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Chaperone-Assisted Folding of Newly Synthesized Proteins in the Cytosol

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The way in which a newly synthesized polypeptide chain folds into its unique three-dimensional structure remains one of the fundamental questions in molecular biology. Protein folding in the cell is a problematic process and, in many cases, requires the assistance of a network of molecular chaperones to support productive protein folding *in vivo*. During protein biosynthesis, ribosome-associated chaperones guide the folding of the nascent polypeptide emerging from the ribosomal tunnel. In this review we summarize the basic principles of the protein-folding process and the involved chaperones, and focus on the role of ribosome-associated chaperones. Our discussion emphasizes the bacterial Trigger Factor, which is the best studied chaperone of this type. Recent advances have determined the atomic structure of the Trigger Factor, providing new, exciting insights into the role of ribosome-associated chaperones in co-translational protein folding.

Keywords trigger factor, GroEL, DnaK, nascent chains, aggregation

INTRODUCTION

In all organisms, messenger-directed protein synthesis is achieved via the ribosome, a complex cellular machine that operates with remarkable efficiency. A rapidly growing *Escherichia coli* cell, for example, uses 20,000 ribosomes to produce an estimated total of 30,000 polypeptides per minute, with average elongation rates for polypeptides of about 10 to 20 residues per ribosome per second. Thousands of different proteins synthesized in a cell within a minute must then fold into a large variety of specific structures. During the past several decades, the process of protein folding has been investigated intensively, both in the test tube and in living cells. Although the biophysical principles governing protein folding are the same *in vitro* as *in vivo*, important differences exist. This review focuses on the folding of proteins in the cytosol, with particular emphasis on the early folding events involving ribosome-associated chaperones. For more detailed information on protein folding *in vitro* and on folding of organellar and secretory proteins, the reader is referred to the following reviews: Dill and Chan, 1997; Dinner *et al*., 2000; Fersht and Daggett, 2002; Ito, 1996; Molinari and Helenius, 2000; Neupert, 1997; Pfanner and Geissler, 2001; Pfanner and Wiedemann, 2002; Radford, 2003; and Schatz and Dobberstein, 1996.

PROTEIN FOLDING *IN VITRO* VS. *IN VIVO*

All the information needed to specify the three-dimensional conformation of a protein is encoded by the protein's amino acid sequence (Anfinsen, 1973). *In vitro* protein folding is studied primarily using small-model proteins consisting of 60 or fewer amino acids. These small-model proteins can be unfolded (e.g., by heat, pressure, or denaturating chemicals), and they spontaneously fold back into the native structure upon removal of the denaturant. In such *in vitro* folding assays, however, only the model protein is present in the aqueous solution, and at a low concentration, seldom exceeding 1 mg/mL.

Several features define the basic differences between the folding of proteins *in vivo* and *in vitro*. First, the cytosol is an extremely crowded environment, with macromolecule concentrations reaching 200 to 300 mg/mL in *E. coli* (Zimmerman & Trach, 1991). Theoretically, such macromolecular crowding leads to excluded volume effects, which strongly affect biochemical rates by increasing protein association constants and thereby increasing the propensity of intermolecular interactions, including aggregation (Zimmerman & Minton, 1993). Consistent with this theory are the results of experiments performed *in vitro* using unfolded reduced lysozyme (van den Berg *et al*., 1999). In the presence of different crowding agents, the yield of correctly folded enzyme was shown to decrease

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dramatically, and aggregation to increase concomitantly. This effect was not due to a reduction in folding rates, and did not occur at oxidizing conditions that preserve native disulfside bonds in lysozyme and increase the folding rate by two to three orders of magnitude. The folding rate of lysozyme thus determines whether the folding yield is sensitive to crowding agents. It is unclear whether this finding can be generalized, particularly because earlier experiments revealed an opposing result, indicating that crowding agents, including proteins, decrease the tendency of several refolding proteins to aggregate (Minton, 2000; Minton *et al*., 1982). Such an effect may result from transient hydrophobic or electrostatic interactions between the folding proteins and other proteins. Another factor that influences folding is the high viscosity of the cytosol (Elowitz *et al*., 1999). *In vitro* analysis should that high viscosity can decelerate folding rates (Jacob *et al*., 1997), and may thereby increase the time frame in which misfolding and aggregation can occur. Although all these experiments demonstrate that crowding and viscosity can influence the folding of proteins *in vitro*, they do not clarify the importance of these factors for the folding of proteins *in vivo*. In fact, they may affect folding in opposing ways, depending on the protein, by increasing or decreasing aggregation.

Second, about one third of newly synthesized proteins must be targeted to an organelle or are secreted to an extracellular compartment where they fulfill their functions. These proteins are targeted as nascent or loosely folded polypeptides to their translocation machinery, and only after reaching their intended destination they fold to their native states. Targeting is achieved primarily through the co-translational action of *cis*-acting signal sequences and *trans*-acting protein factors, such as the signal recognition particle (SRP), and through molecular chaperones, including bacterial SecB and SecA (Driessen *et al*., 2001; Walter & Johnson, 1994) and eukaryotic Hsp90 and Hsp70 (Hartl *et al*., 1990; Schatz & Dobberstein, 1996).

Third, all proteins are synthesized by the ribosome in a vectorial manner, from the N-to the C-terminus, implying that, as long as polypeptide synthesis proceeds, the folding information remains transient and incomplete. This raises the question of exactly how and when the folding of a polypeptide chain to its structure is achieved in living cells. Three different models address this question (Figure 1). They are not mutually exclusive, and the pathway used to obtain a properly folded protein may depend on the specific protein and on environmental conditions.

The first model suggests that folding of the growing polypeptide chain is postponed by chaperone binding until its synthesis is completed (Agashe *et al*., 2004; Fedorov & Baldwin, 1997). In this model, folding is initiated only upon release of the protein from the ribosome, thus making the entire polypeptide sequence available, comparable to the refolding of a chemically unfolded protein *in vitro*.

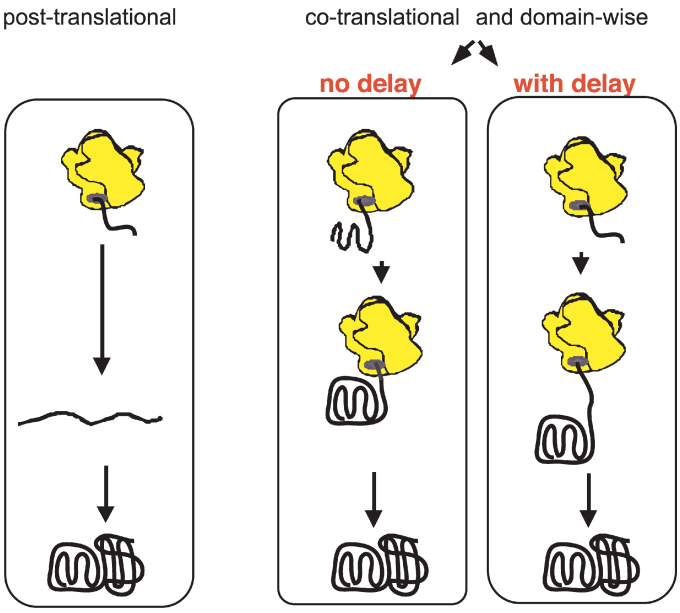

FIG. 1. Potential modes of *de novo* protein folding. Newly synthesized proteins may exclusively fold post-translationally after their release from the ribosome (*left*), or folding may already start co-translationally (*right*), with or without delay, to allow the domain-wise folding of proteins during protein biosynthesis.

The major benefit of such a post-translational folding route would be a possible reduction in inappropriate intramolecular interactions of nascent polypeptide stretches. On the other hand, the prolonged exposure of numerous hydrophobic stretches of an unfolded intermediate in the cytosol may enhance unproductive intermolecular interactions, and thus trigger misfolding and aggregation. It is therefore conceivable that chaperones must mediate post-translational folding; binding of presumably several chaperones to a nascent chain would be required to postpone its folding and to shield its many hydrophobic stretches. A remaining question then is how the dissociation of these chaperones is mediated, and the rebinding of chaperones prevented, to allow the unfolded polypeptide to proceed with folding.

The second model suggests that formation of secondary and tertiary structure begins co-translationally, as soon as the polypeptide chain emerges from the ribosomal exit tunnel. This folding mode would minimize intermolecular off-pathway reactions, given that hydrophobic side chain interactions are immediately formed. For some proteins with complicated folds that require long-distance interactions within the polypeptide sequence, however, unproductive folding intermediates might occur due to the limited availability of folding information.

The third model proposes a stepwise co-translational folding, in which co-translational folding is initally

delayed, and is allowed to proceed only when sufficient sequence information is available for the generation of a folded core or domain. This domain-wise folding could be particularly advantageous for the folding of multi-domain proteins, because this process limits possible unproductive inter- and intramolecular interactions during the early folding steps (Bukau *et al*., 2000; Netzer & Hartl, 1997). As for the post-translational folding mode, molecular chaperones would need to associate with the nascent chains to initially postpone their folding, but for a shorter time and by shielding only a smaller stretch of the polypeptide, corresponding to a domain that typically comprises of 100 to 150 amino acids.

CO-TRANSLATIONAL VS. POST-TRANSLATIONAL PROTEIN FOLDING

Recent advances have defined the complete atomic structures of the large (50S) and small (30S) ribosomal subunits of bacterial and archaeal ribosomes, providing new and important insights into this sophisticated macromolecular machine (Ban *et al*., 1999, 2000; Schluenzen *et al*., 2000; Wimberly *et al*., 2000).

The structure of the *Haloarcula marismortui* large ribosomal subunit revealed a tunnel (Figure 2) of approximately 100 Å in length, through which the polypeptide emerges to reach the tunnel exit (Ban *et al*., 2000; Nissen *et al*., 2000). The tunnel is large enough to protect a polypeptide segment of 30 to 35 aa in length from protease digestion (Hardesty & Kramer, 2001).

The polypeptide exit tunnel is largely formed by rRNA, but has significant contributions from two ribosomal proteins, L22 and L4 (Nissen *et al*., 2000). These two proteins protrude into the interior of the tunnel and, together with the RNA moieties, form the narrowest constriction of the exit tunnel. Until recently, the tunnel was considered to be inert, not interacting with the nascent polypeptide. A recent study by Ito and coworkers, however, provided evidence that the ribosome can discriminate among certain

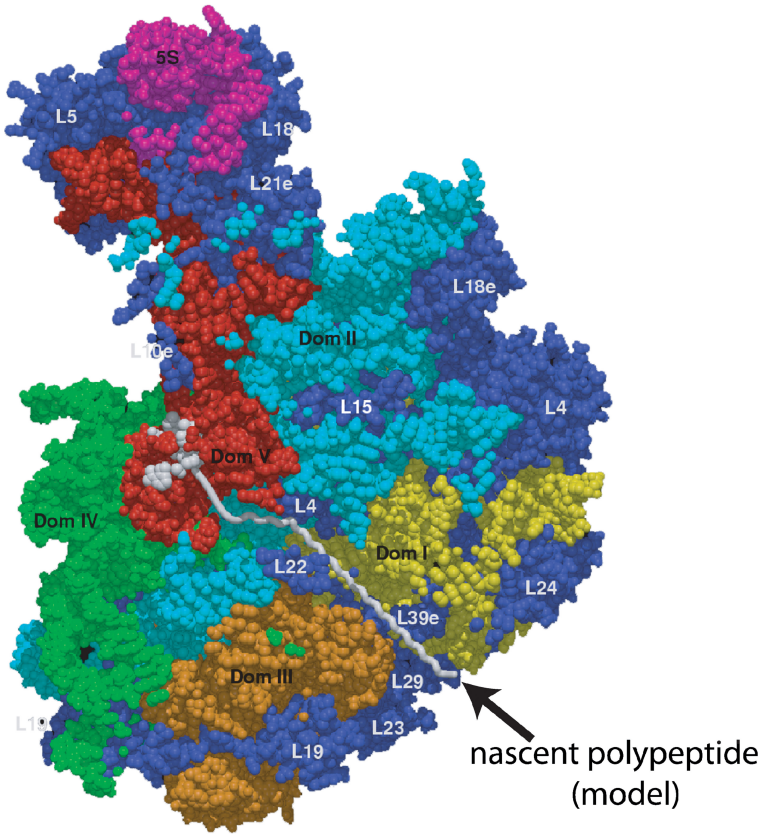

FIG. 2. Structure of the large ribsosomal subunit from *Haloarcula marismortui.* The ribosomal subunit is cut in half to reveal the interior of the 50S complex. A polypeptide running through the ribosomal tunnel is modelled in white. The tunnel surface is shown with backbone atoms of the RNA color coded by domains. Domain I (*yellow*), II (*light blue*), III (*orange*), IV (*green*), V (*light red*), 5S (*pink*), and proteins are blue. (Source: Nissen *et al*., 2000. With permission.)

amino acid sequences as they travel through the tunnel, thus reprogramming the ribosome during protein biogenesis (Nakatogawa & Ito, 2002). In *E. coli*, a specific sequence motif within the C-terminus of the SecM polypeptide (called an arrest sequence, aa 150 to 166 of SecM: FXXXXWIXXXXGIRAGP) interacts with L22 and rRNA at the narrow constriction site within the ribosomal tunnel and causes translational arrest (Nakatogawa & Ito, 2002). This elongation arrest is transient and can be overcome by SecA binding at the exposed N-terminus and subsequent export of SecM into the periplasm. The translational arrest of SecM was the first example to show the interactive nature of a nascent polypeptide within the ribosomal tunnel in *E. coli*.

Little is known about the conformation of the nascent polypeptide in the tunnel. The average diameter of the ribosomal tunnel is approximately 15 Å and might allow formation of an α-helix, but the tunnel is too narrow for assembly of β-sheet or tertiary structures (Ban *et al*., 1999, 2000; Nissen *et al*., 2000). Therefore, the polypeptide chains exiting the ribosomal tunnel are largely in an extended conformation. Interestingly, a recent cryo-EM study suggests that small segments of a stalled nascent polypeptide could fold in a globular conformation within an expanded tunnel (Gilbert *et al*., 2004). The importance and universal validity of this finding for protein folding is unclear, however, and some concerns remain with respect to the interpretation of the cryo-EM data.

Several *in vitro* and *in vivo* studies provide evidence that protein folding can start co-translationally during biosynthesis. Goldberg and coworkers used antibodies against three specific folding states of the trimeric P22 tailspike protein (Friguet *et al*., 1994). The antibody, which recognized the earliest folding intermediate corresponding to the folded monomeric species, could be used to precipitate nascent tailspike protein together with ribosomes from P22-infected *Salmonella* cells, whereas the antibodies directed against the protrimeric form and the mature trimer, respectively, did not. This finding suggests co-translational folding of the P22 tailspike protein but shows that release from the ribosome is necessary for complete folding to the active state. In an independent study, Hartl and coworkers (Frydman *et al*., 1994) found similar results for the luciferase protein from the firefly *Photinus pyralis*. In a translation-active reticulozyte lysate, they showed the N-terminal domain of this enzyme folds and becomes proteinase K-resistant when still associated with the ribosome. Full enzymatic activity of the protein could, only be observed however, after release from the ribosome.

A study that analyzed folding of the Semliki Forest virus capsid protein (C protein) in the cytosol of living cells provided evidence for co-translational folding *in vivo* (Nicola *et al*., 1999). The C protein is at the N-terminus of five proteins, which are translated as a polyprotein precursor. It contains a chymotrypsin-like domain, which, once folded to native structure, acts in *cis* to cleave itself from the precursor. In both hamster cells and *E. coli*, this domain folds rapidly during translation, well before termination of polyprotein synthesis. Although the C protein might be optimized to fold very rapidly (Sanchez *et al*., 2004), the results establish the principle that protein domains can fold co-translationally in the cytosol of prokaryotic and mammalian cells.

The absolute amount of proteins that fold co-translationally remains unclear. In fact several studies showed that the folding of newly synthesized large-model proteins occurs mainly post-translationally in bacteria, but not in reticulozyte lysate. This was shown for an artifical Ras–DHFR fusion protein (Netzer & Hartl, 1997) and for firefly luciferase (Agashe *et al*., 2004). These findings were interpreted to indicate that the folding process is fundamentally different in pro- and eukaryotes. Rapid and efficient folding of multi-domain model proteins occurs co-translationally in the eukaryotic system, while the bacterial system lacks the capacity for folding these model proteins co-translationally. Therefore, the production of eukaryotic multi-domain proteins in bacterial cells produces misfolded species that form inclusion bodies (Agashe *et al*., 2004; Netzer & Hartl, 1997).

One additional feature of the folding process is important to note. It has been shown, at least for one model protein, that no general built-in code exists for a vectorial, N- to C-terminal, deciphering of the folding information *in vivo*. This was shown by random circular permutation experiments in which N-terminal sequences of various lengths were genetically moved to the C-terminus of DsbA (Hennecke *et al*., 1999). Many of the circular permutated DsbA derivatives with new N- and C-termini showed wild-type–like folding efficiencies and thermodynamic stabilities. It remains unclear, however, whether this result also holds true for other proteins with more complicated domain folds.

A NETWORK OF MOLECULAR CHAPERONES ASSISTS PROTEIN FOLDING *IN VIVO*

Molecular chaperones exist in all organisms and in all cellular compartments. They include the heat shock protein (Hsp) families Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and the small HSPs (the numbers indicate the typical molecular mass of the family members). These families are conserved among the vast majority of prokaryotic and eukaryotic organisms. Others, like the Trigger Factor, are present only in bacteria and chloroplasts.

A common feature of all chaperones is the stoichiometric and transient binding of folding intermediates. Chaperones prevent inappropiate inter- and intra-molecular

interactions by binding to hydrophobic patches of nonnative proteins thereby influencing the partitioning between productive and unproductive folding steps. Importantly, chaperones are not part of the final structures of the folded proteins. Instead substrates are released from the chaperones, thereby providing nonnative proteins a new opportunity for productive folding (Buchner, 1996; Bukau & Horwich, 1998; Ellis & Hartl, 1999).

Chaperones' "holder" and "folder" functions depend on their abilities to either prevent protein aggregation or to support proper protein (re)folding. The "holder" function can be ATP-dependent, as in the case of Hsp70 and Hsp60 chaperones, or ATP-independent, as in the case of small HSPs and Hsp40. In contrast, active assistance of folding (e.g., by the Hsp70 and Hsp60 chaperones) is considered to be strictly ATP-dependent (Bukau & Horwich, 1998).

It has been firmly established that many newly synthesized proteins require the assistance of molecular chaperones to fold to the native state (Bukau *et al*., 2000; Frydman, 2001; Hartl & Hayer-Hartl, 2002; Welch *et al*., 1999). These chaperones can be divided into two classes according to their cellular localization. The first class is composed of soluble chaperones of the cytosol that associate co- and post-translationally with newly synthesized proteins. They include prokaryotic and eukaryotic members of the Hsp60 and Hsp70 families. The second class of chaperones forms a "welcoming committee" for nascent chains on the ribosome by binding to both the ribosome and to the nascent chains, thereby assisting early steps of co-translational protein folding. The existence of ribosome-associated chaperones seems to be highly evident in eukaryotes and prokaryotes, although they belong to unrelated protein families (Bukau *et al*., 2000; Frydman, 2001; Hartl & Hayer-Hartl, 2002).

The role of chaperones in the folding of newly synthesized proteins has thus far been best analyzed in bacteria. In *E. coli*, nascent peptides associate with the ribosome-associated chaperone Trigger Factor as soon as they leave the exit tunnel of the ribosome (Figure 3). It is assumed that Trigger Factor interacts with all nascent chains emerging from the ribosomal exit tunnel (Hesterkamp *et al*., 1996; Stoller *et al*., 1995; Valent *et al*., 1995). After release from Trigger Factor, the nascent polypeptide can start or continue its folding to the native state (Agashe *et al*., 2004; Mogk *et al*., 2002). Small proteins, which show fast folding kinetics *in vitro*, may not require additional chaperones to fold into their native structures. In contrast, multidomain proteins, which fold more slowly *in vitro* and may even become trapped in non-native conformations that are prone to aggregation, may need further assistence by ATP-consuming cytosolic chaperones (Figure 3). Such proteins are typical substrates for the DnaK (Hsp70) and GroEL (Hsp60) chaperone systems. The GroEL chaperone interacts with newly synthesized proteins exclusively after their release from the ribosome, whereas DnaK interacts with both nascent polypeptides and polypeptides released from the ribosome (Deuerling *et al*., 1999, 2003; Ewalt *et al*., 1997; Houry *et al*., 1999; Teter *et al*., 1999).

In both prokaryotic and eukaryotic cells, cytosolic Hsp70 and Hsp60 family members act downstream of ribosome-associated chaperones and associate co- or post-translationally with the newly synthesized proteins to promote folding to the final native state (Bukau *et al*., 2000; Frydman, 2001; Hartl & Hayer-Hartl, 2002). Although Trigger Factor homologs are missing in the cytosol of eukaryotes, other ribosome-associated chaperones exist. The yeast *Saccharomyces cerevisiae* harbors a ribosomal chaperone triad comprising of the two Hsp70-type chaperones Ssb and Ssz, and the DnaJ co-chaperone of Hsp70, Zuotin (Zuo) (Gautschi *et al*., 2001; Pfund *et al*., 1998; Yan *et al*., 1998). Moreover, the "nascent chain-associated complex" (NAC) is found in eukaryotes including those of yeast and mouse (Rospert *et al*., 2002). Although no homology exists on the amino acid sequence level, all these eukaryotic proteins share some characteristics with bacterial Trigger Factor. They are abundant monomeric or dimeric proteins in the cytosol that reversibly associate with large subunits of ribosomes, and they can interact with nascent chains of very short length. Their intriguing positioning near the ribosomal exit site for nascent chains qualifies these proteins as the first line of chaperones protecting nascent chains from misfolding and aggregation. However, with the exception of Trigger Factor, such a role remains speculative.

Functional cooperation between different chaperones seems to be a central principle of protein folding in the cell. In bacteria, Trigger Factor and DnaK cooperate in the folding of newly synthesized proteins. Mutant cells lacking the *dnaK* gene, or the Trigger Factor encoding gene *tig*, are viable at regular growth temperatures, whereas double $\Delta dnaK\,\Delta tig$ mutants fail to grow at temperatures above approximately 30°C (Deuerling *et al*., 1999; Teter *et al*., 1999). Cells compensate for the absence of Trigger Factor by induction of the heat shock response, which leads to increased levels of other chaperones and proteases (Deuerling *et al*., 2003). Furthermore, the absence of Trigger Factor in Δtig cells leads to increased association of DnaK with nascent polypeptides (Deuerling *et al*., 1999; Teter *et al*., 1999). DnaK may thus directly replace Trigger Factor by associating with the same hydrophobic stretches within the nascent polypeptide. Peptide library screening showed that Trigger Factor and DnaK indeed exhibit high overlap in their binding specificities (Deuerling *et al*., 2003; Patzelt *et al*., 2001). Alternatively, but not exclusively, DnaK may act as a backup system that rescues misfolded proteins that accumulate in Δtig cells. Recent studies showed that the SecB and GroEL/ES chaperones are further backup systems for Trigger Factor and DnaK (Genevaux *et al*., 2004; Ullers *et al*., 2004;

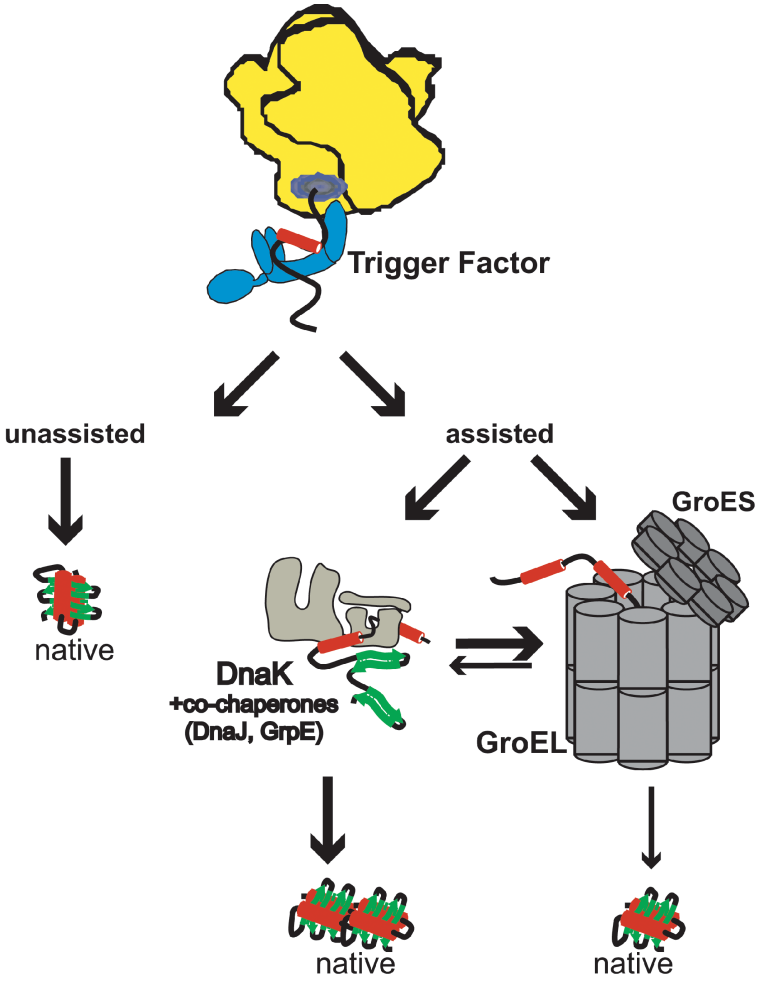

FIG. 3. Model of the role of bacterial chaperones in the folding of newly synthesized proteins. Ribosome-associated Trigger Factor interacts with emerging nascent polypeptides. Subsequently, multiple pathways exist that provide a flexible network for folding of nascent chains to native structures. Wherease some newly synthesized proteins fold autonomously to their native state, others associated post-translationally with the DnaK-system and/or with the GroEL-machine to reach their correct conformation.

Vorderwulbecke *et al*., 2004). DnaK and GroEL also form a functional network that allows substrates to be transferred bidirectionally from one chaperone to another, through a soluble intermediate (Buchberger *et al*., 1996). However, GroEL requires a longer stretch of a substrate polypeptide for efficient binding, and perhaps a more folded overall state of the substrate, as compared to DnaK. Thus, a hierarchy of chaperone interactions seems to exist among newly synthesized proteins, with ribosome-associated Trigger Factor preceeding DnaK, and DnaK preceeding GroEL. It should be emphasized that not all substrates must interact with all these chaperones, with the possible exception of Trigger Factor.

In the following discussions we will focus primarily on the function and mechanism of bacterial chaperones involved in protein folding. First, we will address the molecular mechanisms of GroEL and DnaK, two chaperones that have been functionally and mechanistically well studied. Then we will describe the role of ribosome-associated chaperones, with emphasis on the function and mechanism of Trigger Factor.

THE HSP60 AND HSP70 CHAPERONE MACHINES

Hsp60

GroEL and its co-chaperone GroES form a highly sophisticated chaperone machine in bacteria, mitochondria, and chloroplasts (Ellis, 1987; Fenton, 1997; Ostermann *et al*., 1989). The TriC or CCT chaperones found in eukaryotic cells and archaea are distantly related to GroEL (Frydman *et al*., 1992; Gao *et al*., 1992; Lewis *et al*., 1992). GroEL

and GroES are the only chaperones that are essential for the growth of *E. coli* (Fayet *et al*., 1989). The GroEL system is involved in many cellular processes, including the *de novo* folding of newly synthesized polypeptides (Ewalt *et al*., 1997; Horwich *et al*., 1993), conformational maintenance of pre-existing proteins, secretion, and proteolysis (Houry *et al*., 1999; Kandror *et al*., 1994; Kusukawa *et al*., 1989; Laminet *et al*., 1990). Although the required presence of GroEL for cell growth may result from any of these cellular functions, its role in the *de novo* folding of proteins may be the most critical, given that some substrates are entirely dependent on folding assistance by GroEL.

About 10% to 15% of the newly synthesized polypeptides isolated from growing *E. coli* cells are found to be in transient association with GroEL. Most of these polypeptides interact post-translationally with GroEL (Ewalt *et al*., 1997; Houry *et al*., 1999). Most of the GroEL interacting proteins have molecular weights between 20 and 60 kDa, with a minority ($\leq$20%) having higher molecular weights (Houry *et al*., 1999). Interestingly, many identified GroEL substrates show a common domain architecture, with multiple $\alpha\beta$ domains having α-helices and β-sheets with extensive hydrophobic surfaces. Several stringent model substrates of GroEL—including ornithine transcarbamylase, malate dehydrogenase, rhodanese, and rubisco—belong to this category of proteins (Cheng *et al*., 1989; Ewalt *et al*., 1997; Rye *et al*., 1997; Viitanen *et al*., 1990).

GroEL exhibits a complex overall structure that is composed of two heptameric rings of the large subunit GroEL (57 kDa), stacking back to back and forming a 14-subunit hollow cylinder with two identical binding sites for nonnative proteins (Figure 4A). The smaller co-chaperone GroES (10 kDa) forms a seven-membered, dome-shaped single ring. The interaction between GroEL and GroES

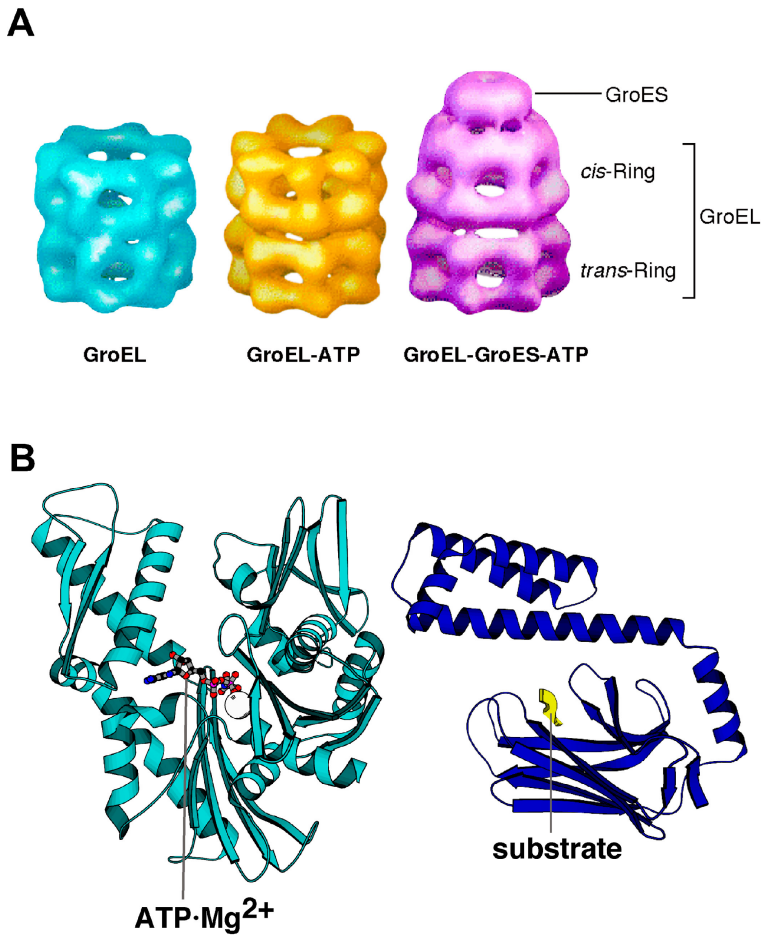

FIG. 4. Structures of GroEL/ES and DnaK. (*A*) Reconstruction of the GroEL structure with (purple) and without (cyan and yellow) the GroES "lid" from cryo-electron microscopic pictures. The images originate from (Roseman *et al*., 1996) with granted permission for reproduction. (*B*) Left: Homology model based on Hsc70 (Flaherty *et al*., 1990) showing a secondary structure representation of the ATPase domain of DnaK in complex with ATP and Mg^{2+}. Right: secondary structure representation of the substrate binding domain of DnaK in complex with a substrate peptide derived from (Zhu *et al*., 1996).

allows certain proteins to fold under otherwise prohibitive conditions. Nonnative substrates bind through hydrophobic interactions to the apical domains of the unoccupied ring of a GroEL-GroES asymmetric complex. ATP and GroES then bind to the same ring occupied by the substrate, forming a *cis* ternary complex that encapsulates the polypeptide within the GroEL-GroES structure. Binding of ATP and GroES induces large conformational changes in GroEL. The apical substrate-binding domains of GroEL, which hold the substrate by multiple interactions, show strong upward and outward motions upon GroES and ATP binding, thereby greatly expanding the distance between the polypeptide binding regions (Fenton, 1997; Horwich *et al*., 1999; Saibil, 2000; Xu & Sigler, 1998). Substrates bound to multiple apical domains may therefore be subjected to stretching forces ("mechanical stress"), which can induce their unfolding as indicated for the model substrates hen lysozyme and rhodanese (Coyle *et al*., 1999; Shtilerman *et al*., 1999). In other cases, however, no evidence for unfolding of bound substrate has been found (Chen *et al*., 2001; Saibil *et al*., 2001). Binding of GroES approximately doubles the volume of the central cavity and obscures GroEL's hydrophobic polypeptide recognition regions (Figure 4A). As a consequence, the substrate polypeptide is encapsulated within a relatively polar environment that favors folding (Xu *et al*., 1997). Thus, the central cavity provides an environment equivalent to infinite dilution of the substrate, thereby preventing nonproductive aggregation. The combination of both principles may ensure proper folding, especially of kinetically trapped folding intermediates that are caught in a stable nonnative conformation. Nucleotide binding to GroEL is responsible for the transition from the high- to the low-affinity state of the chaperone for its substrates and thereby controls substrate dissociation and rebinding. ATP hydrolysis provides a timer, giving the substrate at least 15 seconds to fold (Bukau & Horwich, 1998). ATP hydrolysis primes GroEL to release GroES, allowing the substrate to exit into the bulk solution. Binding of ATP to the *trans* ring induces GroES and substrate release. Substrates that have not reached their native states are then either recaptured by GroEL or another chaperone system, or are eventually targeted for proteolysis or aggregation.

It is unclear which fraction of the GroEL-interacting proteins depends on GroEL for folding *in vivo*. Because proteins larger than approximately 60 kDa do not fit into the folding chamber of this chaperone underneath GroES, proteins with high molecular weights may not be folded productively by GroEL/GroES. A surprising recent finding is that protein folding by GroEL can also occur in a *trans* complex, in which the substrate is bound to the apical substrate-binding domain of the ring opposite to the ring which has GroES bound (Chaudhuri *et al*., 2001; Farr *et al*., 2003). This mode of substrate binding allows GroEL in principle to fold proteins that are too large (<65 kDa) to be accommodated within the folding chamber of the *cis*-complex formed by GroEL-GroES. The *trans* folding mode is important *in vivo*, because it allows partial rescue of *groEL* mutant phenotypes in *E. coli.* Thus, a *trans* mechanism can be generally productive, although, where size permits, *cis* encapsulation supports more efficient folding (Farr *et al*., 2003).

Hsp70

Hsp70 chaperones constitute a family of highly conserved proteins that exist in most prokaryotic and all eukaryotic organisms. Some cell compartments can contain mutiple Hsp70 homologs and orthologs (e.g., the cytosol of yeast cells contains several functionally redundant Hsp70 homologs, such as Ssa1 to Ssa4, as well as the ribosome-associated Ssb and Ssz homologs).

Hsp70 chaperones are involved in a large variety of folding processes, including the folding of newly synthesized polypeptides, the refolding of stress-denatured proteins, the disaggregation of protein aggregates, the translocation of organellar and secretory proteins across membranes, the assembly and disassembly of oligomeric structures, and the control over the biological activity and stability of regulatory proteins (Bukau *et al*., 2000; Craig *et al*., 1999; Gething, 1999; Hartl & Hayer-Hartl, 2002; Neupert & Brunner, 2002; Pfanner & Wiedemann, 2002; Shi *et al*., 1998).

In *E. coli*, the Hsp70 homolog DnaK with its co-chaperones DnaJ and GrpE transiently associates with about 9% to 18% of newly synthesized proteins, including polypeptides that are still in *statu nascendi*, indicating that DnaK may act co- and post-translationally (Deuerling *et al*., 1999; Teter *et al*., 1999). The molecular weight spectrum of the DnaK-interacting polypeptides is much broader as compared to the spectrum of GroEL-associated polypeptides (Deuerling *et al*., 1999; Teter *et al*., 1999). Genetic analyses have shown that, although GroEL is essential for growth of *E. coli* at all temperatures, DnaK is only essential at temperatures above 37°C and below 25°C (Bukau & Walker, 1989; Deuerling *et al*., 1999; Fayet *et al*., 1989; Teter *et al*., 1999). This shows that, whatever the role of DnaK in regular protein folding, it is not strictly essential for viability.

In contrast to GroEL, DnaK does not enclose its substrate completely, but instead acts as a monomer to bind to a single short hydrophobic peptide segment of about five amino acids (Zhu *et al*., 1996). DnaK consists of an N-terminal ATPase-domain and a C-terminal substrate binding domain (Figure 4B). Substrate binding and release is controlled by ATP hydrolysis and ADP/ATP exchange

processes, which are regulated by the co-chaperones DnaJ and GrpE (Liberek *et al*., 1991; McCarty *et al*., 1995; Schmid *et al*., 1994). In the ATP state, substrates associate with and dissociate from DnaK with high rates, but affinity of DnaK for substrates is low. In the ADP state, DnaK binds substrates with high affinity but substrate exchange rates are low. The DnaJ co-chaperone, which alone also interacts with substrates through hydrophobic interactions, stimulates DnaK's ATPase activity. Experimental evidence suggests that DnaJ recognizes the substrate and hands it over to DnaK, whereby ATP is hydrolyzed and a segment of the substrate is tightly enclosed (Laufen *et al*., 1999; Misselwitz *et al*., 1998).

Under physiological conditions, substrate dissociation is controlled by ADP/ATP exchange that, in turn, is stimulated by the GrpE co-chaperone (Liberek *et al*., 1991; Packschies *et al*., 1997). The cycles of substrate binding and release are very short (about 1s per cycle) and probably must be repeated several times before a protein is refolded and no longer recognized as substrate (Mayer *et al*., 2001). It is not yet clear, however, how these binding cycles promote the folding of an unfolded or misfolded protein. According to one hypothesis, DnaK unfolds the substrate locally (Mayer *et al*., 2000; Pierpaoli *et al*., 1997; Slepenkov & Witt, 2002), a mechanism that has the advantage of being independent of the size of the protein substrate. It was shown that among the Hsp70 substrates, proteins of all sizes are found, and that multidomain proteins larger than 60 kDa are particularly dependent on Hsp70 assistance for their folding.

Using a library of cellulose-bound peptides that scanned the sequences of natural proteins, investigators were able to elucidate the binding motif for DnaK (Rüdiger *et al*., 1997). This motif consists of a core of five amino acids enriched in hydrophobic residues, flanked on both sides by a region favorable to positively charged residues. Such motifs occur in virtually all proteins at on average every 30 to 40 residues. In folded proteins, they are mostly found in the hydrophobic core, explaining the promiscuous binding to unfolded proteins.

RIBOSOME-ASSOCIATED CHAPERONES

Trigger Factor

Trigger Factor is not essential for viability in *E. coli* (Deuerling *et al*., 1999; Teter *et al*., 1999) or in *B. subtilis* (Göthel *et al*., 1998). Deletion of the *tig* gene (codes for Trigger Factor) in *E. coli* does not affect growth at all temperatures tested between 15 and 42°C and does not cause defects in protein folding (Deuerling *et al*., 1999; Teter *et al*., 1999). In the absence of DnaK and DnaJ, however, the deletion of the *tig* gene causes cell death at temperatures above 30°C and is accompanied by the massive aggregation of about 340 newly synthesized protein species (Deuerling *et al*., 1999; Teter *et al*., 1999). Thus, either of the two chaperones is sufficient for cell viability at temperatures above 30°C. However, the lack of either of the two chaperones must be compensated for the cell. Thus, in wild type *E. coli* cells, about 9% to 18% of newly synthesized proteins interact transiently with DnaK, but in the absence of Trigger Factor, this level is increased 2- to 3-fold (to 26% to 36%), indicating that, upon loss of Trigger Factor, DnaK can associate with the nascent polypeptide (Deuerling *et al*., 1999; Schaffitzel *et al*., 2001; Teter *et al*., 1999). In addition, it was shown that deletion of the *tig* gene induces the heat-shock response in *E. coli*, thereby leading to a compensatory increase in the steady state levels of chaperones, including DnaK (Deuerling *et al*., 2003). Thus, DnaK may serve as a potent back-up system in the absence of Trigger Factor.

Trigger Factor binds to peptides enriched in aromatic and basic amino acids, whereas peptides with acidic amino acids are disfavored (Patzelt *et al*., 2001). The Trigger Factor binding motif consists of eight amino acids, whereby the positioning of basic and aromatic residues within this motif seems non-essential for binding. Comparing the binding motif of Trigger Factor and DnaK showed that both prefer to interact with hydrophobic and positively charged segments in proteins (Patzelt *et al*., 2001; Rüdiger *et al*., 1997). The overlap in their substrate specificity might be the prerequisite that both chaperones, despite their different localization and mechanisms of action, can protect similar hydrophobic segments in unfolded protein species and therefore cooperate in protein folding.

The aggregated protein species found in cells lacking Trigger Factor and DnaK range between 16 kDa and 167 kDa, whereby large multi-domain proteins (>60 kDa) are enriched. Unfolded conformers of large proteins expose statistically more hydrophobic surface patches than do those of small proteins, and as a consequence may have a greater chance of undergoing intra- and intermolecular aggregation. Moreover, larged proteins may fold more slowly to the native state, increasing the time frame during which aggregation of folding intermediates may occur. The identified aggregation-prone proteins are involved in a large variety of cellular processes—including transcription, translation, and metabolism—but they do not show any common features regarding pI, specific secondary structural elements. Many essential proteins such as the elongation factor EF-Tu and the RNA-polymerase subunit RpoB, are found among the aggregation-prone protein species, which could explain the synthetic lethality of $\Delta tig\Delta dnaK$ mutant cells (Deuerling *et al*., 2003).

NAC and the Yeast Triad Ssb/Ssz/Zuo

The role of the eukaryotic ribosome-associated chaperones is less understood than that of bacterial Trigger Factor. Inactivation of the yeast chaperone triad, consisting of the Hsp70 homologs Ssb and Ssz and the DnaJ homolog Zuotin (Zuo), through deletion of either one or all of its members leads to the appearance of three phenotypes. Cells grow poorly under osmotic stress; they are cold-sensitive and hypersensitive to aminoglycosides, such as paromomycin, that block ribosomal protein synthesis and impair translational fidelity (Gautschi *et al*., 2001; Hundley *et al*., 2002; Yan *et al*., 1998). The function of the ribosome-associated triad and the molecular basis of the known phenotypes of the yeast triad mutants remain unknown. The role of the triad in co-translational protein folding is therefore elusive. This holds also true for the second ribsosome-associated chaperone complex in yeast, the nascent chain-associated complex (NAC) (Lauring *et al*., 1995; Rospert *et al*., 2002; Wiedmann & Prehn, 1999). The NAC is not essential in *S. cerevisiae* (George *et al*., 1998; Reimann *et al*., 1999; Rospert *et al*., 2002). Two beta-NAC homologs exist in yeast, encoded by the *EGD1* and *BTT1* genes. Deletion of both genes causes a growth defect at 37°C. Interestingly, the temperature sensitivity is suppressed when the *EGD2* gene encoding alpha-NAC is additionally deleted, indicating that uncomplexed alpha NAC has a negative effect on yeast growth (Reimann *et al*., 1999). The reason for the inhibitory effect of the alpha subunit is unclear. Although NAC's role in *de novo* protein folding has not been proven, the importance of its *in vivo* function is emphasized by the embryonically lethal phenotype of NAC mutants in mice and fruit flies (Deng & Behringer, 1995; Markesich *et al*., 2000). In addition, a role in targeting of new proteins into mitochondria and the ER has been suggested, which may also suggest a chaperone-like function of NAC (Beatrix *et al*., 2000; Fünfschilling & Rospert, 1999; George *et al*., 1998; Möller *et al*., 1998; Wiedmann & Prehn, 1999; Wiedmann *et al*., 1994).

INTERACTION WITH NASCENT POLYPEPTIDES AND RIBOSOMES

Although no sequence homology exists between the different ribosome-associated chaperones found in prokaryotes and eukaryotes, they do show intriguing similarities in the ways that they interact with ribosomes and nascent polypeptides. They all associate with the large ribosomal subunit in a 1:1 stoichiometry and crosslink to short nascent polypeptides (Hesterkamp *et al*., 1996; Pfund *et al*., 1998; Stoller *et al*., 1995; Valent *et al*., 1995; Wiedmann *et al*., 1994). The presence and absence of nascent chains alters the interaction of these chaperones with the ribosome, as indicated by the differences in the salt resistance of the chaperone-ribosome interaction. Moreover, the interaction of these chaperones with their substrates critically depends on their ribosomal attachement, given that the affinities for unfolded polypeptides in the absence of ribosomes are low.

NAC's association with the ribosome is mediated by its beta subunit. Deletion of the N-terminal 11 amino acid residues of beta-NAC in yeast results in the loss of ribosome association. Whereas only beta-NAC associates with the ribosome, both subunits were shown to crosslink to nascent polypeptide chains in a distance of 17 to 100 amino acids from the ribosomal peptidyl-transferase center (Wang *et al*., 1995; Wiedmann *et al*., 1994).

In the yeast chaperone triad, only Ssb has been shown to crosslink to nascent polypeptide chains of between 54 and 152 amino acids. The interaction of Ssb requires the presence of the Zuo/Ssz complex, which is believed to be the co-chaperone of Ssb (Gautschi *et al*., 2002; Hundley *et al*., 2002; Pfund *et al*., 1998). Zuo tethers Ssz to the ribosome, whereas Ssb contacts the ribosome autonomously (Gautschi *et al*., 2001; Pfund *et al*., 1998; Yan *et al*., 1998).

Trigger Factor was shown to crosslink to nascent polypeptides as short as 57aa in length (Valent *et al*., 1997; Valent *et al*., 1995). The N-terminal domain of Trigger Factor (amino acids 1 to 144) is both necessary and sufficient for the specific binding of Trigger Factor to ribosomes (Hesterkamp *et al*., 1997). Sequence alignments of several Trigger Factor homologs showed a highly conserved GFRXGXXP motif (TF-signature) within this domain (Kramer *et al*., 2002). Mutational analysis provided evidence that the TF-signature is involved in the binding to ribosomes. Introduction of three alanine residues at positions 44 to 46 (FRK to AAA) within the *E. coli* TF-signature results in a strong ribosome-binding deficiency.

The localization of eukaryotic chaperones on the ribosome is unknown. However, the Trigger Factor docking site on the *E. coli* ribosome has been mapped recently (Kramer *et al*., 2002). The tunnel exit where the nascent polypeptide emerges is surrounded by a proteinaceous ring of five ribosomal proteins, including L32, L22, L24, L29, and L23 (Nissen *et al*., 2000). Using an engineered Trigger Factor mutant with a UV-activatable crosslinker located directly adjacent to the TF-signature, Trigger Factor was crosslinked to the neighboring ribosomal proteins L23 and L29 of the large ribosomal subunit. Analyses of *E. coli* strains carrying mutations in ribosomal protein genes provided evidence that Trigger Factor binds to L23 but does not associate with L29. Binding of Trigger Factor occurs via the conserved residue Glu18 of L23. Mutational alteration of Glu18 resulted in a strong binding deficiency for Trigger Factor (Kramer *et al*., 2002). The association of Trigger Factor with L23 is crucial for its association with nascent polypeptides and its *in vivo* function as a chaperone for newly synthesized proteins. This feature was

demonstrated using an *in vitro* transcription/translation system, in which the use of L23 mutant ribosomes leads to a drastic reduction of the crosslinking efficiency of Trigger Factor toward nascent polypeptides, compared to wild type ribosomes. *Escherichia coli* strains carrying L23 mutant ribosomes showed conditional lethality at 34°C in the absence of the cooperating DnaK chaperone (Kramer *et al*., 2002), providing evidence that the interaction with L23 is essential for the chaperone function of Trigger Factor *in vivo*.

STRUCTURE AND MECHANISM OF TRIGGER FACTOR: A CRADLE FOR NEWLY SYNTHESIZED PROTEINS

Aside from the detailed biochemical and functional analyses of Trigger Factor, this protein's mechanism of action was enigmatic. How does a ribosome-associated chaperone promote the co-translational folding of newly synthesized proteins? To account for the folding activity of Trigger Factor, several possible different modes of action exist. These are not mutually exclusive and correlate with the different models suggested for *de novo* folding as discussed previously (Figure 1). By transiently binding to unfolded nascent polypeptides, Trigger Factor may act as either a "holder" chaperone, thereby preventing folding during biosynthesis, or as a "folder" chaperone, promoting the co-translational domain-wise folding of nascent polypeptides. Until recently, the holder theory of Trigger Factor was favored, because Trigger Factor does not work under ATP-consumption like other folder chaperones (e.g., DnaK or GroEL). This assumption was supported by a recent study indicating that Trigger Factor shifts the folding of newly synthesized proteins toward the post-translational route (Agashe *et al*., 2004). This hypothesis was based on the finding that large multi-domain model proteins like bacterial β-galactosidase and firefly luciferase were found to fold rapidly but inefficiently in the absence of Trigger Factor *in vitro* and *in vivo*. In the presence of Trigger Factor, the folding of these model proteins was delayed, but the total yield of active protein was higher when synthesized in an *in vitro*, *E. coli*-based translation system.

Very recently, the crystal structure of *E. coli* Trigger Factor, as well as that of an N-terminal Trigger Factor fragment bound to *Haloarcula marismortui* 50S, were solved by Ban and coworkers. These structures now shed new light on our understanding of co-translational protein folding and suggest an unexpected mechanism of action for this ribosome-associated chaperone (Ferbitz *et al*., 2004). Trigger Factor has a unique structure and a very unusual extended shape (Figure 5). The N-terminal domain that mediates ribosome binding builts the "tail" of the molecule. Surprisingly, the second domain in the linear sequence of Trigger Factor, harboring the PPIase activity, is located at the "head" of the molecule, and is connected to the "tail" by an 80 Å link that extends along the "back" of the Trigger Factor. Curiously, the alpha-helical C-terminal domain inserts itself between the first and the second domain in the three-dimensional structure to build the "back" and the "arms" of Trigger Factor. The surface charge distribution illustration of this molecule highlights two striking structural features (Figure 5). First, Trigger Factor resembles a crouching dragon in its overall shape, with the PPIase domain as its head, the C-terminal domain as its body, and with two extending arms and the tail formed by the N-terminus. Second, a large cavity is formed between the N-terminal ribosome binding "tail" and the "arms" of the molecule. The interior of this "cradle" is characterized by the exposure of numerous hydrophobic side chains, offering multiple contact sides for an unfolded polypeptide chain.

Based on the crystal structure of a heterologous complex between *E. coli* Trigger Factor and the *H. marismortui* 50S subunit, Ban and coworkers could accurately place the full Trigger Factor on the ribosome through superposition of the ribosome-binding region visible in both structures (Ferbitz *et al*., 2004). Remarkably, Trigger Factor hunches over the polypeptide exit of the ribosome and extends its sticky, hydrophobic, inner face of the cradle toward the area where the nascent polypeptides emerge (Figure 6). In agreement with a biochemical and genetic analysis performed using *E. coli* ribosomes, binding to the ribosome is mediated exclusively through the signature motif in the ribosome binding domain, and without any other domain of Trigger Factor contacting the ribosomal surface (Hesterkamp *et al*., 1997; Kramer *et al*., 2002).

These structural insights into Trigger Factor, in combination with results obtained from its extensive biochemical and genetic characterization provide a new picture of Trigger Factor's mode of action. These findings suggest a model (Figure 6) in which Trigger Factor provides a shielded folding environment for nascent polypeptide with its hydrophobic cradle, ideally positioned on top of the ribosomal exit site to capture emerging chains. Several lines of evidence have suggested the role of the cradle as the major substrate-binding site of Trigger Factor. By analyzing the contributions of inidvidual Trigger Factor domains to its chaperone function, investigators recently showed that the fused N-terminal and C-terminal domains provide Trigger Factor with almost wild-type–like chaperone activity *in vivo* and *in vitro*, as well as peptide binding activity, even in the absence of the PPIase domain (Genevaux *et al*., 2004; Kramer *et al*., 2004). Moreover, the cradle is structurally similar to a domain of the SurA chaperone, which also has substrate-binding ability (Behrens *et al*., 2001). Within the hydrophobic cradle, multiple but transient and low-affinity interactions with the growing nascent polypeptide may occur. This finding is consistent

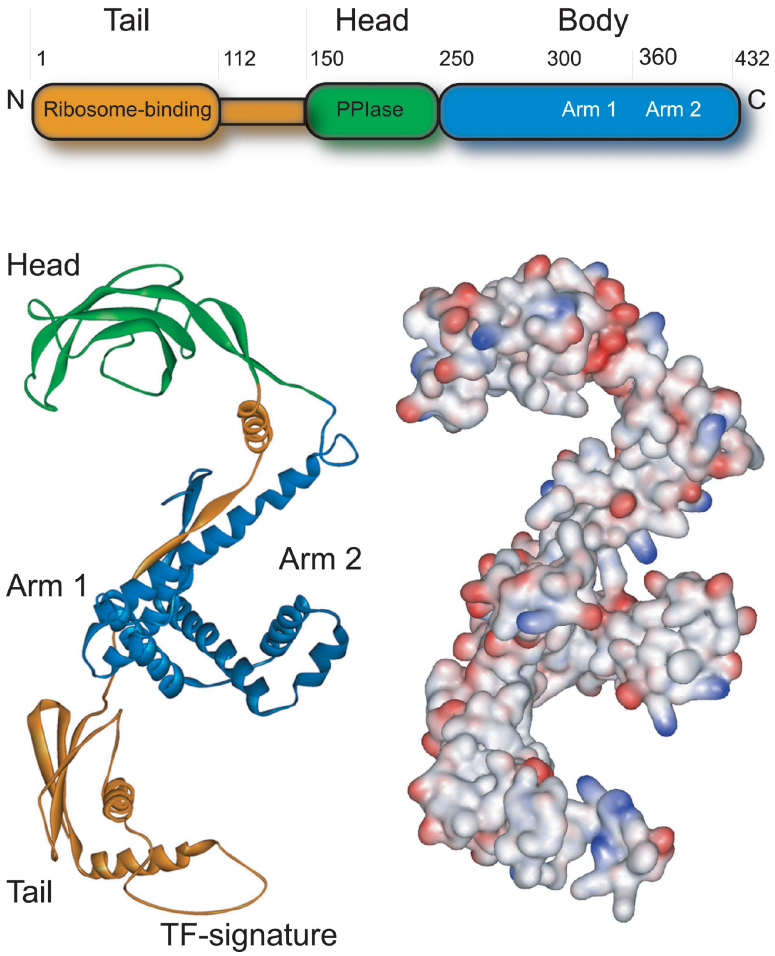

FIG. 5. Structure of *Escherichia coli* Trigger Factor. The upper panel shows the domain outline of Trigger Factor. The lower panel shows the three dimensional structure of Trigger Factor. Structural data are derived from (Ferbitz *et al*., 2004) PDB entry PDB1W26. Left: Ribbon diagram of the Trigger Factor fold. Right: Solvent accessible surface of Trigger Factor colored by electrostatic potential (blue = positive, red = negative).

with the observation that the association between Trigger Factor and the translating ribosomes becomes salt insensitive, presumably because of hydrophobic interactions (Hesterkamp *et al*., 1996). The interactions with Trigger Factor's cradle may initially delay the folding of the newly made polypeptide, but may allow for the formation of a folded core as soon as sufficient folding information is synthesized. Consequently, hydrophobic interactions are lost, and the folded domain is detached from the chaperone. This idea is in contrast to the previous assumption that Trigger Factor can only bind small segments of unfolded peptides but cannot provide a shielded folding space. The two modes of action may not be mutually exclusive and may depend on the nature of the nascent polypeptide. The folded domain may either escape from the Trigger Factor through the gaps in the cradle, or alternative substrate folding may trigger the release of Trigger Factor from the ribosome and allow rebinding to occur when a significant new portion of the nascent chains is exposed at the ribosomal exit site. Not all proteins may be able to fold in such a manner, e.g., when the domain size would exceed the space limit within the Trigger Factor cradle. In such cases Trigger Factor might shift the folding of these substrates to a post-translational mode, as observed for the folding of firefly luciferase and β-galactosidase (Agashe *et al*., 2004). Trigger Factor has inherent affinity for ribosomes, which is increased when the ribosomes display nascent peptides Hesterkamp *et al*., 1996; Lill *et al*., 1988). The relatively weak binding of the Trigger Factor to ribosomes ($K_D = 1\ \mu$ M) (Maier *et al*., 2003; Patzelt *et al*., 2002) and its small interaction surface at the ribosome via its signature motif may be ideally suited for its role in protein folding. By maintaining a precise balance of binding affinities, Trigger Factor dissociates from vacant ribosomes within

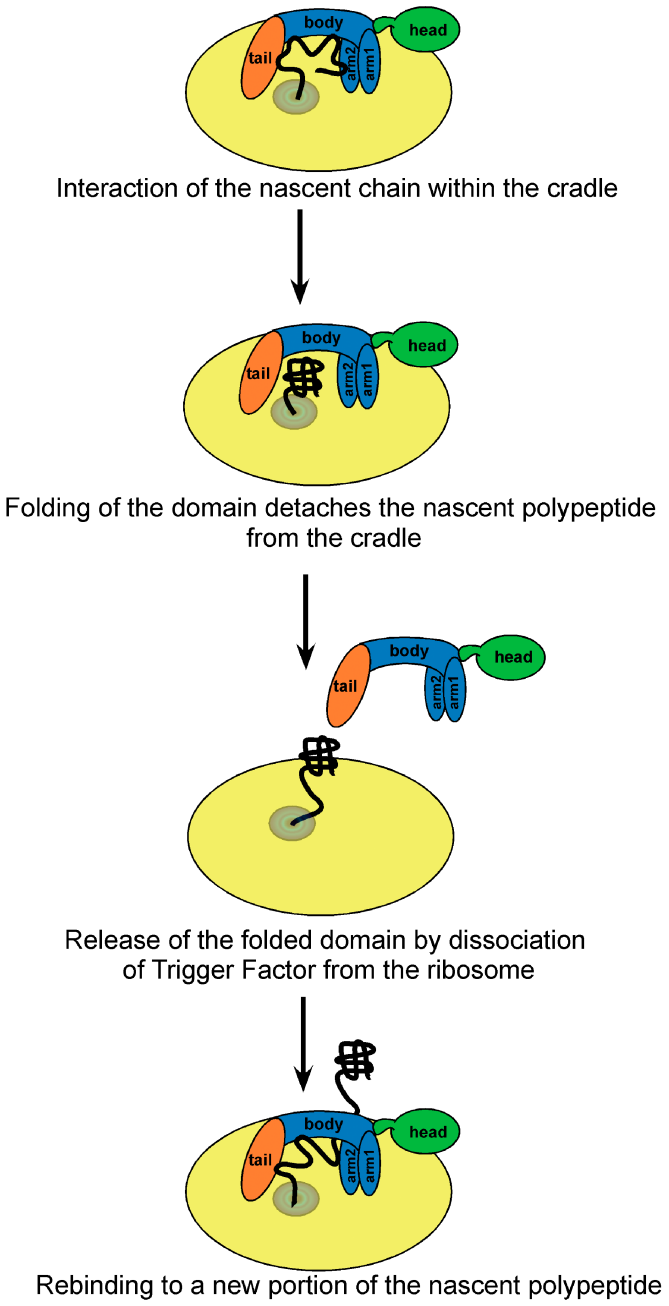

FIG. 6. Model of the Trigger Factor mechanism based on (Ferbitz *et al*., 2004) with permission. Ribosome-bound Trigger Factor (color code is as in Figure 5) interacts with an unfolded nascent polypeptide by transient hydrophobic contacts. Upon folding of this domain contacts between the Trigger Factor and the newly synthesized peptide are weakened and Trigger Factor dissociates from the ribosome. The Trigger Factor rebinds to the ribosome when the next stretch of newly synthesized, unfolded polypeptide becomes exposed.

seconds (Maier *et al*., 2003), but may become stabilized by hydrophobic interactions with an emerging, unfolded nascent peptide. Still puzzling is the role of the PPIase domain. This domain is located peripherally to the region responsible for the Trigger Factor chaperone cavity and to the exit of the ribosomal tunnel. (Ferbitz *et al*., 2004). As a consequence of the tether length, the PPIase activity is limited to the region above the "arms" and behind the "back" of the Trigger Factor, where partially folded proteins go after escaping from beneath the Trigger Factor. This idea is consistent with the finding that the PPIase domain is dispensable *in vivo* and *in vitro* for the folding of proteins (Genevaux *et al*., 2004; Kramer *et al*., 2004). The availability of the crystal structure of Trigger Factor will now allow us to investigate in more detail the molecular events during co-translational folding of newly synthesized proteins in *E. coli*.

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REFERENCES

Agashe, V.R., Guha, S., Chang, H.C., Genevaux, P., Hayer-Hartl, M., Stemp, M., Georgopoulos, C., Hartl, F.U., and Barral, J.M. 2004. Function of trigger factor and DnaK in multidomain protein folding: increase in yield at the expense of folding speed. *Cell* **117**(2):199.

Anfinsen, C.B. 1973. Principles that govern the folding of protein chains. *Science* **181:**223.

Ban, N., Nissen, P., Hansen, J., Capel, M., Moore, P.B., and Steitz, T.A. 1999. Placement of protein and RNA structures into a 5 Å-resolution map of the 50S ribosomal subunit. *Nature* **400:**841.

Ban, N., Nissen, P., Hansen, J., Moore, P.B., and Steitz, T.A. 2000. The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* **289:**905.

Beatrix, B., Sakai, H., and Wiedmann, M. 2000. The alpha and beta subunit of the nascent polypeptide-associated complex have distinct functions. *J Biol Chem* **275**(48):37838.

Behrens, S., Maier, R., de Cock, H., Schmid, F.X., and Gross, C.A. 2001. The SurA periplasmic PPIase lacking its parvulin domains functions *in vivo* and has chaperone activity. *EMBO J* **20**(1):285.

Buchberger, A., Schröder, H., Hesterkamp, T., Schönfeld, H.-J., and Bukau, B. 1996. Substrate shuttling between the DnaK and GroEL systems indicates a chaperone network promoting protein folding. *J Mol Biol* **261:**328.

Buchner, J. 1996. Supervising the fold: functional principles of molecular chaperones. *FASEB J* **10:**10.

Bukau, B., Deuerling, E., Pfund, C., and Craig, E.A. 2000. Getting newly synthesized proteins into shape. *Cell* **101:**119.

Bukau, B. and Horwich, A.L. 1998. The Hsp70 and Hsp60 chaperone machines. *Cell* **92:**351.

Bukau, B. and Walker, G.C. 1989. Cellular Defects caused by deletion of the *Escherichia coli dnaK* gene indicates roles for heat shock protein in normal metabolism. *J Bact* **171**(5):2337.

Chaudhuri, T.K., Farr, G.W., Fenton, W.A., Rospert, S., and Horwich, A.L. 2001. GroEL/GroES-mediated folding of a protein too large to be encapsulated. *Cell* **107**(2):235.

Chen, J., Walter, S., Horwich, A.L., and Smith, D.L. 2001. Folding of malate dehydrogenase inside the GroEL-GroES cavity. *Nat Struct Biol* **8**(8):721.

Cheng, M.Y., Hartl, F.U., Martin, J., Pollock, R.A., Kalousek, F., Neupert, W., Hallberg, E.M., Hallberg, R.L., and Horwich, A.L. 1989. Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria. *Nature* **337**(6208):620.

Coyle, J.E., Texter, F.L., Ashcroft, A.E., Masselos, D., Robinson, C.V., and Radford, S.E. 1999. GroEL accelerates the refolding of hen lysozyme without changing its folding mechanism. *Nature Struct Biol* **6:**683.

Craig, E., Yan, W., and James, P. 1999. Genetic dissection of the Hsp70 chaperone system of yeast. In: *Molecular Chaperones and Folding Catalysts. Regulation, Cellular Function and Mechanisms*, Bukau, B., Ed. Amsterdam, Harwood Academic Publishers: 139.

Deng, J.M. and Behringer, R.R. 1995. An insertional mutation in the BTF3 transcription factor gene leads to an early postimplantation lethality in mice. *Transgenic Res* **4**(4):264.

Deuerling, E., Patzelt, H., Vorderwülbecke, S., Rauch, T., Kramer, G., Schaffitzel, E., Mogk, A., Schulze-Specking, A., Langen, H., and Bukau, B. 2003. Trigger Factor and DnaK possess overlapping substrate pools and binding specificities. *Mol Microbiol* **47**(5):1317.

Deuerling, E., Schulze-Specking, A., Tomoyasu, T., Mogk, A., and Bukau, B. 1999. Trigger factor and DnaK cooperate in folding of newly synthesized proteins. *Nature* **400:**693.

Dill, K.A. and Chan, H.S. 1997. From Levinthal to pathways to funnels. *Nature Struct Biol* **4:**10.

Dinner, A.R., Sali, A., Smith, L.J., Dobson, C.M., and Karplus, M. 2000. Understanding protein folding via free-energy surfaces from theory and experiment. *Trends Biochem Sci* **25**(7):331.

Driessen, A.J., Manting, E.H., and van der Does, C. 2001. The structural basis of protein targeting and translocation in bacteria. *Nat Struct Biol* **8**(6):492.

Ellis, J. 1987. Proteins as molecular chaperones. *Nature (London)* **328:**378.

Ellis, R.J. and Hartl, F.U. 1999. Principles of protein folding in the cellular environment. *Curr Opin Struct Biol* **9:**102.

Elowitz, M.B., Surette, M.G., Wolf, P.-E., Stock, J.B., and Leibler, S. 1999. Protein mobility in the cytoplasm of *Escherichia coli*. *J Bacteriol* **181:**197.

Ewalt, K.L., Hendrick, J.P., Houry, W.A., and Hartl, F.U. 1997. *In vivo* observation of polypeptide flux through the bacterial chaperonin system. *Cell* **90:**491.

Farr, G.W., Fenton, W.A., Chaudhuri, T.K., Clare, D.K., Saibil, H.R., and Horwich, A.L. 2003. Folding with and without encapsulation by cis- and trans-only GroEL-GroES complexes. *Embo J* **22**(13): 3220.

Fayet, O., Ziegelhoffer, T., and Georgopoulos, C. 1989. The *groES* and *groEL* heat shock gene products of *Escherichia coli* are essential for bacterial growth at all temperatures. *J Bacteriol* **171**(3):1379.

Fedorov, A.N. and Baldwin, T.O. 1997. Cotranslational protein folding. *J Biol Chem* **272:**32175.

Fenton, W.A.a.H. and Arthur, L. 1997. GroEL-mediated protein folding. *Protein Sci* **6:**743.

Ferbitz, L., Maier, T., Patzelt, H., Bukau, B., Deuerling, E., and Ban, N. 2004. Trigger factor in complex with the ribosome forms a molecular cradle for nascent proteins. *Nature* **431:**590.

Fersht, A.R. and Daggett, V. 2002. Protein folding and unfolding at atomic resolution. *Cell* **108**(4):573.

Flaherty, K.M., Deluca-Flaherty, C., and McKay, D.B. 1990. Three-dimensional structure of the ATPase fragment of a 70K heat-shock cognate protein. *Nature* **346:**623.

Friguet, B., Djavadi-Ohaniance, L., King, J., and Goldberg, M.E. 1994. In vitro and ribosome-bound folding intermediates of P22 tailspike protein detected with monoclonal antibodies. *J Biol Chem* **269**(22):15945.

Frydman, J. 2001. Folding of newly translated proteins in vivo: The role of molecular chaperones. *Annu Rev Biochem* **70:**603.

Frydman, J., Nimmesgern, E., Erdjument-Bromage, H., Wall, J.S., Tempst, P., and Hartl, F.-U. 1992. Function in protein folding of TRiC, a cytosolic ring complex containing TCP-1 and structurally related subunits. *EMBO J* **11:**4767.

Frydman, J., Nimmesgern, E., Ohtsuka, K., and Hartl, F.U. 1994. Folding of nascent polypeptide chains in a high molecular mass assembly with molecular chaperones. *Nature* **370:**111.

Fünfschilling, U. and Rospert, S. 1999. Nascent polypeptide-associated complex stimulates protein import into yeast mitochondria. *Mol Biol Cell* **10:**3289.

Gao, Y., Thomas, J.O., Chow, R.L., Lee, G.-H., and Cowan, N.J. 1992. A cytoplasmic chaperonin that catalyzes β-actin folding. *Cell* **69:** 1043.

Gautschi, M., Lilie, H., Fünfschilling, U., Mun, A., Ross, S., Lithgow, T., Rucknagel, P., and Rospert, S. 2001. RAC, a stable ribosome-associated complex in yeast formed by the DnaK-DnaJ homologs Ssz1p and zuotin. *Proc Natl Acad Sci USA* **98:**3762.

Gautschi, M., Mun, A., Ross, S., and Rospert, S. 2002. A functional chaperone triad on the yeast ribosome. *Proc Natl Acad Sci USA* **99**(7):4209.

Genevaux, P., Keppel, F., Schwager, F., Langendijk-Genevaux, P.S., Hartl, F.U., and Georgopoulos, C. 2004. In vivo analysis of the overlapping functions of DnaK and trigger factor. *EMBO Rep* **5**(2):195.

George, R., Beddoe, T., Landl, K., and Lithgow, T. 1998. The yeast nascent polypeptide-associated complex initiates protein targeting to mitochondria *in vivo*. *Proc Natl Acad Sci USA* **95:**2296.

Gething, M.J. 1999. Role and regulation of the ER chaperone BiP. *Semin Cell Dev Biol* **10**(5):465.

Gilbert, R.J., Fucini, P., Connell, S., Fuller, S.D., Nierhaus, K.H., Robinson, C.V., Dobson, C.M., and Stuart, D.I. 2004. Three-dimensional structures of translating ribosomes by Cryo-EM, *Mol Cell* **14**(1):57.

Göthel, S.F., Scholz, C., Schmid, F.X., and Marahiel, M.A. 1998. Cyclophilin and trigger factor from *Bacillus subtilis* catalyze in vitro protein folding and are necessary for viability under starvation conditions. *Biochemistry* **37:**13392.

Hardesty, B. and Kramer, G. 2001. Folding of a nascent peptide on the ribosome. *Prog Nucleic Acid Res Mol Biol* **66:**41.

Hartl, F.U. and Hayer-Hartl, M. 2002. Molecular chaperones in the cytosol: From nascent chain to folded protein. *Science* **295**(5561): 1852.

Hartl, F.U., Lecker, S., Schiebel, E., Hendrick, J.P., and Wickner, W. 1990. The binding cascade of SecB to SecA to SecY/E mediates preprotein targeting to the *E. coli* plasma membrane. *Cell* **63:** 269.

Hennecke, J., Sebbel, P., and Glockshuber, R. 1999. Random Circular Permutation of DsbA Reveals Segments that are Essential for Protein Folding and Stability. *J Mol Biol* **286:**1197.

Hesterkamp, T., Deuerling, E., and Bukau, B. 1997. The amino-terminal 118 amino acids of *Escherichia coli* trigger factor constitute a domain that is necessary and sufficient for binding to ribosomes. *J Biol Chem* **272:**21865.

Hesterkamp, T., Hauser, S., Lütcke, H., and Bukau, B. 1996. *Escherichia coli* trigger factor is a prolyl isomerase that associates with nascent polypeptide chains. *Proc Natl Acad Sci USA* **93:**4437.

Horwich, A.L., Brooks Low, K., Fenton, W.A., Hirshfield, I.N., and Furtak, K. 1993. Folding in vivo of bacterial cytoplasmic proteins: role of GroEL. *Cell* **74:**909.

Horwich, A.L., Weber-Ban, E.U., and Finley, D. 1999. Chaperone rings in protein folding and degradation. *Proc Natl Acad Sci USA* 1**96:**11033.

Houry, W.A., Frishman, D., Eckerskorn, C., Lottspeich, F., and Hartl, F.U. 1999. Identification of *in vivo* substrates of the chaperonin GroEL. *Nature* **402:**147.

Hundley, H., Eisenman, H., Walter, W., Evans, T., Hotokezaka, Y., Wiedmann, M., and Craig, E. 2002. The in vivo function of the ribosome-associated Hsp70, Ssz1, does not require its putative peptide-binding domain. *Proc Natl Acad Sci USA* **99**(7):4203.

Ito, K. 1996. The major pathways of protein translocation across membranes. *Genes Cells* **1:**337.

Jacob, M., Schindler, T., Balbach, J., and Schmid, F.X. 1997. Diffusion control in an elementary protein folding reaction. *Proc Natl Acad Sci USA* **94:**5622.

Kandror, O., Busconi, L., Sherman, M., and Goldberg, A.L. 1994. Rapid degradation of an abnormal protein in *Escherichia coli* involves the chaperones GroEL and GroES. *J Biol Chem* **269:**23575.

Kramer, G., Rauch, T., Rist, W., Vorderwülbecke, S., Patzelt, H., Schulze-Specking, A., Ban, N., Deuerling, E., and Bukau, B. 2002. L23 protein functions as a chaperone docking site on the ribosome. *Nature* **419:**171.

Kramer, G., Rutkowska, A., Wegrzyn, R.D., Patzelt, H., Kurz, T.A., Merz, F., Rauch, T., Vorderwülbecke, S., Deuerling, E., and Bukau, B. 2004. Functional dissection of Escherichia coli Trigger Factor: Unraveling the function of individual domains. *J Bacteriol* **186**(12):3777.

Kusukawa, N., Yura, T., Ueguchi, C., Akiyama, Y., and Ito, K. 1989. Effects of mutations in heat-shock genes *groES* and *groEL* on protein export in *Escherichia coli*. *EMBO J* **8**(11):3517.

Laminet, A.A., Ziegelhoffer, T., Georgopoulos, C., and Pluckthun, A. 1990. The *Escherichia coli* heat shock proteins GroEL and GroES modulate the folding of the beta-lactamase precursor. *EMBO J* **9**(7):2315.

Laufen, T., Mayer, M.P., Beisel, C., Klostermeier, D., Reinstein, J., and Bukau, B. 1999. Mechanism of regulation of Hsp70 chaperones by DnaJ co-chaperones. *Proc Natl Acad Sci USA* **96:**5452.

Lauring, B., Wang, S., Sakai, H., Davis, T.A., Wiedmann, B., Kreibich, G., and Wiedmann, M. 1995. Nascent-polypeptide-associated complex: A bridge between ribosome and cytosol. *Cold Spring Harb Symp Quant Biol* **60:**47.

Lewis, V.A., Hynes, G.M., Zheng, D., Saibil, H., and Willison, K. 1992. T-complex polypeptide-1 is a subunit of a heteromeric particle in the eukaryotic cytosol. *Nature* **358:**249.

Liberek, K., Marszalek, J., Ang, D., Georgopoulos, C., and Zylicz, M. 1991. *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. *Proc Natl Acad Sci USA* **88:**2874.

Lill, R., Crooke, E., Guthrie, B., and Wickner, W. 1988. The "Trigger factor cycle" includes ribosomes, presecretory proteins and the plasma membrane. *Cell* **54:**1013.

Maier, R., Eckert, B., Scholz, C., Lilie, H., and Schmid, F.X. 2003. Interaction of trigger factor with the ribosome. *J Mol Biol* **326**(2):585.

Markesich, D.C., Gajewski, K.M., Nazimiec, M.E., and Beckingham, K. 2000. Bicaudal encodes the Drosophila beta NAC homolog, a component of the ribosomal translational machinery. *Development* **127**(3):559.

Mayer, M.P., Brehmer, D., Gassler, C.S., and Bukau, B. 2001. Hsp70 chaperone machines. *Adv Protein Chem* **59:**1.

Mayer, M.P., Rüdiger, S., and Bukau, B. 2000. Molecular basis for interactions of the DnaK chaperone with substrates. *Biol Chem* **381:**877.

McCarty, J.S., Buchberger, A., Reinstein, J., and Bukau, B. 1995. The role of ATP in the functional cycle of the DnaK chaperone system. *J Mol Biol* **249:**126.

Minton, A.P. 2000. Protein folding: thickening the broth. *Curr Biol* **10:**R97.

Minton, K.W., Karmin, P., Hahn, G.M., and Minton, A.P. 1982. Nonspecific stabilization of stress-susceptible proteins by stress-resistant proteins: a model for the biological role of heat shock proteins. *Proc Natl Acad Sci USA* **79:**7107.

Misselwitz, B., Staeck, O., and Rapoport, T.A. 1998. J proteins catalytically activate Hsp70 molecules to trap a wide range of peptide sequences. *Mol Cell* **2:**593.

Mogk, A., Mayer, M.P., and Deuerling, E. 2002. Mechanisms of Protein Folding: molecular chaperones and their application in biotechnology. *ChemBioChem* **3:**807.

Molinari, M. and Helenius, A. 2000. Chaperone selection during glycoprotein translocation into the endoplasmic reticulum. *Science* **288:**331.

Möller, I., Beatrix, B., Kreibich, G., Sakai, H., Lauring, B., and Wiedmann, M. 1998. Unregulated exposure of the ribosomal M-site caused by NAC depletion results in delivery of non-secretory polypeptides to the Sec61 complex. *FEBS Letters* **441:**1.

Nakatogawa, H. and Ito, K. 2002. The ribosomal exit tunnel functions as a discriminating gate. *Cell* **108**(5):629.

Netzer, W.J. and Hartl, F.U. 1997. Recombination of protein domains facilitated by co-translational folding in eukaryotes. *Nature* **388:**343.

Neupert, W. 1997. Protein import into mitochondria. *Annu Rev Biochem* **66:**863.

Neupert, W. and Brunner, M. 2002. The protein import motor of mitochondria. *Nat Rev Mol Cell Biol* **3**(8):555.

Nicola, A.V., Chen, W., and Helenius, A. 1999. Co-translational folding of an alphavirus capsid protein in the cytosol of living cells. *Nature Cell Biol* **1:**341.

Nissen, P., Hansen, J., Ban, N., Moore, P.B., and Steitz, T.A. 2000. The structural basis of ribosome activity in peptide bond synthesis. *Science* **289:**920.

Ostermann, J., Horwich, A.L., Neupert, W., and Hartl, F.U. 1989. Protein folding in mitochondria requires complex formation with hsp60 and ATP hydrolysis. *Nature* **341**(6238):125.

Packschies, L., Theyssen, H., Buchberger, A., Bukau, B., Goody, R.S., and Reinstein, J. 1997. GrpE accelerates nucleotide exchange of the molecular chaperone DnaK with an associative displacement mechanism. *Biochemistry* **36**(12):3417.

Patzelt, H., Kramer, G., Rauch, T., Schönfeld, H.J., Bukau, B., and Deuerling, E. 2002. Three-sate equilibrium of *Escherichi coli* trigger Factor. *Biol Chem* **383:**1611.

Patzelt, H., Rudiger, S., Brehmer, D., Kramer, G., Vorderwulbecke, S., Schaffitzel, E., Waitz, A., Hesterkamp, T., Dong, L., Schneider-Mergener, J., Bukau, B., and Deuerling, E. 2001. Binding specificity of Escherichia coli trigger factor. *Proc Natl Acad Sci USA* **98**(25):14244.

Pfanner, N. and Geissler, A. 2001. Versatility of the mitochondrial protein import machinery. *Nat Rev Mol Cell Biol* **2**(5):339.

Pfanner, N. and Wiedemann, N. 2002. Mitochondrial protein import: two membranes, three translocases. *Curr Opin Cell Biol* **14**(4):400.

Pfund, C., Lopez-Hoyo, N., Ziegelhoffer, T., Schilke, B.A., Lopez-Buesa, P., Walter, W.A., Wiedmann, M., and Craig, E.A. 1998. The molecular chaperone Ssb from Saccharomyces cerevisiae is a component of the ribosome-nascent chain complex. *EMBO J* **17:**3981.

Pierpaoli, E.V., Sandmeier, E., Baici, A., Schönfeld, H.-J., Gisler, S., and Christen, P. 1997. The power stroke of the DnaK/DnaJ/GrpE molecular chaperone system. *J Mol Biol* **269:**757.

Radford, S.E. 2003. Co-translocational misfolding in the ER of living cells. *Nat Struct Biol* **10**(3):153.

Reimann, B., Bradsher, J., Franke, J., Hartmann, E., Wiedmann, M., Prehn, S., and Wiedmann, B. 1999. Initial characterization of the nascent polypeptide-associated complex in yeast. *Yeast* **15**(5):397.

Roseman, A.M., Chen, S., White, H., Braig, K., and Saibil, H.R. 1996. The chaperonin ATPase cycle: Mechanism of allosteric switching and movements of substrate-binding domains in GroEL. *Cell* **87**: 241.

Rospert, S., Dubaquie, Y., and Gautschi, M. 2002. Nascent-polypeptide-associated complex. *Cell Mol Life Sci* **59**(10):1632.

Rüdiger, S., Buchberger, A., and Bukau, B. 1997. Interaction of Hsp70 chaperones with substrates. *Nat Struct Biol* **4:**342.

Rüdiger, S., Germeroth, L., Schneider-Mergener, J., and Bukau, B. 1997. Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries. *EMBO J* **16:**1501.

Rye, H.S., Burston, S.G., Fenton, W.A., Beechem, J.M., Xu, Z., Sigler, P.B., and Horwich, A.L. 1997. Distinct actions of cis and trans ATP within the double ring of the chaperonin GroEL. *Nature* **388**(6644):792.

Saibil, H.R. 2000. Conformational changes studied by cryo-electron microscopy. *Nature Struct Biol* **7:**711.

Saibil, H.R., Horwich, A.L., and Fenton, W.A. 2001. Allostery and protein substrate conformational change during GroEL/GroES-mediated protein folding. *Adv Protein Chem* **59:**45.

Sanchez, I.E., Morillas, M., Zobeley, E., Kiefhaber, T., and Glockshuber, R. 2004. Fast folding of the two-domain semliki forest virus capsid protein explains co-translational proteolytic activity. *J Mol Biol* **338**(1):159.

Schaffitzel, E., Rüdiger, S., Bukau, B., and Deuerling, E. 2001. Functional dissection of trigger factor and DnaK: interactions with nascent polypeptides and thermally denatured proteins. *Biological Chemistry* **382:**1235.

Schatz, G. and Dobberstein, B. 1996. Common principles of protein translocation across membranes. *Science* **271:**1519.

Schluenzen, F., Tocilj, A., Zarivach, R., Harms, J., Gluehmann, M., Janell, D., Bashan, A., Bartels, H., Agmon, I., Franceschi, F., and Yonath, A. 2000. Structure of functionally activated small ribosomal subunit at 3.3 angstroms resolution. *Cell* **102**(5):615.

Schmid, D., Baici, A., Gehring, H., and Christen, P. 1994. Kinetics of molecular chaperone action. *Science* **263:**971.

Shi, Y., Mosser, D.D., and Morimoto, R.I. 1998. Molecular chaperones as HSF-specific transcriptional repressors. *Genes & Development* **12:**654.

Shtilerman, M., Lorimer, G.H., and Englander, S.W. 1999. Chaperonin function: Folding by forced unfolding. *Science* **284:**822.

Slepenkov, S.V. and Witt, S.N. 2002. The unfolding story of the Escherichia coli Hsp70 DnaK: Is DnaK a holdase or an unfoldase?. *Mol Microbiol* **45**(5):1197.

Stoller, G., Ruecknagel, K.P., Nierhaus, K.H., Schmid, F.X., Fischer, G., and Rahfeld, J.-U. 1995. A ribosome-associated peptidyl-prolyl *cis/trans* isomerase identified as the trigger factor. *EMBO J* **14:** 4939.

Teter, S.A., Houry, W.A., Ang, D., Tradler, T., Rockabrand, D., Fischer, G., Blum, P., Georgopoulos, C., and Hartl, F.U. 1999. Polypeptide flux through bacterial Hsp70: DnaK cooperates with Trigger Factor in chaperoning nascent chains. *Cell* **97:**755.

Ullers, R.S., Luirink, J., Harms, N., Schwager, F., Georgopoulos, C., and Genevaux, P. 2004. SecB is a bona fide generalized chaperone in *Escherichia coli*. *Proc Natl Acad Sci USA* **101**(20):7583.

Valent, Q.A., de Gier, J.-W.L., von Heijne, G., Kendall, D.A., ten Hagen-Jongman, C.M., Oudega, B., and Luirink, J. 1997. Nascent membrane and presecretory proteins synthesized in *Escherichia coli* associate with signal recognition particle and trigger factor. *Molecular Microbiology* **25**(1):53.

Valent, Q.A., Kendall, D.A., High, S., Kusters, R., Oudega, B., and Luirink, J. 1995. Early events in preprotein recognition in *E. coli*: Interaction of SRP and trigger factor with nascent polypeptides. *EMBO J* **14:**5494.

van den Berg, B., Ellis, R.J., and Dobson, C.M. 1999. Effects of macromolecular crowding on protein folding and aggregation. *EMBO J* **18:**6927.

Viitanen, P.V., Lubben, T.H., Reed, J., Goloubinoff, P., O'Keefe, D.P., and Lorimer, G.H. 1990. Chaperonin-facilitated refolding of ribulosebisphosphate carboxylase and ATP hydrolysis by chaperonin 60 (groEL) are K^+ dependent. *Biochemistry* **29**(24):5665.

Vorderwulbecke, S., Kramer, G., Merz, F., Kurz, T.A., Rauch, T., Zachmann-Brand, B., Bukau, B., and Deuerling, E. 2004. Low temperature or GroEL/ES overproduction permits growth of *Escherichia coli* cells lacking trigger factor and DnaK. *FEBS Lett* **559**(1–3): 181.

Walter, P. and Johnson, A.E. 1994. Signal sequence recognition and protein targeting to the endoplasmic reticulum membrane. *Annu Rev Cell Biol* **10:**87.

Wang, S., Sakai, H., and Wiedmann, M. 1995. NAC covers ribosome-associated nascent chains thereby forming a protective environment for regions of nascent chains just emerging from the peptidyl transferase center. *J Cell Biol* **130:**519.

Welch, W.J., Eggers, D.K., Hansen, W.J., and Nagata, H. 1999. Early events in the synthesis and maturation of polypeptides. In: *Molecular Chaperones and Folding Catalysts. Regulation, Cellular Function and Mechanism*, Bukau, B., Ed., Amsterdam, Harwood Academic Publishers, 177.

Wiedmann, B. and Prehn, S. 1999. The nascent polypeptide-associated complex (NAC) of yeast functions in the targeting process of ribosomes to the ER membrane. *FEBS Letters* **458:**51.

Wiedmann, B., Sakai, H., Davis, T.A., and Wiedmann, M. 1994. A protein complex required for signal-sequence-specific sorting and translocation. *Nature* **370:**434.

Wiedmann, B., Sakai, H., Davis, T.A., and Wiedmann, M. 1994. A protein complex required for signal-sequence-specific sorting and translocation. *Nature* **370**(6489):434.

Wimberly, B.T., Brodersen, D.E., Clemons Jr., W.M., Morgan-Warren, R.J., Carter, A.P., Vonrhein, C., Hartsch, T., and Ramakrishnan, V. 2000. Structure of the 30S ribosomal subunit. *Nature* **407:**327.

Xu, Z., Horwich, A.L., and Sigler, P.B. 1997. The crystal structure of the asymmetric GroEL-GroES-(ADP)7 chaperonin complex. *Nature* **388:**741.

Xu, Z. and Sigler, P.B. 1998. GorEL/GroES: structure and function of a two-stroke folding machine. *J Struct Biol* **124:**129.

Yan, W., Schilke, B., Pfund, C., Walter, W., Kim, S., and Craig, E.A. 1998. Zuotin, a ribosome-associated DnaJ molecular chaperone. *EMBO J* **17:**4809.

Zhu, X., Zhao, X., Burkholder, W.F., Gragerov, A., Ogata, C.M., Gottesman, M., and Hendrickson, W.A. 1996. Structural analysis of substrate binding by the molecular chaperone DnaK. *Science* **272:**1606.

Zimmerman, S.B. and Minton, A.P. 1993. Macromolecular crowding: biochemical, biophysical, and physiological consequences. *Annu Rev Biophys Biomol Struct* **22:**27.

Zimmerman, S.B. and Trach, S.O. 1991. Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli*. *J Mol Biol* **222:**599.