release) and high affinity for substrates. In other words, DnaK in its ADP state captures substrates, and ATP inhibits the capture [40]. The ATPase activity thus constitutes a switch regulating the velocity and stability of substrate binding by DnaK.

An interesting difference between proteins of the DnaK/HSP70 family and the GTPases is that the latter are activated to bind proteins when they contain bound nucleotide triphosphate (GTP) [41b], whereas HSP70 forms stable complexes with protein substrates when nucleotide diphosphate (ADP) is bound.

The ATPase activity of DnaK is itself under tight control of two cofactors: DnaJ, which markedly stimulates the ATPase activity [42, 43], and GrpE, which facilitates the ADP/ATP exchange since DnaK binds ADP more tightly than ATP [44]. The stimulation of the ATPase activity requires the conserved J domain [45] of DnaJ (residues 2–78 in *E. coli*; [46]). DnaJ tightly couples ATP hydrolysis with binding of protein substrate by DnaK [47] through a mechanism that involves communication between the ATPase and the substrate-binding domains of DnaK. But DnaJ itself is also capable of associating with unfolded substrates and preventing aggregation, having most binding motifs in common with DnaK [48]. This qualifies DnaJ as a chaperone in its own right and as a targeting partner for DnaK [42].

The DnaK reaction cycle in protein folding is therefore the following: DnaJ acts on the ATP-bound DnaK (rapid substrate binding and release, low affinity, so-called T state) to stimulate the hydrolysis of ATP, resulting in the ADP-bound form of DnaK, which binds the substrate tightly (so-called R state). DnaK probably transduces free energy from ATP binding and hydrolysis to produce a conformational change in the substrate protein that increases the probability of proper folding. Substrate ejection then requires the dissociation of ADP, which is catalyzed by GrpE, and which occurs in concert with binding of a new ATP molecule. Therefore, under *in vivo* conditions with an estimated chaperone ratio of DnaK/DnaJ/GrpE = 10/1/3, both DnaJ and GrpE appear to control the chaperone cycle by transient interactions with DnaK [49a].

In mammalian cells, a network of co-operating and competing chaperone cofactors, such as the DnaJ-like HSP40s, HAP46/BAG-1[49b] (Sn1 in yeast; [50]), Hip, Hop (Sti 1 in yeast; [51]), and CHIP [52] modulates the chaperone activity of the heat-shock cognate protein HSC70 [53] (see Fig. 13.12). For a synopsis of the *E. coli*, yeast and mammalian HSP40s proteins see Table IV in Ref. [42]. The large tumor antigen (T antigen) of simian virus 40 (SV40) is also a DnaJ molecular chaperone [54]).

Concerning the role of DnaK in assisting folding of newly synthesized cytosolic proteins, it had been thought for long time that DnaK plays a critical role in *de novo* folding (see Sect. 13.3.1). Indeed, DnaK is present in the *E. coli* cytosol at $\sim 50 \mu\text{M}$, roughly equivalent to the concentration of ribosomes. However, the fraction of newly translated proteins that is recovered in a complex with DnaK is only 5–10% of the total soluble *E. coli* proteins at 30°C, preferentially in the size range of 30–75 kDa [55]. Also a study with a mutant lacking the function of DnaK (ΔdnaK) indicates that, at 30°C at least, DnaK is not essential for bacterial viability, nor for *de novo* folding of the majority of *E. coli* proteins [56]. However, DnaK is essential for *E. coli* growth at 37°C and above, and a large set of thermolabile *E. coli* proteins are substrates of DnaK and of two other chaperones, ClpB [57] and trigger factor [58] during heat stress, both *in vivo* and in cell extracts.

In eukaryotes, the so-called heat-shock “cognate” proteins HSC70s are non-inducible but constitutively expressed homologs of the HSP70s. Interestingly, the HSP70 genes (but not the HSC70 genes) are intronless. Although unusual (other genes that lack intervening sequences include histones and α -interferon), this feature is perhaps significant for genes that are rapidly activated at the transcriptional level. Two DnaK homologs have been found in *E. coli*: Hsc66 (=HscA) which seems to be specialized for the assembly of iron–sulfur cluster proteins [59], and Hsc62 (=HscC) [60], the function of which is less clear: it forms a complex with the transcription factor σ^{70} , and may function as its negative modulator [61], but it may also be involved in the repair of damage induced by radiation and cadmium since mutations in this protein have been identified that are hypersensitive to ultraviolet light and cadmium. Triple knock-outs of all *E. coli* genes encoding HSP70 proteins ($\Delta\text{dnaK} \Delta\text{hscA} \Delta\text{hscC}$) are viable, indicating that HSP70 proteins are not strictly essential for viability. There are also four additional DnaJ proteins in *E. coli*: (i) CbpA, (ii) DjlA, both of which interact with DnaK, (iii) Hsc20 (=HscB), which interacts with HscA, and (iv) DjlC, which seems to be the appropriate DnaJ cochaperone for HscC. The unique common feature of all the DnaJ homologs is a short sequence of about 75 amino acid residues called the J domain, which is essential for the interaction with an HSP70 chaperone partner and for the stimulation of its ATPase activity [62].

13.2.3

The GroEL/GroES System

Early genetic studies identified the *E. coli* *groES* and *groEL* genes because mutations in them blocked the growth of bacteriophages λ , T4 and T5. GroEL/GroES (HSP60/HSP10) are also known as Group I chaperonins, to make a distinction with Group II chaperonins of archaea and eukarya (also called TRiC/CCT) [63]) (see Fig. 13.7): the latter have a few substrates including the cytoskeletal proteins actin and tubulin [64–66], Cdc20 [67a], the Von Hippel-Landau tumor suppressor complex [67b] and the WD-repeat proteins [67c]. GroEL/ES are large cylindrical complexes that promote protein folding in the sequestered environment of their central cavity. Group I chaperonins are present not only in all eubacteria [67d], but also in mitochondria and chloroplasts [68a, 68b].

GroEL/GroES is the only chaperone system in *E. coli* cytoplasm essential under all growth conditions [69]. GroEL is organized in two stacked rings, each composed of seven 60 kDa subunits [70, 71] (see Fig. 13.5). However, mutants of GroEL that are fully functional as single rings have been recently isolated [72].

The co-chaperonin GroES, a single ring of seven 10 kDa subunits, forms the lid on a folding cage (Group II chaperonins have not such a detachable GroES-like co-chaperonin [73], see Fig. 13.7). Non-native proteins are encapsulated in this cage, or passive box, which, when capped by GroES in the presence of ATP, creates an environment of infinite dilution inside its central cavity where individual polypeptide chains are free to fold without risk of aggregation. Then the dissociation of GroES allows release of the trapped protein from the cavity [74] (see Fig. 13.6). However it remains a matter of debate whether the chaperonin cage plays only a passive role in protecting the protein substrate from aggregation, or an active role in accelerating folding rates [75].

Typical GroEL substrates consist of two or more domains with $\alpha\beta$ -folds, which contain α -helices and buried β -sheets with extensive hydrophobic surfaces. Binding takes place between the hydrophobic residues in the apical domain of GroEL and the hydrophobic faces exposed by the β -sheets in the $\alpha\beta$ -domains of protein

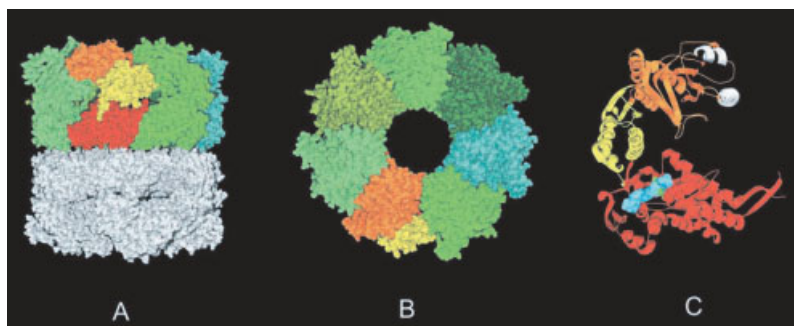


Figure 13.5 Structure of the GroEL chaperonin from *E. coli*. (A) Side-view of the GroEL tetradecamer. Subunits comprising the top ring are shown in colour, subunits of the bottom ring are shown in grey. Each subunit can be dissected into three domains: apical (orange), intermediate (yellow) and equatorial (red). (B) Top view of the GroEL tetradecamer. The seven subunits of the ring are shown in shades of green. For one subunit, the apical and the intermediate domains are highlighted in orange and yellow, respectively. (C) Ribbon representation of a GroEL subunit. The equatorial domain (red) consists almost exclusively of α helices and contains the nucleotide-binding site, which is occupied by ATP γ S (blue). The intermediate domain (yellow) serves as a molecular hinge that connects the equatorial domain with the apical domain (orange). Binding of GroES and polypeptides occurs in a hydrophobic groove formed by the two helices (white) facing the central cavity. Reprinted with permission from Walter [71].

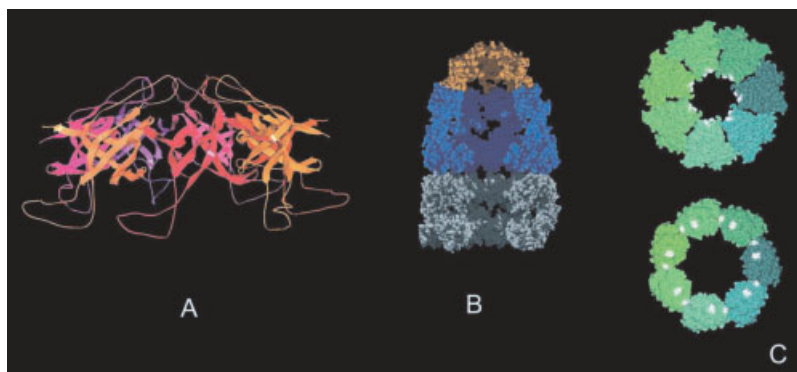


Figure 13.6 Structure of the GroELS chaperonin from *E. coli*.

(A) Sideview of the GroES heptamer. The individual subunits (in shades of red) consist mainly of β -sheets and form a dome. The flexible extensions on the bottom are the so-called mobile loops that mediate binding to GroEL. (B) Cross-section of a GroEL "bullet". Each GroEL ring encloses a cavity that serves as a folding compartment for a polypeptide substrate. Binding of GroES (orange) to the top GroEL ring (blue) blocks the access to the upper cavity and concomitantly induces an *en bloc* movement of the apical domains. (C) Changes in the GroEL structure upon binding of GroES. In this top view, the seven subunits comprising one ring of GroEL are shown in shades of green and blue. The hydrophobic residues in the apical domains important for binding of polypeptide and GroES are shown in white. In the absence of GroES (top panel), these residues coat the inside of the central cavity and account for the high affinity for unfolded polypeptides. Upon binding of GroES (lower panel) the apical domains rotate outwards by 90°. The hydrophobic patches become buried in the subunit interfaces, rendering the inner surface of the cavity mainly hydrophilic and causing the release of a bound polypeptide. Concomitantly, the diameter of the cavity increases from 45 to 80 Å. Reprinted with permission from Walter [71].

substrates [76]. Binding stimulates ATP binding and hydrolysis, causing a conformational change in the box. The transmission of an allosteric signal between the two rings of the GroEL complex is a key aspect of the reaction mechanism [77, 78].

Only 10–15% of all newly synthesized polypeptides transit GroEL post-translationally [79, 76], which agrees with the intracellular concentrations of 2.6 and 5.1 μM reported for GroEL and GroES, respectively, since this is sufficient to facilitate the folding of no more than 5% of all of the proteins within the *E. coli* cell [80]. GroEL is absolutely essential for the correct folding of *E. coli* dihydropicolinate synthase, the first enzyme in the diaminopimelic acid synthesis pathway, and therefore also for cell-wall synthesis [81].

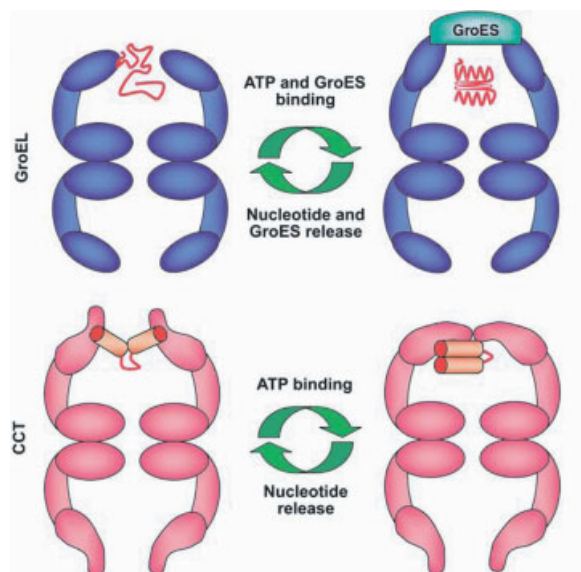


Figure 13.7 Schematic model of the folding mechanism of GroEL and CCT. Both chaperonins cycle between an open, substrate-receptive conformation and a closed conformation. In GroEL, substrate recognition and binding is performed in the open conformation by a hydrophobic region in the apical domain of the GroEL subunits. The closed conformation is generated upon ATP binding in the presence of the cochaperonin GroES, and the unfolded polypeptide is liberated in the GroEL cavity where folding takes place. The polypeptide is then liberated from the GroEL cavity after GroES release, which is induced upon ATP hydrolysis.

In CCT, built up by two superimposed rings, each ring is constituted by eight different, albeit homologous subunits (30% identity). The apical domains of specific subunits recognize the substrate. The sealing of the CCT cavity, carried out by the movements of the apical domains induced upon ATP binding, is performed by the helical protrusions present at the tip of the apical domains, and the substrate is not liberated in the CCT cavity but remains bound to the apical domains and forced to acquire a more compact, native conformation. Reprinted with permission from Valpuesta et al. [66].

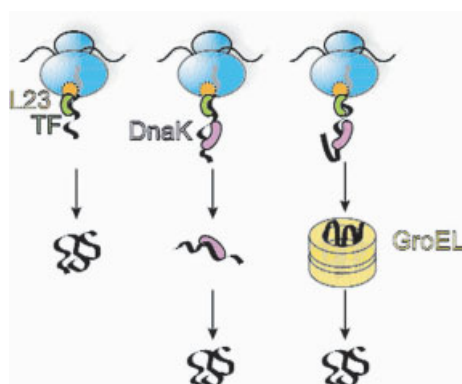


Figure 13.8 Three bacterial chaperones participating in *de novo* protein folding. The Trigger Factor interacts with emerging nascent chains via its interaction with ribosomal protein L23 at the ribosomal exit site. Some polypeptides then interact with DnaK and GroEL, which assist the folding of selected subsets of cellular proteins. Reprinted with permission from Albanese and Frydman [142].

13.2.4

Other Chaperones

13.2.4.1 HSP90

HSP90 is one of the most abundant chaperones in the cell, and is already at high levels prior to cellular stress. HSP90 is a constitutive homodimer and has only recently been recognized as an ATP-dependent chaperone [82] (see Fig. 13.9).

HtpG, the bacterial HSP90 homolog is non-essential in *E. coli* [83], but is essential for the thermal stress management in cyanobacteria [84]. In eukaryotes, HSP90-null mutants are lethal. In yeast, HSP90 does not act generally in nascent protein folding [85], but some substrates (i.e., the α -complemented β -galactosidase) depend on HSP90 for folding, assembly and/or stabilization. In higher eukaryotes, most HSP90 substrates are signal transduction proteins, such as steroid hormone receptors and signaling kinases. Interaction of the glucocorticoid receptor with HSP90 is essential for its activity. HSP90 operates as part of a multichaperone family which includes HSP70 and several cochaperones such as p23 [85b], Hop, CHIP, Cdc37, Aha1 (see [86] for a Table of the HSP90 interacting proteins). Ansamycin drugs which specifically target HSP90, the most representative being geldanamycin, makes HSP90, involved in many growth-regulatory pathways, an attractive target for cancer therapeutics [87a, 87b]. For another astonishing property of HSP90, see Sect. 13.7.1.

13.2.4.2 Clp/HSP100 Family

ClpB/HSP104 and ClpA, ClpX and ClpY (=HslU) are ATP-dependent chaperones associated with disassembly and degradation of protein complexes. They are members of the AAA⁺ family of proteins, which are ATPases Associated with a variety of cellular Activities [88–90].

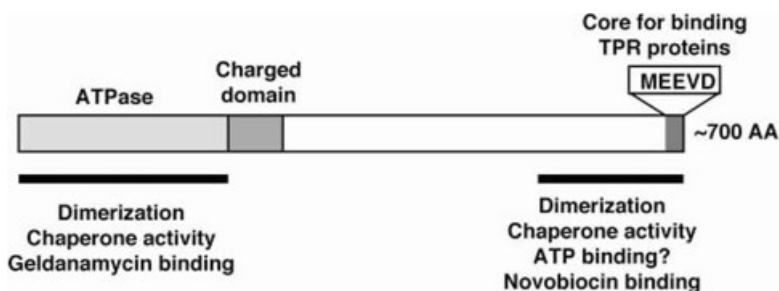


Figure 13.9 Schematic representation of the domain structure of HSP90. ATP/ADP and the HSP90 inhibitors geldanamycin and radicicol bind to the same pocket inside the N-terminal domain. Following the N-terminal ATPase domain, all eukaryotic HSP90 proteins have a charged domain of varying size. They end at the very

C-terminus with the pentapeptide MEEVD, which constitutes the core of the HSP90 interaction surface for the tetratricopeptide repeats (TPR) of HSP90 co-chaperones. Neither the charged domain nor the pentapeptide is required for viability in *S. cerevisiae*. Reprinted with permission from Picard [86].

The common physical feature of *E. coli* ClpA, ClpX and ClpY is a ring structure formed by their ATPase subunits, which arrange into ring-shaped hexameric or heptameric complexes enclosing a central cavity. They act as regulatory subunits of proteases, for example, the homohexameric ClpA, or ClpX, associate with each side of the heptameric double-ring protease ClpP, delivering recognized substrates to it for degradation [91]. This structure (ClpA₆, ClpP₇×2, ClpA₆) resembles that of the eukaryotic 20S core proteasome (=28 subunits) arranged in four homoheptameric rings, as $\alpha 7$, $\beta 7$, $\beta 7$ and $\alpha 7$ [92]. Similarly, in the *E. coli* HslUV complex, each HslU (=ClpY) hexamer binds to opposite ends of the dodecamer HslV (=CplQ) protease component [93]. In the absence of association with the protease partner ClpP, ClpA or ClpX can mediate disassembly of oligomeric substrate proteins, such as the ClpX-mediated disassembly of the Mu transposase tetramer [94] and the ClpA-mediated remodeling of bacteriophage P1 RepA dimers (inactive) into active monomers [95] (see Fig. 13.13) or the *in vitro* unfoldase activity of ClpA [96]. Similarly, without its protease partner HslV (=ClpQ), the HslU (=ClpY) ATPase acts as a molecular chaperone to prevent aggregation of Sula, an inhibitor of cell division in *E. coli* [97].

Unlike ClpA, ClpX and HslU, the protein ClpB is unique among the HSP100 proteins of *E. coli* since it does not interact with a proteolytic partner. *E. coli* cells deleted of *ClpB* show a higher rate of death above 50°C, indicating that ClpB is essential for cell survival at high temperatures, like HSP104 [98] in yeast. ClpB, which has two ATPase domains [99] but whose physiological oligomeric state is unclear at the moment (most probably an hexamer)[100a–c], does not assist itself in protein folding, but disaggregates preformed protein aggregates before transferring them to HSP70 (see Sect. 13.3.2).

13.2.4.3 DegP

E. coli DegP (=HtrA) is a periplasmic heat-shock protein, which possesses both chaperone and protease activities. The chaperone function dominates at low temperatures and the protease function, at elevated temperatures [101]. DegP becomes necessary for *E. coli* growth at temperatures above 39°C for the removal of the misfolded outer-membrane proteins [102]. The proteolytic sites are located in the central cavity of the DegP hexamer formed by the staggered association of trimeric rings [103]. This unique structural organization indicates a new type of protease chaperone machine.

13.2.4.4 Periplasmic Chaperones

Aside from DegP (see Sect. 13.2.4.3) and pili chaperones (see Sect. 13.2.4.5), the *E. coli* periplasm contains many other chaperones involved in the folding and targeting specifically of outer-membrane proteins [104] with the exception of TolC [105]. Some of these chaperones have redundant functions in protein folding [106], perhaps because of the particular conditions (absence of ATP and a relatively more

oxidizing state than in the cytoplasm) prevailing in the periplasm [107]. Misfolding of proteins in the cell envelope of *E. coli* induces a cascade of particular signaling pathways mediated by the Rse proteins and the transcription factor σ^E [108].

13.2.4.5 Pili Chaperones

Some periplasmic chaperones such as PapD and FimC are specialized in the assembly of bacterial pili (the adhesive structure that enables bacteria to bind to host cells). The priming action of the chaperone drives subunit assembly into the fiber [109]. The integration of the next pilin, the building element of the *growing* pilus, requires the removal of the chaperone sitting at the growing end. Each pilin is also complexed with a chaperone which promotes a partially unfolded state of the pilin that is required for assembly (see Fig. 13.10); in other words, the chaperone provides the missing information for folding of the pilin [110] and this case of structural complementation represents therefore an interesting extension of the Anfinsen dogma described in Sect. 13.1.

13.2.4.6 Small HSPs

The α -crystallins [111, 112] are the most representative members of the small heat-shock protein family (sHSPs) which form dynamic oligomeric structures [113]. Their chaperone activity, required to prevent aggregation of intermediate filaments [114], is of potential importance in human disease [115]: for example, a mutation in the α B-crystallin chaperone gene causes a desmin-related myopathy [116a–d]. There is another example of a mutation in a gene encoding a putative chaperonin, which causes the McKusick–Kaufman syndrome [117a], and of mutations in the sHSP22 [117b] and sHSP27[117c] which cause neurodegenerative disorders.

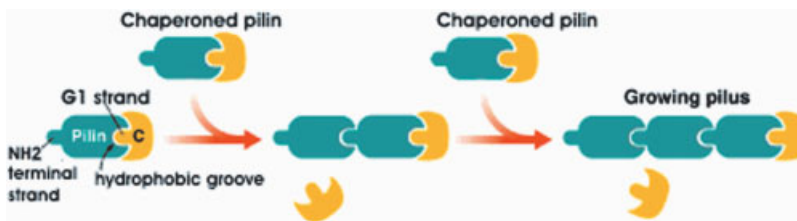


Figure 13.10 Benefits of a chaperone for a growing pilus. The pilus is the adhesive structure that enables bacteria to bind to host cells. A chaperoned pilin protein is added to a growing pilus in the following way: the chaperone carries the pilin subunit to the large pore subunit (called the usher) where the pilin subunit is released by the chaperone and becomes attached to the end of the growing pilus rod. Therefore, the pilin can be complemented by either a different molecule (the chaperone) or the same molecule (another pilin). Reprinted with permission from Eisenberg [298].

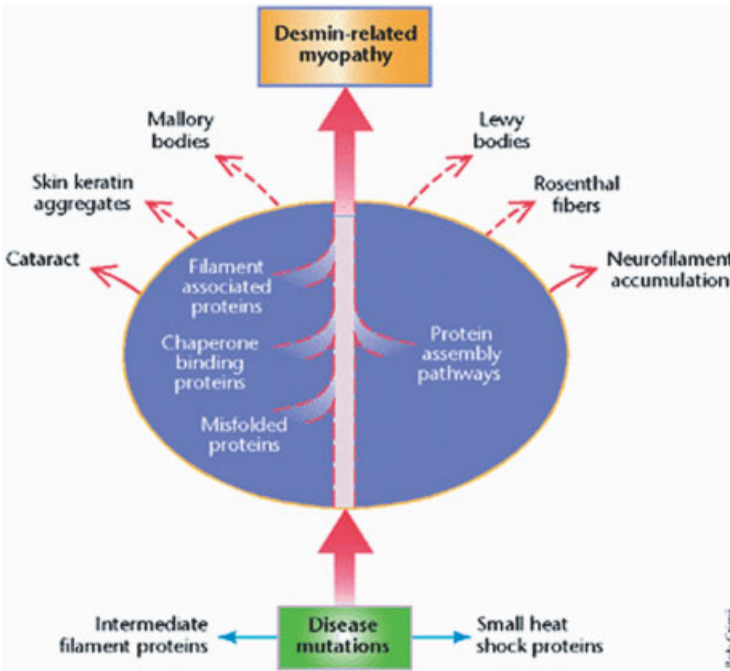


Figure 13.11 Small heat-shock protein chaperones for the intermediate filaments. Mutations in either small heat-shock proteins, such as α B-crystallin, or intermediate filament proteins lead to collapse and aggregation of cellular intermediate filament networks resulting in skeletal muscle and cardiac myopathy. Reprinted with permission from Quinlan and Van Den Ijssel [114].

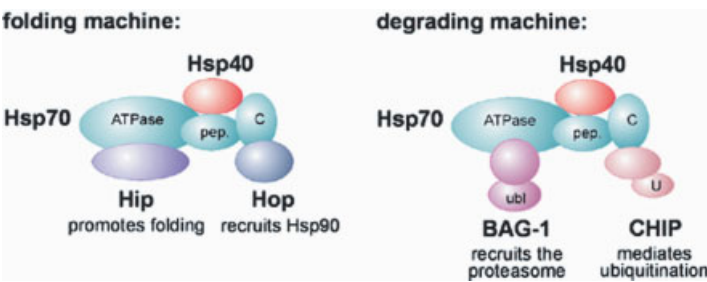


Figure 13.12 HSP70 as a folding machine or a degrading machine. Binding of distinct co-chaperones to the N-terminal ATPase domain of HSP70 (ATPase) and to its C-terminus (C) gives rise to chaperones machines involved in protein folding or in protein degradation. The co-chaperones Hip and BAG-1 compete

for binding to the ATPase domain, whereas Hop and CHIP associate with the C-terminus in a competitive manner. (pep = peptide-binding domain of HSP70; ubl = ubiquitin-like domain of BAG-1; U = U-box of CHIP). Reprinted with permission from Höhfeld [195].

In *E. coli*, the genes encoding HSP15, a ribosome-associated heat-shock protein [118], and HSP33, a redox-regulated molecular chaperone [119a, 119b] form part of a heat-shock-regulated multigene operon. HSP31, the *yedU* (*hchA*) gene product, alleviates protein misfolding by interacting with early unfolding intermediates [120a–f]. The 16 kDa IbpA and IbpB (for inclusion-body associated heat-shock proteins) are dispensable in *E. coli*, but cooperate with ClpB and DnaK/DnaJ/GrpE (and not with GroEL/GroES) *in vivo* at high temperatures [121, 122] and *in vitro* [123]. A novel sHSP named AgsA, which suppresses protein aggregation, has been recently discovered in *Salmonella enterica* [124].

In plant cells, sHSPs, which are the predominant proteins synthesized under stress conditions, exhibit chaperone activity in cooperation with HSP70 [125, 126a, 126b]. sHSPs which are incorporated into protein aggregates help to the disaggregation reaction mediated by ClpB/DnaK, highlighting a role of sHSPs in cellular protein quality control [127] (see section 13.3.2).

13.2.4.7 Endoplasmic Reticulum (ER) Chaperones

Several ER chaperones, including calnexin and calreticulin, co-operate to ensure the correct biogenesis of glycosylated proteins [128, 129a, 129b].

13.2.4.8 Intramolecular Chaperones

The prosequences of some proteases act as intramolecular chaperones for the correct folding of the polypeptide chains to which they are covalently but transiently attached: this is the case for the 77-residue propeptide of subtilisin [130]. Similar folding mechanisms are used by several prokaryotic and eukaryotic proteins, including prohormone convertases. In the *Bordetella pertussis* autotransporter BrkA, which contributes to adherence of the bacterium to host cells, a conserved region acts as an intramolecular chaperone to effect folding of the 73 kDa domain (referred to as the passenger domain) and ferry it to the bacterial surface [131].

13.3 Chaperone Networks

13.3.1

De novo Protein Folding

It is important to know whether chaperones act independently, or are organized in a universal chaperone pathway, through which proteins, especially newly translated polypeptides, will be channelled while they are still ribosome-bound. Alternatively, unfolded polypeptides following translation will be discharged into the soluble cytosolic medium and then cycle between the endogenous chaperones (kinetic partitioning) [132]. To address that question, a mutant form of GroEL called “trap GroEL”, which irreversibly captures unfolded polypeptides, when overexpressed in growing yeast and mammalian cells, did not interrupt the productive folding pathway, indicating a high level of organization in folding reactions [133, 65, 134], i.e., the existence of

an integrated folding compartment, directly coupled with the translation machinery [135]. Residing on the yeast ribosome, the HSP70 Ssb [136, 137] forms a chaperone triad with the HSP70 homolog Ssz1 and zuotin, a DnaJ-related HSP40, to act on nascent chains emerging from the ribosome [138]. During *in vitro* translation, a nascent polypeptide-associated complex (NAC) has been shown to form a protective environment for regions of nascent chains just emerging from the ribosomal tunnel [139, 140a]. In the endoplasmic reticulum too, a subset of chaperones and folding enzymes form multiprotein complexes to bind nascent proteins [140b].

In the case of prokaryotes, such a rigid sequential pathway is less obvious [140c], but nascent chains emerging at the peptide exit tunnel of the ribosome are awaited by a “welcoming committee” consisting of three chaperones [141]: the sequence of interactions of newly synthesized proteins with chaperones is proposed to be trigger factor (TF), then the HSP70 system, and then the GroEL/ES system [142] (see Fig. 13.8). TF interacts with relatively short emerging nascent chains, via its interaction with ribosomal protein L23 at the ribosomal exit site [21, 22], and may then migrate transiently with the nascent chain, followed by rapid dissociation and rebinding to ribosomes. DnaK, which has partially overlapping functions with TF [58, 143, 55], binds to longer chains and allows the larger polypeptides to fold. Although complementary, the mechanisms of DnaK and TF in protein folding are distinct from each other [144–146]. Indeed, DnaK is not recruited to translating ribosomes in the absence of TF [147].

Finally, GroEL functions post-translationally to assist folding of a subset of cytosolic proteins [76]. DnaK and GroEL have been shown to co-operate in many folding pathways [148–152, 20c] and in ribosome biogenesis at high temperature [153]. However, DnaK and GroEL do not obligatorily act in succession by promoting earlier and later folding steps, respectively: rather, they can form a lateral network, for example for a large protein that does not fit the GroEL/GroES cavity [154]. ClpB and HtpG also participate in *de novo* folding in mildly stressed *E. coli* cells [155], and in the eubacterium *Thermus thermophilus*, a protein, DafA, cycles between the DnaK chaperone system and translational machinery, although its role is unknown at the moment [301].

However, the ribosome itself could be the first player in facilitating cotranslational and sequential *domain folding*, in the case of eucaryotic proteins composed of multiple domains [156]. Indeed, co-translational folding of a virus capsid protein has been observed without release from the ribosome and without the assistance of HSP70: folding is more favorable kinetically if it occurs at the same time as protein synthesis, but it remains to be seen whether this protein is exceptional in its capacity to fold so efficiently while still bound to ribosomes [157]. The ribosome may play a key role [158], either because the vectorial aspect of protein biosynthesis is critical in the process of protein folding, or in recruiting the folding machinery [159], or in controlling the subsequent fate of the translated polypeptide, through its interaction with the peptide exit tunnel as shown in *E. coli* [160–162] or even in actively refolding unfolded proteins, as shown *in vitro* [163].

13.3.2

Protein Disaggregation

During prolonged heat shock of *Saccharomyces cerevisiae* cells, the high-molecular-weight aggregated proteins that form are rapidly eliminated in wild-type cells, while they persist in cells lacking the stress tolerance factor HSP104. Refolding from the aggregated state requires not only HSP104, but also the HSP70 homolog Ssa and the DnaJ homolog YDJ1 [164]. A close functional relationship between HSP104 and HSP70 has also been shown *in vivo* [165], and in their ability to restore mRNA splicing after heat inactivation [166]. In maintenance of mitochondrial function, HSP78, the mitochondrial ClpB homolog, co-operates with matrix HSP70 [167, 168]. However, HSP104 and HSP70 have antagonistic interactions in yeast prion curing [169] (see Sect. 13.7.2).

In *Thermus thermophilus* [170, 171] as well as *E. coli* [172–176, 57, 177], a multi-chaperone system composed of ClpB and DnaK/DnaJ/GrpE has been shown to prevent and revert protein aggregation both *in vivo* and in cell extracts. There is a recent indication that ClpB acts prior to DnaK on protein substrates [178]. Therefore, the sequential mechanism could be the following: (i) ClpB exposes new DnaK-binding sites on the surface of the aggregates; (ii) DnaK binds the aggregate surfaces and melts the incorrect hydrophobic associations; and (iii) DnaK and GroEL complete refolding of solubilized proteins [179]. Small HSPs, which are incorporated into protein aggregates, help the disaggregation reaction mediated by ClpB and the DnaK system [123, 127].

The interactions of chaperones with disease-causing misfolded proteins [180a, 180b] and toxic aggregates, such as polyglutamine-rich proteins [181a, 181b], neurodegenerative amyloid plaques and prions is therefore a promising field of research [182–186].

ClpB also cooperates with HtpG in facilitating *de novo* protein folding in mildly stressed *E. coli* cells [155], and with DnaK/DnaJ/GrpE in the activation of the plasmid RK2 replication initiation protein TrfA by converting inactive dimers to an active monomer form [187].

13.3.3

Posttranslational Quality Control

Proteins that cannot fold properly because of mutations, errors in translation or stress damage are degraded by the ubiquitin–proteasome system in eukaryotes [188], or by specific proteases (FtsH, Lon, DegP, HslUV, ClpAP and ClpXP) in bacteria. Co-operation between chaperones and proteases, both of which recognize hydrophobic regions exposed on unfolded polypeptides, ensure post-translational quality control, i.e., whether a damaged protein will be destroyed before it folds properly [189]. The kinetics of partitioning of non-native proteins between chaperones and proteases, in other words, the competition between these two sets of proteins has been proposed to underlie the basis for protein quality control [190, 191].

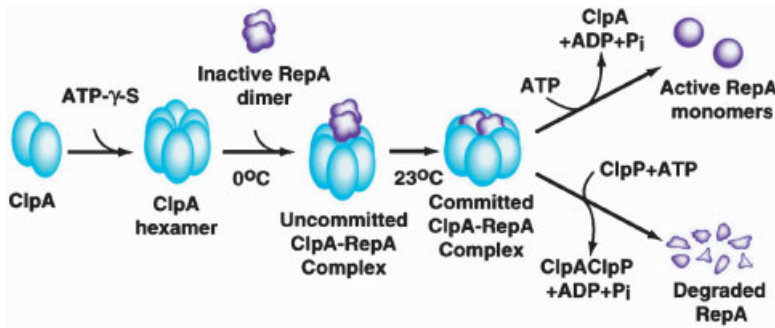


Figure 13.13 Dual function of the *E. coli* ClpA chaperone in RepA monomerization and in the ClpAP protease activity. For remodeling the bacteriophage P1 RepA dimers into monomers, the initial step is the self-assembly of ClpA and its association with inactive RepA dimers. Upon ATP binding, stable complexes

containing 1 mol of RepA dimer per mol of ClpA hexamer are formed and are committed to activating RepA. Finally, active RepA monomer is released upon ATP hydrolysis. The alternate fate for RepA is the degradation by ClpAP protease. Reprinted with permission from Pak and Wickner [95].

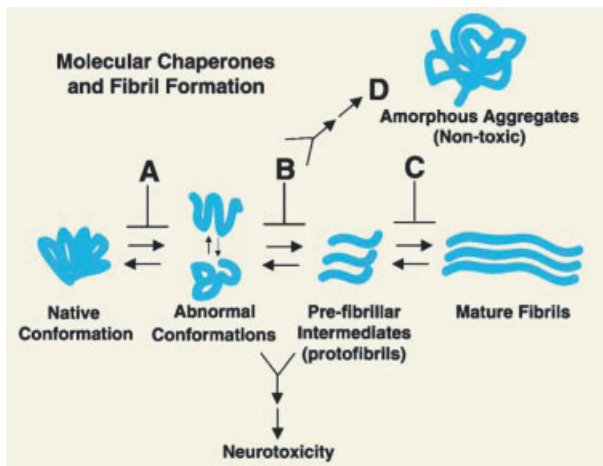


Figure 13.14 A model for molecular chaperone suppression of neurotoxicity. Amyloid is an ordered fibrillar structure arising from partial unfolding and exposure of hydrophobic surfaces that are normally buried in the core of a folded protein. Amyloid is also formed by proteins containing an expanded series of glutamine repeats (polyQ). Molecular chaperones may suppress neurotoxicity of amyloid-forming

proteins by preventing their conversion between native conformations and toxic conformations (A), or the formation of pre-fibrillar intermediates (B), or the conversion between pre-fibrillar intermediates and mature fibrils (C), or by facilitating the conversion of toxic intermediates into nontoxic amorphous aggregates (D), readily degraded by the proteolytic machinery. Reprinted with permission from Muchowski [186].

In *E. coli*, a synergistic action of two proteases, Lon and ClpXP, and the DnaK system is revealed at 42°C, as proteases became essential for survival at low DnaK levels [192]. Recent results suggest that DnaK operates as a functional coupling between the posttranslational quality control of proteins and the late stages of *E. coli* ribosome biogenesis [193] (see section 13.7.3 and Fig. 13.15).

In eukaryotes, misfolded proteins are targeted for proteosomal degradation through polyubiquitination by the ubiquitin ligase CHIP, which is also a co-chaperone of HSP70 and HSP90, therefore linking chaperones to the degradation machinery [194, 195]. The fact that an increased ubiquitin-dependent degradation can replace the requirement for HSPs [196] is also indicative of the protein quality control exerted by the chaperones, and furthermore suggests that heat-stressed cells do not die because of the loss of protein activity, but because of the inherent toxicity of denatured or aggregated proteins.

Another quality control mechanism in *E. coli*, triggered when protein synthesis on the ribosome stalls for any of a variety of reasons, is the tmRNA-mediated tagging of incomplete nascent proteins, which targets them for degradation by specific proteases including ClpXP and ClpAP [197]. When normal protein synthesis is compromised, a ribosomal A-site mRNA cleavage also contributes to ribosome rescue and protein quality control [198].

13.4

Chaperones and Stress

13.4.1

The Heat-shock Response and its Regulation

Cells respond to stress by transcriptional activation of heat-shock genes, most of which encode molecular chaperones. Instead of increasing the concentration of a transcriptional activator, the heat-shock response in eukaryotes is mediated by activation of a pre-existing pool of transcription factors, the family of heat-shock factors (HSF1–4). In unstressed cells, HSFs are present in both the cytoplasm and nucleus as inert monomers that have no DNA-binding activity. In response to heat shock, HSFs assemble into trimers, which accumulate within the nucleus, bind to the heat-shock elements (HSE) located in the heat-shock-responsive gene promoters and become phosphorylated. This in turn leads to increased levels of HSP70. The attenuation of the heat shock occurs through conversion of the active trimeric form of HSF to the non-DNA-binding monomer. This negative regulation of HSF is mediated by direct binding of HSP70 and by another negative regulator, the heat-shock factor binding protein 1 (HSBP1), which interacts with both the trimeric state of HSF and HSP70, leading finally to the dissociation of HSF trimers [199].

It has been observed that HSP70/HSC70, which shuttle between nucleus and cytoplasm, localize to the nucleus and to the nucleolus upon exposure to heat stress, where they bind to incompletely folded proteins in the pre-ribosome assembly unit to protect them from irreversible denaturation [200]. Starvation also promotes nuclear

accumulation of the HSP70 Ssa4 in yeast cells [201]. However, neither HSC70 nor HSP70 is involved in nucleolar transcription in the amphibian oocyte [202].

In Gram-negative bacteria such as *E. coli*, the transcription factor σ^{32} , product of the *rpoH* gene [203], associates with RNA polymerase to direct transcription of heat-shock genes. Under normal conditions, DnaK and DnaJ sequester σ^{32} through direct binding, inhibiting its association with RNA polymerase and promoting its degradation (σ^{32} is an extremely unstable protein with a half-life of less than 1 min) [204]. Following heat shock, DnaK/DnaJ is titrated by misfolded proteins, allowing σ^{32} to recruit RNA polymerase to the heat-shock promoters. Then, as more non-native proteins are removed, DnaK becomes once again available to bind σ^{32} , thereby turning-off the heat-shock response [205, 206]. But heat-shock regulatory mechanisms exhibit great diversity among bacteria and are quite different in Gram-positive, proteobacteria and cyanobacteria, where both σ^{32} and another mechanism (the HrcA/CIRCE repressor-operator system) monitor the levels of unfolded protein in the cell to determine the need for the expression of major cellular chaperones [207].

13.4.2

Thermotolerance

Thermophilic organisms from the three phylogenetic domains (eubacteria, archaea and eukarya) acquire thermotolerance, i.e., an enhanced survival at lethal temperatures, after a brief exposure to near-lethal temperatures [208]. This led to the hypothesis that thermotolerance depends on one or more of the HSPs synthesized after heat shock [209]. Indeed, HSP104 expression plays a central role in thermotolerance in yeast [98]. However, uncoupling thermotolerance from the induction of the HSPs has been observed [210, 211], indicating that other processes outside the heat-shock response are essential to the development of thermotolerance. For example, constitutive expression of HSP70 increases heat resistance [212, 213], but HSP70 alone is not able to confer the degree of resistance to heat killing seen with heat-induced thermotolerance [214].

In *E. coli*, DnaK plays an essential role in protection against protein oxidative damage and starvation-induced thermotolerance [215–217], probably in co-ordinating the sigma factors σ^{32} and σ^S levels [218, 219].

13.4.3

Who Detects Stress?

Temperature controls the expression of many bacterial genes; for instance, the transcription of genes encoding virulence factors which are expressed at 37°C, the host temperature, but are turned off at 30°C or below. Changes in DNA supercoiling, mRNA conformation, protein conformation, and chaperone-mediated capture of regulators have been implicated in thermosensing [220]. Highly conserved RNA sequences within 3' untranslated regions have been postulated as sensors of environmental stress [221]. An mRNA-based thermosensor controls expression of rhizobial

heat-shock genes [222], of the virulence genes in *Listeria monocytogenes* [223] and of the primary step of the heat-shock response in *E. coli*, i.e. the translational induction of σ^{32} synthesis [224]. Temperature control of the *E. coli* transcription factor RpoS (σ^S) also depends on the synthesis and stability of the untranslated RNA DsrA [225] through temperature sensing by the *dsrA* promoter [226].

Many cases of temperature sensing by a protein are also known. The virulence plasmid-encoded protein TlpA in *Salmonella typhimurium* [227], the RheA repressor in *Streptomyces albus* [228a], the HrcA repressor in *Bacillus thermoglucosidasius* [228b], the small chaperone HSP26 in *Saccharomyces cerevisiae* [229], the DegP protease/chaperone in *E. coli* [101] (see Sect. 13.2.4.3) and DnaK itself [230, 231] serve as a cellular thermometer. The co-chaperone GrpE may act as a thermosensor, and when the temperature increases its reduced ADP/ATP exchange factor activity increases the time in which DnaK binds its substrates [232] and in this way adapts the DnaK/DnaJ/GrpE system to heat-shock conditions [233]. In eukaryotes also, HSP70 functions as a sensor in a Bag1/HSP70-mediated stress signalling pathway [234], and the regulatory domain of human heat shock factor HSF is sufficient to sense heat stress [235].

13.5

Assembly and Disassembly of Macromolecular Complexes

The first chaperone to be described was nucleoplasmin, a pentameric nuclear protein that mediates the formation of nucleosomes during early development [236]. Then it was shown that the *E. coli* GroEL/GroES chaperonin acts at an early stage in the head assembly pathways of bacteriophages λ [237], T4, T5 and μ [238]. GroEL/GroES also promotes assembly of the plastid ribulose-1,5-bisphosphate carboxylase (rubisco) [239], and of the molybdenum-iron protein of nitrogenase [240]. Moreover, it mediates iterative annealing of non-productive assembly intermediates at the quaternary structure level [241a]. Some histone chaperones assist chromatin assembly [241b] and small HSPs (see section 13.2.4.6), control the polymerisation of microtubules [184], microfilaments and intermediate filaments, i.e. the formation of the cytoskeleton [241c].

There are many examples in which DnaK or HSP70 plays a role in protein quaternary structure changes: disentanglement of the λ DNA- λ O- λ P-DnaB preprimosomal complex found at the origin of λ DNA replication [242], multimerization of the C protein, a positive regulator of bacteriophage μ late transcription [243], monomerization of the replication initiation protein RepE of mini-F plasmid, mostly present as inactive RepE dimers [244]; monomerization of the replication initiation protein RepA of P1 plasmid, mostly present as inactive RepA dimers [245], monomerization of the replication initiation protein TrfA of RK2 plasmid, mostly present as inactive dimers [187] and disassembly of clathrin-coated vesicles in cooperation with the cochaperone auxilin [246].

DnaK has also been implicated in the assembly of polyomavirus capsids [247], of bacterial ribosomes [193, 248] (see Fig. 13.15) and in the α -complementation

of β -galactosidase in *E. coli* [249] (the same is true for HSP90 in yeast) [250]. *In vitro*, denatured β -galactosidase forms active tetramers upon addition of HSP70 [251].

HSP70, which is associated with a distinct cytoplasmic aggregate during lymphocyte activation [252] and in *E. coli* with the insoluble (aggregated) proteins under conditions in which proteins tend to aggregate (severe heat shock) [56], dissociates hydrophobic protein aggregates, for example DnaA [253], alone or in cooperation with ClpB [179].

Finally, HSP70 participates, although transiently, in chaperone-mediated telomerase assembly (in contrast to HSP90 and its cochaperone p23 which remain associated with the active telomerase) [254]. It also works together with HSP90 in the formation of a ribonucleoprotein complex necessary for hepadnavirus assembly [255], in the biogenesis of the heme-regulated kinase of the α subunit of eukaryotic translation initiation factor 2 [256], and in the assembly and disassembly of steroid hormone receptors [257], whereas it blocks the assembly of a functional apoptosome (anti-apoptotic effect of HSP70) [258, 259].

Two other chaperones specifically suited for disaggregating proteins are members of the Clp family, ClpX, which disassembles the Mu transposase tetramer [94], and ClpA, which remodels bacteriophage P1 RepA dimers into monomers [95] (see Fig. 13.13), when they do not function with their collaborating protease ClpP (see ClpXP and ClpAP in Sect. 13.2.4.2).

Some proteins have their 'private chaperone', such as the LipA lipase of *Pseudomonas cepacia* [260] and myosin [261]: the protein UNC-45 functions both as a molecular chaperone and as an HSP90 co-chaperone for assembly of myosin into motile cellular structures essential for cell division, cell motility, and muscle contraction [262]. CcmE is a heme chaperone that binds heme transiently in the periplasm of *E. coli* and delivers it to newly synthesized and exported c-type cytochromes [263].

13.6

Protein Translocation Across Membranes

Some molecular chaperones are necessary for postranslational protein secretion in all three kingdoms of life (bacteria, archaea and eukaryotes) [264].

In yeast, HSP70 is involved in the routing of proteins to mitochondria, as it assists mitochondrial precursors to achieve "import-competence", probably by stabilizing translocation-competent conformations until the outer mitochondrial membrane is contacted [265]. In mammals, both the chaperones HSP90 and HSP70 bind to the newly synthesized preproteins in the cytosol to target them to the import receptor Tom70 at the outer mitochondrial membrane [266].

Also, in the bacterial type III protein secretion system (used by many bacterial pathogens to deliver virulence effector proteins directly into the host-cell cytosol), specific chaperones are required to maintain their substrates in a secretion-competent state [267].

In Gram-negative bacteria such as *E. coli*, an export-specific molecular chaperone, SecB, keeps preproteins destined to be posttranslationally translocated in a partially unfolded conformation, and pilots them to a membrane-associated receptor, SecA, which provides the link to the translocon complex. SecB (a non-essential protein)

differs from other chaperones in that it is a homotetramer and functions independently of nucleotide triphosphate hydrolysis [139]. Discrimination between the SecA/SecB-dependent targeting and the signal recognition particle (SRP)-dependent targeting to the (same) translocon complex (SecYE) involves the ribosome-associated chaperone TF (see Sect. 13.2.1), which interacts with sequences near the N-terminus of the mature regions of presecretory proteins and therefore occludes SRP binding [268a, 268b]. But other data argue against a role for TF in pathway discrimination [269]. Nevertheless, TF has a unique ability to sequester nascent polypeptides for a relatively prolonged period, and in this way retards protein export [144]. Other chaperones not specific for secretory proteins such as DnaK also maintain the transport competence of presecretory proteins [270]. The functional redundancy of chaperones in the protein export pathway may explain why SecB is not essential for cell viability. In Gram-negative bacteria, distinct molecular chaperones of the periplasm, such as Skp, SurA and PpiD, are involved in the biogenesis of outer membrane proteins [104].

13.7

New Horizons in Chaperone Research

13.7.1

HSP90 and the Pandora's Box of Hidden Mutations

Morphological mutations are stored without expressing phenotypes because they are “buffered” by the molecular chaperone HSP90 in *Drosophila melanogaster* [271] and in *Arabidopsis thaliana* [272]. HSP90 functions as a chaperone for mutated abnormal proteins to enable them to “behave” functionally like normal proteins. Decreasing the levels of HSP90, whether by mutation or by an HSP90 inhibitor, uncovers a Pandora's box of developmental abnormalities [257]. HSP90 therefore appears as a capacitor of morphological evolution and phenotypic variation. Interestingly, the yeast prion ψ^+ also provides a mechanism, although totally different, for genetic variation and phenotypic diversity [273]. Also in bacteria, GroEL buffers against deleterious mutations [274].

13.7.2

Chaperones and Prions

By analogy with the mode of transmission of the abnormal prion protein, the causative agent of neurodegenerative diseases [275], two self-propagating proteins, ψ^+ and [URE3], abnormal isoforms of SUP35 and URE2 respectively, have been characterized and named prions, in the yeast *S. cerevisiae* [276]. The importance of yeast chaperones HSP70 and HSP104 in the propagation and maintenance of ψ^+ [277], their antagonistic interactions in prion curing [169] is a promising research topic. The conformational conversion of the normal prion protein into its abnormal infectious isoform could therefore involve a chaperone-like factor [278].

13.7.3

Chaperones and Ribosome Biogenesis

DnaK is necessary for the late stages of ribosome assembly in *E. coli* at high temperature (above 37°C), but not at 30°C. Several observations [279] suggest that an extra-ribosomal factor, naturally thermosensitive and therefore DnaK-dependent *only* at high temperature, would mediate ribosome assembly, rather than DnaK itself. The benefit for the bacterial cell would be to have, through DnaK, a regulatory link between the late stages of ribosome biogenesis and the protein quality control. The titration of DnaK by the thermodenatured proteins during thermal stress would both mediate the heat-shock response (see Sect. 13.4.1) and prevent the late steps of ribosome biogenesis, providing an additional control level of ribosome biogenesis (for the other well-known control mechanisms, see Fig. 13.15). In other words, DnaK would play a role of thermometer in the late stages of ribosome biogenesis [193]. Such a link between the function of mitochondrial chaperones (HSP70–HSP78) and the naturally thermosensitive mitochondrial DNA synthesis has been shown in the yeast *S. cerevisiae* [280].

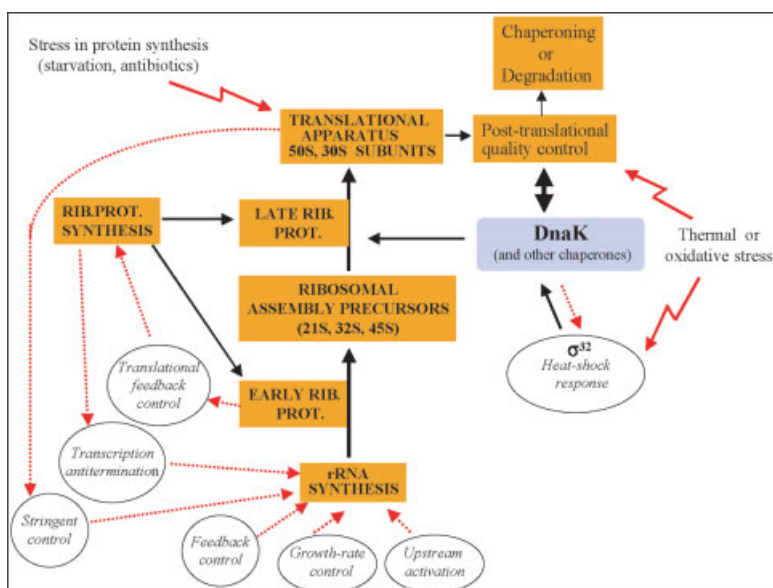


Figure 13.15 An additional level of control of ribosome bio-genesis in *E. coli*: the DnaK-assisted late steps of ribosome assembly at high temperature. Biosynthetic pathways are symbolized by rectangles and full arrows; control mechanisms by circles and dotted arrows. Ribosomal RNA synthesis is known to be regulated by different mechanisms [302],

such as transcription antitermination [303], stringent control, feedback control, growthrate control and upstream activation. Ribosomal protein synthesis is translationally auto-controlled [304].

A possible mechanism for the DnaK-assisted ribosome bio-genesis at high temperature is described in Sect. 13.7.3.

13.7.4

RNA Chaperones

There is a growing body of evidence for RNA recognition and binding by molecular chaperones [281]. The chaperonin of the archeon *Sulfolobus solfataricus* is an RNA-binding protein that participates in ribosomal RNA processing [282]. The existence of RNA chaperones that resolve misfolded RNA structures *in vivo* has been shown in many cases [283, 284].

13.7.5

Chemical Chaperones

Some low-molecular-weight compounds are able to stabilize the conformation of proteins that are defective in patients of inherited diseases. Therefore, “chemical-chaperone” is a new concept in drug research [285]. For example, some chemical chaperones increase the activity of N370S β -glucosidase, the most common mutation causing Gaucher disease [286]. A designed peptide is able to rescue mutants of the tumor suppressor p53 in cancer cells (chaperoning strategy) [287].

The osmolyte trimethylamine-oxide stimulates the *in vitro* reconstitution of functional 50S ribosomes [288] and decreases the *E. coli* homoserine *trans*-succinylase aggregation at high temperature [289]. Some hydrogel nanoparticles (nanogels) assist protein refolding in a manner similar to the mechanism of molecular chaperones, namely by catching and releasing proteins [290].

13.7.6

Medical implications

The therapeutic applications of heat shock/stress proteins and chaperone inducers for medicine constitute a promising field [291a, 291b], particularly as far as neurodegenerative disorders are concerned [186, 292]. Two HSP70 family members are expressed in atherosclerotic lesions [293]. HSP70s have also been implicated in many pathways in immunology (presentation of the antigen [294a, 294b] and in molecular cancerology [295a, 31]. For example, when secreted by viable immunocompetent cells, HSP70 in the extracellular milieu acts as a powerful cytokine [295b].

13.7.7

Chaperoning the chaperones

But, and this is an unavoidable final question, who chaperones the chaperones? Is it a self-assembly process? [296, 297a]. There is no doubt that the study of molecular chaperones will continue to surprise us. A recent example [297b] shows that it is even possible to mutationally improve the efficiency of a chaperonin, as soon as an efficient method of selection can be devised!

Table 13.1 Major chaperone families

In prokaryotes	In eukaryotes	Function
DnaK	HSP70, HSC70	<i>De novo</i> protein folding.
Co-chaperones: DnaJ and GrpE (DnaK homologs =HscA and HscC)	Co-chaperones : HSP40, BAG-1, HIP, HOP, CHIP	Protein refolding; prevention and reversion of aggregation
GroEL	HSP60 (mitochondria)	Protein quality control, in cooperation with proteases
Co-chaperonin : GroES	= Cpn60 (chloroplasts) co-chaperonin: HSP10 = Cpn10 (same localization)	Protein translocation across membranes
Trigger factor		Oligomerization; assemblies and disassemblies.
		Modulation of the heat-shock response
	TCP-1, CCT/TriC NAC GimC = prefoldin	Protein folding (in particular actin and tubulin)
HtpG	HSP90	Signal transduction proteins: steroid hormone receptors, signaling kinases.
ClpB	HSP100 / HSP104	Thermotolerance; protein desaggregation
ClpA, ClpX (without ClpP)		Disassembly of quaternary structures
ClpA, ClpX (with ClpP)	Proteasome	Proteolysis
HslU (with HslV = ClpQ) = ClpY (without HslV = ClpQ)		Prevention of aggregation
IbpA, IbpB	HSP26, α -crystallin and other small HSPs	Protein folding and thermoprotection
SecB		Protein export via the general secretory pathway
	Nucleoplasmin	Assembly of nucleosomes
	Calnexin	Folding of glycosylated proteins in the ER
	Calreticulin	

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