Cell, Vol. 97, 755–765, June 11, 1999, Copyright ©1999 by Cell Press

Polypeptide Flux through Bacterial Hsp70: DnaK Cooperates with Trigger Factor in Chaperoning Nascent Chains

Sarah A. Teter,* Walid A. Houry,* Debbie Ang,† Thomas Tradler,‡ David Rockabrand,§ Gunter Fischer,‡ Paul Blum,§ Costa Georgopoulos,† and F. Ulrich Hartl*‖
*Max-Planck-Institut für Biochemie
Department of Cellular Biochemistry
Am Klopferspitz 18A
D-82152 Martinsried
Germany
†Departement de Biochimie Medicale
Centre Medical Universitaire
1 rue Michel-Servet
1211 Geneve
Switzerland
‡Max-Planck-Gesellschaft Forschungsstelle
"Enzymologie der Proteinfaltung"
Kurt-Mothes-Str. 3
D-06120 Halle/Saale
Germany
§School of Biological Sciences
University of Nebraska
Lincoln, Nebraska 68588-0118

Summary

A role for DnaK, the major *E. coli* Hsp70, in chaperoning de novo protein folding has remained elusive. Here we show that under nonstress conditions DnaK transiently associates with a wide variety of nascent and newly synthesized polypeptides, with a preference for chains larger than 30 kDa. Deletion of the nonessential gene encoding trigger factor, a ribosome-associated chaperone, results in a doubling of the fraction of nascent polypeptides interacting with DnaK. Combined deletion of the trigger factor and DnaK genes is lethal under normal growth conditions. These findings indicate important, partially overlapping functions of DnaK and trigger factor in de novo protein folding and explain why the loss of either chaperone can be tolerated by *E. coli*.

Introduction

Although the native structure of a protein is dictated by its amino acid sequence, efficient protein folding in vivo requires assistance by molecular chaperones, at least for a significant fraction of polypeptides (Gething and Sambrook, 1992; Ellis, 1993; Hartl, 1996). Molecular chaperones typically bind to hydrophobic amino acid residues exposed in unfolded polypeptides and release their substrates in a controlled manner, thereby preventing aggregation and promoting proper folding. The mechanisms of two major chaperone systems that act in de novo folding, the Hsp70s and the cylindrical chaperonins, are now well understood from in vitro studies (Bukau and Horwich, 1998). In contrast, the contributions of the same components to protein folding in vivo are as yet poorly characterized.

In present models of in vivo protein folding, Hsp70-type chaperones are assigned an important role in binding hydrophobic regions of nascent polypeptides on ribosomes (Gething and Sambrook, 1992; Georgopoulos and Welch, 1993; Hartl, 1996; Johnson and Craig, 1997). Because folding requires synthesis of an entire polypeptide domain, incomplete domains that exist in partially folded states may expose hydrophobic residues during translation. Given the high concentration of nascent chains in polyribosomes, unproductive interchain associations might be favored over intramolecular folding. For this reason, it has been argued that nascent chains must be protected from misfolding and aggregation. While Hsp70 homologs in eukaryotic cells interact with a wide range of nascent chains (Beckmann et al., 1990; James et al., 1997; Pfund et al., 1998; Thulasiraman et al., 1999), the role of the bacterial Hsp70 system in de novo protein folding has remained elusive, raising the question of whether the mechanisms of protein folding in bacteria differ fundamentally from those in eukaryotes.

Polypeptide binding and release by DnaK, the major Hsp70 in *E. coli*, is regulated in an ATP-dependent reaction cycle by the chaperone DnaJ and the nucleotide exchange protein GrpE (Bukau and Horwich, 1998). In addition to DnaK, *Escherichia coli* contains two less abundant Hsp70 proteins, Hsc66 and Hsc62 (Vickery et al., 1997; Yoshimune et al., 1998). Under standard growth conditions at 37°C, DnaK is present in the *E. coli* cytosol at ~50 μM (Hesterkamp and Bukau, 1998), roughly equivalent to the concentration of ribosomes, and would thus be able to interact with many newly synthesized polypeptides. Yet recent studies led to the conclusion that DnaK is unlikely to play a critical role in de novo folding (Hesterkamp et al., 1996; Hesterkamp and Bukau, 1998), on the basis that *E. coli* cells lacking DnaK (and DnaJ) grow normally at 30°C–37°C, provided they possess a suppressor mutation in σ^{32} that prevents the uncontrolled overexpression of other heat shock proteins (Bukau and Walker, 1990). Furthermore, attempts to detect an interaction of DnaK with short nascent chains by cross-linking in an in vitro translation system failed (Hesterkamp et al., 1996). DnaK was, however, found to be required for the survival of cells under various stress conditions and for the refolding of certain stress-denatured polypeptides (Bukau and Walker, 1990; Hesterkamp and Bukau, 1998; Rockabrand et al., 1998).

Association with nascent chains has been observed for *E. coli* trigger factor (TF), an abundant chaperone and peptidyl-prolyl *cis/trans* isomerase that binds to ribosomes (Lill et al., 1988; Stoller et al., 1995; Valent et al., 1995, 1997; Hesterkamp et al., 1996); and like DnaK, TF may also be dispensable under normal growth conditions (Gothel et al., 1998). So far, GroEL/GroES is the only chaperone system known to be absolutely essential

‖To whom correspondence should be addressed (e-mail: uhartl@biochem.mpg.de).

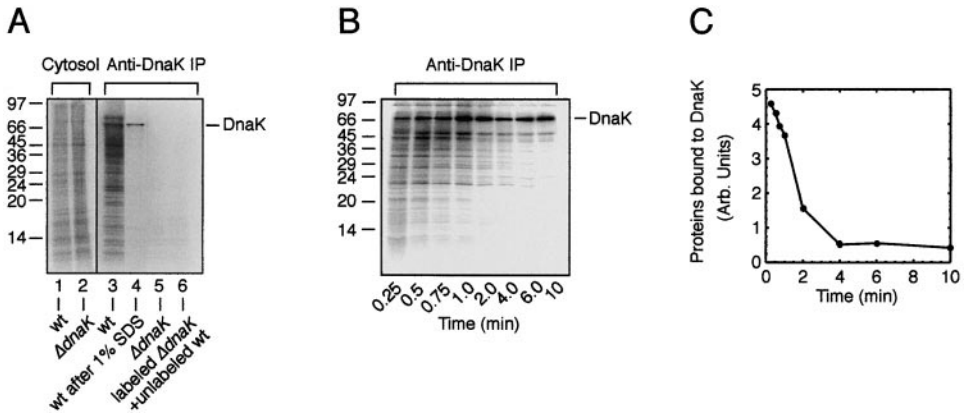

Figure 1. Transient Interaction of Newly Synthesized Proteins with DnaK

E. coli spheroplasts were pulse–chase labeled at 30°C, followed by lysis and coimmunoprecipitation (co-IP) of DnaK–polypeptide complexes. (A) SDS-PAGE of DnaK and associated polypeptides. A 15 s pulse of [^{35}S]methionine was followed by addition of excess nonradioactive methionine for 15 s, followed by lysis. Total radiolabeled proteins in the soluble fraction (cytosol) of wild-type (MC4100) (lane 1) and Δ*dnaK* (BB1553) spheroplasts (lane 2) were immunoprecipitated with anti-DnaK antibody (lanes 3 and 5; equivalent to 15 times the amount loaded in lanes 1 and 2). IP was performed following denaturation with SDS and subsequent dilution (lane 4). IP (as in lanes 3 and 5) was performed, but from a 1:1 mixture of radiolabeled Δ*dnaK* and unlabeled wild-type spheroplasts (lane 6).

(B) SDS-PAGE of DnaK and associated polypeptides labeled and immunoprecipitated as in (A), with excess methionine added at time 0.

(C) Quantification of the data in (B).

in *E. coli* (Fayet et al., 1989), but only 10%–15% of all newly synthesized polypeptides transit GroEL posttranslationally (Ewalt et al., 1997). Thus, the possibility arises that the vast majority of bacterial proteins may fold in a chaperone-independent manner.

Here, we analyzed the interaction of DnaK with newly synthesized polypeptides in live *E. coli* cells. Our findings establish this Hsp70 as a major nascent chain–binding chaperone whose in vivo function in protein folding overlaps partially with that of TF. The fact that deletion of the genes encoding TF and DnaK is synthetically lethal suggests that chaperone binding to nascent chains is critical for efficient protein folding in bacteria in addition to the essential posttranslational chaperone action of GroEL.

Results

A Wide Spectrum of Proteins Interact Transiently with DnaK

DnaK–substrate complexes were analyzed by coimmunoprecipitation from pulse-chase-labeled *E. coli* spheroplasts. Rapid lysis of spheroplasts on ice proved to be efficient in halting ATP-dependent DnaK cycling when combined with depletion of ATP from the extract by apyrase. Spheroplasts of the wild-type strain MC4100 were labeled with [^{35}S]methionine for 15 s at 30°C, a time sufficient to synthesize an average *E. coli* protein of 300 amino acid residues and to complete the synthesis of partially elongated larger proteins in labeled form, thus avoiding the overrepresentation of larger polypeptides that contain a greater number of methionine residues (see Ewalt et al., 1997) (Figure 1A, lanes 1 and 2). A monoclonal antibody, mAb2G5, which recognizes a region close to the ATP-binding cleft of the N-terminal DnaK domain (Krska et al., 1993), coprecipitated DnaK and a wide variety of newly synthesized polypeptides from the lysed spheroplasts (Figure 1A, lane 3). These proteins were strongly diminished in amount when cell lysates were incubated with ATP in the absence of apyrase prior to antibody addition (not shown) and represented authentic in vivo substrates of DnaK for the following reasons. When, prior to immunoprecipitation, DnaK–substrate interactions were disrupted with SDS, followed by subsequent dilution of the denaturant, only labeled DnaK itself was precipitated (Figure 1A, lane 4). Precipitation of newly synthesized polypeptides with anti-DnaK antibody was not observed in a MC4100-derived DnaK deletion strain (Δ*dnaK*) that exhibited nearly normal growth rates at 30°C–37°C (Figure 1A, lanes 2 and 5) (Bukau and Walker, 1990). Furthermore, when labeled cells from the Δ*dnaK* strain and unlabeled wild-type cells were mixed during lysis, no radiolabeled polypeptides were associated with DnaK, indicating that complex formation between DnaK and newly synthesized polypeptides occurred in the intact spheroplasts and not during or after cell lysis (Figure 1A, lane 6).

The profile of polypeptides bound to DnaK extended from small chains of less than 14 kDa to polypeptides larger than 90 kDa, but in comparison to the total labeled proteins (Figure 1A, lane 1), DnaK bound preferentially to chains of ~30 to 75 kDa (Figure 1A, lane 3). Taking the efficiency of DnaK precipitation (~45%) into account, the DnaK antibody precipitated 5%–10% of the total soluble polypeptides and 15%–20% of all polypeptides larger than 30 kDa that were synthesized within 15 s of labeling. However, the actual fraction of newly translated polypeptides interacting with DnaK is probably significantly greater, because Hsp70–substrate complexes are known to be labile under the conditions of immunoprecipitation (Manning-Krieg et al., 1991; Frydman et al., 1994). Since high-affinity peptide sites for DnaK binding occur on average every 40 amino acids in unfolded polypeptides (Rüdiger et al., 1997) and even large unfolded proteins, such as firefly luciferase (60 kDa), form chaperone complexes containing no more

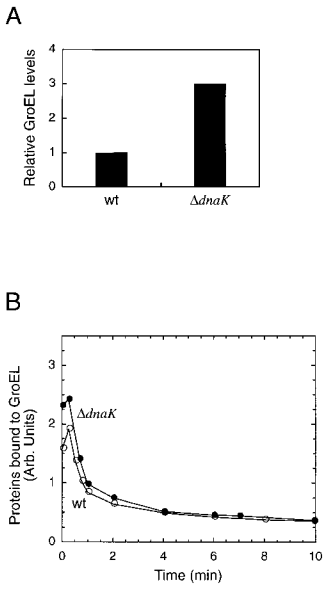

Figure 2. Interaction of Newly Synthesized Proteins with GroEL in Wild-Type and Δ*dnaK E. coli* Strains

(A) GroEL levels in wild-type (wt) (MC4100) and Δ*dnaK* (BB1553) cells were measured by quantitative Western blot analysis.

(B) Pulse–chase experiments followed by co-IP of GroEL–substrate complexes with affinity-purified anti-GroEL antibodies were carried out on wt and Δ*dnaK* spheroplasts as described in Figure 1. The flux through GroEL of newly synthesized proteins with molecular weight less than 60 kDa is shown and expressed per total GroEL present in the cells.

than two molecules of DnaK (Szabo et al., 1994), the preference of DnaK for chains larger than 30 kDa is probably functionally relevant.

To examine the kinetics of DnaK–substrate interactions in vivo, spheroplasts were first labeled for 15 s, followed by the addition of excess unlabeled methionine during a chase period (Figure 1B). These experiments showed that DnaK substrates associate with the chaperone immediately after, or concurrently with, their synthesis on ribosomes. The bulk of these proteins interact transiently and leave DnaK rapidly within 2 min of chase (Figure 1C), consistent with a role of DnaK in the folding of newly synthesized polypeptides. Since incorporation of radiolabel into protein stopped completely only at ~2 min after addition of unlabeled amino acid (not shown), some labeled polypeptides continue to accumulate on DnaK during the early phase of the chase.

DnaK Can Cooperate with GroEL in Protein Folding

It has been proposed that DnaK can stabilize newly synthesized polypeptides in a nonaggregated state for subsequent folding in the central cavity of the chaperonin GroEL (Langer et al., 1992). In assessing the possible significance of an Hsp70-to-chaperonin relay in vivo, we first determined the levels of GroEL in the wild-type and DnaK deletion strains. Under normal growth conditions, Δ*dnaK* cells of strain BB1553 contain three times more GroEL than wild-type cells (Figure 2A), although they contain a suppressor mutation in σ^{32} (also see Bukau and Walker, 1990). Pulse–chase labeling experiments followed by immunoprecipitation of GroEL–polypeptide complexes were performed to measure the flux of newly synthesized polypeptides through GroEL. As shown recently (Ewalt et al., 1997), in wild-type cells about 10% of newly synthesized polypeptides associated transiently with GroEL. Interestingly, the amount (Figure 2B) and pattern (data not shown) of total synthesized polypeptides that transit GroEL was essentially unchanged in Δ*dnaK* cells, consistent with the near normal growth rate of the DnaK deletion strain under these conditions. Thus, Δ*dnaK* cells achieve the same flux of newly translated polypeptides through GroEL as wild type, albeit at 3-fold higher GroEL levels.

Efficient transfer from the ribosome to GroEL in Δ*dnaK* cells was also observed for rhodanese, a 33 kDa protein that is highly dependent on GroEL for folding (Langer et al., 1992; Ewalt et al., 1997). Under conditions of low-level expression from the *trc* promoter, only soluble rhodanese was produced. The flux rates of rhodanese through GroEL in wild-type and Δ*dnaK* cells were very similar, indicating that interaction with DnaK is not obligatory for this protein to reach GroEL (data not shown).

To explore the chaperone interactions of rhodanese in more detail, the protein was expressed in wild-type cells from a T7-controlled plasmid to relatively high levels (~3% of newly labeled protein) where only 50% of rhodanese remained soluble (Ewalt et al., 1997). Under these conditions, between 5% and 10% of the total rhodanese synthesized could be coimmunoprecipitated with DnaK (Figure 3A). Rhodanese bound to DnaK with kinetics reflecting its rate of synthesis but decayed from DnaK more slowly than most endogenous proteins, following a pronounced plateau phase (Figure 3B). Interestingly, rhodanese accumulated on GroEL with a delay relative to its binding to DnaK and was discharged from GroEL with similar slow kinetics as from DnaK (Figure 3B). The slow dissociation of rhodanese from DnaK was due to limiting concentrations of GroEL, as demonstrated in cells that overexpressed GroEL/GroES ~4-fold from an arabinose-controlled plasmid (Figure 3C). Under these conditions, 85% of rhodanese was soluble (not shown). The maximum amount of rhodanese detected in the complex with GroEL exceeded that recovered with DnaK, and the newly synthesized polypeptide dissociated from GroEL with faster kinetics, similar to those measured for the chaperonin-assisted refolding of rhodanese in vitro (Langer et al., 1992). Significantly, the dissociation of rhodanese from DnaK was also markedly accelerated upon overexpression of GroEL/GroES (Figure 3D), reflecting an increased flux rate for rhodanese from DnaK to GroEL.

These results are consistent with the view that DnaK, presumably together with DnaJ and GrpE, is normally involved in mediating efficient protein flux through GroEL. Loss of DnaK under nonstress conditions is apparently fully compensated by the increased expression of GroEL and perhaps by the action of other chaperones. By cycling on and off DnaK, newly synthesized, GroEL-dependent proteins may be maintained in a conformation competent for productive interaction with GroEL for a limited time. Such a mechanism is consistent with the finding that overexpression of DnaK/DnaJ increases the soluble expression of certain GroEL substrates (Checa and Viale, 1997). Whether protein transfer from DnaK to GroEL is strictly unidirectional remains to be addressed.

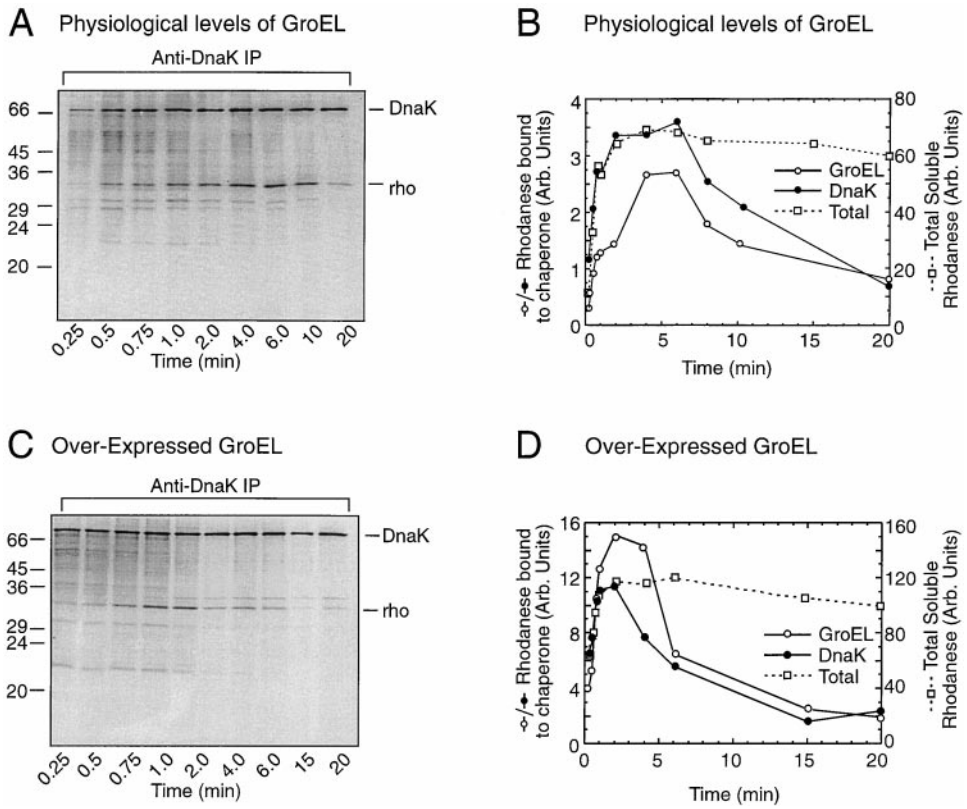

Figure 3. Transit of Rhodanese through DnaK and GroEL In Vivo

Spheroplasts were prepared from *E. coli* expressing rhodanese, labeled, and lysed as in Figure 1. Lysates were subjected to anti-DnaK and anti-GroEL IP.

(A and B) Pulse–chase labeling of spheroplasts at physiological levels of GroEL/GroES.

(A) SDS-PAGE of DnaK coimmunoprecipitates from lysates prepared at various times during the chase with unlabeled methionine.

(B) Kinetics of incorporation of [^{35}S]methionine into total full-length rhodanese and binding and release of radiolabeled rhodanese from DnaK and GroEL. Protein amounts were determined by phosphorimaging quantitation of experiments in (A) and are expressed in arbitrary units. For time points after [^{35}S]methionine incorporation was complete, DnaK served as a standard for equal loading, and rhodanese intensities were standardized to this value.

(C and D) *E. coli* cells contained the plasmids pBADGroESL and pET-rhodanese. Prior to induction of rhodanese, cells were grown in the presence of arabinose to induce overexpression of GroEL/GroES.

(C) Labeling, lysis, IP, and SDS-PAGE analysis were performed as in (A).

(D) Quantification of the data as in (B).

DnaK Interacts with Proteins that Are Not Substrates of GroEL

It seemed plausible that DnaK also interacts with newly translated proteins whose folding is not mediated by GroEL. This subset of proteins might be expected to include large polypeptides that exceed the space limitations of the central cavity of GroEL. To test this hypothesis, two such proteins, firefly luciferase (60 kDa) and bacterial β-galactosidase (β-gal; 116 kDa), were expressed in *E. coli*. Firefly luciferase is a well-studied substrate of the Hsp70 system that does not interact productively with GroEL (Frydman et al., 1992). Stress-denatured luciferase requires DnaK, DnaJ, and GrpE for its refolding in vivo (Schröder et al., 1993), but newly synthesized luciferase, expressed from a T7 promoter, was reported to fold with wild-type efficiency (~50% of total synthesized) in a Δ*dnaK* strain under nonstress conditions (Hesterkamp and Bukau, 1998).

When expressed at 30°C to about 6% of total newly labeled proteins, 60% of luciferase accumulated in inclusion bodies. Strikingly, in pulse-chase-labeled spheroplasts, up to 30% of the soluble, newly synthesized luciferase was recovered as a complex with DnaK (Figure 4A). Luciferase was released slowly from this complex over 10–15 min of chase (Figure 4B) in a soluble, folded state (data not shown). Note that at 6 min after the chase, most endogenous proteins have already dissociated from DnaK and luciferase is the predominant DnaK-bound polypeptide (Figure 4A). Interestingly, in eukaryotic systems, luciferase folding initiates cotranslationally and is completed rapidly upon synthesis (Frydman et al., 1994). In contrast, our results suggest that upon expression in *E. coli*, a large fraction of luciferase folds posttranslationally with much slower kinetics.

Next, we examined the interaction of DnaK with β-gal. This bacterial protein was expressed under the control of an arabinose promoter largely in soluble form. About 15%–20% of full-length β-gal was recovered in a com-

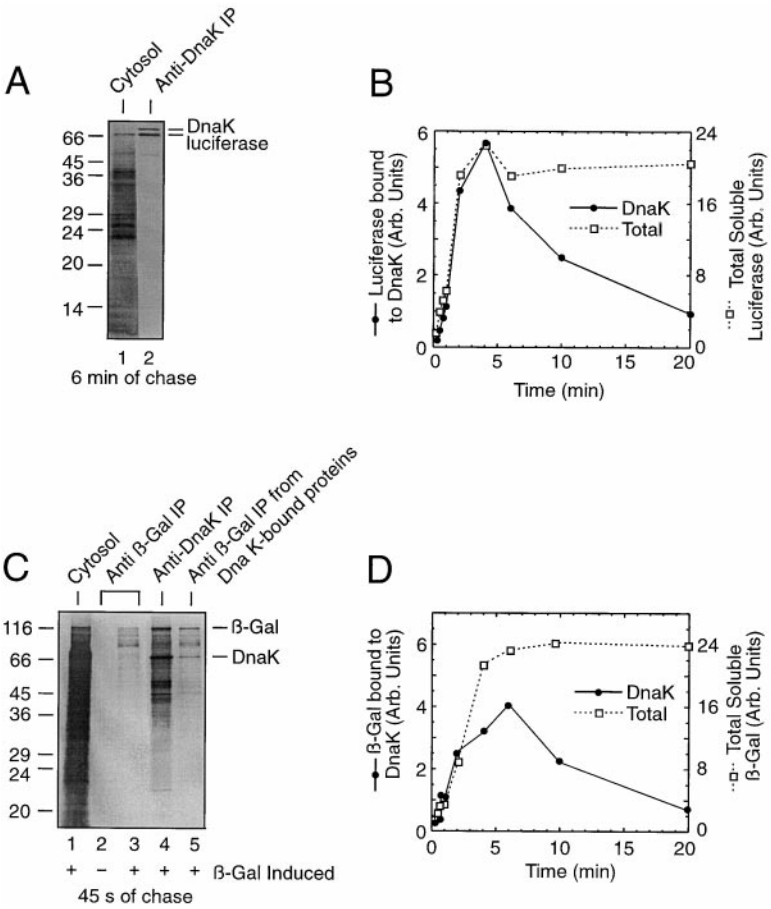

Figure 4. Interaction of DnaK with Newly Translated Firefly Luciferase and β-Galactosidase In Vivo

(A) Anti-DnaK IP was performed from pulse-chase-labeled spheroplasts expressing luciferase. SDS-PAGE analysis of the immunoprecipitate from a lysate taken at 6 min following the chase is shown (lane 2, 15 equivalents loaded), in addition to a fraction of cytosol from this time point (lane 1, 1 equivalent).

(B) Kinetics of incorporation of [^{35}S]methionine into total full-length luciferase in the soluble fraction as well as its association and release from DnaK as determined by phosphorimaging analysis (see Figure 2).

(C) β-galactosidase (β-gal) was expressed from an arabinose-controlled plasmid. Spheroplasts were labeled for 15 s in the presence of an inducer (0.2% arabinose) or repressor (0.2% glucose), followed by a 45 s chase with unlabeled methionine, anti-DnaK IP, and SDS-PAGE/fluorography. Lane 1, total cytosol (1 equivalent); lanes 2 and 3, anti-β-gal IP under denaturing conditions (15 equivalents); lane 4, anti-DnaK IP (15 equivalents); lane 5, re-IP of anti-DnaK precipitate with anti-β-gal antibodies after SDS denaturation and dilution of the denaturant (10 equivalents).

(D) Kinetics of incorporation of [^{35}S]methionine into total full-length β-gal in the soluble fraction as well as its association and release from DnaK during the chase, determined as in (B).

plex with DnaK immediately upon translation and released with slow kinetics (Figure 4D), consistent with a recent report that newly synthesized β-gal reaches its enzymatically active state within 5 to 10 min of synthesis (Chattopadhyay et al., 1999). Although luciferase and β-gal are not dependent on DnaK for folding and assembly under the conditions tested (Hesterkamp and Bukau, 1998), both proteins nevertheless interact with DnaK during biogenesis for prolonged periods. Taking into consideration that unassisted refolding of these proteins in vitro is very inefficient (Szabo et al., 1994; Freeman and Morimoto, 1996), it seems likely that in the Δ*dnaK* deletion strain, other chaperones may fulfill the function of DnaK in assisting their folding.

DnaK Interacts with Ribosome-Bound Polypeptide Chains

For all three model proteins analyzed, full-length polypeptides were detected in a complex with DnaK immediately upon synthesis (Figures 3 and 4). The expression of the 116 kDa protein β-gal presented an ideal opportunity to test whether the interaction of DnaK with its substrates initiates cotranslationally. Spheroplasts were labeled for 15 s and chased briefly (45 s). This chase time allowed β-gal nascent chains and some full-length labeled protein to accumulate, as shown by immunoprecipitation under denaturing conditions with a specific β-gal antibody (Figure 4C, lanes 2 and 3). To determine whether nascent β-gal chains were bound to DnaK, DnaK–polypeptide complexes were first isolated by anti-DnaK precipitation (Figure 4C, lane 4), subsequently denatured with SDS, and then reimmunoprecipitated with anti-β-gal antibody (Figure 4C, lane 5). In addition to the full-length protein, a set of incomplete, presumably nascent, chains of β-gal were reprecipitated. A small amount of denatured DnaK adsorbed nonspecifically to the Sepharose beads.

Ribosome-bound polypeptides were analyzed to further investigate the possibility that DnaK interacts with its substrates cotranslationally. Spheroplasts were labeled for 30 s and rapidly lysed in the presence of apyrase to prevent the cycling of substrates off DnaK. The population of ribosome-bound polypeptides was then isolated by centrifugation through sucrose (Figure 5, lane 1). Most of these nascent polypeptides were released from the ribosomes by puromycin (Figure 5, lane 2). DnaK immunoprecipitations from the ribosomal fraction demonstrated the association of DnaK with a wide range of nascent chains from below 14 kDa in size up to at least 80 kDa (Figure 5, lane 3). As in DnaK immunoprecipitates from total lysates, there was a clear preference for nascent chains in the size window of ~30 to 75 kDa, with a notable accumulation of distinct chains between 45 and 66 kDa. These products may represent translationally paused chains of specific proteins. Approximately 20% of the total DnaK-precipitable proteins in these experiments were nascent chains. It is important to emphasize that these DnaK substrates were bound

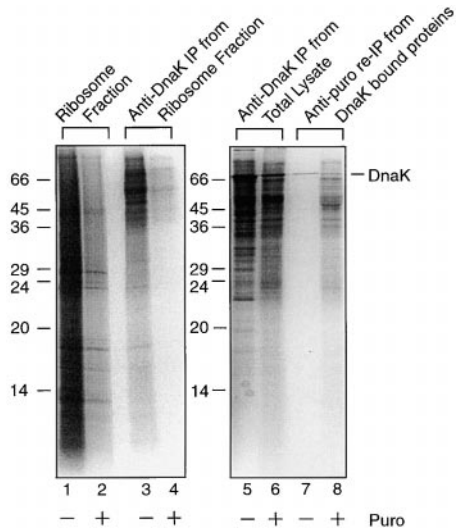

Figure 5. Interaction of DnaK with Ribosome-Associated Nascent Chains

E. coli spheroplasts were labeled for 30 s with [^{35}S]methionine and then lysed with or without puromycin in the presence of 400 mM KOAc. Newly labeled ribosome-bound polypeptides without (lane 1) and with puromycin (puro) treatment (lane 2; 1 equivalent loaded) are displayed on SDS-PAGE. Samples without puromycin (−puro) were treated with chloramphenicol to stabilize ribosome-bound nascent chains. Lanes 3 and 4, anti-DnaK IPs from ribosome fractions (15 equivalents). Lanes 5 and 6, anti-DnaK IPs from total lysates (1.5 equivalents). Lanes 7 and 8, re-IP of anti-DnaK precipitates with puromycin antibodies (6 equivalents).

to DnaK before cell lysis and remained DnaK bound throughout the isolation of ribosomes by ultracentrifugation in the presence of high salt. Thus, these experiments may substantially underestimate the fraction of nascent chains among the total DnaK substrates.

The nascent chain–binding activity of DnaK was confirmed, taking advantage of the fact that puromycin-released nascent chains become C-terminally tagged with puromycin and can be precipitated with a specific anti-puromycin antibody (Eggers et al., 1997). DnaK-bound polypeptides, precipitated from puromycin-treated cell lysates (Figure 5, lane 6), were dissociated with SDS and then reprecipitated with anti-puromycin antibodies (Figure 5, lanes 7 and 8). Again, at least 20% of the total DnaK-bound polypeptides were identified as nascent chains by this procedure. This experiment also confirmed the preferential association of DnaK with nascent chains in the size range of ~30–75 kDa. Collectively, these results identify DnaK as a nascent chain–binding chaperone with a preference for chains larger than the average length of an *E. coli* protein.

Nascent Chain Binding of DnaK Is Modulated by Trigger Factor

Only one other chaperone component, the abundant TF, is thought to act as a major nascent chain–binding protein in *E. coli* (Stoller et al., 1995; Valent et al., 1995; Hesterkamp et al., 1996). We considered the possibility that DnaK and TF might have partially overlapping functions in protecting nascent and newly synthesized polypeptides. To examine the effect of TF on the interaction

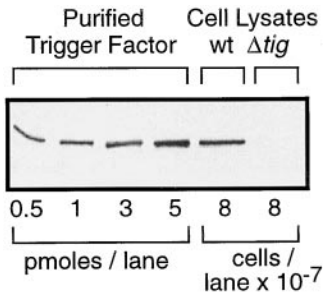

Figure 6. Deletion of Trigger Factor (TF) and Estimation of TF Protein Content in Wild-Type *E. coli*

Purified TF (Stoller et al., 1995) and lysates from JC7623 cells, as well as cells lacking the gene for TF (Δ*tig*), were analyzed by SDS-PAGE and Western blotting using anti–trigger factor antibodies.

of DnaK with newly made polypeptides, the *tig* gene was deleted by homologous recombination in *E. coli* strain JC7623. Although the resulting Δ*tig* strain does not express TF (Figure 6), it nevertheless grows normally at 30°C–42°C (data not shown). In wild-type cells, TF is present at ~20,000 molecules per cell, compared to 15,000 ribosomes (Ellis and Hartl, 1996). TF is, however, required for the survival of cells at temperatures below 8°C (data not shown), confirming an observation by Kandror and Goldberg (1997). Interestingly, loss of the peptidyl-prolyl isomerase activity (PPIase) of TF is responsible for this effect, based on the inability of a PPIase-deficient mutant form of TF to restore resistance to low temperatures (T. T. and G. F., unpublished observations). Thus, during growth at 30°C–42°C, both the chaperone and PPIase activities of TF are dispensable.

Next, we studied the effect of TF deletion on the function of DnaK. Spheroplasts of the Δ*tig* strain and the respective wild-type parent strain incorporated [^{35}S]methionine into protein with equal efficiency at 30°C. Aggregation of newly synthesized polypeptides was not observed (not shown). The time course of DnaK binding to newly made polypeptides was determined in pulse–chase experiments (Figures 7A and 7B). Wild-type cells of strain JC7623 showed an even more pronounced exclusion of polypeptides below 30 kDa from DnaK binding (Figure 7A) than observed in the MC4100 *E. coli* strain used above (see Figure 1). Interestingly, the interaction of DnaK with newly synthesized polypeptides in the Δ*tig* strain differed in two aspects from that in wild type. First, the fraction of newly translated polypeptides of ~30 to 75 kDa recovered in a complex with DnaK was increased 2-fold (Figures 7B and 7C). Second, it was noted that the spectrum of polypeptides coimmunoprecipitating with DnaK in the Δ*tig* strain included an increased representation of shorter chains (Figure 7B; also see Figure 7D below).

Due to its specific association with the large ribosomal subunit (Stoller et al., 1995), TF is able to bind to nascent chains immediately upon their exit from the ribosome (Valent et al., 1997). Thus, TF may normally prohibit the interaction of DnaK with short nascent chains. To address this possibility, cotranslationally bound substrates were analyzed specifically. DnaK-bound polypeptides were first coimmunoprecipitated from puromycin-treated

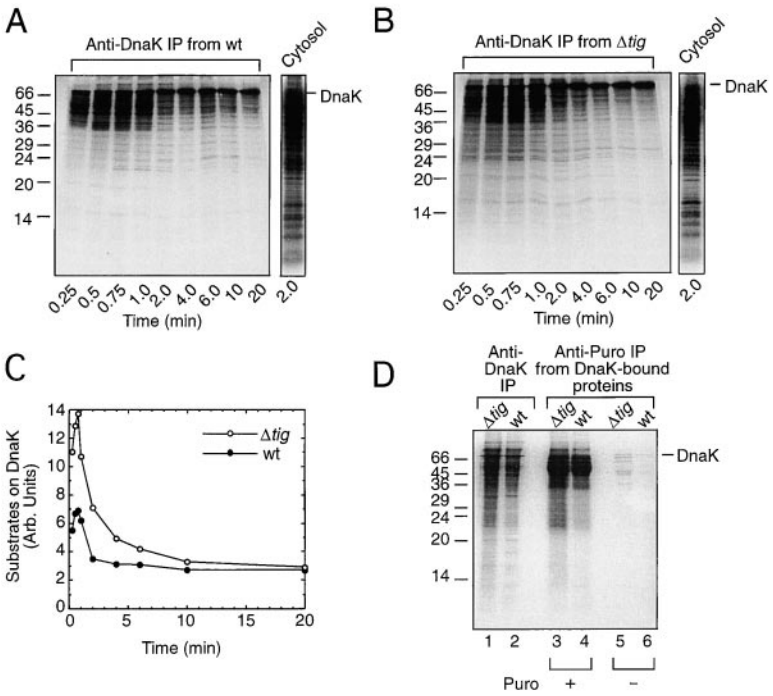

Figure 7. Effect of TF Deletion on Association of DnaK with Newly Synthesized and Nascent Polypeptides

Wild-type (wt) *E. coli* (JC7623) were compared to *E. coli* lacking the gene for TF (Δ*tig*). Pulse labeling and IP of DnaK–polypeptide complexes was as in Figure 1. Cytosol extracts from wt and Δ*tig* spehroplasts that had incorporated equal amounts of radiolabel were analyzed.

(A and B) SDS-PAGE/fluorography of total soluble proteins in wt and Δ*tig* strains upon 15 s of labeling and 2 min chase (cytosol; 1 equivalent), as well as of anti-DnaK IPs at different times during chase (15 equivalents loaded).

(C) Quantification of newly labeled proteins coimmunoprecipitating with DnaK from the experiment shown in (A) and (B).

(D) Lysates from Δ*tig* and wt cells (labeled as in Figure 4) were treated with or without puromycin and immunoprecipitated with anti-DnaK antibody (lanes 1 and 2, reactions without puromycin), followed by re-IP of DnaK-bound nascent chains with anti-puromycin antibody (lanes 3–6; amount loaded corresponds to four times that in lanes 1 and 2).

extracts of pulse-labeled wild-type and Δ*tig* spheroplasts and then reprecipitated with puromycin antibody (Figure 7D, lanes 3–6). The total amount of DnaK-associated nascent chains was 2-fold greater in the *tig*-deleted cells than in wild type. Moreover, in the absence of TF, the binding of DnaK to shorter nascent chains between ~22 and 35 kDa and below 22 kDa was significantly enhanced.

These results indicate that upon loss of TF, *E. coli* cells make more extensive use of the capacity of DnaK to bind to nascent and newly synthesized polypeptides. The relative exclusion of DnaK from nascent chains below ~35 kDa in wild-type cells appears to be due, at least in part, to the association of these chains with TF.

Synthetic Lethality of the *tig* and *dnaK* Genes

The results described above suggested that the loss of TF under normal growth conditions might be compensated by DnaK and vice versa. Assuming that this chaperone redundancy provides a necessary function, one would expect that the combined deletion of both the *tig* and *dnaK* genes results in a significant growth defect or even in lethality in *E. coli*.

In testing this hypothesis, we first confirmed that the genes encoding DnaK and its cofactor DnaJ, the *dnaK dnaJ* operon, can be deleted in the *E. coli* MC4100 genetic background at the most physiologically relevant growth temperature of 37°C (Bukau and Walker, 1989). This result was indicated by the growth of colonies that were P1 transduced to kanamycin resistance (Kan^R) using the allele (*dnaK dnaJ*)::Kan^R, a deletion/substitution of the *dnaK dnaJ* operon by Kan^R. The ~50% cotransducible allele *thr*::Tet^R, conferring tetracycline resistance, was included in the analysis as an internal control (Table 1). As previously observed, such *dnaK dnaJ*–deleted strains grow slowly at 37°C with a tendency to rapidly accumulate extragenic suppressors and do not grow at all at 42°C (Bukau and Walker, 1989). Unexpectedly, we found that in other *E. coli* genetic backgrounds, such as W3110, the *dnaK dnaJ* operon cannot be deleted under the conditions analyzed at 37°C, indicated by the absence of Kan^R transductants, unless DnaK and DnaJ are expressed from a plasmid (pDM38) in the same strain (Table 1). Thus, it appears that the requirement for the *dnaK dnaJ* operon under nonstress conditions is variable among *E. coli* K-12 strains. In contrast, the *tig* gene could be deleted in all wild-type strains tested at 37°C and 42°C (not shown).

Strikingly, under the same experimental conditions in which the *dnaK dnaJ* operon or the *tig* gene can be readily deleted in the MC4100 background, sequential deletion of *tig* and *dnaK dnaJ* resulted in strict synthetic lethality, based on the complete inability of a *tig*-deleted MC4100 strain (MC4100 *tig*::cam^R) to accept the *dnaK dnaJ* deletion (Table 1). This genetic result strongly supports the conclusions of our functional analysis that DnaK and TF have partially overlapping functions in protein folding. Clearly, under nonstress conditions at 37°C, *E. coli* can tolerate the loss of either function but not both, suggesting that chaperone binding to nascent and newly synthesized chains is critical for *E. coli* growth. The synthetic lethality of the *dnaK dnaJ* deletion in the Δ*tig* background can be caused by the loss of the *dnaK* gene product alone, because it is still observed in the presence of a plasmid that produces functional DnaJ protein (data not shown). Moreover, when trigger factor was deleted by introducing the *tig*::Cam^R-inactivated allele into various *dnaK* mutant backgrounds that express

Table 1. Synthetic Lethality of the *dnaK dnaJ* Operon and *tig* Genes in *E. coli*

	Recipient Strains				
	W3110	W3110 *tig*::Cam^R	W3110 (pDM38 *dnaK*$^+$ *dnaJ*$^+$)	MC4100	MC4100 *tig*::Cam^R
Kan^R transductants	0	0	114	149[a]	0
Tet^R transductants	105	117	98	158	150
	(0/105)	(0/117)	(47/98)	(42/100)	(0/103)

A bacteriophage P1 lysate was grown on strain PK101 *thr*::Tet^R (*dnaK dnaJ*)::Kan^R previously transformed with plasmid pDM38, encoding the intact *dnaK dnaJ* operon. The transduction plates (L-agar containing 3 mM Na citrate and either 10 μg/mL of tetracycline or 70 μg/mL kanamycin) were scored following a 40 hr incubation at 37°C. Recipient strain W3110 (pDM38) was used as a control to demonstrate that the disrupted (*dnaK dnaJ*)::Kan^R allele can be transduced easily.

[a] These Kan^R transductants grew much more slowly than the ones using the W3110 (pDM38 *dnaK*$^+$ *dnaJ*$^+$) recipient strain. They did not form colonies at 42°C, and at 37°C, faster-growing variants readily accumulated. Following a 40 hr incubation at 37°C, the Tet^R transductants were cross-streaked on kanamycin-containing L-plates to test for the inheritance of the (*dnaK dnaJ*)::Kan^R allele. The ratio of Kan^R Tet^R transductants to total Tet^R transductants is shown in parentheses.

DnaJ (such as *dnaK*756), the corresponding double mutants were unable to form colonies at temperatures permissive for the parent *dnaK* mutants.

Discussion

This study provides evidence that DnaK interacts with a wide range of nascent and newly synthesized polypeptides and defines this bacterial Hsp70 as a major chaperone acting early in de novo protein folding. The finding that DnaK is dispensable under nonstress conditions in certain strain backgrounds can be explained by our observation of partially redundant functions of DnaK and the chaperone TF in binding newly synthesized polypeptides. This conclusion is strongly supported by the genetic result that the combined deletion of *tig* and *dnaK* genes causes lethality under conditions where loss of either gene is tolerated. Thus, under nonstress conditions, *E. coli* depends on at least two essential chaperone functions, provided predominantly by the Hsp70 system in cooperation with TF and by GroEL/GroES.

Sequence of Chaperone Interactions with Nascent Chains

Polypeptide chains that will eventually reside in the cytosol are likely to interact first with TF and then may associate co- or posttranslationally with DnaK (Figure 8). Other chaperones, possibly including the less abundant *E. coli* Hsp70s, may have access to nascent polypeptides as well. Whereas most polypeptides leave DnaK rapidly, certain newly synthesized chains persist on DnaK posttranslationally, reflecting slower intrinsic rates of folding or the need of these polypeptides to be transferred to other chaperones in a timely fashion. In the absence of TF, the chaperone function of DnaK is used more extensively and is modulated to include shorter nascent chain substrates. The normal chain length preference of DnaK for polypeptides larger than 30 kDa (~270 amino acids) appears to be the result, at least in part, of the primary interaction of TF with nascent chains (Stoller et al., 1995; Valent et al., 1995; Hesterkamp et al., 1996). Binding to the large ribosomal subunit close to the polypeptide exit site allows TF to associate with chains as short as 57 amino acids (Valent et al., 1997) and seems to exclude DnaK from binding. Due to an irregular surface topology of the ribosome at the polypeptide exit site (Frank et al., 1995), binding of TF to the ribosome may effectively block access of DnaK. Moreover, very short nascent chains may be relatively inaccessible to the peptide-binding domain of DnaK, even in the absence of TF (see Figure 7D).

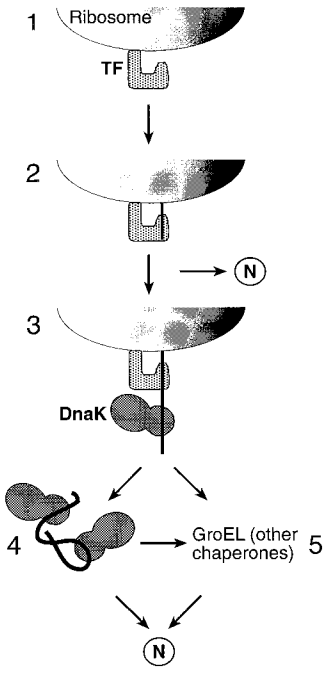

Figure 8. Proposed Sequence of Chaperone Interactions with Newly Made Cytosolic Proteins in *E. coli*

(1) Trigger factor (TF) associates with the ribosome close to the polypeptide exit site and (2) interacts with the chain very early in translation. Most short polypeptides may only associate with TF and fold rapidly to the native state (N) upon completion of synthesis. TF is drawn to schematically represent the three-domain structure of the protein, consisting of ribosome-binding, PPIase, and chaperone domains. (3) Longer polypeptides may first interact with TF and then with DnaK (only cotranslational binding is shown). DnaK is known to cooperate with DnaJ and GrpE, of which DnaJ may also transiently interact with the substrate polypeptide. (4) The DnaK system may posttranslationally assist in folding/assembly or (5) stabilize folding intermediates for transfer to GroEL (or other chaperones). GroEL can also receive substrates without prior interaction with DnaK (not shown).

Posttranslational Functions of DnaK in Folding

The preference of DnaK for larger *E. coli* proteins may reflect the propensity of polypeptides with complex domain structures to bury hydrophobic residues slowly during folding. DnaK associates co- and posttranslationally with newly synthesized β-gal and firefly luciferase. These multidomain proteins are too large to interact productively with GroEL/GroES (Frydman et al., 1992; Ayling and Baneyx, 1996). They decay from DnaK slowly and probably undergo multiple cycles of DnaK binding and release during folding and assembly. Although the unassisted refolding of these proteins in vitro is inefficient (Szabo et al., 1994; Freeman and Morimoto, 1996), their de novo folding in *E. coli* is not absolutely dependent on DnaK or Hsc66 (Hesterkamp and Bukau, 1998). Based on the functional redundancy between DnaK and TF, either component may be sufficient to mediate the folding of these substrates under nonstress conditions, perhaps in conjunction with other chaperones. Indeed, TF may also act posttranslationally, given that only 30% of total TF is ribosome associated (Lill et al., 1988) and that isolated TF binds unfolded proteins in vitro (Scholz et al., 1997).

Another potential role attributed to DnaK (in conjunction with DnaJ and GrpE) is the stabilization of newly made polypeptides for subsequent chaperonin-assisted folding (Langer et al., 1992). Our results are consistent with a role of the DnaK system in mediating the efficient transfer of polypeptides from the ribosome to GroEL. This pathway is not obligatory, however, since the DnaK-deleted cells can achieve a normal substrate flux through GroEL by a compensatory increase in chaperonin levels. We also consider the possibility that TF may support substrate transfer to GroEL, based on a reported interaction between TF and GroEL that is mediated by unfolded polypeptide (Kandror et al., 1997).

Quantitative Significance of Chaperone Interactions

A mechanistic and quantitative understanding of the minimal set of cellular chaperones acting in protein folding in bacteria is emerging. These components include TF, the Hsp70 system (DnaK, DnaJ, GrpE), and GroEL/GroES, which are encoded even in the smallest bacterial genomes (Fraser et al., 1995). Under normal growth conditions, GroEL binds transiently to about 10%–15% of all newly made proteins (Ewalt et al., 1997). The fraction of newly translated proteins that is recovered in a complex with DnaK is also about 5%–10% of the total but comprises ~20% of the chains larger than 30 kDa. The actual fraction of newly made polypeptides interacting with DnaK may be significantly greater, considering the known instability of Hsp70–polypeptide complexes during immunoprecipitation. The fraction of proteins interacting with TF remains to be determined but may be within the same range as that interacting with DnaK. Assuming that TF also associates with shorter substrates not bound by DnaK, we estimate that 50% or more of all cytosolic proteins interact with either or both of these components during biogenesis.

Experimental Procedures

Bacterial Strains and Plasmids

Strains used are MC4100 (Casadaban, 1976), BB1553 (MC4100 Δ*dnaK52::cat sidB1*; Bukau and Walker, 1990), BL21(DE3), and JC7623 (AB1157 *recB21 recC22 sbcB15, sbcC201*; Lloyd and Buckman, 1985). The Δ*tig* strain was constructed by insertionally inactivating the gene in strain JC7623 with a fragment containing the *cat* gene flanked by its neighbors *bolA* and *clpP*. Homologous recombinants with *cat* replacing the *tig* gene were identified by chloramphenicol resistance and confirmed by Southern blotting and Western analysis.

Plasmids used are pT7-rhodanese, pBADESL (Ewalt et al., 1997), pBAD/*myc*-His/*lacZ* (InVitrogen), and pET/*luc*/*myc*-His (Schneider et al., 1996).

Genetic Analysis

Bacteriophage P1 transduction experiments were carried out as described (Ang and Georgopoulos, 1989). Strain PK101 (from Dr. E. Craig via Dr. W. Kelley) carries a deletion/substitution of the *dnaK dnaJ* operon with a Kan^R cassette (Kang and Craig, 1990), and as a consequence, grows poorly. This strain was transformed with plasmid pDM38 (Missiakas et al., 1993), encoding the intact *dnaK dnaJ* operon, thus ensuring good bacterial and bacteriophage P1 growth. Strain PK101 (pDM38) was transduced to *thr*::Tet^R using the appropriate P1 lysate, resulting in a PK101 *thr*::Tet^R (*dnaK dnaJ*)::Kan^R (pDM38) strain. The *thr*::Tet^R marker, which is ~50% cotransducible with the (*dnaK dnaJ*)::Kan^R allele, was included in the transductional analysis as an internal control. Transduction to Tet^R ensures that the transduction was successful without introducing selective pressure to accept the *dnaK dnaJ* deletion. A bacteriophage P1 lysate grown on this strain was used to transduce *E. coli* W3110 (strain B178; Ang and Georgopoulos, 1989) or *E. coli* MC4100 (from Dr. E. Bremer via Dr. S. Raina) to either Tet^R or Kan^R. The *tig*::Cam^R allele was introduced into the above W3110 and MC4100 genetic backgrounds by using the appropriate P1 lysate and selecting for Cam^R at 37°C on L-agar plates (10 μg/mL chloramphenicol).

Protein Expression

Cells were grown at 30°C or 37°C to midlog phase in M63 medium, 0.2% glycerol, 0.2% glucose. For expression of rhodanese and luciferase, IPTG was added at 0.4 mM and growth continued for 30 min prior to pulse labeling. For induction of β-gal, cells were grown to midlog phase, harvested and transferred to M63, and supplemented with 0.2% glycerol and 0.2% arabinose 30 min prior to pulse labeling. Cells that carried the pBADESL plasmid were grown to midlog phase in M63 medium, 0.2% glycerol, 0.2% arabinose.

Pulse Labeling

Cells were converted to spheroplasts and pulse–chase labeled using [^{35}S]methionine (60 μCi/mL) followed by 1 mM unlabeled methionine (Ewalt et al., 1997). When necessary, spheroplasts were resuspended in media containing 0.4 mM IPTG, 0.2% arabinose, and carbenicillin (100 μg/mL) and/or chloramphenicol (30 μg/mL). Spheroplasts were diluted into an equal volume of ice-cold hypoosmotic lysis buffer (final concentrations of 10 mM Tris [pH 7.5], 0.1% Triton X-100, 0.1% deoxycholate [DOC], 1 mM Pefabloc, 10 U/mL apyrase, 1 U/mL RNase-free DNase I) and incubated on ice for 10 min. When noted, lysis was performed with prior addition of 1.5 mM puromycin (Sigma; individual batches were tested for activity in nascent chain release) or 0.1 mg/mL chloramphenicol; after 10 min, 400 mM KOAc was added to puromycin- and chloramphenicol-treated lysates, followed by a 30 min incubation on ice.

Immunoprecipitations

Anti-DnaK monoclonal antibody (mAb2G5) was purified as described (Krska et al., 1993). Spheroplast lysates were clarified by centrifugation (14,000 g, 10 min, 4°C) and adjusted to 150 mM NaCl. Extracts were first incubated with protein G–Sepharose (10 μL beads per 800 μL lysate, blocked with 2% BSA in PBS) for 45 min at 4°C. For anti-DnaK immunoprecipitations, extracts were incubated with 5 μg of mAb2G5 per sample (750 μL) for 1 hr at 4°C, followed by addition of protein G–Sepharose (10 μL, 2% BSA in PBS) for 30 min at 4°C. Beads were washed as described for immunoprecipitation of GroEL complexes (Ewalt et al., 1997). DnaK immunoprecipitation was ~45% efficient, based on quantitative immunoblotting. Control DnaK immunoprecipitation and immunoprecipitation of nascent

chains of β-gal under denaturing conditions was performed by treating cleared lysate with 2% SDS for 10 min at 25°C, followed by 40-fold dilution in lysis buffer containing 1% Triton X-100 and 0.5% DOC. GroEL immunoprecipitations were performed as described (Ewalt et al., 1997). Proteins were eluted from beads using SDS sample buffer. For sequential immunoprecipitations, samples were eluted with 2% SDS and then diluted to 0.05% SDS in lysis buffer containing 150 mM NaCl, 1% Triton X-100, and 0.5% DOC, followed by addition of polyclonal anti-β-gal (Sigma) or anti-puromycin antibodies (a kind gift from Dr. P. Walter). The eluted proteins were analyzed by SDS-PAGE and phosphorimaging analysis.

Ribosome Fractionation

Spheroplasts were labeled with [^{35}S]methionine (60 μCi/mL) for 30 s and treated as in the immunoprecipitation experiments except that 10 mM $MgCl_2$, 60 mM NH_4Cl, and 1:1000 diluted RNAse inhibitor (Pharmacia) was added to the lysis buffer. Lysates were cleared and an aliquot subjected to anti-DnaK immunoprecipitation after addition of 150 mM NaCl. Ribosomes were collected by centrifugation (60,000 rpm, Beckman Ti70, 108 min, 4°C) through a 3-fold or greater volume of sucrose (0.7 M sucrose, 10 mM Tris [pH 7.5], 10 mM $MgCl_2$, 60 mM NH_4Cl, 400 mM KOAc, 1:1000 dilution of RNase inhibitor). Ribosomal fractions were resuspended in 150 mM NaCl-containing buffer and DnaK–polypeptide complexes isolated by immunoprecipitation. Ribosomal pellets were analyzed by spectroscopy (A_{260}) to ensure that reactions contained equivalent units of ribosomal material.

Acknowledgment

This work was supported in part by a grant from the Swiss National Foundation to C. G.

Received March 16, 1999; revised May 12, 1999.

References

Ang, D., and Georgopoulos, C. (1989). The heat-shock-regulated grpE gene of Escherichia coli is required for bacterial growth at all temperatures but is dispensable in certain mutant backgrounds. J. Bacteriol. *171*, 2748–2755.

Ayling, A., and Baneyx, F. (1996). Influence of the GroE molecular chaperone machine on the in vitro refolding of Escherichia coli beta-galactosidase. Protein Sci. *5*, 478–487.

Beckmann, R.P., Mizzen, L.E., and Welch, W.J. (1990). Interaction of Hsp 70 with newly synthesized proteins: implications for protein folding and assembly. Science *248*, 850–854.

Bukau, B., and Walker, G.C. (1989). Cellular defects caused by deletion of the Escherichia coli *dnaK* gene indicate roles for heat shock protein in normal metabolism. J. Bacteriol. *171*, 2337–2346.

Bukau, B., and Walker, G.C. (1990). Mutations altering heat shock specific subunit of RNA polymerase suppress major cellular defects of E. coli mutants lacking the DnaK chaperone. EMBO J. *9*, 4027–4036.

Bukau, B., and Horwich, A.L. (1998). The Hsp70 and Hsp60 chaperone machines. Cell *92*, 351–366.

Casadabon, M.J. (1976). Transposition and fusion of the lac genes to selected promoters in Escherichia coli using bacteriophage lambda and mu. J. Mol. Biol. *104*, 541–555.

Chattopadhyay, S., Pal, S., Pal, D., Sarkar, D., Chandra, S., and Das Gupta, C. (1999). Protein folding in Escherichia coli: role of 23S ribosomal RNA. Biochim. Biophys. Acta *1429*, 293–298.

Checa, S.K., and Viale, A.M. (1997). The 70-Kda heat-shock protein DnaK chaperone system is required for the productive folding of ribulose-bisphosphate carboxylase subunits in Escherichia coli. Eur. J. Biochem. *248*, 848–855.

Eggers, D.K., Welch, W.J., and Hansen, W.J. (1997). Complexes between nascent polypeptides and their molecular chaperones in the cytosol of mammalian cells. Mol. Biol. Cell *8*, 1559–1573.

Ellis, R.J. (1993). The general concept of molecular chaperones. Philos. Trans. R. Soc. Lond. B Biol. Sci. *339*, 257–261.

Ellis, R.J., and Hartl, F.U. (1996). Protein folding in the cell: competing models of chaperonin function. FASEB J. *10*, 20–26.

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*, 491–500.

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*, 1379–1385.

Frank, J., Zhu, J., Penczek, P., Li, Y., Srivastava, S., Verschoor, A., Radermacher, M., Grassucci, R., Lata, R.K., and Agrawal, R.K. (1995). A model of protein synthesis based on cryo-electron microscopy of the E. coli ribosome. Nature *376*, 441–444.

Fraser, C.M., Gocayne, J.D., White, O., Adams, M.D., Clayton, R.A., Fleischmann, R.D., Bult, C.J., Kerlavage, A.R., Sutton, G., Kelley, J.M., et al. (1995). The minimal gene complement of Mycoplasma genitalium. Science *270*, 397–403.

Freeman, B.C., and Morimoto, R.I. (1996). The human cytosolic molecular chaperones hsp90, Hsp70 (hsc70) and hdj-1 have distinct roles in recognition of a non-native protein and protein refolding. EMBO J. *15*, 2969–2979.

Frydman, J., Nimmesgern, E., Erdjument, B.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*, 4767–4778.

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*, 111–117.

Georgopoulos, C., and Welch, W.J. (1993). Role of the major heat shock proteins as molecular chaperones. Annu. Rev. Cell Biol. *9*, 601–634.

Gething, M.-J., and Sambrook, J. (1992). Protein folding in the cell. Nature *355*, 33–45.

Gothel, 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*, 13392–13399.

Hartl, F.U. (1996). Molecular chaperones in cellular protein folding. Nature *381*, 571–580.

Hesterkamp, T., and Bukau, B. (1998). Role of the DnaK and HscA homologs of Hsp70 chaperones in protein folding in E.coli. EMBO J. *17*, 4818–4828.

Hesterkamp, T., Hauser, S., Lutcke, 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*, 4437–4441.

James, P., Pfund, C., and Craig, E.A. (1997). Functional specificity among hsp70 molecular chaperones. Science *275*, 387–389.

Johnson, J.L., and Craig, E.A. (1997). Protein folding in vivo: unraveling complex pathways. Cell *90*, 201–204.

Kandror, O., and Goldberg, A.L. (1997). Trigger factor is induced upon cold shock and enhances viability of Escherichia coli at low temperatures. Proc. Natl. Acad. Sci. USA *94*, 4978–4981.

Kandror, O., Sherman, M., Moerschell, R., and Goldberg, A.L. (1997). Trigger factor associates with GroEL in vivo and promotes its binding to certain polypeptides. J. Biol. Chem. *272*, 1730–1734.

Kang, P.J., and Craig, E.A. (1990). Identification and characterization of a new Escherichia coli gene that is a dosage-dependent suppressor of a *dnaK* deletion mutation. J. Bacteriol. *172*, 2055–2064.

Krska, J., Elthon, T., and Blum, P. (1993). Monoclonal antibody recognition and function of a DnaK (HSP70) epitope found in gram-negative bacteria. J. Bacteriol. *175*, 6433–6440.

Langer, T., Lu, C., Echols, H., Flanagan, J., Hayer, M.K., and Hartl, F.U. (1992). Successive action of DnaK, DnaJ and GroEL along the pathway of chaperone-mediated protein folding. Nature *356*, 683–689.

Lill, R., Crooke, E., Guthrie, B., and Wickner, W. (1988). The "trigger factor cycle" includes ribosomes, presecretory proteins, and the plasma membrane. Cell *54*, 1013–1018.

Lloyd, R.G., and Buckman, C. (1985). Identification and genetic analysis of sbcC mutations in commonly used recBC sbcB strains of Escherichia coli K-12. J. Bacteriol. *164*, 836–844.

Manning-Krieg, U.C., Scherer, P.E., and Schatz, G. (1991). Sequential action of mitochondrial chaperones in protein import into the matrix. EMBO J. *10*, 3273–3280.

Missiakas, D., Georgopoulos, C., and Raina, S. (1993). The *Escherichia coli* heat shock gene *htpY* : mutational analysis, cloning, sequencing, and transcriptional regulation. J. Bacteriol. *175*, 2613–2624.

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*, 3981–3989.

Rockabrand, D., Livers, K., Austin, T., Kaiser, R., Jensen, D., Burgess, R., and Blum, P. (1998). Roles of DnaK and RpoS in starvation-induced thermotolerance of Escherichia coli. J. Bacteriol. *180*, 846–854.

Rüdiger, S., Germeroth, L., Schneider-Mergener, J., and Bukau, B. (1997). Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide. EMBO J. *16*, 1501–1507.

Schneider, C., Sepplorenzino, L., Nimmesgern, E., Ouerfelli, O., Danishefsky, S., Rosen, N., and Hartl, F.U. (1996). Pharmacologic shifting of a balance between protein refolding and degradation mediated by hsp90. Proc. Natl. Acad. Sci. USA *93*, 14536–14541.

Scholz, C., Stoller, G., Zarnt, T., Fischer, G., and Schmid, F.X. (1997). Cooperation of enzymatic and chaperone functions of trigger factor in the catalysis of protein folding. EMBO J. *16*, 54–58.

Schröder, H., Langer, T., Hartl, F.U., and Bukau, B. (1993). DnaK, DnaJ and GrpE form a cellular chaperone machinery capable of repairing heat-induced protein damage. EMBO J. *12*, 4137–4144.

Stoller, G., Rucknagel, 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*, 4939–4948.

Szabo, A., Langer, T., Schroder, H., Flanagan, J., Bukau, B., and Hartl, F.U. (1994). The ATP hydrolysis-dependent reaction cycle of the Escherichia coli Hsp70 system DnaK, DnaJ, and GrpE. Proc. Natl. Acad. Sci. USA *91*, 10345–10349.

Thulasiraman, V., Yang, C.F., and Frydman, J. (1999). In vivo newly translated polypeptides are sequestered in a protected folding environment. EMBO J. *18*, 85–95.

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*, 5494–5505.

Valent, Q.A., Degier, J.W.L., Vonheijne, G., Kendall, D.A., Tenhagenjongman, 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. Mol. Microbiol. *25*, 53–64.

Vickery, L.E., Silberg, J.J., and Ta, D.T. (1997). Hsc66 and Hsc20, a new heat shock cognate molecular chaperone system from Escherichia coli. Protein Sci. *6*, 1047–1056.

Yoshimune, K., Yoshimura, T., and Esaki, N. (1998). Hsc62, a new DnaK homologue of Escherichia coli. Biochem. Biophys. Res. Commun. *250*, 115–118.