nature structural & molecular biology

# Converging concepts of protein folding *in vitro* and *in vivo*

F Ulrich Hartl & Manajit Hayer-Hartl

**Most proteins must fold into precise three-dimensional conformations to fulfill their biological functions. Here we review recent concepts emerging from studies of protein folding *in vitro* and *in vivo*, with a focus on how proteins navigate the complex folding energy landscape inside cells with the aid of molecular chaperones. Understanding these reactions is also of considerable medical relevance, as the aggregation of misfolding proteins that escape the cellular quality-control machinery underlies a range of debilitating diseases, including many age-onset neurodegenerative disorders.**



Numerous proteins have been shown to fold spontaneously *in vitro*, confirming Anfinsen's pioneering insight that the linear sequence of the polypeptide chain contains all the necessary information to specify a protein's three-dimensional structure[1]. Although protein folding has been studied intensely for almost 50 years, how the final fold (and the folding process) is determined by the amino acid sequence remains one of the most important problems in biology. Moreover, in the more recent past it has become clear that, in the cell, a large fraction of newly synthesized proteins require assistance by molecular chaperones to reach their folded states efficiently and on a biologically relevant timescale[2]. Clearly, proteins in the test tube and in the cell are subject to the same laws of physics, so what is special about folding under cellular conditions, and why are chaperones necessary? The increasing availability of highly sensitive biophysical techniques to study folding *in vitro* and in cellular systems is now providing new insights into these issues (see the Review by Bartlett and Radford[3] in this issue). These studies also shed light on the process of aggregation, a potentially dangerous off-pathway reaction that can cause disease and must be prevented by molecular chaperones.

## Folding and aggregation

Folding intermediates are the rule for larger proteins of >100 amino acids (~90% of all proteins in a cell), which have a greater tendency to rapidly collapse in aqueous solution into compact non-native conformations[4]. As shown recently by a combination of rapid mixing techniques and sensitive spectroscopic measurements, even small proteins that fold on a subsecond timescale may pass through structural intermediates *en route* to the native state[3,4]. Such intermediates either represent on-pathway 'stepping stones' toward the native state or kinetically stable, misfolded conformations that may require substantial reorganization before the native state can be reached. The formation of metastable, non-native interactions during folding is interpreted as a consequence of the ruggedness of the funnel-shaped folding energy landscape[5,6] (**Fig. 1**), irrespective of whether proteins are thought to fold through multiple downhill routes or through preferred pathways defined by the sequential assembly of elementary folding units, so-called 'foldons'[7,8]. Examples of such minimal nucleation motifs are the two-stranded-helix motifs found in α/β domain proteins. Multistate folding behavior with populated intermediates would be observed when multiple foldons are separated and do not act cooperatively[8] or when foldons misassemble, resulting in a kinetic block of folding. The propensity to misfold increases with topologically complex fold types that are stabilized by long-range interactions (for example, α/β domain architectures) or when proteins contain multiple domains that are separate in the native state but may interact during folding[9,10].

Partially folded or misfolded states often tend to aggregate, particularly when they represent major kinetic traps in the folding pathway. This is due to the fact that these forms typically expose hydrophobic amino acid residues and regions of unstructured polypeptide backbone, features that are mostly buried in the native state. Like intramolecular folding, aggregation—the association of two or more non-native protein molecules—is largely driven by hydrophobic forces and primarily results in the formation of amorphous structures (**Fig. 1**). Alternatively, aggregation can lead to the formation of highly ordered, fibrillar aggregates called amyloid, in which β-strands run perpendicular to the long fibril axis (cross-β structure) (**Fig. 1**). Although apparently restricted to a subset of proteins under physiological conditions, these thermodynamically highly stable structures are accessible to many proteins under denaturing conditions, largely independent of sequence, suggesting that their formation is an inherent property of the polypeptide chain[11]. The formation of amyloid fibrils is usually toxic to cells and may give rise to some of the most debilitating neurodegenerative diseases.

## Folding in the cell—the molecular chaperone concept

New, fluorescence-based techniques now allow protein folding and aggregation to be observed *in vivo* in real time[12]. These and other studies indicate that the tendency of partially folded proteins to

Max Planck Institute of Biochemistry, Department of Cellular Biochemistry, Martinsried, Germany. Correspondence should be addressed to F.U.H. (uhartl@biochem.mpg.de) or M.H.-H. (mhartl@biochem.mpg.de).

Published online 3 June 2009; doi:10.1038/nsmb.1591

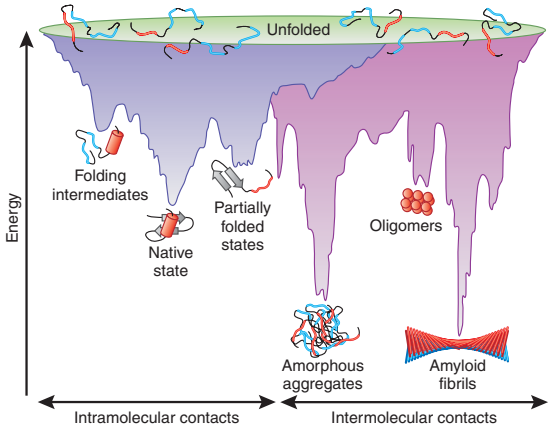


**Figure 1** Energy landscape scheme of protein folding and aggregation. The purple surface shows the multitude of conformations 'funneling' to the native state via intramolecular contacts and the pink area shows the conformations moving toward amorphous aggregates or amyloid fibrils via intermolecular contacts. Both parts of the energy surface overlap. Aggregate formation can occur from intermediates populated during *de novo* folding or by destabilization of the native state into partially folded states and is normally prevented by molecular chaperones. Cell-toxic oligomers may occur as off-pathway intermediates of amyloid fibril formation.

aggregate is greatly enhanced in the highly crowded environment of the cell, largely explaining the requirement of molecular chaperones[13]. Whereas folding experiments *in vitro* are typically performed in dilute solution to minimize aggregation, in the cell, folding occurs in the presence of 300–400 g $l^{-1}$ of protein and other macromolecules. The resulting excluded volume effects substantially enhance the affinities between interacting protein molecules, including folding intermediates. The translation process can potentially further increase the risk of misfolding and aggregation, because incomplete polypeptide chains cannot fold into stable native conformations. Additionally, the exit channel of the large ribosomal subunit, which is ~100 Å long but, at most, 20 Å wide[14], largely precludes folding beyond the formation of α-helical elements[15,16] and thus prevents the C-terminal 40–60 residues of the chain from participating in long-range interactions (see the Review by Bukau and colleagues[17] in this issue). As a consequence, productive folding can occur only after a complete protein or at least a domain (~50–300 amino acids) has emerged from the ribosome, consistent with the general rules of folding established *in vitro* and supported by recent simulations of nascent chain folding[18]. Because translation is relatively slow (~15–75 s for a 300-amino-acid protein), nascent chains are exposed in partially folded, aggregation-sensitive states for prolonged periods of time. Moreover, non-native intrachain contacts formed during translation could block folding upon completion of synthesis. Molecular chaperones therefore interact co-translationally with nascent polypeptides and inhibit their premature (mis)folding. For example, the chaperone Trigger factor binds to the small titin I27 chain (~120 amino acids) until its complete β-sandwich domain has emerged from the ribosome[19].

The cellular chaperone machinery ensures that folding is efficient for most proteins[20]. We define a molecular chaperone as any protein which interacts, stabilizes or helps a non-native protein to acquire its native conformation but is not present in the final functional structure. Chaperones are involved in a multitude of cellular functions, including *de novo* folding, refolding of stress-denatured proteins, oligomeric assembly, intracellular protein transport and assistance in proteolytic degradation. Chaperones that participate broadly in protein biogenesis, such as the heat-shock protein (Hsp)-70s and chaperonins (Hsp60s), primarily recognize hydrophobic amino acid side-chains exposed by non-native proteins and promote their folding through ATP-regulated cycles of binding and release (**Fig. 2**). Binding to chaperone blocks aggregation and reduces the concentration of folding intermediates, whereas transient release of bound hydrophobic regions is necessary for folding to proceed. It is important to realize that chaperones act not by contributing steric information to the folding process but rather by optimizing the efficiency of folding. Notably, a number of essential proteins have extremely low intrinsic folding efficiencies and essentially do not fold in the absence of chaperones. For example, actins and tubulins seem to have highly energetically frustrated folding pathways and can overcome kinetic folding barriers only through assistance by chaperones. As mutations often disrupt a protein's ability to fold, it follows that the chaperone system is also important in buffering such deleterious mutations[21,22]. This buffering function is thought to be crucial in the evolution of new protein functions and phenotypic traits[21,23,24].

Numerous classes of structurally unrelated chaperones have been described[25,26]. Many of these are known as stress proteins or heat-shock proteins, as they are upregulated by cells under conditions of conformational stress in which the concentration of aggregation-prone folding intermediates increases. Chaperones are usually classified

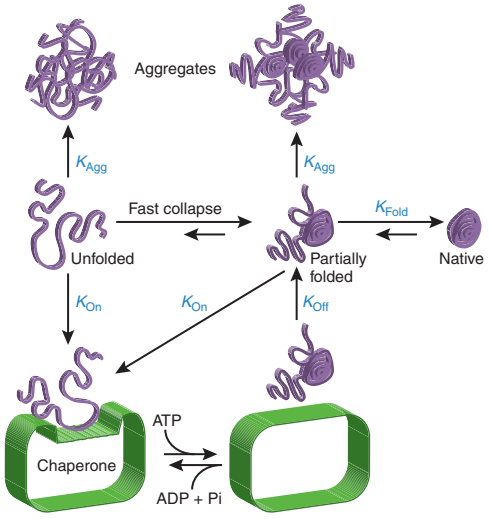


**Figure 2** Chaperone mechanism in promoting folding through kinetic partitioning. Many chaperones, such as the Hsp70s, are switched between high- and low-affinity states for unfolded and partially folded protein by ATP binding and hydrolysis. ATP binding causes substrate release, allowing folding toward the native state to proceed; hydrolysis is necessary for chaperone cycling. Folding is promoted and aggregation prevented when $K_{Fold}$ is greater than $K_{On}$ for chaperone binding (or rebinding) of partially folded states and when $K_{On}$ is greater than intermolecular association by the higher-order rate constant $K_{Agg}$ ($K_{Fold} > K_{On} > K_{Agg}$). Multiple cycles of substrate binding and release will slow folding relative to spontaneous folding in the absence of aggregation, assuming that the chaperone does not accelerate the rearrangement of kinetically trapped states. Note that for proteins that populate such misfolded states, $K_{On}$ may be greater than $K_{Fold}$ ($K_{Fold} \leq K_{On} > K_{Agg}$). These proteins are stabilized in a non-aggregated state, but fail to fold through chaperone cycling. They may require transfer into the chaperonin cage for folding. Under certain conditions (overproduction of slow folding proteins, conformational stress), $K_{Agg}$ may become faster than $K_{On}$ and aggregation occurs ($K_{Agg} > K_{On} \geq K_{Fold}$), unless chaperone expression is induced via the stress-response pathway.

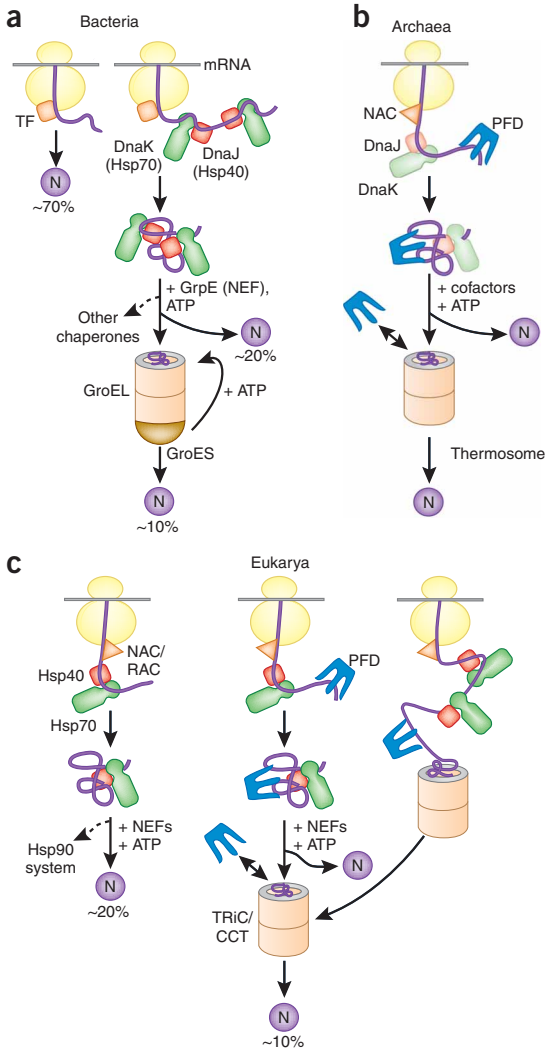


**Figure 3** Protein folding in the cytosol. Models for the chaperone-assisted folding of newly synthesized polypeptides in the cytosol. (**a**) Bacteria. Nascent chains probably interact generally with Trigger factor (TF), and most small proteins (~70% of total) may fold rapidly upon synthesis without further assistance. Longer chains interact subsequently with DnaK and DnaJ (Hsp70 system) and fold upon one or several cycles of ATP-dependent binding and release (~20% of total). About 10% of chains transit the chaperonin system (GroEL and GroES) for folding. N, native protein. (**b**) Archaea. PFD, prefoldin; NAC, nascent chain–associated complex. Note that only some archaeal species contain DnaK and DnaJ. (**c**) Eukarya. Like TF, NAC probably interacts generally with nascent chains, but the role of NAC in folding is not yet clear. About 20% of chains reach their native states in a reaction assisted by RAC (ribosome-associated complex), Hsp70 and Hsp40. A fraction of these must be transferred to Hsp90 for folding. About 10% of chains are co- or post-translationally passed on to the chaperonin TRiC/CCT in a reaction mediated by Hsp70 and PFD, both of which interact directly with TRiC/CCT. PFD recognizes the nascent chains of certain TRiC substrates, including actin and tubulins.

according to their molecular weight (Hsp40, Hsp60, Hsp70, Hsp90, Hsp100 and the so-called small Hsp proteins). Although all these components have the capacity to prevent aggregation, only certain members of the Hsp100 family in bacteria and fungi can actively dissociate aggregates for subsequent protein refolding or degradation (reviewed in ref. 27). The cellular chaperone machinery forms complex networks that are indispensable for protein quality control and maintenance of protein homeostasis[28].

## Principles of chaperone pathways in *de novo* folding

The chaperone pathways and networks acting in protein folding in the cytosol follow general organizational rules[29,30]. In all three domains of life, we find two major principles of chaperone action, represented by (i) machinery that functions in stabilizing nascent polypeptides on ribosomes and initiating folding (also see ref. 17 in this issue) and (ii) components that act downstream in completing the folding process[31,32] (**Fig. 3**). Both systems cooperate in coherent pathways. The number of interacting substrates and the degree of functional redundancy among chaperone components decreases from upstream to downstream.

The first category of factors includes chaperones that bind directly to the large ribosomal subunit in close proximity to the polypeptide exit site, such as bacterial Trigger factor (**Fig. 3a**) and a specialized Hsp70 system called RAC (ribosome-associated complex) in eukaryotes (**Fig. 3c**). In *S. cerevisiae*, RAC consists of the Hsp70 homologs Ssb1 (or Ssb2), Ssz1 and the Hsp40 homolog zuotin, whereas mammalian RAC consists of Hsp70L1 and Mpp11, homologs of Ssz1 and zuotin[33,34]. Additionally, archaea and eukarya contain the nascent chain–associated complex, NAC[17,25,26] (**Fig. 3b,c**). These ribosome-bound chaperones are in a privileged position to interact with the majority of nascent chains destined for the cytosol. They bind linear chain segments enriched with hydrophobic amino acids, thus delaying premature chain compaction and maintaining the elongating polypeptide in a non-aggregated state until sufficient structural information for productive folding is available. Based on a recent analysis by cryo–electron tomography, the three-dimensional organization of polyribosomes additionally seems to minimize the probability of nascent chain contacts between adjacent ribosomes[35].

Members of the Hsp70 family (DnaK in bacteria, Hsc70 in higher eukaryotes) function as second-tier chaperones for longer nascent chains. These factors do not bind directly to the ribosome and mediate co- or post-translational folding through ATP-regulated binding cycles. Co-translational domain folding serves to avoid non-native interdomain contacts, thus smoothing the energy landscape for large proteins[9,36]. This mechanism is essential for the folding of the large number of eukaryotic multidomain proteins and is facilitated by the slower translation speed in eukaryotes (~4 amino acids per second versus ~20 amino acids per second in bacteria). The Hsp70s are also the central organizers of the chaperone network and distribute subsets of proteins to chaperones that act downstream, such as the chaperonins (Hsp60s) (GroEL in bacteria and TRiC/CCT in eukarya)[24,31,37] and Hsp90 (ref. 38). The chaperonins are large, cylindrical complexes that function by enclosing protein molecules, one at a time, in a cage-like nanocompartment, so that folding can occur unimpaired by aggregation. Interestingly, the eukaryotic chaperonin interacts directly with Hsp70 (ref. 39) and other upstream factors, such as prefoldin[40], and thus can be recruited to nascent chains that are unable to fold with Hsp70 alone[41]. This functional coupling by direct interaction between chaperones facilitates co-translational folding and avoids the partitioning of non-native protein into the bulk cytosol[42,43]. Hsp90 is another important chaperone system that functions downstream of Hsp70 in the folding and conformational regulation of many signaling molecules (reviewed in refs. 38,44). Additional machinery may be required for the assembly of folded protein subunits into large oligomeric complexes[45], as shown recently for RbcX, a specific assembly chaperone of hexadecameric Rubisco[46].

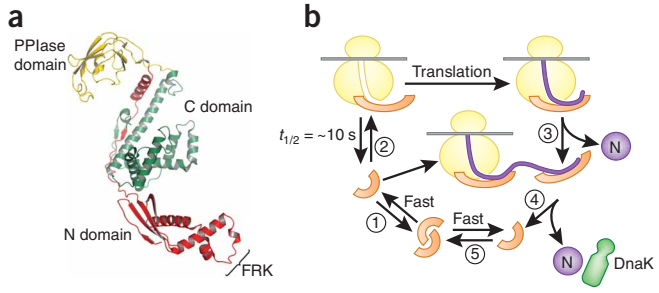


**Figure 4** Structure and function of Trigger factor (TF). (**a**) The domain structure of TF, including the FRK ribosome-binding loop in the N domain (PDB 1W26)[54]. (**b**) A model of the TF reaction cycle (modified from ref. 18). (1) Free TF is in rapid equilibrium between monomeric and dimeric states. (2) TF monomer binds to nontranslating ribosomes with a $K_d$ of ~1 μM and a mean residence time of ~10 s. Ribosome binding causes a conformational expansion of TF and may activate TF for interaction with nascent chains. (3) Nascent chains that interact weakly with TF may begin to compact co-translationally in the vicinity of TF. Release of TF from the nascent chain coincides with TF dissociation from the ribosome and allows completion of folding to native state (N). (4) Structurally more complex proteins may interact strongly with TF. TF remains bound to the nascent chain after dissociating from the ribosome and a new TF molecule can enter at the ribosome. Eventual chain dissociation from TF facilitates transfer to DnaK or folding. (5) Released TF enters the monomer-dimer pool.

The flux of newly synthesized proteins through the chaperone system is currently best understood for the bacterial cytosol (**Fig. 3a**). In *Eschrichia coli*, most of the ~2,400 cytosolic proteins are thought to interact first with ribosome-bound Trigger factor, which is restricted to bacteria. Although most small, single-domain proteins probably do not require further chaperone interactions, longer chains (>20–30 kDa) may interact subsequently with the ATP-regulated Hsp70 DnaK and its Hsp40 cochaperone, DnaJ. Co-immunoprecipitation experiments indicate that ~20% by mass of newly synthesized polypeptides fold through cycles of Hsp70 binding and release[47]. Trigger factor and DnaK have partially overlapping functions, but their combined deletion is lethal at temperatures above 30 °C[47,48]. The GroEL chaperonin acts downstream of Trigger factor/DnaK and is involved in the folding of ~10% of cytosolic proteins[24,49]. GroEL and GroES are essential at all growth conditions, because a number of essential proteins are crucially GroEL dependent for folding[24]. Similar fractions of total protein have been shown to transit the eukaryotic Hsp70 and chaperonin systems[42,50,51].

The chaperone pathways operating in the endoplasmic reticulum (ER) follow analogous organizational principles, but specialized machinery is used in disulfide bond formation and sugar modification of many secretory proteins (reviewed in ref. 52). Likewise, the biogenesis and folding of membrane proteins uses specialized machinery for insertion and assembly of the membrane-integrated parts, whereas cytosolic and ER luminal chaperones assist in the folding of exposed domains (for review, see the Perspective by Skach[53] in this issue).

## Chaperone paradigms

Several mechanistic paradigms of chaperone action in protein folding have been defined. The following sections provide a brief discussion of Trigger factor, an ATP-independent chaperone, the ATP-regulated Hsp70 system and the chaperonins, which mediate folding in the cytosol. For each of these systems, there are mechanistic models that are well supported by structural and functional data.

**Trigger factor.** Trigger factor is an abundant ~50-kDa protein, consisting of an N-terminal ribosome-binding domain, a peptidyl-prolyl isomerase (PPIase) domain and a C-terminal domain, the latter being positioned in the structure between the N and PPIase domains[54] (**Fig. 4a**). Trigger factor exists in two forms: a monomer when bound to the ribosome and a dimer when free in the cytosol. The PPIase domain, which can catalyze prolyl *cis-trans* isomerization *in vitro*, functions as an auxiliary chaperone site independent of proline residues[19,55,56]. The C domain, containing two arm-like protrusions, is the major binding region for hydrophobic nascent chain segments[55,57]. The N domain binds to ribosomal proteins L23 and L29 next to the polypeptide exit site[54], with a mean residence time of ~10–15 s (ref. 19). Ribosome binding causes a conformational opening of Trigger factors, presumably activating it for nascent chain interaction[19,58,59] (**Fig. 4b**). Chain release from Trigger factor is ATP-independent, and is probably driven by the tendency of the bound polypeptide to bury hydrophobic regions. Nascent chains that interact only weakly may begin to fold co-translationally, perhaps with the ribosome-bound Trigger factor providing a protected environment[54,56]. However, when the nascent chain exposes strongly hydrophobic segments, Trigger factor leaves the ribosome but remains associated with the elongating chain, explaining how Trigger factor delays the folding of certain multidomain proteins relative to translation[36]. The eventual dissociation of Trigger factor facilitates folding or polypeptide transfer to downstream chaperones such as DnaK.

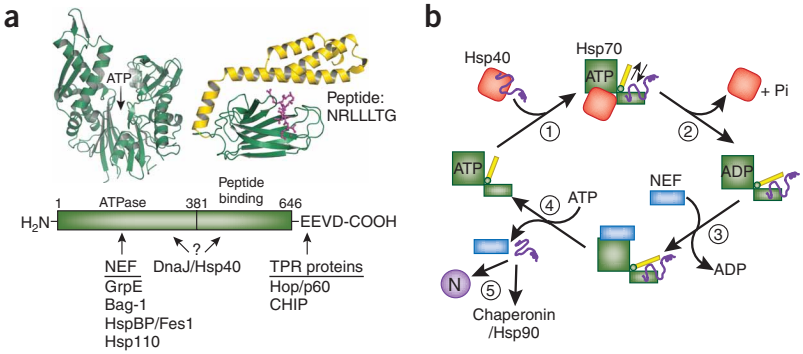


**Figure 5** Hsp70 system. (**a**) Structures of the ATPase domain (PDB 1DKG)[104] and the peptide-binding domain (PDB 1DKZ)[61] of Hsp70 shown representatively for *E. coli* DnaK. The α-helical lid of the peptide binding domain is shown in yellow and the extended peptide substrate as a ball-and-stick model in pink. ATP indicates the position of the nucleotide-binding site. The amino acid sequence of the peptide is indicated. The interaction of prokaryotic and eukaryotic cofactors with Hsp70 is shown schematically. Residue numbers refer to human Hsp70. Only the Hsp70 proteins of the eukaryotic cytosol have the COOH-terminal sequence EEVD, which is involved in binding of tetratricopeptide repeat (TPR) cofactors[103]. (**b**) Hsp70 reaction cycle. NEF, nucleotide-exchange factor (GrpE in case of *E. coli* DnaK; Bag, HspBP1 and Hsp110 in case of eukaryotic cytosolic Hsp70). (1) Hsp40-mediated delivery of substrate to ATP-bound Hsp70. (2) Hydrolysis of ATP to ADP, accelerated by Hsp40, results in closing of the α-helical lid and tight binding of substrate by Hsp70. Hsp40 dissociates from Hsp70. (3) Dissociation of ADP catalyzed by NEF. (4) Opening of the α-helical lid, induced by ATP binding, results in substrate release. (5) Released substrate either folds to native state (N), is transferred to downstream chaperones or rebinds to Hsp70.

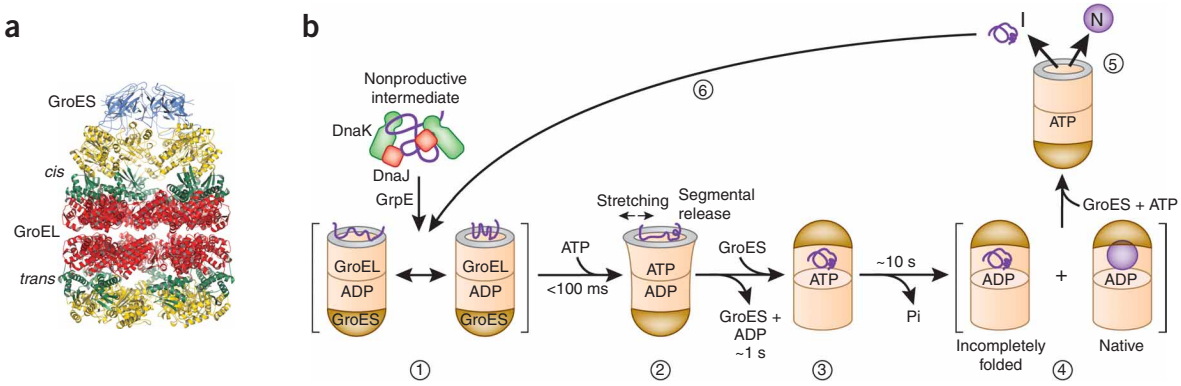


**Figure 6** The GroEL-GroES chaperonin. (**a**) Crystal structure of the asymmetric GroEL–GroES complex (PDB 1AON)[73], showing the GroES-bound chamber of GroEL (*cis*) and the opposite GroEL ring (*trans*). (**b**) Working model summarizing the conformational changes in a substrate protein upon transfer from DnaK–DnaJ (Hsp70 system) to GroEL and during GroEL–GroES-mediated folding. (1) Substrate protein may be delivered to GroEL by DnaK–DnaJ in a non-aggregated, but kinetically trapped, state. Upon binding to GroEL it undergoes local unfolding to an ensemble of expanded and more compact conformations. (2) ATP-dependent domain movement of the apical GroEL domains result in stretching of tightly bound regions of substrate and in release and partial compaction of less stably bound regions. (3) Compaction is completed upon substrate encapsulation by GroES. (4) Folding in the chaperonin cage. (5) Substrate release upon GroES dissociation. (6) Rebinding of incompletely folded states. Note that binding of a second substrate molecule to the open ring of GroEL in steps (4) and (5) as well as the transient formation of a symmetrical GroEL–$GroES_2$ complex is omitted for simplicity. N, native state; I, folding intermediate.

**The Hsp70 system.** The Hsp70 proteins are the most versatile chaperones and occur both as constitutively expressed and stress-inducible forms[25]. Besides broadly assisting in *de novo* folding, they have various other functions, including protein trafficking and assistance in the proteolytic degradation of terminally misfolded proteins. Hsp70s generally collaborate with chaperones of the Hsp40 (DnaJ) family and nucleotide-exchange factors (NEFs) in the ATP-regulated binding and release of non-native proteins[60]. Their role in *de novo* folding begins by binding to nascent chains, but they generally do not interact directly with the ribosome (with the exception of the specialized Hsp70s Ssb1, Ssb2 and Ssz1 in fungi and Hsp70L1 in mammalian cells). Binding and release by Hsp70 is achieved through the allosteric coupling of a conserved N-terminal ATPase domain (~40 kDa) with a C-terminal peptide-binding domain (PBD) (~25 kDa), the latter consisting of a β-sandwich subdomain and an α-helical lid segment[61] (**Fig. 5a**). The β-sandwich recognizes extended, ~7-residue segments enriched with hydrophobic amino acids[62]. Such segments occur on average every 50–100 residues in proteins. The α-helical lid and a conformational change in the β-sandwich domain regulate the affinity state for peptide in an ATP-dependent manner[60,63]. In the ATP-bound state, the lid adopts an open conformation, resulting in high on- and off-rates (low affinity) for peptide. Hydrolysis of ATP to ADP is strongly accelerated by Hsp40, leading to lid closure and stable peptide binding (low on- and off-rates; high affinity) (**Fig. 5b**). Interaction of substrate with Hsp70 is mediated by the so-called J domain, which is present in all Hsp40s[60]. Hsp40s also interact directly with unfolded polypeptides and can recruit Hsp70 to protein substrates[64]. Following ATP-hydrolysis, various NEFs (GrpE in bacteria; Bag, HspBP1 or Hsp110 in eukaryotes) bind to the Hsp70 ATPase domain and catalyze ADP-ATP exchange, which results in lid opening and substrate release, thereby completing the reaction cycle.

Hsp70-mediated folding and prevention of aggregation may be explained by a process of kinetic partitioning, as shown in **Figure 2**: binding of Hsp70 to non-native substrate hinders aggregation by transiently shielding exposed hydrophobic segments and at the same time reducing the concentration of aggregation-prone species. Release allows fast-folding molecules (or domains) to bury hydrophobic residues, whereas molecules that need longer than a few seconds to fold will rebind to Hsp70 and avoid aggregation. Binding to Hsp70 may result in conformational remodeling[65], perhaps removing kinetic barriers to fast folding. A subset of proteins that are unable to partition to fast-folding trajectories upon Hsp70 cycling remain stabilized in a non-aggregated state and must be transferred into the specialized environment of the chaperonin cage for folding[24,31]. Interestingly, the recently described NEFs of the Hsp110 family in eukaryotes are themselves Hsp70 homologs[66–70] and thus may cooperate with conventional Hsp70s in protein folding beyond catalyzing nucleotide exchange[69]. Such a cooperation could provide functional coordination between multiple bound Hsp70 molecules and facilitate folding for proteins with complex domain topologies.

**The chaperonins.** Chaperonins are large, double-ringed complexes of ~800 kDa. There are two groups of chaperonin[26,71]. Members of group I (also called Hsp60s) occur in bacteria (GroEL), mitochondria and chloroplasts. They have seven-membered rings and functionally cooperate with Hsp10 proteins (bacterial GroES), which form the lid of the folding cage. The group II chaperonins in archaea (thermosome) and in the eukaryotic cytosol (TRiC/CCT) consist of eight- or nine-membered rings. They are independent of Hsp10 factors, their lid function being built into the chaperonin ring in the form of specialized α-helical extensions. As in the case of Hsp70, substrate binding by chaperonin is ATP-regulated, but, unlike the Hsp70s, the chaperonins promote folding through cycles of global protein encapsulation.

The group I chaperonin of *E. coli* (GroEL–GroES) has been studied extensively[2,30,71] (**Fig. 6**). GroEL interacts with at least ~250 different cytosolic proteins, of which ~85 are predicted to be obligate GroEL substrates. Most of these proteins are between 20 kDa and 50 kDa and have complex α/β or α+β domain topologies, with a distinct enrichment of the $(\beta/\alpha)_8$ TIM barrel fold[24]. Such proteins are stabilized by many long-range interactions and therefore tend to populate kinetically trapped folding intermediates[72]. The GroEL double-ring is composed of ~57-kDa subunits that consist of an equatorial ATPase domain, an intermediate hinge domain and an apical substrate-binding domain. The apical domains expose hydrophobic amino acid residues for substrate binding toward the ring

center. GroES is a heptameric ring of ~10-kDa subunits that covers the ends of the GroEL cylinder (**Fig. 6a**)[71,73]. Based on recent analyses by NMR, single-molecule Förster resonance energy transfer (FRET) and EM, GroEL-bound substrates populate an ensemble of compact and locally expanded states that lack stable tertiary interactions (**Fig. 6b**)[74–77]. Whereas unfolding upon binding may help to resolve non-native interactions in trapped intermediates[75], folding critically depends on the global encapsulation of the substrate in the chaperonin ring by GroES[22,71,78]. Binding of GroES is preceded by ATP binding to GroEL and causes a pronounced conformational change that leads to the formation of a cage with a highly hydrophilic, net negatively charged inner wall. Encapsulated protein up to ~60 kDa is free to fold in this environment for 10–15 s, the time needed for ATP hydrolysis in the GroES-bound ring (*cis*-ring). Protein substrate leaves the cage upon GroES dissociation, which is induced by ATP binding in the opposite ring (*trans*-ring). Substrate that has not yet folded rapidly rebinds to GroEL for further folding attempts. Proteins that exceed the size limit of the chaperonin cage either use the Hsp70 system for folding[24,36] or may reach native state through binding and release from GroEL without encapsulation[79].

Enclosing unfolded protein, one molecule at a time, in a specialized folding compartment provides an effective solution to the aggregation problem. However, the physical properties of the compartment are likely to have additional effects on folding beyond prevention of aggregation. Notably, larger substrates fully occupy the limited volume of the GroEL and GroES nanocage, as shown impressively by recent cryo-EM analysis of chaperonin complexes[80]. The constraints resulting from this high degree of geometric confinement will inevitably affect the folding energy landscape of such proteins. Although it has been suggested that the chaperonin merely functions as a passive aggregation-prevention device[81], other studies provided evidence that encapsulation can accelerate folding up to tenfold over the rate of spontaneous folding (measured in the absence of aggregation)[22,78,82]. This rate acceleration may be attributed to steric confinement by the cage, entropically destabilizing misfolded states and promoting the acquisition of more compact, native-like conformations, consistent with confinement theory[83,84]. Mutational analysis showed further that the polar residues of the cavity wall are crucial for rapid folding[22,85,86]. According to molecular dynamics simulations, these polar residues are expected to promote folding by accumulating ordered water molecules in their vicinity, thereby generating a local environment in which a substrate protein is forced to bury exposed hydrophobic residues more effectively[87]. This effect would be significant only with proteins that approach the size limit of the cage, consistent with the finding that folding of smaller substrates of 25–30 kDa is not accelerated[78,88].

Other elements of the chaperonin mechanism may also contribute to accelerating folding. It has been proposed that repeated unfolding events in successive binding-and-release cycles help to reverse kinetically trapped states ('iterative annealing')[89,90]. Moreover, the release of substrate from the GroEL apical domains during encapsulation may follow a stepwise mechanism, with less tightly bound hydrophobic regions being released first[75] (**Fig. 6b**). This stepwise release might facilitate the folding of proteins that fail to fold through spontaneous hydrophobic collapse. The contribution of these effects to chaperonin-assisted folding as well as the exact coordination of the two GroEL rings in the folding cycle remain to be clarified[91].

## Implications in understanding human disease

An increasing number of diseases are now recognized to be associated with aberrant protein folding, with pathomechanistic considerations converging on chaperone functions and related quality-control pathways. Of particular interest in this regard are pathological states in which aberrant folding results in a toxic gain of function. These disorders include, among others, Alzheimer's, Parkinson's and Huntington's disease as well as the prion diseases, all of which are associated with the deposition of fibrillar aggregates (amyloid) either within or around neurons in specific brain regions (also the Perspective by Tessier and Lindquist[92] in this issue). Although the toxic principle operating in these disorders is far from being understood, a consensus is emerging that oligomeric, soluble states of the respective disease proteins are the primary cytotoxic species[93]. These intermediates may interact aberrantly with other proteins or with membrane surfaces, altering their functional properties[94]. Interestingly, increasing the levels of molecular chaperones, most prominently members of the Hsp70 family, has been shown to inhibit the formation of such oligomers and to prevent the formation of amyloid aggregates for different disease proteins[94–97]. In the case of polyglutamine-repeat proteins, which cause Huntington's disease and several other related disorders, Hsp70 cooperates with the chaperonin TRiC/CCT in preventing the accumulation of toxic oligomers[98–100], reminiscent of the functional cooperation between these chaperone systems in *de novo* protein folding.

These observations raise the question of why, at a certain point in life, the protein quality-control machinery ceases to cope with the formation of potentially toxic, misfolded proteins. Recent advances in understanding the genetic programs underlying the aging process indicate that the functional capacity of molecular chaperones and other aspects of protein quality control decrease during aging[101]. Although the underlying mechanisms of this decline are not yet understood in detail, this important insight would provide a plausible explanation for the late onset of many neurodegenerative diseases caused by aberrant protein folding. It also suggests that searching for ways to re-establish protein homeostasis, for example by upregulating the expression of chaperones, may offer promising venues for therapeutic intervention[28,102]. A more detailed understanding of the complexities of protein folding and cellular chaperone networks will be required for these approaches to ultimately be of clinical benefit.

ACKNOWLEDGMENTS

Work in our laboratory is supported by the Max-Planck-Gesellschaft, the Deutsche Forschungsgemeinschaft, The European Commission, The Körber Foundation and the Ernst-Jung-Foundation.

Published online at http://www.nature.com/nsmb/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Anfinsen, C.B. Principles that govern the folding of protein chains. *Science* **181**, 223–230 (1973).
2. Hartl, F.U. Molecular chaperones in cellular protein folding. *Nature* **381**, 571–579 (1996).
3. Bartlett, A.L. & Radford, S.E. An expanding arsenal of experimental methods yields an explosion of insights into protein folding mechanisms. *Nat. Struct. Mol. Biol.* **16**, 582–588 (2009).
4. Brockwell, D.J. & Radford, S.E. Intermediates: ubiquitous species on folding energy landscapes? *Curr. Opin. Struct. Biol.* **17**, 30–37 (2007).
5. Onuchic, J.N. & Wolynes, P.G. Theory of protein folding. *Curr. Opin. Struct. Biol.* **14**, 70–75 (2004).
6. Jahn, T.R. & Radford, S.E. The Yin and Yang of protein folding. *FEBS J.* **272**, 5962–5970 (2005).
7. Englander, S.W., Mayne, L. & Krishna, M.M. Protein folding and misfolding: mechanism and principles. *Q. Rev. Biophys.* **40**, 287–326 (2007).
8. Lindberg, M.O. & Oliveberg, M. Malleability of protein folding pathways: a simple reason for complex behaviour. *Curr. Opin. Struct. Biol.* **17**, 21–29 (2007).
9. Netzer, W.J. & Hartl, F.U. Recombination of protein domains facilitated by co-translational folding in eukaryotes. *Nature* **388**, 343–349 (1997).
10. Wright, C.F., Teichmann, S.A., Clarke, J. & Dobson, C.M. The importance of sequence diversity in the aggregation and evolution of proteins. *Nature* **438**, 878–881 (2005).
11. Dobson, C.M. Protein folding and misfolding. *Nature* **426**, 884–890 (2003).

12. Ignatova, Z. & Gierasch, L.M. Monitoring protein stability and aggregation *in vivo* by real-time fluorescent labeling. *Proc. Natl. Acad. Sci. USA* **101**, 523–528 (2004).
13. Ellis, R.J. & Minton, A.P. Protein aggregation in crowded environments. *Biol. Chem.* **387**, 485–497 (2006).
14. Ban, N., Nissen, P., Hansen, J., Moore, P.B. & Steitz, T.A. The complete atomic structure of the large ribosomal subunit at 2.4 angstrom resolution. *Science* **289**, 905–920 (2000).
15. Lu, J.L. & Deutsch, C. Folding zones inside the ribosomal exit tunnel. *Nat. Struct. Mol. Biol.* **12**, 1123–1129 (2005).
16. Woolhead, C.A., McCormick, P.J. & Johnson, A.E. Nascent membrane and secretory proteins differ in FRET-detected folding far inside the ribosome and in their exposure to ribosomal proteins. *Cell* **116**, 725–736 (2004).
17. Kramer, G., Boehringer, D., Ban, N. & Bukau, B. The ribosome as a platform for cotranslational processing, folding and targeting of newly synthesized proteins. *Nat. Struct. Mol. Biol.* **16**, 589–597 (2009).
18. Elcock, A.H. Molecular simulations of cotranslational protein folding: fragment stabilities, folding cooperativity, and trapping in the ribosome. *PLOS Comput. Biol.* **2**, e98 (2006).
19. Kaiser, C.M. *et al.* Real-time observation of trigger factor function on translating ribosomes. *Nature* **444**, 455–460 (2006).
20. Vabulas, R.M. & Hartl, F.U. Protein synthesis upon acute nutrient restriction relies on proteasome function. *Science* **310**, 1960–1963 (2005).
21. Maisnier-Patin, S. *et al.* Genomic buffering mitigates the effects of deleterious mutations in bacteria. *Nat. Genet.* **37**, 1376–1379 (2005).
22. Tang, Y.C. *et al.* Structural features of the GroEL-GroES nano-cage required for rapid folding of encapsulated protein. *Cell* **125**, 903–914 (2006).
23. Rutherford, S.L. & Lindquist, S. Hsp90 as a capacitor for morphological evolution. *Nature* **396**, 336–342 (1998).
24. Kerner, M.J. *et al.* Proteome-wide analysis of chaperonin-dependent protein folding in *Escherichia coli*. *Cell* **122**, 209–220 (2005).
25. Chang, H.C., Tang, Y.C., Hayer-Hartl, M. & Hartl, F.U. SnapShot: molecular chaperones, Part I. *Cell* **128**, 212 (2007).
26. Tang, Y.C., Chang, H.C., Hayer-Hartl, M. & Hartl, F.U. SnapShot: molecular chaperones, Part II. *Cell* **128**, 412 (2007).
27. Weibezahn, J., Schlieker, C., Tessarz, P., Mogk, A. & Bukau, B. Novel insights into the mechanism of chaperone-assisted protein disaggregation. *Biol. Chem.* **386**, 739–744 (2005).
28. Balch, W.E., Morimoto, R.I., Dillin, A. & Kelly, J.W. Adapting proteostasis for disease intervention. *Science* **319**, 916–919 (2008).
29. Frydman, J. Folding of newly translated proteins *in vivo*: The role of molecular chaperones. *Annu. Rev. Biochem.* **70**, 603–647 (2001).
30. Hartl, F.U. & Hayer-Hartl, M. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* **295**, 1852–1858 (2002).
31. Langer, T. *et al.* Successive action of DnaK, DnaJ and GroEL along the pathway of chaperone-mediated protein folding. *Nature* **356**, 683–689 (1992).
32. Albanèse, V., Yam, A.Y.W., Baughman, J., Parnot, C. & Frydman, J. Systems analyses reveal two chaperone networks with distinct functions in eukaryotic cells. *Cell* **124**, 75–88 (2006).
33. Otto, H. *et al.* The chaperones MPP11 and Hsp70L1 form the mammalian ribosome-associated complex. *Proc. Natl. Acad. Sci. USA* **102**, 10064–10069 (2005).
34. Hundley, H.A., Walter, W., Bairstow, S. & Craig, E.A. Human Mpp11 J protein: ribosome-tethered molecular chaperones are ubiquitous. *Science* **308**, 1032–1034 (2005).
35. Brandt, F. *et al.* The native 3D organization of bacterial polysomes. *Cell* **136**, 261–271 (2009).
36. Agashe, V.R. *et al.* Function of trigger factor and DnaK in multidomain protein folding: increase in yield at the expense of folding speed. *Cell* **117**, 199–209 (2004).
37. Frydman, J., Nimmesgern, E., Ohtsuka, K. & Hartl, F.U. Folding of nascent polypeptide chains in a high molecular mass assembly with molecular chaperones. *Nature* **370**, 111–117 (1994).
38. Wandinger, S.K., Richter, K. & Buchner, J. The Hsp90 chaperone machinery. *J. Biol. Chem.* **283**, 18473–18477 (2008).
39. Cuéllar, J. *et al.* The structure of CCT-Hsc70 NBD suggests a mechanism for Hsp70 delivery of substrates to the chaperonin. *Nat. Struct. Mol. Biol.* **15**, 858–864 (2008).
40. Vainberg, I.E. *et al.* Prefoldin, a chaperone that delivers unfolded proteins to cytosolic chaperonin. *Cell* **93**, 863–873 (1998).
41. McCallum, C.D., Do, H., Johnson, A.E. & Frydman, J. The interaction of the chaperonin tailless complex polypeptide 1 (TCP1) ring complex (TRiC) with ribosome-bound nascent chains examined using photo-cross-linking. *J. Cell Biol.* **149**, 591–602 (2000).
42. Thulasiraman, V., Yang, C.F. & Frydman, J. *In vivo* newly translated polypeptides are sequestered in a protected folding environment. *EMBO J.* **18**, 85–95 (1999).
43. Siegers, K. *et al.* Compartmentation of protein folding *in vivo*: sequestration of non-native polypeptide by the chaperonin-GimC system. *EMBO J.* **18**, 75–84 (1999).
44. Zhao, R. & Houry, W.A. Molecular interaction network of the Hsp90 chaperone system. *Adv. Exp. Med. Biol.* **594**, 27–36 (2007).
45. Ellis, R.J. Molecular chaperones: assisting assembly in addition to folding. *Trends Biochem. Sci.* **31**, 395–401 (2006).
46. Saschenbrecker, S. *et al.* Structure and function of RbcX, an assembly chaperone for hexadecameric rubisco. *Cell* **129**, 1189–1200 (2007).
47. Teter, S.A. *et al.* Polypeptide flux through bacterial Hsp70: DnaK cooperates with trigger factor in chaperoning nascent chains. *Cell* **97**, 755–765 (1999).
48. Deuerling, E., Schulze-Specking, A., Tomoyasu, T., Mogk, A. & Bukau, B. Trigger factor and DnaK cooperate in folding of newly synthesized proteins. *Nature* **400**, 693–696 (1999).
49. Ewalt, K.L., Hendrick, J.P., Houry, W.A. & Hartl, F.U. *In vivo* observation of polypeptide flux through the bacterial chaperonin system. *Cell* **90**, 491–500 (1997).
50. Dekker, C. *et al.* The interaction network of the chaperonin CCT. *EMBO J* **27**, 1827–1839 (2008).
51. Yam, A.Y. *et al.* Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. *Nat. Struct. Mol. Biol.* **15**, 1255–1262 (2008).
52. Kleizen, B. & Braakman, I. Protein folding and quality control in the endoplasmic reticulum. *Curr. Opin. Cell Biol.* **16**, 343–349 (2004).
53. Skach, W.R. Cellular mechanisms of membrane protein folding. *Nat. Struct. Mol. Biol.* **16**, 606–612 (2009).
54. Ferbitz, L. *et al.* Trigger factor in complex with the ribosome forms a molecular cradle for nascent proteins. *Nature* **431**, 590–596 (2004).
55. Lakshmipathy, S.K. *et al.* Identification of nascent chain interaction sites on Trigger factor. *J. Biol. Chem.* **282**, 12186–12193 (2007).
56. Merz, F. *et al.* Molecular mechanism and structure of Trigger factor bound to the translating ribosome. *EMBO J.* **27**, 1622–1632 (2008).
57. Merz, F. *et al.* The C-terminal domain of *Escherichia coli* Trigger factor represents the central module of its chaperone activity. *J. Biol. Chem.* **281**, 31963–31971 (2006).
58. Schlünzen, F. *et al.* The binding mode of the Trigger factor on the ribosome: implications for protein folding and SRP interaction. *Structure* **13**, 1685–1694 (2005).
59. Baram, D. *et al.* Structure of Trigger factor binding domain in biologically homologous complex with eubacterial ribosome reveals its chaperone action. *Proc. Natl. Acad. Sci. USA* **102**, 12017–12022 (2005).
60. Mayer, M.P., Rudiger, S. & Bukau, B. Molecular basis for interactions of the DnaK chaperone with substrates. *Biol. Chem.* **381**, 877–885 (2000).
61. Zhu, X. *et al.* Structural analysis of substrate binding by the molecular chaperone DnaK. *Science* **272**, 1606–1614 (1996).
62. Rüdiger, S., Buchberger, A. & Bukau, B. Interaction of Hsp70 chaperones with substrates. *Nat. Struct. Biol.* **4**, 342–349 (1997).
63. Pellecchia, M. *et al.* Structural insights into substrate binding by the molecular chaperone DnaK. *Nat. Struct. Biol.* **7**, 298–303 (2000).
64. Young, J.C., Barral, J.M. & Hartl, F.U. More than folding: localized functions of cytosolic chaperones. *Trends Biochem. Sci.* **28**, 541–547 (2003).
65. Schiene-Fischer, C., Habazettl, J., Schmid, F.X. & Fischer, G. The Hsp70 chaperone DnaK is a secondary amide peptide bond *cis-trans* isomerase. *Nat. Struct. Biol.* **9**, 419–424 (2002).
66. Dragovic, Z., Broadley, S.A., Shomura, Y., Bracher, A. & Hartl, F.U. Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. *EMBO J.* **25**, 2519–2528 (2006).
67. Raviol, H., Sadlish, H., Rodriguez, F., Mayer, M.P. & Bukau, B. Chaperone network in the yeast cytosol: Hsp110 is revealed as an Hsp70 nucleotide exchange factor. *EMBO J.* **25**, 2510–2518 (2006).
68. Liu, Q. & Hendrickson, W.A. Insights into Hsp70 chaperone activity from a crystal structure of the yeast Hsp110 Sse1. *Cell* **131**, 106–120 (2007).
69. Polier, S., Dragovic, Z., Hartl, F.U. & Bracher, A. Structural basis for the cooperation of Hsp70 and Hsp110 chaperones in protein folding. *Cell* **133**, 1068–1079 (2008).
70. Schuermann, J.P. *et al.* Structure of the Hsp110: Hsc70 nucleotide exchange machine. *Mol. Cell* **31**, 232–243 (2008).
71. Horwich, A.L., Fenton, W.A., Chapman, E. & Farr, G.W. Two families of chaperonin: physiology and mechanism. *Annu. Rev. Cell Dev. Biol.* **23**, 115–145 (2007).
72. Gromiha, M.M. & Selvaraj, S. Inter-residue interactions in protein folding and stability. *Prog. Biophys. Mol. Biol.* **86**, 235–277 (2004).
73. Xu, Z., Horwich, A.L. & Sigler, P.B. The crystal structure of the asymmetric GroEL-GroES-(ADP)7 chaperonin complex. *Nature* **388**, 741–750 (1997).
74. Horst, R. *et al.* Direct NMR observation of a substrate protein bound to the chaperonin GroEL. *Proc. Natl. Acad. Sci. USA* **102**, 12748–12753 (2005).
75. Sharma, S. *et al.* Monitoring protein conformation along the pathway of chaperonin-assisted folding. *Cell* **133**, 142–153 (2008).
76. Hillger, F. *et al.* Probing protein-chaperone interactions with single-molecule fluorescence spectroscopy. *Angew. Chem. Int. Ed.* **47**, 6184–6188 (2008).
77. Elad, N. *et al.* Topologies of a substrate protein bound to the chaperonin GroEL. *Mol. Cell* **26**, 415–426 (2007).
78. Brinker, A. *et al.* Dual function of protein confinement in chaperonin-assisted protein folding. *Cell* **107**, 223–233 (2001).
79. Chaudhuri, T.K., Farr, G.W., Fenton, W.A., Rospert, S. & Horwich, A.L. GroEL/GroES-mediated folding of a protein too large to be encapsulated. *Cell* **107**, 235–246 (2001).
80. Clare, D.K., Bakkes, P.J., van Heerikhuizen, H., van der Vies, S.M. & Saibil, H.R. Chaperonin complex with a newly folded protein encapsulated in the folding chamber. *Nature* **457**, 107–110 (2009).
81. Apetri, A.C. & Horwich, A.L. Chaperonin chamber accelerates protein folding through passive action of preventing aggregation. *Proc. Natl. Acad. Sci. USA* **105**, 17351–17355 (2008).
82. Lin, Z. & Rye, H.S. Expansion and compression of a protein folding intermediate by GroEL. *Mol. Cell* **16**, 23–34 (2004).

83. Baumketner, A., Jewett, A. & Shea, J.E. Effects of confinement in chaperonin assisted protein folding: rate enhancement by decreasing the roughness of the folding energy landscape. *J. Mol. Biol.* **332**, 701–713 (2003).
84. Hayer-Hartl, M. & Minton, A.P. A simple semiempirical model for the effect of molecular confinement upon the rate of protein folding. *Biochemistry* **45**, 13356–13360 (2006).
85. Tang, Y.C., Chang, H.C., Chakraborty, K., Hartl, F.U. & Hayer-Hartl, M. Essential role of the chaperonin folding compartment *in vivo*. *EMBO J.* **27**, 1458–1468 (2008).
86. Wang, J.D., Herman, C., Tipton, K.A., Gross, C.A. & Weissman, J.S. Directed evolution of substrate-optimized GroEL/S chaperonins. *Cell* **111**, 1027–1039 (2002).
87. England, J.L., Lucent, D. & Pande, V.S. A role for confined water in chaperonin function. *J. Am. Chem. Soc.* **130**, 11838–11839 (2008).
88. Horst, R., Fenton, W.A., Englander, S.W., Wuthrich, K. & Horwich, A.L. Folding trajectories of human dihydrofolate reductase inside the GroEL GroES chaperonin cavity and free in solution. *Proc. Natl. Acad. Sci. USA* **104**, 20788–20792 (2007).
89. Thirumalai, D. & Lorimer, G.H. Chaperonin-mediated protein folding. *Annu. Rev. Biophys. Biomol. Struct.* **30**, 245–269 (2001).
90. Lin, Z., Madan, D. & Rye, H.S. GroEL stimulates protein folding through forced unfolding. *Nat. Struct. Mol. Biol.* **15**, 303–311 (2008).
91. Koike-Takeshita, A., Yoshida, M. & Taguchi, H. Revisiting the GroEL-GroES reaction cycle via the symmetric intermediate implied by novel aspects of the GroEL(D398A) mutant. *J. Biol. Chem.* **283**, 23774–23781 (2008).
92. Tessier, P. & Lindquist, S. Unraveling molecular interactions and structures of self-perpetuating prions. *Nat. Struct. Mol. Biol.* **16**, 598–605 (2009).
93. Chiti, F. & Dobson, C.M. Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* **75**, 333–366 (2006).
94. Barral, J.M., Broadley, S.A., Schaffar, G. & Hartl, F.U. Roles of molecular chaperones in protein misfolding diseases. *Semin. Cell Dev. Biol.* **15**, 17–29 (2004).
95. Muchowski, P.J. *et al.* Hsp70 and Hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc. Natl. Acad. Sci. USA* **97**, 7841–7846 (2000).
96. Muchowski, P.J. & Wacker, J.L. Modulation of neurodegeneration by molecular chaperones. *Nat. Rev. Neurosci.* **6**, 11–22 (2005).
97. Auluck, P.K., Chan, H.Y.E., Trojanowski, J.Q., Lee, V.M.Y. & Bonini, N.M. Chaperone suppression of α-synuclein toxicity in a *Drosophila* model for Parkinson's disease. *Science* **295**, 865–868 (2002).
98. Behrends, C. *et al.* Chaperonin TRiC promotes the assembly of polyQ expansion proteins into nontoxic oligomers. *Mol. Cell* **23**, 887–897 (2006).
99. Tam, S., Geller, R., Spiess, C. & Frydman, J. The chaperonin TRiC controls polyglutamine aggregation and toxicity through subunit-specific interactions. *Nat. Cell Biol.* **8**, 1155–1162 (2006).
100. Kitamura, A. *et al.* Cytosolic chaperonin prevents polyglutamine toxicity with altering the aggregation state. *Nat. Cell Biol.* **8**, 1163–1170 (2006).
101. Morley, J.F. & Morimoto, R.I. Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. *Mol. Biol. Cell* **15**, 657–664 (2004).
102. Sittler, A. *et al.* Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. *Hum. Mol. Genet.* **10**, 1307–1315 (2001).
103. Scheufler, C. *et al.* Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell* **101**, 199–210 (2000).
104. Harrison, C.J., Hayer-Hartl, M., Di Liberto, M., Hartl, F.U. & Kuriyan, J. Crystal structure of the nucleotide exchange factor GrpE to the ATPase domain of the molecular chaperone DnaK. *Science* **276**, 431–435 (1997).